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Emerging spread of β-thalassemia trait in Nigeria

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ABSTRACT

Background: Chronic anaemia mainly thalassemia and sickle cell anaemia are inherited disorders of haemoglobin. Presently about 7% of the world's populations are carriers of a potentially pathological haemoglobin gene. Sickle cell disease is a common haemoglobinopathy in Nigeria but recently cases of β-thalassemia traits are becoming prominent. This study aimed at screening for β-thalassemia in adults and children with chronic anaemia in Nigeria by assessing the patients' level of haemoglobin F, haemoglobin A_2 and red cell indices. **Materials and methods:** Haemoglobin F and HbA_2 were determined in the chronic anaemia patients by Alkaline Denaturation Method and Beta-Thal HbA_2 Quick Column Procedure respectively. Haemoglobin genotype was determined by Haemoglobin Electrophoresis at alkaline medium while Complete Blood count was estimated using Sysmex KX-2IN Autoanalyser. **Results:** The mean HbF, HbA_2, HCT, MCV, MCH and MCHC of the children and adults are 2.56 ± 0.46 and 2.45 ± 0.87 (%); 2.05 ± 0.25 and 1.89 ± 0.60 (%); 0.21 ± 0.31 and 0.21 ± 0.36 (L/L); 81.58 ± 12.59 and 78.69 ± 14.11 (fL); 22.74 ± 5.39 and 23.07 ± 7.36 (pg); 27.52 ± 3.84 and 31.23 ± 14.32 (g/l) respectively. Four percent (2 subjects) of each adult and children population had increased HbF level (>1.5%) and HbA_2 levels (>2.8%) and these subjects are composed of 2 children with haemoglobin genotype AA and two adult with haemoglobin genotypes SS. **Conclusions:** The outcome of this study reiterates the emergence of β-thalassemia traits and iron deficiency anaemia in different parts of Nigeria irrespective of their haemoglobin genotype status. This requires adequate specialized intervention for their diagnosis and treatment. There is therefore the need for subsequent molecular analysis to determine the β-thalassemia genes present in the studied community.

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Introduction

Anaemia is one of the most common disorders affecting humans in the world today and it still remains a problem not only in the developing countries, but also in developed countries [1]. Chronic anaemia is a form of anaemia which usually persists longer than two to six months. There are more than 400 possible causes of anaemia; effective treatment therefore depends on the underlying cause [2].

Inherited haemoglobin disorders are the most common genetic disorders, estimate by the World Health Organization to be carried by approximately 7% of the world's population [1]. Some of these diseases, particularly sickle-cell anaemia, and the more severe forms of thalassemia, cause life-threatening medical emergencies, chronic disability to families, and a major drain on health resources [3].

Beta thalassemias which are due to mutations in the HBB gene on chromosome 11, inherited in an autosomal-recessive fashion [4] results in reduced synthesis of beta chains, and a relative excess of α chains causing damage to the red cells leading to profound anaemia which in turn causes expansion of the ineffective marrow, with severe effects on development, bone formation, and growth [5].

Sickle beta-thalassemia (S/beta-thalassemia) is a condition which results from coinheritance of a sickle cell gene and a beta-thalassemia gene. The clinical phenotype depends on the type of beta-thalassemia gene (beta ($^+$) or beta ($^\circ$)) inherited. The clinical and haematological features have several similarities and these sometimes pose difficulty in correct diagnosis of the condition. A definitive diagnosis is required in order to initiate early supportive treatment in patients with homozygous sickle cell disease (SS disease) and to define the later clinical course [6].

In β°/β°, the haemoglobin produced is mainly HbF (98%) and HbA_2 (1.5%). There is a small amount of HbA (0.5%) when the genotype is β°/β^+ or $\beta^+\beta^+$. HbS/β° thalassemia resembles sickle cell anaemia. However, the MCV, and MCH are lower in HbS/β° and HbA_2 is raised [7]. This study screened for β-thalassemia by determining and correlating the haemoglobin A_2, haemoglobin F levels and red cell indices in children and adults with chronic anaemia.

Materials and methods

Subjects

A total of 100 chronic anaemic patients (comprising of 50 children ages Birth to 15 years and 50 adults ages 16 years and above) attending Haematology clinic or hospitalised in various male, female and children wards of Ladoke Akintola University Of Technology Teaching Hospital, Osogbo, Nigeria were recruited for this study within a period of 6 months. Informed consent was obtained from the patients or their parent. Ethical clearance was obtained from the Ethical committee of the Ladoke Akintola University of Technology Teaching Hospital, Osogbo, Osun State.

