

# Beta thalassaemia trait in western Nigeria

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## Abstract

**Background:** Genes for thalassaemia, haemoglobin S, Glucose-6-phosphate dehydrogenase which confer resistance to malaria are found in high frequencies in Nigeria, 25% of the population being carriers of the sickle cell trait while another 25% are hemizygous for the G6PD gene. The frequency of alpha thalassaemia is equally high among Nigerians but there is little information on beta thalassaemia in this population. A recent study however suggest a high prevalence of beta thalassaemia in the same population, hence the need for this study.

**Methods:** Haemoglobin A<sub>2</sub> and HbF were determined in healthy adults who have haemoglobin A genotype by elution after electrophoresis and alkaline denaturation methods respectively.

**Results:** The mean HbA<sub>2</sub> among the subjects was 3.3% (range 2.0-5.6%) while the mean HbF was 2.6% (range 0.4-8.8%). Twenty-six percent of the subjects had HbA<sub>2</sub> values higher than 3.9% while 86% had HbF values greater than 1%, twenty-four percent had elevated HbA<sub>2</sub> and HbF. The mean HbA<sub>2</sub> value was 2.7% among those with HbF <1%, 3.6% among those with HbF 1-3% and 3.1% among those with HbF >3%.

**Conclusion:** These findings confirm that the frequency of beta thalassaemia in western Nigeria is higher than previously thought and that many of the individuals studied may be silent carriers of the beta thalassaemia trait. Its presence may also have been masked by the high prevalence of alpha thalassaemia in the same environment. It is therefore important to consider beta thalassaemia trait as a differential diagnosis in patients who present with haemolytic anaemia in this environment

**Key words:** Beta thalassaemia trait, haemoglobin A<sub>2</sub>, haemoglobin F, silent carrier.

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## Introduction

Genes that confer resistance to malaria (including thalassaemia, haemoglobin S, glucose-6-phosphate dehydrogenase) are found in high frequencies in areas of high malaria endemicity. These genes differ in relative frequency depending on the location; the sickle cell gene is most prevalent in sub Saharan Africa while thalassaemia is most prevalent in the Mediterranean and Asia. The thalassaemias occur in a broad geographical area including the Mediterranean, Middle East, India and South-east Asia but they occur sporadically in other populations. The a<sup>0</sup> thalassaemias are found most commonly in the Mediterranean and Orientals but are extremely rare in Africa and the Middle East. The deletional forms of a<sup>+</sup> thalassaemia however occur with great frequency throughout West Africa, the Mediterranean, the Middle East and South East Asia.

Earlier studies in West Africa showed a beta thalassaemia trait frequency of about 9% in Liberia<sup>1</sup> and similar frequencies have been reported from Ghana<sup>2</sup>

and Ivory Coast.<sup>3</sup> These findings contrasted sharply with a reported prevalence of only 0.8% in Nigeria,<sup>4</sup> despite the fact that all these countries share the same climate and have very similar levels of malaria endemicity and also, 25% of the Nigerian population are carriers of the sickle cell trait while another 25% are hemizygous of the G6PD gene.<sup>5,6</sup> In view of this disparity and the suggestion by a recent study of a high prevalence of beta thalassaemia among sickle cell trait persons who present with symptoms of a haemoglobinopathy<sup>7</sup>, this study was designed to re-assess the presence of beta thalassaemia among Nigerians.

## Subjects and Methods

The subjects were fifty healthy Nigerian adults living in Ibadan, a large city in western Nigeria. Malaria is holoendemic in the area and the prevalence of the sickle cell trait (Hb A + S) in the populations is about 25%. Inclusion criteria included: (i) apparently healthy adult, (ii) no known chronic illness, (iii) no known risk factor for iron deficiency such as chronic bleeding, peptic ulcer disease and pregnancy, (iv) haemoglobin A type by electrophoresis, (v) verbal consent to participate in the study. Only those with haemoglobin type A were studied in order to avoid the technical difficulty of separating HbS from HbA<sub>2</sub> on electrophoresis.<sup>8</sup> Individuals with true heterozygous states are almost symptom free except

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when in double heterozygous state (such as Hb S/ $\beta$  thalassaemia) or during periods of stress (e.g. pregnancy). Therefore, this study was conducted among healthy adults only.

HbA<sub>2</sub> level was determined by elution after electrophoresis on cellulose acetate paper while HbF determination was by the alkaline denaturation method.<sup>9</sup> The coexistence of an elevated HbA<sub>2</sub> level (> 3.9%) with an elevated HbF level (>1%) was considered indicative of classical  $\beta$  thalassaemia trait.<sup>8,10</sup>

## Results

The 50 subjects comprised 25 women and 25 men. Their haematocrit ranged from 0.31 – 0.49 with a mean haematocrit of 0.388 (SD 0.044). The mean HbA<sub>2</sub> among the subjects was 3.3% (SD 0.8%) with a range of 2.0-5.6%. Thirteen (26%) of the subjects had HbA<sub>2</sub> values higher than 3.9%. The mean HbF was 2.6 (SD 1.7%) with a range 0.4-8.8%. Forty-three (86%) of them had HbF values greater than 1%. Only 13 (26%) had values over 3% while 7 (14%) had values below 1%.

