*Afr. J. Med. med. Sci.* (2013) 42, 355-358

# *Beta thalassaemia in Nigeria: Myth or Fact?

**TR Kotila**

*Department of Haematology, College of Medicine, University of Ibadan, Ibadan, Nigeria*

**Abstract**

*Background*: The high prevalence of sickle cell disorders and the mild deletional α-thalassaemia among Nigerians is well known, but β-thalassaemia is believed to be almost nonexistent. Beta thalassaemia trait (BTT) was screened for in patients with unexplained recurrent haemolytic anaemia and healthy individuals.

*Methods:* β-thalassaemia trait (BTT) was screened for using MCH and $HbA_2$ of 27pg and 3.5% respectively as cut off in 151 Nigerians which included 29 patients and 122 apparently healthy individuals. The subjects were categorized into four, Group I (high $HbA_2$, low MCH), Group II (low $HbA_2$ and MCH), Group III (high $HbA_2$ and high/normal MCH) and Group IV (normal $HbA_2$ and MCH).

*Results:* Group I are possible carriers of BTT while group IV are least likely to carry either alpha or beta thalassaemia genes. There were 36 (26%), 39 (28%), 27 (19%) and 38 (27%) in groups I-IV respectively. The mean MCH, MCV, haematocrit and $HbA_2$ for the study population were 26±2.8, 81±7.9, 37.5±6.8 and 3.4±1.7 respectively. The mean MCV and haematocrit were significantly lower for group I compared to group IV (76.9 Vs 86.6, p=0.00) and (36.5Vs39.7, p=0.03) respectively. Group II had significantly lower MCV and haematocrit than group IV (75.4(p=0.00) and 36.4(p=0.01) respectively. There was a positive correlation between the MCH and MCV with the haematocrit (p=0.004, p=0.001 respectively) but $HbA_2$ showed a stronger negative correlation with the haematocrit (p<0.0001).

*Conclusion:* This does not only show the presence of BTT, but a higher prevalence than previously thought, mutations responsible for it should therefore be characterized.

**Keywords:** *β-thalassaemia, red cell indices, haemoglobin, haemolytic anaemia*

**Résumé**

*Contexte*: La prévalence élevée de la drépanocytose troubles et de mild deletional α-thalassémie entre Nigérians est bien connue, mais β-thalassémie est presque inexistant. Version bêta thalassémie attribut (TTC) a été projeté pour les patients avec récurrentes inexpliquées anémie hémolytique et individus en bonne santé.

*Méthodes: une taxe* a été projeté pour l'utilisation SMI et HBA2 de 27pg et 3,5 % respectivement, de couper de 151 Nigérians, qui comprenait 29 patients et 122 individus apparemment en bonne santé. Les sujets ont été répartis en quatre, le Groupe I (HbA2 élevée, faible SMI), Groupe II (faible HbA2 & SMI), du groupe III (haute HbA2 & haute/normal SMI) et du groupe IV (normal HbA2 & SMI).

*Résultats : Groupe I* sont possibles les transporteurs de la TOC tandis que le groupe IV sont moins susceptibles de transporter alpha ou bêta thalassémie gènes. Il y a eu 36 (26%), 39 (28%), 27 (19%) et 38 (27%) dans les groupes I-IV, respectivement. La moyenne SMI, MCV, hématocrite et HBA2 pour la population de l'étude était de 26 ± 2,8 , 81 ± 7,9 , 37,5 ± 6,8 et 3,4 ± 1,7 respectivement. Les MCV et hématocrite étaient sensiblement inférieurs pour le groupe I par rapport au groupe IV (76,9 vs 86,6 , p=0.00 ) et ( 36,5 Vs39.7, p=0.03 ) respectivement. Groupe de travail II avait sensiblement inférieur MCV et de l'hématocrite de groupe IV (75,4 (p=0.00 ) et 36,4 (p=0.01 ) respectivement. Il y avait une corrélation positive entre la santé maternelle et infantile et MCV avec l' hématocrite (p=0.004 , p=0.001 respectivement) mais HbA2 a montré une forte corrélation négative avec l'hématocrite (p < 0,0001 ).

*Conclusion : Ce* n'est pas seulement montrer la présence de la TOC, mais une prévalence plus élevée qu'on ne le pensait auparavant, des mutations responsables devraient donc être caractérisée.