Methods

Five ml of venous blood was collected into an EDTA bottle. The Complete blood count (Haematocrit, MCV and MCH) was estimated with Sysmex KX-21N autoanalyser [8]. Cellulose Acetate Electrophoresis was performed to determine various genotypes of the patients [9]; HbF was estimated using the Alkaline Denaturation Method [10] while HbA_2 was estimated with the Beta-Thal HbA_2 Quick Column Procedure by Helena Laboratories (Catalogue No. 5341) [11, 12]. The Beta-Thal HbA_2 Quick Column is quantitative method in which 50 μl of whole blood collected was added to 200 μl of haemolysates reagent-C provided, mixed vigorously and allowed to stand at least 5 minutes for complete hemolysis to occur. Then 100 μl of the sample haemolysate was slowly applied to the Sickle-Thal quick column and another 100 μl of the sample preparation was added to a large collection tube labeled Total Fraction (TF) and filled up to 15 ml mark. The haemolysates appeared glossy when viewed from above until the sample is completely absorbed by the resin. Then 3.0 mL of Sickle-Thal A_2 developer (provided by the manufacturer) was slowly applied to the column, allowed passing through the column into a small collection tube (approximately 30 minutes to 1 hour) and this eluate contains the HbA_2. The percentage of HbA_2 was determined spectrophotometrically at 415 nm by measuring the absorbance of each eluate and each Total Fraction (TF).

Subjects with increased HbF (>1.5%) and increased HbA_2 (>2.8%, according to the Beta-Thal HbA_2 Quick Column Procedure Manufacturer) were considered to be indicative of β-thalassemia trait. Statistical analysis was done using the SPSS version 20. $P < 0.05$ denotes a significant difference.

Results

A total of 100 subjects comprising of 50 adults (28 males and 22 females) and 50 (26 males and 24 females) children with packed cell volume less than 25% were used in this study.

Table I shows the mean and standard deviation (SD) of the studied parameters and age. The mean HbF levels of the children and adults are 2.56 ± 0.46 and 2.45 ± 0.87 while their mean HbA_2 levels are 2.05 ± 0.25 and 1.89 ± 0.60 respectively.

Table II shows the cross tabulation of HbF and HbA_2 values in the children and Adult patients. The data obtained

Table I – Mean and standard deviation of the haematological parameters

Variable	Mean ± SD Children	Mean ± SD Adults	P value
PCV (l/l)	0.21 ± 0.31	0.21 ± 0.36	0.773
MCV (fL)	81.58 ± 12.59	78.69 ± 14.11	0.283
MCH (pg)	22.74 ± 5.39	23.07 ± 7.36	0.796
MCHC (g/l)	27.52 ± 3.84	31.23 ± 14.32	0.081
HbF (%)	2.56 ± 0.46	2.45 ± 0.85	0.442
HbA_2 (%)	2.05 ± 0.25	1.89 ± 0.60	0.181

Table II – Cross tabulation of HbA_2 and HbF in the children and adult patients

	HbA_2		P value
	HbA_2 <2.8%	HbA_2 >2.8%	
Children			
HbF<1.5	31	0	0.003
HbF ≥1.5	17	2	
Adults			
HbF <1.5	34	0	
HbF ≥1.5	14	2	

Table III – Comparison of HbA_2 levels with the MCV, MCH values in the Children and Adult subjects

	HbA_2		P value
	A_2 <1.8%	A_2 >1.8%	
Children			
<MCV (fL), MCH (pg)*	14	7	0.001
>MCV (fL), MCH (pg)**	21	8	
Total	35	15	
Adults			
<MCV (fL), MCH (pg)	8	6	0.001
>MCV (fL), MCH (pg)	29	7	
Total	37	13	

* <MCV (fL), MCH (pg) – decreased level of both mean cell volume (in femtolitre) and mean cell haemoglobin (in picogram).
** >MCV (fL), MCH (pg) – increased level of both mean cell volume (in femtolitre) and mean cell haemoglobin (in picogram).

showed that 31 children had HbF level <1.5% and HbA_2 <2.8%, 17 children had HbF ≥1.5% and HbA_2 <2.8% with just 2 children having HbF level >1.5% and HbA_2 levels >2.8% while 34 adult subjects had HbF level <1.5% and HbA_2 < 2.8%, 14 had HbF ≥1.5% and HbA_2 <2.8% with 2 adult subjects having HbF level >1.5% and HbA_2 levels >2.8%.

