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Emerging spread of β-thalassaemia trait in Nigeria

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ABSTRACT

Background: Chronic anaemia mainly thalassaemia 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-thalassaemia traits are becoming prominent. This study aimed at screening for β-thalassaemia 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; (20.96 ± 3.56) and (21.15 ± 3.12); (78.69 ± 14.11) and (81.58 ± 12.59); (23.07 ± 7.36) (22.74 ± 5.39); (31.23 ± 14.32) and (27.52 ± 3.84) 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-thalassaemia 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-thalassaemia 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 thalassaemia, cause life-threatening medical emergencies, chronic disability to families, and a major drain on health resources [3].

Beta thalassaemias 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-thalassaemia (S/beta-thalassaemia) is a condition which results from coinheritance of a sickle cell gene and a beta-thalassaemia gene. The clinical phenotype depends on the type of beta-thalassaemia gene (beta (+) or beta (°)) 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 $\beta^{\circ}/\beta^{\circ}$, 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° thalassaemia 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-thalassaemia 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-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 min 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 labelled 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 min to 1 h) 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-thalassaemia 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 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,

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