## Introduction

The thalassaemias are a group of inherited disorders of globin chain synthesis with a high incidence in many parts of the world. The geographical overlap between endemic malaria and the thalassaemias establishes the hypothesis that the thalassaemias are a consequence of malarial selection. Other genetic diseases associated with the malaria hypothesis include sickle cell disease and Glucose-6-phosphate

Correspondence: Dr. T.R. Kotila, Department of Haematology, College of Medicine, University of Ibadan, Ibadan, Nigeria.
E-mail: tkotila@comui.edu.ng

**This article has been published in our last edition, Vol.42 No.3 pages 261 to 264 but due to errors of symbols it has to be republished in this issue. The errors are regretted.*

frequency of 0.008 [4]. The disparity in the prevalence of β-thalassaemia and other genetic disorders associated with the malaria selection is surprising more especially since some Nigerian patients have presented with symptoms and signs of a haemoglobinopathy in the absence of sickle cell disease [5]. Alpha thalassaemia also does not explain these symptoms since only the $-\alpha^{3.7}$ deletion which is of little or no clinical significance is observed in Nigerians [3,6]. This discrepancy has led to the misdiagnosis of β-thalassaemia and thus subjecting such patients to unnecessary investigations. The high prevalence of hypochromic microcytic anaemia in patients is thought to be due to iron deficiency despite normal iron studies in the general populace [7,8] and failure of most of these patients to respond to iron therapy. The presence of BTT was sought for in some patients referred for recurrent haemolytic anaemia and some apparently healthy individuals by screening them using red cell indices and $HbA_2$.

## Materials and methods

The study population included 29 patients who were referred on account of unexplained recurrent haemolytic anaemia and 122 apparently healthy individuals who were mostly blood donors.

*Haematologic parameters*

All the subjects were screened for β-thalassaemia using red cell indices (MCV and MCH) obtained by automation from a particle counter (Sysmex KX-ZI), a fully automated 3-part differential cell counter. $HbA_2$ was estimated by electrophoresis using cellulose acetate membrane at pH 8.6; $HbA_2$ was separated from the other haemoglobins and eluted into a buffer. The absorbance of $HbA_2$ and the other haemoglobins were measured at 413nm and the amount of $HbA_2$ was calculated as a percentage of the total haemoglobin. The MCH and $HbA_2$ were used to categorize all the subjects into four groups, the cut off used for the MCH and $HbA_2$ are 27pg and 3.5% respectively. Group I (high $HbA_2$, low MCH), Group II (low $HbA_2$ and MCH), Group III (high $HbA_2$ and MCH) Group IV (Normal $HbA_2$ and MCH).

## DNA analysis

DNA was extracted from peripheral blood leukocytes of the 151 individuals by standard methods [9,10]. All the samples were tested for the $-\alpha^{3.7}$ deletion, 43 and 12 individuals (which included ten and six patients respectively) who were normal for the $-\alpha^{3.7}$ deletion were tested for the $-\alpha^{4.2}$ deletion and the triple gene rearrangement respectively (The results of which has been previously reported) [6].

## Results

Individuals with HbC band on electrophoresis were excluded from the analysis because of the technical difficulty of quantifying $HbA_2$ in them, therefore only results of 140 individuals were analysed.

