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Foetal haemoglobin levels in sickle cell anaemia in Nigerians

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Summary

Many patients with sickle cell anaemia (SCA) are known to synthesize increased amounts of foetal haemoglobin (Hb F). In some situations, the levels attained are so high that the course of the disease is ameliorated since Hb F does not participate in the polymerization process characteristic of the sickling phenomenon. It has also been reported that the simultaneous inheritance of an α-thalassaemia gene reduces the severity of SCA. We have examined the levels of Hb F in relation to the erythrocyte indices and the coinheritance of the deletion type α-thalassaemia in SCA patients in Nigeria. The concentration of Hb F in peripheral blood was measured by the alkali denaturation technique of Betke *et al.* [15], whilst erythrocyte indices were determined on a Coulter S plus II counter. Alpha-thalassaemia was detected by the restriction endonuclease analysis of DNA obtained from peripheral white blood cells (WBC) and nucleated red cells using α-globin gene-specific probes.

The mean Hb F level in 130 SCA subjects was 5.9 ± 3.8% (range 0.9–16%). Males had significantly lower levels than females. Hb concentration, haematocrit, and Hb A_2 did not differ in subjects with Hb F levels lower than 2% (Group I) when compared with those whose Hb F levels were higher than 8% (Group II). The mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH) were lower in Group I. Globin analysis in 30 of these subjects showed that 20 had four, eight had three, and two had two α-globin genes. The mean Hb F level (6.4 ± 3.7%) in the four alpha-gene group was significantly higher than that in the three alpha-gene group (2.7 ± 1.6%). These findings did not influence the severity of the disease.

Résumé

Il arrive souvent que les patients atteint de l'anémie du drépanocyte synthétisent des quantités encore plus importantes d'hémoglobine foetal. Parfois les niveaux atteints sont assez et si élevés pour influer sur le cours de la maladie puisque Hb F ne participe pas au processus de polymérisation qui caractérise le phénomène du drépanocyte. Il a été également rapporté que l'héritage simultané du gène de l'alpha thalassémie réduit la sévérité du drépanocyte. Nous avons examiné le rapport entre les niveau du Hb F en relation aux indices d'érythrocyte et à la présence du type 'suppression' d'alpha thalassémie dans les patients de l'anémie du drépanocyte au Nigéria. On a mesuré la concentration du Hb F dans le sang périphérique en utilisant la méthode de la dénaturation de l'alkali de Betke *et al.* [15] tandis que les indices d'erythrocyte ont été déterminés sur un compteur Coulter S plus II. Nous avons détécté l'alpha thalassémie par l'analyse de restriction d'endonucléase obtenu à partir du WBC péripherique et des cellules rouges nucléés en utilisant les sondes spécifiques des gènes d'alpha globine.

Le niveau moyen de Hb F chez les 130 cas du drépanocyte était de 5.9 ± 3.8% (allant de 0.9 à 16%). Les niveaux étaient plus bas chez les hommes. Il n'y avait pas de différence dans la concentration d'Hb, de l'hématocrit et de l'Hb A_2 quand on a comparé des cas aux niveaux de Hb F audessous de 2% (Groupe I) à ceux dont les niveaux de Hb F étaient au-dessus de 8% (Groupe II). Les MCV et MCH étaient plus bas dans le groupe I. L'analyse du gène de globine des 30 de ces cas a donné les résultats suivants;

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20 d'entre eux avaient quatre gènes d'alpha globine, huit en avaient trois, et deux en avaient deux. Le niveau moyen d'Hb F (6.4 ± 3.7%) dans le groupe de quatre gènes d'alpha globine était beaucoup plus élevé que celui du groupe de trois gènes d'alpha globine (2.7 ± 1.6%). Ces données ni'influent pas sur la sévérité de la maladie.

Introduction

The sickle haemoglobin occurs in very high frequencies across a wide belt in tropical Africa. In Nigeria, heterozygote frequencies are as high as 25% [1,2], while sickle cell anaemia (SCA) is the commonest genetic disorder in the population. The clinical manifestations have been found to vary tremendously among the patients [3] and this has led to attempts to unravel the causes of this variation. Extrinsic and intrinsic factors are known to ameliorate the severity of SCA. The extrinsic factors include the social class of the patient [4], which in turn determines the access to medical care, and the presence or absence of infections, particularly malaria.

