AFRICAN JOURNAL OF MEDICINE
and medical sciences

VOLUME 31, NUMBER 2 JUNE 2002

EDITOR:
B. O. OSOTIMEHIN

ASSISTANT EDITOR:
A. O. UWAIFO

ISSN 1116 — 4077

Afr J. Med. med. Sci. (2002) **31**, 155-158

Trends in haemoglobin S, F, and A2 levels in West African and Caribbean sickle cell anaemia patients

PO Olatunji

Department of Haematology, Faculty of Health Sciences, University of Ilorin. Ilorin, Nigeria

Summary

The levels of Haemoglobins S, F and A_2, as well as Haemoglobin concentration, age, and average admissions per year (AVEADM) were compared in sickle cell anaemia patients of West African and Caribbean origins. Correlations of the haemoglobin levels with age, and the trends were also determined. The West African patients who comprised of 42 Nigerians, 23 Ghanaians and 13 Sierra Leoneans were compared to determine any differences in trend within them. One hundred and thirty-four patients, made up of 78 West Africans and 56 Caribbeans were analysed. The Caribbean patients were significantly older than the West African patients ($P = 0.023$). Haemoglobins S and F showed positive and negative significant correlations, respectively, with age in West African patients whereas there was no significant correlation in the Caribbean patients. Scatter charts and trendlines show that Haemoglobin S continued to increase while Haemoglobin F declined with age up to the age of 30 years in West Africans, Nigerians showing the steepest slope, while it remained stable with increasing age in the Caribbean patients. These findings may be due to the fact that the Caribbeans are genetic and socio-cultural mixtures of the different West African peoples, and the absence of indigenous malaria pressure.

Keywords: *Haemoglobin S,F, and A2 Levels, caribbean , sickle cell anaemia*

Résumé

Le niveau d'hémoglobine S,F et A2 aussi bien que la concentration d'hémoglobine, l'âge et l'admission moyen per an se sont comparés chez les malades de drépanocytose d'origine d'Afrique occidentale et des Caraïbes. Les corrélations de niveau d'hémoglobine avec l'âge et les tendances ont été aussi déterminées les malades de l'Ouest Afrique dont 42 Nigérians, 23 Ghanéens, et 13 viennent de Sierra Leone se sont comparés pour déterminer aucune différences de tendances parmi eux. Une analyse est faite de cent trente quatre malades dont 78 sont d'origine de l'ouest africain et 56 des caraïbes. Les malades Caraïbes étaient considérablement plus âgé que ceux de l'Afrique d'Ouest (p=0,023). Les hémoglobines s et f ont montré des corrélations positive et négative signifiant avec l'âge chez les malades de l'Ouest Africain tandis que il n'y a pas une corrélation signifiant chez les malades caraïbes. Les courbes dispersés et les lignes de tendance montrent que l'hémoglobine F réduit avec l'âge jusqu'à 30 ans chez les Africains de l'Ouest, avec les Nigérians qui montrent la pente la plus forte, lors qu'il reste stationnaire avec le vieillissement chez les malades caraïbes. Ces conclusions peut être dû au fait que les caraïbes sont d'une mélange génétique et socioculturel des différents peuple de l'Afrique de l'Ouest et l'absence de la tension malaria indigène.

Introduiction

In a normal individual, foetal haemoglobin (HbF) makes up 60-80% of total haemoglobin at birth. The switch from the foetal to adult haemoglobin is associated with increase in the production of β globin chain resulting in an increasing level of haemoglobin A (HbA) and a progressive fall in the level of HbF. It reduces to about 3% at 16 to 20 weeks of life and falls to 0.5 to 1% in adult life. This switch is believed to be complete at about two years of life when haemoglobin is about 98% of adult type [1]

The molecular abnormality is a single point mutation on the β-globin gene resulting in the production of structurally abnormal β globin polypeptide chain [2]. Therefore, the disease does not produce a phenotype during intrauterine life when the predominant haemoglobin is the foetal (HbF) type. Since the discovery of the laboratory, clinical and genetic features associated with homozygous inheritance of the sickle gene [2,3,4], geographical variations in its clinical manifestations have been documented [5] These variations have been found to be due to socioeconomic [6] and gene cluster haplotype [7] factors which determine, among other things, the level of haemoglobin F to be found in each patient. The major haplotypes include the Benin (# 19), the Senegal (# 3) and the Bantu (Central African Republic) (# 20) types, with the Benin haplotype being associated with Nigerian patients [8,9] In patients with sickle cell anaemia, not only is HbA replaced with HbS, the switch from foetal to adult haemoglobins occurs more gradually with HbF remaining higher than 2% in the majority of patients. In addition, a small amount of another minor haemoglobin, HbA_2, is produced.