The relationship between the HbF and HbA_2 levels with the various Haemoglobin variants is represented in Figure 1. Subjects with reduced HbF (<1.5%) are 22 HbAA, 7 HbAS, 5HbSS adults and 21 HbAA, 7 HbAS and 3HbSS children while those with increased HbF (>1.5%) 12 HbAA, 1 HbAS, 6HbSS adults and 26 HbAA, 15 HbAS and 7HbSS children. Also, subjects with reduced HbA_2 (<2.8%) are 34 HbAA, 8 HbAS, 6HbSS adults and 26 HbAA, 15 HbAS and 7HbSS children while those with increased HbA_2 (>2.8%) are 2 SS adult and 2 AS children (Table III).

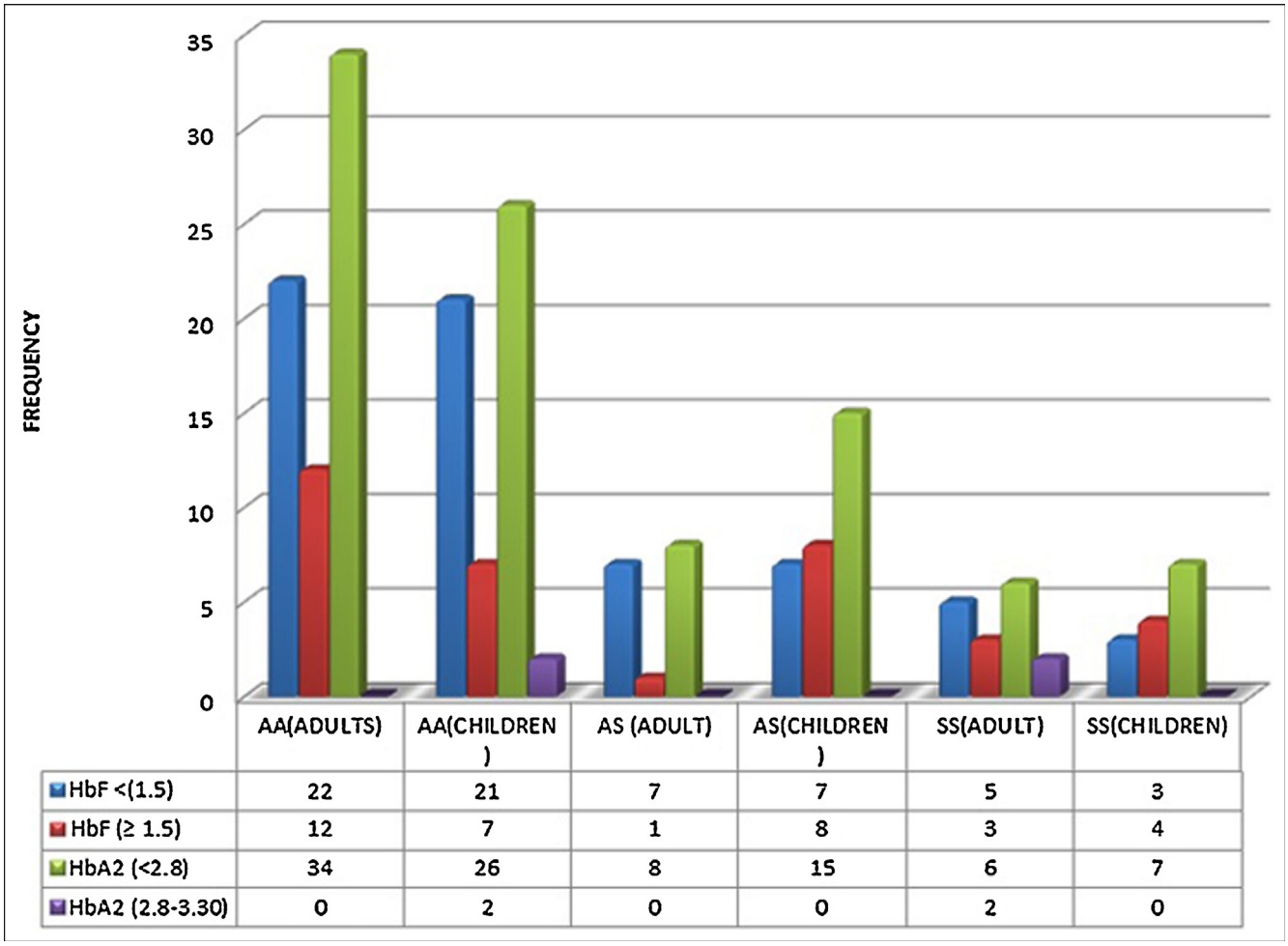

Fig. 1 – HbF, HbA_2 levels and the different haemoglobin variants of the subjects