There were 36 (26%), 39 (28%), 27 (19%) and 38(27%) in groups I-IV respectively, group I are individuals with low MCH (<27pg) and high $HbA_2$ (>3.5%) and were designated possible carriers of the β-thalassaemia trait, group II have both low MCH and $HbA_2$ and were designated likely carriers of α-thalassaemia while group III with high $HbA_2$ and MCH may be silent carriers of the β-thalassaemia trait and group IV with low $HbA_2$ and high (normal) MCH are likely to be "normal". The mean MCH, MCV, haematocrit and $HbA_2$ for the study population were 26±2.8, 81±7.9, 37.5±6.8 and 3.4±1.7 respectively while that for the "normal" group are 28±1.2, 86±4.1, 39±4.1 and 2.0±0.8 respectively which could be taken as reference range for the Nigerian population. The mean MCV and haematocrit were significantly lower for the β-thalassaemia group than for the "normal" group (76.9 Vs 86.6, p=0.00) and (36.5Vs39.7, p=0.03) respectively. Similarly, the α-thalassaemia group had significantly lower MCV and haematocrit than the "normal" group (75.4(p=0.00) and 36.4(p=0.01) respectively. All those in group I have low MCV (<80fl), 59% of group II have low MCV, while all those in groups III and IV have normal MCV (>80fl). Two people who had high MCV (≥"100) were in group III. There were however no significant difference between those who are supposedly silent carrier and the "normal" group. The result of the alpha thalassaemia status is compared with the β-thalassaemia grouping in Table 1. The α-thalassaemia genotypes of groups I and II (β and α-thalassaemia groups (groups I and II) are similar but contrast sharply with those of groups III and IV (silent carrier and "normal" groups) (Table 1). Ninety percent of the patients were either in the alpha or β-thalassaemia group (Table 2). There was a positive correlation between the MCH and MCV with the haematocrit (p=0.004, p=0.001 respectively) while $HbA_2$ showed a stronger negative correlation with the haematocrit (p<0.0001). In order to control for α-thalassaemia as a confounding factor correlation was sought for among individuals who are normal for the $-\alpha^{3.7}$ deletion, the MCV and MCH still showed good positive correlations, but with low positive correlations and outliers at both extremes of the scatter plot while high positive correlations were observed around MCH of 26-30 and MCV of 80-90. The level of $HbA_2$ was not affected by the

haemoglobin type, 51% of HbAS individuals had haemoglobin type, 51% of HbAS individuals had elevated $HbA_2$ which is similar to 53% of people with HbAA. Twenty one percent of all the people with elevated $HbA_2$ have HbAS.

$HbA^2$ [11]. The MCV has also been found to correctly predict the severity of the β-thalassaemia mutation [12] thus making MCV a guide to the mutation and a phenotypic characteristic related directly to the genotype. Thus using both red cell

**Table 1:** The alpha thalassaemia status of the different groups

| Alpha thalassaemia status | Heterozygote(-α/αα) | Homozygote(-α/-α) | Normal | Total |
|---|---|---|---|---|
| Group I(β Thal) | 17 | 5 | 11 | 33 |
| Group II(α Thal) | 19 | 6 | 12 | 37 |
| Group III('Silent Carriers') | 4 | 2 | 17 | 23 |
| Group IV('Normal') | 10 | 0 | 26 | 36 |
| Total | 50 | 13 | 66 | 129 |

*Alpha thalassaemia status was not determined in 11 individuals because of low DNA concentration*

**Table 2:** Mean haematological parameters of the patient group

| Thalassaemia Groups | Number of Cases | MCH (Fl) | MCV (pg) | Haematocrit (%) | RDW | HbA2 |
|---|---|---|---|---|---|---|
| Alpha Thal | 14 | 23.8 | 70.2 | 31.1 | 43.3 | 2.7 |
| Beta Thal | 12 | 22.2 | 71.9 | 31.2 | 50.6 | 4.6 |
| Silent Carrier | 2 | 28.1 | 86.4 | 18.5 | 65.1 | 7.7 |
| Normal | 1 | 30.6 | 86.3 | 34.0 | 47.2 | 3.0 |

## Discussion

The results may appear not to have shown a clear cut demarcation of the patients or "healthy" group into beta or α-thalassaemia groups using red cell indices and $HbA_2$ as screening parameters. This will suggest the high likelihood of a great overlap between the groups which is not unexpected. With a high prevalence of α-thalassaemia in the same region [3], it is expected that the phenotypic expression of β-thalassaemia will be masked by coinheritance of α-thalassaemia which may partly explain the difficulty in making clinical diagnosis of β-thalassaemia in the region before now. The high prevalence of sickle cell disease in the same environment and its similarity to β-thalassaemia in its clinical manifestations may further compound the delay in diagnosis, in a setting with inadequate diagnostic tools.

Red cell indices is an accepted screening criterion in the diagnosis of β-thalassaemia especially now that cell counters are widely used in routine practice, it is often used as a screening tool before the application of molecular diagnostic techniques where such facilities are available. Successful prevention programs for BTT in Greece and Italy have also relied on screening by red cell indices and indices and $HbA_2$, BTT was found to be as high as 26% in the Nigerian population, a similar prevalence of 26% was obtained when fifty healthy Nigerians were screened for β-thalassaemia using $HbA_2$ and HbF only. The cut off for the diagnosis in the later study was $HbA_2$ and HbF of greater than 3.9% and 1% respectively [13], all these individuals were asymptomatic at the time of diagnosis.