The multicentric origin of the sickle mutation as shown by the different β^s gene cluster haplotype is associated with varying level of haemoglobin F in the patients [10]. This varying level of HbF partly accounts for the variability of the clinical course of sickle cell anaemia, with those having higher level being associated with milder clinical course and experiencing longer survival [11]. It is easily assumed that patients with the same haplotype such as the Benin type found in countries around Nigeria and patients from the Caribbean Islands who migrated from the West African countries will have comparable level of HbF during lifetime.

The present study was based in a hospital in London with one of the highest heterogeneous population of sickle cell anaemia patients from the standpoint of West African and Caribbean origins. It therefore affords an opportunity to compare the variation of the various haemoglobin types in peoples of these areas and how they might affect interpretation and extrapolation of data originating therefrom. The changes, relationships, and possible influences of the levels of the various haemoglobin types with advancing age will be studied in the patients.

Materials and methods

One hundred and fourty-eight patients with sickle cell anaemia attending the sickle cell clinic of the Central Middlesex Hospital in North London, were entered into the study. These included 88 patients of West African origin and sixty of Caribbean origin aged 1-44 and 1-49 years, respectively. Patients were assigned origins corresponding to the birthplace of their parents and where they lived before emigration to Britain. Reasons for emigration included emigration of parents, education and better health care services

Correspondence: Dr. P.O. Olatunji, Department of Haematology, Faculty of Health Sciences, University of Ilorin, P.M.B. 1515, Ilorin, Nigeria.

The demographic data (including age, sex, place of origin) of the patients were recorded both from clinical records and during attendance at the sickle cell clinics between January 1993 and June 1994. Average admissions per year were determined from previously documented clinical records over the period of attendance. The average admissions per year (AVEADM) was computed from the average annual frequencies of painful crisis, acute chest syndrome, severe haemolytic, sequestration, and aplastic crises, and acute stroke requiring hospital admissions.

The haemoglobin genotype of patients were determined using Agar Gel Electrophoresis at acid pH with kit from Helena Laboratories, Iso Electric Focusing, Polymerase Chain Reaction (PCR) [12] and Restriction Fragment Length Polymorphism (RFLP) [13] using Dde I as the restriction endonuclease. Blood samples were taken, after obtaining informed consent, on normal clinic days provided that the patients were in steady state and had not experienced any episode of crisis in the previous four weeks before the clinic day. Samples were collected into sequestrene bottles (containing EDTA) and haematological parameters including PCV, red cell indices, WBC and platelet counts were determined on the Coulter STKS electronic counter. Haemoglobins F, S and A_2 levels were determined by high pressure liquid chromatography (HPLC). The data were stored and the analyses were carried out using the EPI Info Version 5 statistical package supplied by the CDC, Atlanta, Georgia. The results are presented in form of tables and charts. Means were compared with the Student's t test, proportions were compared using the chi-squared method while regression was used to test associations.

Results

Out of the 148 patients seen analyses could be carried out on 134 comprising 78 West Africans aged 1-44 years and 56 Caribbeans age 3-49 years. The West Africans included 42 Nigerians, 23 Ghanaians and 13 Sierra Leoneans. The mean ± standard deviation of age, average admissions per year (AVEADM) and Haemoglobin concentration of the West African patients were 19.54 ± 11.44, 1.015 ± 1.65 and 8.75 ± 1.13 respectively while the Caribbeans had 23.82 ± 11.44, 0.95 ± 1.29 and 8.97 ± 1.33 respectively. The Caribbean patients were significantly older ($P = 0.023$) than the West Africans.

Table 1: Mean haemoglobin levels, age and AVEADM in different patient populations

Parameters	Patient Group					
	Nigerian n = 42	Ghana n =23	S/Leone n = 13	W. Africa n =78	Caribbean n = 56	*P
HbS %	89.2±7.1	86.7±9.3	84.8±11.4	87.7±8.6	85.6±11.3	>0.05
HbF %	8.4±6.9	11.2±9.0	12.6±11.0	9.9±8.4	9.3±7.8	>0.05
HbA_2 %	2.6±0.8	2.6±0.8	2.4±0.6	2.6±0.6	2.7±0.7	>0.05
Hb gm/dl	8.6±1.1	9.1±1.0	8.8±1.4	8.8±1.1	9.0±1.3	>0.05
Age yrs	19.3±10.1	22.6±14.0	18.1±9.5	19.5±11.4	23.8±11.4	0.023
Aveadm	0.95±1.13	1.3±1.8	1.1±2.6	1.0±1.65	0.95±1.3	>0.05