Discussion

β-thalassemia is a major inherited haemoglobin disorder which is being misdiagnosed in this part of the world especially when presenting with major symptoms such as chronic anaemia. A diagnosis of thalassemia may be suspected based on complete blood count (CBC) and peripheral blood film examination, Haemoglobin Electrophoresis, Isoelectric focusing, Haemoglobin A_2 and Haemoglobin F quantification and Genetic testing [13]. However CBC, Haemoglobin Electrophoresis, Haemoglobin A_2 and Haemoglobin F quantification methods that are readily available in this setting were used for this study. It should also be noted that while molecular methods are now being used for the diagnosis of thalassemia, they are used for confirmation after the quantitation of HbA_2 and estimation of HbF levels as was done in this study and this criteria are also being used in resolving difficult cases. Therefore, our diagnostic methods and procedures are appropriate in this setting.

This study therefore screens for β-thalassemia in chronic anaemic adults and children based on increased level of HbF and HbA_2 and reduced red cell indices. Out of a total of 100 chronic anaemic subjects recruited for this study, 4 individuals comprising of 2 adults and 2 children had increased HbA_2 (>2.8%) and HbF (>1.5%) levels which is indicative of β-Thalassemia trait according to Kotila et al. [14]. A similar study carried out by Omoti in 2005, in which HbA_2 and HbF were estimated by modified Betke and micro column method respectively, in sickle cell anaemia patients was observed to have 6 patients (2.4%) out of 246 patients with increased HbA_2 (>3.5%) and HbF (>1.5%) [15].

HbSS subjects were observed to have the highest mean ± SD HbF level (2.09 ± 1.94%), followed by HbSC (0.85 ± 0.54%), HbAA (0.69 ± 0.46%), HbAS (0.52 ± 0.31%), and HbAC (0.57 ± 0.26%), with statistically significant differences among the haemoglobin types ($P < 0.05$) in a study conducted by Akanni et al. in 2011 [16]. This study also shows that the 4 subjects with thalassemia features (increased HbA_2 and HbF levels) in this study are composed of 2 female children with haemoglobin genotype AA, a male and a female adult with haemoglobin genotypes SS respectively.

In addition, increased HbF level (>1.5%) in this study was found to be higher in females than in males, with female HbAS subjects having the highest mean HbF level. Similar work was done by Mason et al. in 1982 [17], whereby the mean HbF levels was higher in females than in males, with HbSS and HbSC subjects having the highest mean HbF level. In addition, Mason's study in 1992 also shows that after the age of 10, HbF levels were consistently higher in females than in males.

The red cell indices play important roles and are widely used in the classification of anaemia. They are used in screening programme for the detection of carriers of β^+ thalassemia in population surveys. However it has been discovered that individual values for MCV, MCH and MCHC occasionally overlap with those in the normal population thereby casting doubt on the adequacy of these criteria alone in identifying all cases of the heterozygous thalassemia in various haemoglobin variants [18].

The lowest level of HbA_2 is in association with the most severe Iron deficiency anaemia. Iron and folate deficiency each suppressed HbA_2 levels in β-thalassemia heterozygous, microcytosis which is MCV <65.9 fl, HbA_2 (range 1.3–1.8) was useful in the differential diagnosis of Iron deficiency anaemia [19]. Hence, thalassemia, iron deficiency anaemia, and sickle cell disease remain the leading chronic forms of anaemia depending on the levels of foetal haemoglobin and haemoglobin A_2. Out of the 100 subjects analyzed, 14 children and 8 adults had reduced MCV (<77 fl), MCH (<21 pg) and reduced HbA_2 (1.3–1.8% according to the Helena Beta-Thal HbA_2 Quick Column) and these subjects are categorized to be Iron deficient anaemic subjects.

HbF and HbA_2 levels in conjunction with the red cell indices remains a reliable diagnostic tool in β-thalassemia diagnosis provided adequate diagnostic methods are engaged.

In conclusion, the result of the screening shows increased HbA_2 and HbF levels in 2 adult and children subjects each with different haemoglobin variants AA and SS. Further genetic tests are recommended to identify the mutations in those with features of thalassemia (with increased HbA_2 and HbF) among these patients who have already developed chronic anaemia.

Authors' contributions/ Wkład autorów

AEO – idea and design of the study, interpreted the findings in the study. ATT – data analysis and acquisition. BEO – data analysis and acquisition interpreted the findings in the study.

Conflict of interest/ Konflikt interesu

None declared.

Financial support/ Finansowanie

None declared.

Ethics/ Etyka

The work described in this article have been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans; EU Directive 2010/63/EU for animal experiments; Uniform Requirements for manuscripts submitted to Biomedical journals.

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