

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 $\hat{\alpha}$ -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: $\hat{\alpha}$ -thalassaemia trait (BTT) was screened for using MCH and HbA₂ 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 HbA₂, low MCH), Group II (low HbA₂ and MCH), Group III (high HbA₂ and high/normal MCH) and Group IV (normal HbA₂ 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 HbA₂ for the study population were 26 \pm 2.8, 81 \pm 7.9, 37.5 \pm 6.8 and 3.4 \pm 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 HbA₂ 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: $\hat{\alpha}$ -thalassaemia, red cell indices, haemoglobin, haemolytic anaemia

Résumé

Contexte: La prévalence élevée de la drépanocytose troubles et de mild deletional α -thalassémie entre Nigériens est bien connue, mais

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

$\hat{\alpha}$ -thalassémie est presque inexistant. Version bêta thalassémie attribut (TTC) a été projeté pour les patients avec récurrentes inexplicables 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 Nigériens, 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 \pm 2,8 , 81 \pm 7,9 , 37,5 \pm 6,8 et 3,4 \pm 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 dehydrogenase (G6PD) deficiency. All these genetic disorders have a high frequency in the Nigerian population with a gene frequency of 0.25-0.30 [1-3] in the heterozygote state except $\hat{\alpha}$ -thalassaemia trait (BTT) which before now was thought to have a gene

frequency of 0.008 [4]. The disparity in the prevalence of $\hat{\alpha}$ -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 $-\hat{\alpha}^{3,7}$ deletion which is of little or no clinical significance is observed in Nigerians [3,6]. This discrepancy has led to the misdiagnosis of $\hat{\alpha}$ -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 HbA₂.

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 $\hat{\alpha}$ -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. HbA₂ was estimated by electrophoresis using cellulose acetate membrane at pH 8.6; HbA₂ was separated from the other haemoglobins and eluted into a buffer. The absorbance of HbA₂ and the other haemoglobins were measured at 413nm and the amount of HbA₂ was calculated as a percentage of the total haemoglobin. The MCH and HbA₂ were used to categorize all the subjects into four groups, the cut off used for the MCH and HbA₂ are 27pg and 3.5% respectively. Group I (high HbA₂, low MCH), Group II (low HbA₂ and MCH), Group III (high HbA₂ and MCH) Group IV (Normal HbA₂ 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 $-\hat{\alpha}^{3,7}$ deletion, 43 and 12 individuals (which included ten and six patients respectively) who were normal for the $-\hat{\alpha}^{3,7}$ deletion were tested for the $-\hat{\alpha}^{4,2}$ 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 HbA₂ 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 HbA₂ (>3.5%) and were designated possible carriers of the $\hat{\alpha}$ -thalassaemia trait, group II have both low MCH and HbA₂ and were designated likely carriers of $\hat{\alpha}$ -thalassaemia while group III with high HbA₂ and MCH may be silent carriers of the $\hat{\alpha}$ -thalassaemia trait and group IV with low HbA₂ and high (normal) MCH are likely to be "normal". The mean MCH, MCV, haematocrit and HbA₂ 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 $\hat{\alpha}$ -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 $\hat{\alpha}$ -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 (\geq 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 $\hat{\alpha}$ -thalassaemia grouping in Table 1. The $\hat{\alpha}$ -thalassaemia genotypes of groups I and II ($\hat{\alpha}$ and $\hat{\alpha}$ -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 $\hat{\alpha}$ -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 HbA₂ showed a stronger negative correlation with the haematocrit (p<0.0001). In order to control for $\hat{\alpha}$ -thalassaemia as a confounding factor correlation was sought for among individuals who are normal for the $-\hat{\alpha}^{3,7}$ 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 HbA₂ was not affected by the

haemoglobin type, 51% of HbAS individuals had elevated HbA₂ which is similar to 53% of people with HbAA. Twenty one percent of all the people with elevated HbA₂ have HbAS.

HbA² [11]. The MCV has also been found to correctly predict the severity of the $\hat{\alpha}$ -thalassaemia mutation [12] thus making MCV a guide to the mutation and a phenotypic characteristic related

Table 1: The alpha thalassaemia status of the different groups

Alpha thalassaemia status	Heterozygote(- $\hat{\alpha}$ / $\hat{\alpha}$)	Homozygote(- $\hat{\alpha}$ / $\hat{\alpha}$)	Normal	Total
Group I($\hat{\alpha}$ Thal)	17	5	11	33
Group II($\hat{\alpha}$ 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 (FI)	MCV (pg)	Haematocrit (%)	RDW	HbA ₂
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 $\hat{\alpha}$ -thalassaemia groups using red cell indices and HbA₂ 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 $\hat{\alpha}$ -thalassaemia in the same region [3], it is expected that the phenotypic expression of $\hat{\alpha}$ -thalassaemia will be masked by coinherance of $\hat{\alpha}$ -thalassaemia which may partly explain the difficulty in making clinical diagnosis of $\hat{\alpha}$ -thalassaemia in the region before now. The high prevalence of sickle cell disease in the same environment and its similarity to $\hat{\alpha}$ -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 $\hat{\alpha}$ -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

directly to the genotype. Thus using both red cell indices and HbA₂, 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 $\hat{\alpha}$ -thalassaemia using HbA₂ and HbF only. The cut off for the diagnosis in the later study was HbA₂ 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 HbA₂ in heterozygous $\hat{\alpha}$ -thalassaemia as was observed in this study, ranging from those with unusually high levels of HbA₂ to those with isolated elevated haemoglobin A₂ levels. Sole Elevation of haemoglobin A₂ levels is not uncommon in BTT, as discussed by Weatherall and Clegg, coinherance of $\hat{\alpha}$ with $\hat{\alpha}$ thalassaemia may result in normalization of the red-cell indices and balanced globin synthesis, thus leaving a raised HbA₂ as the sole abnormality [14]. The greater overlap between groups I and II may be as a result of coinherance of both $\hat{\alpha}$ and $\hat{\alpha}$ -thalassaemia in these groups, it is also known that iron deficiency may lower the level of HbA₂ therefore resulting in the possibility of carriers of $\hat{\alpha}$ -thalassaemia trait to be included in group II. The outliers in both extremes of the scatter plot are individuals who though normal

for α^{37} deletion have another abnormality causing a low or high MCH and MCV such as α -thalassaemia. The high MCV may result from megaloblastic haemopoiesis 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 α -thalassaemia as a differential diagnosis and investigate for such in Nigerian patients who present with anaemia especially in the presence of microcytosis and hypochromia.

Acknowledgment

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. *Afr J 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 α -Thalassaemia correlates with the severity of mutations. *Blood* 1992;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