**Significance between W. African and Caribbean patients*

The mean levels of Haemoglobins F, S and A_2 were 9.9 ± 8.41,87.7 ± 38.64 and 2.59 ± 0.63 respectively, for West African patients, and, 9.3 ± 7.8, 85.57 ± 11.2 and 2.74 ± 0.76 for the Caribbeans patients. When Haemoglobins F, S and A_2 were correlated with age, West African patients showed negative correlation (r = -0.38, $P<0.01$) and positive correlation (r = 0.34, $P < 0.01$) for Haemoglobin F and S respectively. On the other hand there were no significant correlations between Heamoglobin F or S and age in the Caribbean patient. The Haemoglobin F or S and age in the Caribbean patient. The Haemoglobin concentration and average admissions per year were not significantly different in the two groups of patients (Tables 1 and 2).

Table 2: Correlations of haemoglobins S, F and A12 versus age in different patient populations.

Parameter	Correlation Coefficients for various populations							
	Nigeria		W. Africans		S/Leone		Caribbean	
	r	p	r	p	r	p	r	p
HbS	0.55	<0.01	0.34	<0.02	0.15	>0.20	0.21	>0.10
HbF	-0.55	<0.01	-0.38	<0.01	-0.26	>0.20	-0.15	>0.20
HbA_2	-0.15	>0.05	-0.21	>0.10	-0.04	>0.20	-0.12	>0.20

r= Correlation Coefficient p= level of significance

Figures 1 and 2 show the scatter diagrams of Haemoglobin levels versus age with the logarithm and linear trend lines in the two groups of patients. In the West African patients, Haemoglobin F level continued to decline until the age of 15 years while Haemoglobin S maintained an upward trend up to the age of 30 years.

Figure 1: Scatter plot and trends in Haemoglobins S, F and A2 versus Age among West African patients with Sickle Cell Anaemia

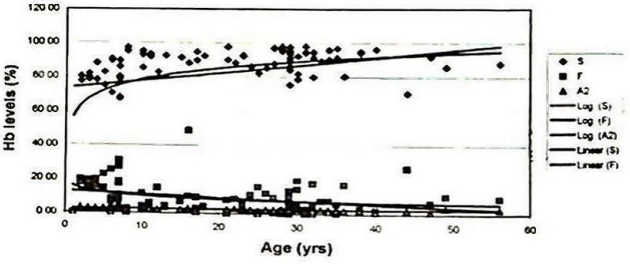
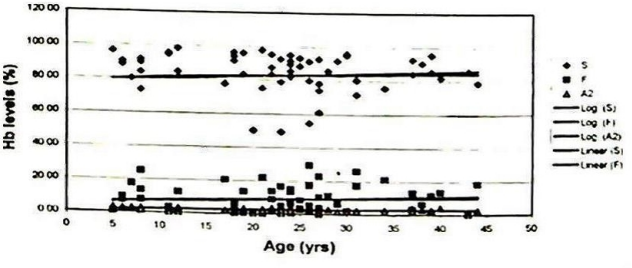


Figure 2: Scatter plot and trends in Haemoglobins S, F and A2 versus Age among Caribbean patients with Sickle Cell Anaemia.



In the Caribbean patients both Haemoglobins F and S remained relatively constant with increasing age. Haemoglobin A_2 remained constant as age increased in both groups of patients. Figures 3 and 4 which are the scatter and trend charts for Nigerians and Sierra Leonean patients, respectively, show that

trends in Nigerian patients is similar to those of the combined West African patients while the trend in Sierra Leoneans resemble that of the Caribbean patients.

Figure 3: Scatter plot and trends in Haemoglobins S, F and A2 versus Age among Nigerian patients with Sickle Cell Anaemia

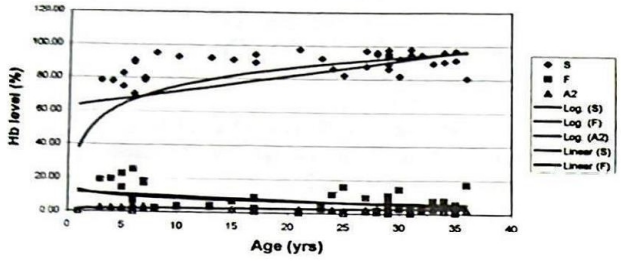
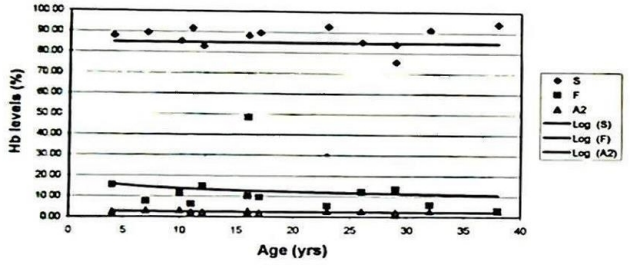