Several studies on the influence of intrinsic factors on SCA have been reported. These include the demonstration that very high levels of foetal haemoglobin lead to benign disease among Saudi Arabians [5,6], in Southern India [7], and also among Jamaicans [8]. Shurafa *et al.* [9] reported that long survivors of SCA had significantly higher foetal haemoglobin levels than short survivors. On the other hand, some studies have not demonstrated such beneficial effects [10,11]. Results of investigations into the influence of α-thalassaemia have been equally controversial, with some showing amelioration [12,13], whilst others could demonstrate no effect [14].

To examine the factors likely to affect the variability of the clinical manifestations of SCA in Nigeria, we determined the levels of foetal haemoglobin and alpha globin genotypes in these patients. As far as we are aware, this is the first definitive study in this region and our findings form the basis of this report.

Subjects and methods

The 130 subjects were randomly selected from the patients attending the paediatric and haematology clinics. The diagnosis of SCA was made by standard clinical and laboratory studies. There were equal numbers of males and females and the ages ranged from 2 to 30 years. Most had been studied for several years, and the haematological parameters are those taken in the steady state, and analysed by a Coulter S plus counter. Foetal haemoglobin was determined by the alkali denaturation technique [15] as modified by Pembrey [16]. The cellular distribution of foetal haemoglobin was assessed using the Kleihauer elution technique [17].

DNA Analysis

Alpha-thalassaemia was identified by restriction endonuclease analysis of DNA. The DNA was obtained from peripheral white and nucleated red blood cells using phenol-chloroform-isoamyl alcohol [18]. The DNA was digested with Bam HI and Bgl II enzymes. The fragments were separated by electrophoresis on 0.8% agarose gels and transferred onto nitrocellulose filters using the technique of Southern [19]. This was followed by hybridization using an alpha globin gene-specific probe labelled with $dCT^{32}P$. The filters were washed under stringent conditions and subjected to autoradiography [20].

Results

The proportion of haemoglobin F (Hb F) in the peripheral blood of SCA patients was found to vary considerably. The frequency distribution is shown in Fig. 1 and it is positively skewed. The mean was 5.9 ± 3.8% with a range of 0.9–16.7%. The mean values in males were significantly lower than in females (Table 1). In all cases, the cellular distribution of Hb F was heterogeneous. The mean values of Hb F in different age groups are shown in Table 2. There was a slight decrease from 6.8%, in those under 5 years of age, to 5.0% in the age group 15–20 years, this was followed by a slight rise to 6.4% in those over 25 years. None of the differences, however, reached statistical significance. A comparison of the haematological parameters was made between those subjects (Group I) with low Hb F levels (≤ 2%) and those (Group II) with higher levels (≥ 8%). The groups had equal numbers of males and

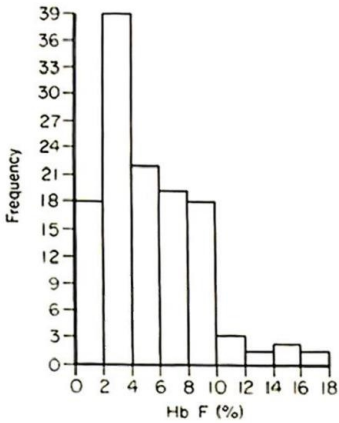

Fig. 1. Distribution of Hb F values in patients with sickle cell anaemia.

Table 1. Percentage levels of Hb F in males and females

	n	Mean*	SD	Range
Males	65	4.9	3.2	0.9–14.3
Females	65	6.2	3.2	1.1–16.7

*$P < 0.05$, between males and females.

Table 2. Percentage levels of Hb F in different age groups

	Age (years)					
	< 5	5–10	10–15	15–20	20–25	> 25
Mean	6.8	5.7	5.5	5.2	5.0	6.4
SD	4.5	3.1	2.5	3.0	3.6	4.8

females, and the results are shown in Table 3. The difference in MCV and MCH reached statistical significance, however, due to logistical difficulties, only one quarter of those studied had values for these parameters.

Globin gene analysis was performed in 30 randomly selected subjects with the results shown in Table 4. The subjects with one alpha gene deletion (α −/α α) had significantly lower MCV, MCH and mean Hb F than those with the normal complement of four alpha globin genes (α α/α α). The other two subjects homozygous for α-thalassaemia (α −/α −) had even lower MCV (69.0 fl) and MCH (21.1 pg) but the group was rather too small to be included in Table 4 for comparison.