Twenty four percent (12/50) had both elevated HbA<sub>2</sub> and HbF (Table). The mean HbA<sub>2</sub> value was 2.7% among those with HbF <1%, 3.6% among those with HbF 1-3% and 3.1% among those with HbF >3%.

**Table 1: Cross tabulation of HbA<sub>2</sub> values versus HbF values**

	HbF (%)	
	£ 1	> 1
HbA <sub>2</sub> (%)	< 4	6(12%) 31(62%)
	<sup>3</sup> 4	1(2%) 12(24%)

## Discussion

This study which shows that the beta thalassaemia trait in western Nigeria is higher than previously thought was based on elevated HbA<sub>2</sub> and HbF levels. Laboratory diagnosis of the beta thalassaemia trait depends primarily on the quantitation of HbA<sub>2</sub> in haemolysates,<sup>11</sup> red cell indices and the level of HbF are also used in conjunction with elevated HbA<sub>2</sub> in making a diagnosis. Red cell indices in iron deficiency and alpha thalassaemia trait will give a similar picture as found in beta thalassaemia trait and so may not be as informative in an environment in which the three pathologies exist concurrently. Various methods are available for the quantitation of HbA<sub>2</sub>, but traditionally electrophoresis is still the method of choice even though it is slow and labour intensive.<sup>11</sup> HPLC which is a better

technology is not without its intrinsic interpretive problems, like in electrophoretic method HbA<sub>2</sub> may be falsely increased by the presence of HbS adducts, it is also expensive. In order to avoid falsely high levels of HbA<sub>2</sub> only patients with HbA were used for this study. It should be noted that while techniques based on the polymerase chain reaction (PCR) are now used for the diagnosis of thalassaemia, they are usually used for confirmation only *after* the quantitation of HbA<sub>2</sub> from cellulose acetate membranes and estimation of HbF levels as was done in this study or in resolving difficult cases. Thus, our diagnostic criteria are appropriate in our setting.

Beta thalassaemia is a heterogeneous disorder which is often classified into classical (characterised by elevated levels of HbA<sub>2</sub> and HbF) and silent (normal HbA<sub>2</sub>  $\pm$  elevated HbF) types.<sup>10</sup> Twelve individuals or 24% of the subjects in this study satisfied the criteria for the diagnosis of classical beta thalassaemia, i.e. high levels of both HbA<sub>2</sub> and HbF. In addition, 31 other individuals (62%) had raised HbF levels but normal levels of HbA<sub>2</sub>, which suggests silent beta thalassaemia trait. These findings suggest that beta thalassaemia may be much more frequent in this locality than previously thought, being much more than the 0.8% reported by Esan in 1970.<sup>4</sup> In the study by Esan only haemolysates found to have elevated HbA<sub>2</sub> on visual inspection were quantified unlike the present study in which HbA<sub>2</sub> was quantified in all samples. This may account for the differences observed.

The frequency of alpha thalassaemia is high in the same population and alpha thalassaemia is known to ameliorate the severity of homozygous beta thalassaemia by reducing the degree of imbalance in alpha and beta globin chain synthesis.<sup>12,13</sup> This imbalance also increases the haemoglobin content of the red cells in the heterozygous state thus making the patients symptom-free. The high frequency of alpha thalassaemia will also affect the diagnosis of beta thalassaemia by distorting the picture of the red cell indices. These will thus explain the difficulties in making this diagnosis before now. Similarly hypochromic and microcytic red cells which are often seen on review of peripheral film in this environment and sometimes thought to be due to iron deficiency are often found to be unresponsive to iron therapy which will suggest the presence of another pathology.

Iron deficiency is a known cause of low levels of HbA<sub>2</sub> in individuals with beta thalassaemia and this might explain the low HbA<sub>2</sub> found in those with HbF greater than 3% in this study. However, iron studies were

not done in this study and, thus, no firm conclusions can therefore be drawn. PCR analysis may have resolved some of these issues but these techniques are not yet commonly available in our setting.

In conclusion, this study has demonstrated that beta thalassaemia trait may be more common than previously thought in western Nigeria. It is therefore important to consider beta thalassaemia trait as a differential diagnosis in patients who present with haemolytic anaemia in this environment. Future studies using molecular methods on an epidemiological level will enable the precise delineation of the true thalassaemias in the population.

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