There are variations in the levels of $HbA_2$ in heterozygous β-thalassaemia as was observed in this study, ranging from those with unusually high levels of $HbA_2$ to those with isolated elevated haemoglobin $A_2$ levels. Sole Elevation of haemoglobin $A_2$ levels is not uncommon in BTT, as discussed by Weatherall and Clegg, coinheritance of α with β thalassaemia may result in normalization of the red-cell indices and balanced globin synthesis, thus leaving a raised $HbA_2$ as the sole abnormality [14]. The greater overlap between groups I and II may be as a result of coinheritance of both α and β-thalassaemia in these groups, it is also known that iron deficiency may lower the level of $HbA_2$ therefore resulting in the possibility of carriers of β-thalassaemia trait to be included in group II. The outliers in both extremes of the scatter plot are individuals who though normal for $-\alpha^{3.7}$ deletion have another abnormality causing a

low or high MCH and MCV such as β-thalassaemia. The high MCV may result from megaloblastic haemopoesis which is not uncommon in haemolytic anaemias, this result from folate deficiency which is in turn due to the recurrent haemolysis.

Therefore it can be said that the prevalence of Beta thalassaemia trait is higher than previously thought, DNA analysis would be required to determine the mutations responsible in this population since these may differ from mutations in other populations. It is also necessary to consider β-thalassaemia as a differential diagnosis and investigate for such in Nigerian patients who present with anaemia especially in the presence of microcytosis and hypochromia.

**Acknowledgments**

This work was supported by the centenary fellowship of the Royal Society of Tropical Medicine and Hygiene.

**References**

1. Omotade OO, Kayode CM, Falade SL, *et al.* Routine screening for sickle cell haemoglobinopathy by electrophoresis in an infant welfare clinic. West Afr J Med.1998;17:91-94.
2. Ademowo OG and Falusi AG. Molecular epidemiology and activity of erythrocyte G6PD variants in a homogenous Nigerian population. E Afr Med J 2002;79:42-44.
3. Falusi AG, Esan GJF, Ayyub H and Higgs DR. α Thalassaemia in Nigeria: its interaction with sickle cell disease. Eur J Haematol. 1987; 38:370-375.
4. Esan GJF. The thalassaemia syndromes in Nigeria. Br J Haematol. 1970; 19:47-56.
5. Kotila TR. When the inheritance of two heterozygote states become a diagnostic problem: Misdiagnosis of the sickle cell trait. Nig. J Med. 2007;16 No2: 173-176.
6. Kotila TR. Phenotypic and genotypic expression of alpha thalassaemia in Ibadan, Nigeria. AfrJ Med med Sci 2012; 41: 283-287.
7. Oluboyede OA and Williams AI. Serum ferritin and other iron studies in adult Nigerians with chronic renal failure-review of management of anaemia. Afr J Med Med Sci 1995;24:231-237.
8. Abudu OO, Maculay K and Oluboyede OA. Serial evaluation of iron stores in pregnant Nigerians with hemoglobin SS or SC. J Natl Med Assoc. 1990;82:41-48.
9. Goossens M and Kan YW. DNA analysis in the diagnosis of hemoglobin disorders. Methods Enzymol 1981;76:805-817.
10. Miller SA, Dykes DD and Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acid Res 1988;16:1215.
11. Tan GB, Aw TC, Dunstan RA *et al.* Evaluation of high performance liquid chromatography for routine estimation of hemoglobins A2 and F. J Clin Pathol. 1993;46: 852-856.
12. Rund D, Filon D, Strauss N, *et al.* Mean corpuscular volume of heterozygotes for β-Thalassaemia correlates with the severity of mutations. Blood1992;79:238-243.
13. Kotila TR, Adeyemo AA, Mewoyeka OO and Shokunbi WA. Beta Thalassaemia trait in Western Nigeria. Afr. Health Sci 2009;9:46-49.
14. Weatherall DJ and Clegg JB. The Thalassaemia Syndromes. Oxford, England, Blackwell Scientific, 2001.

Received: 30/05/13
Accepted: 14/08/13