Discussion

The observations reported here show that the levels of foetal haemoglobin are raised in SCA patients in this population. The levels obtained are similar to those in Jamaicans [5], black Americans [21] and Brazilians [22], but are, however, markedly lower than those observed in Saudi Arabia and parts of India [23,24]. The levels of Hb F observed in our subjects are not very high and the foetal haemoglobin levels observed may not affect the clinical severity of their illness. Foetal haemoglobin does not participate in the polymerization process that is characteristic of the sickling phenomenon. However, when there is a mixture with sickle haemoglobin, Hb F must attain a critical level for it to reduce polymerization by increasing the minimal gelling concentration (MGC). This happens in patients doubly heterozygous for the sickle gene and the negro type of hereditary persistence of foetal haemoglobin (HPFH). Such combinations produce very mild symptoms, and have foetal haemoglobin concentra-

Table 3. Haematological parameters in high and low Hb F groups

	Hb F (%)	Hb (g/dl)	MCV* (fl)	MCH* (pg)	Hb A_2 (%)
Group I	≤ 2	7.9 ± 1.1	82.4 ± 6.2	26.7 ± 2.4	2.9 ± 0.7
Group II	≥ 8	8.0 ± 1.3	100.8 ± 4.0	33.6 ± 2.1	2.6 ± 0.7

*$P < 0.05$.

Table 4. Haematological parameters and α-globin genotype

Genotype	n†	Hb (%)	MCV* (fl)	MCH* (pg)	Hb F* (%)	Hb A_2 (%)
α α/α α	18	7.6 ± 0.8	94 ± 4.4	31.2 ± 1.7	6.4 ± 3.7	3.2 ± 1.1
α –/α α	10	7.8 ±1.4	86.7 ± 4.4	28.5 ± 2.6	2.7 ± 1.6	3.3 ± 0.9

*$P < 0.05$.
†Excludes two with α –/α –.

tions in the range 15–40%. The fact that a few SCA patients whose foetal haemoglobin levels reach beyond 15% are not separated in this study reflects their very small numbers. The heterogeneous distribution of Hb F in the cells also means that perhaps few cells are protected. The lower levels of Hb F found in males compared with females confirms the report of Serjeant [8], although this difference was not observed by Wood [25]. A higher Hb F level would be expected if there was selective loss through haemolysis of cells with low amounts of this haemoglobin. Since there is no evidence that such a situation occurs preferentially in females, this difference remains unexplained.

Our finding of a diminished amount of foetal haemoglobin in sickle cell subjects with coexistent α-thalassaemia agrees with those of Higgs [13,26], however, Embury has reported the opposite, i.e. a higher level of foetal haemoglobin among patients with α-thalassaemia [12]. Another group has demonstrated that patients with SCA and coexistent α-thalassaemia have reduced haemolysis [27]. This is expected to lead to a reduced selection pressure for cells with high foetal haemoglobin levels since the red blood cells may be expected to live longer. This process can therefore explain the reduced levels of Hb F recorded in patients with coexistent α-thalassaemia. It is notable that the Hb F levels and α-thalassaemia observed in these patients modified some erythrocyte indices (MCV and MCH). There is no indication that the level of Hb F is sufficiently raised to modify the course of the disease in the subjects studied.

Although the patients examined had coexistent heterozygous α-thal-2 (α –/α α), a larger number homozygous for α-thal-2 (α –/α –) need to be investigated to demonstrate any meaningful effect on the severity of the disease. The present findings form the basis for further studies.

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References

1. Esan GJF. The thalassaemia syndromes in Nigeria. Br J Haematol 1970;19:47–56.
2. Fleming AF, Storey J, Molineaux L, Iroko EA, Attai EDE. Abnormal haemoglobins in the Sudan savanna of Nigeria. I. Prevalence of haemoglobins and relationships between sickle cell trait, malaria and survival. Ann Trop Med Parasitol 1979;73:161–72.
3. Esan GJF. The clinical spectrum of sickle cell anaemia in Nigerian adults. Br J Haematol 1970; 44:43–50.
4. Akinla O. Abnormal haemoglobins and pregnancy. Nig Med J 1972;2:11–15.
5. Ali SA. Milder variant of sickle cell disease in Arabs in Kuwait associated with unusually high level of fetal haemoglobin. Br J Haematol 1970; 19:613–19.
6. Perrine RP, Pembrey ME, John P, Perrine S. Natural history of sickle cell anaemia in Saudi Arabs: a study of 270 subjects. Ann Int Med 1978;88:1–6.
7. Brittenham G, Lozoff B, Harris JW, Mayson SM, Miller A, Huisman THJ. Sickle cell anaemia and trait in Southern India: further studies. Am J Haematol 1979;6:107–23.
8. Serjeant GR. Fetal haemoglobin in homozygous sickle cell disease. Clin Haematol 1975;4: 109–22.

9. Shurafa MS, Prasad AS, Rucknagel DL, Kan YW. Long survival in sickle cell anaemia. Am J Haematol 1982;12:357–85.
10. Steinberg MH, Dreiling BJ, Morrison FS, Necheles TF. Mild sickle cell disease: clinical and laboratory studies. J Am Med Assoc 1973; 224:317–21.
11. Powars DR, Schroeder WA, Weiss JN, Chan LS, Azen SP. Lack of influence of fetal haemoglobin levels on Erythrocyte Indices on the severity of sickle cell anaemia. J Clin Invest 1980;65:732–40.
12. Embury SH, Dozy AM, Miller J, *et al*. Concurrent sickle cell anaemia and α-thalassemia: effect on severity of anaemia. N Eng J Med 1982;306:270–4.
13. Higgs DR, Aldridge BE, Lamb J, *et al*. The interaction of alpha-thalassaemia and homozygous sickle cell disease. N Engl J Med 1982; 306:1441–6.
14. Steinberg MH, Rosenstock W, Coleman MB *et al*. Effects of thalassaemia and imcrocytosis on the haematologic and vasoocclusive severity of sickle cell anaemia. Blood 1984;63:1353–60.
15. Betke K, Marti HR, Schlight I. Estimation of small percentages of fetal haemoglobin. Nature 1959;184:1877–8.
16. Pembrey MC, McWade P, Weatherall DJ, Reliable routine estimation of small amounts of fetal haemoglobin by alkali denaturation. J Clin Pathol 1972;25:738–40.
17. Kleihauer E. Determination of fetal haemoglobin: elution technique. In: Schmidt RM, Huisman THJ, Lachman H, eds. The Detection of Haemoglobinopathies. Ohio: CRC Press 1974:20–2.
18. Gross Bellard M, Dudet PA, Chambon P. Isolation of high molecular-weight DNA from mammalian cells. Eur J Biochem 1973;36:32–8.
19. Southern EM. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J Mol Biol 1975;98:503–17.
20. Old JM, Higgs DR. The thalassaemias. In: Weatherall DJ ed. Gene Analysis. Edinburgh: Churchill Livingstone, 1983:20–2.
21. Wrightstone RN, Huisman THJ. On the levels of haemoglobin F and A_2 in sickle cell anaemia and some related disorders. Am J Clin Pathol 1974;61:375–81.
22. Hutz MH, Salzano FM, Adam J. Hb F levels longevity of homozygotes and clinical course of sickle cell anaemia in Brazil. An J Med Genet 1983;14:669–76.
23. Prembrey MC, Wood WG, Weatherall DJ, Perrine RP. Fetal haemoglobin production and the sickle gene in the cases of Eastern Saudi Arabia. Br J Haematol 1978;40:415–29.
24. Haghshenass M, Ismail-Beigi F, Clegg JB, Weatherall DJ. Mild sickle cell anaemia in Iran associated with high levels of fetal haemoglobin. J Med Genet 1977;14:168–71.
25. Wood WG, Pembrey ME, Serjeant GR, Perrine RP, Weatherall DJ. Hb F synthesis in sickle cell anaemia: a comparison of Saudi Arab cases with those in African origin. Br J Haematol 1980;45:431–45.
26. Altay C, Gravely ME, Joseph BR, Williams DF Alpha-thalassaemia-$_2$ and the variability of haematologic values in children with sickle cell anaemia. Pediatr Res 1981;15:1093–6.
27. de Ceulaer K, Higgs DR, Weatherall DJ, Hayes JR, Serjeant BE, Serjeant GR. Thalassaemia reduces the hemolytic rate in homozygous sickle cell disease. N Eng J Med 1983;309:189–90.

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