Figure 4: Scatter plot and trends in Haemoglobins S, F and A2 versus Age among Sierra Leonian patients with Sickle Cell Anaemia



Discussion

People from different countries of West Africa who were taken to the Caribbeans during the slave trade have since become one people of the West Indies. Heterogeneous populations are difficult to find except in places where migration for economic, educational and social reasons is still permitted or encouraged. This is the situation with North London where Caribbeans and people from various West African countries live in significant number, thus facilitating the study of sickle cell anaemia patients attending the Central Middlesex Hospital [12]. The number of patients analyzed in this study may appear to be small on its face value. This should, however, be considered in the light of the fact that from the 1991 Census of England and Wales, the total population of blacks (African and Caribbean) in the borough of Brent where the Central Middlesex Hospital is situated, is 15,310 [13]. Even if we take the HBSS patients as 3% of the black population, they will be a total of 459. Therefore, 148 patients can not be said to be too few. It then becomes possible to investigate differences among patients that can be attributed to their different origins, and determine factor in such places responsible for the observed difference. Two factors that may be responsible for differences between the West Africans and Caribbeans in the presentation of sickle cell anaemia include:

1. The maintenance of different genetic and environmental identity in countries of West Africa while the Caribbean people are a mixture of the genetic, social and cultural properties of the West Africans [14].
2. The Caribbean Islands do not fall into the original malaria belt and the people are free from the influence of the malaria pressure.

The first factor can be associated with the level of Haemoglobin F, which is known to influence the clinical course of sickle cell anaemia while the second one is associated with level of Haemoglobin S which influences the rate of sickling. Of particular interest in this study, therefore, are the levels and the rates of increase or decline of Haemoglobins F and S among patients of the different peoples.

Although there is no difference in the level of HbF between the West Africans as a group and the Caribbeans (9.9% and 9.29%, respectively), obvious but non-statistically significant disparity exists in HbF levels for different African countries (Nigeria, Ghana and Sierra Leone, 8.4%, 11.2% and 12.6%, respectively) in this study. Of greater importance is the rate of rise of HbS and decline of HbF signified by the correlation coefficients. While they were significant in the West African patients, they were not in the Caribbean patients. The main reflection of this difference is in the significantly higher mean age of the Caribbean patients (23.8±11.4 vs. 19.5±11.4) which may be an indication of better survival. Among West African patients, the correlation was most significant in the Nigerian patients ($P < 0.01$) and insignificant ($P > 0.05$) among patients from Ghana and Nigeria combined. The charts showing the scatter plots and trend lines present mirror images of HbS and HbF. They also show that while in Africans HbS rose steeply and HbF declined with age up till 30 years, both haemoglobins remained constant regardless of age in the Caribbean patients. This is consistent with the findings of Serjeant and is a factor which has significance for sickling and survival, and which may be related to the absence of indigenous malaria pressure.

In conclusion, this study has shown that Caribbean patients are older than their West African counterparts, and that a steep rise and decline in HbS and HbF respectively only in the West African patients.

It also shows that the attainment of stable adult levels of HbS and HbF in the West African patients except Sierra Leonians does not really exist under the age of 30 years. The implication of this finding is that results of researches in sickle cell anaemia in one population may not automatically be applicable to the other. This underlines the need for serious effort on the part of government and NGOs at funding and supporting major researches in sickle cell diseases in Africa.

References

1. Stomatoyannopoulos G and Neinhuis AW: Haemoglobin switching. In: Athe Molecular Basis of Blood Diseases Stomatoyannopoulos et al 2nd Edition W.B. Saunders Company London 1994; 107-155.
2. Ingram VS: A specific clinical difference between the globin of normal human and sickle cell anaemia haemo globin. Nature 1956; 178: 792-794.
3. Herrick JB: Peculiar, elongated, sickle-shaped red blood corpuscles in a case of sever anaemia. Arch Int. Med. 1910, 6: 517-521
4. Nagel RL and Ranney HM. Genetic epidemiology of structural mutations on the β-globin gene. Seminars in Haematology. 1990; 27: 342-359.
5. el-Hazim MA: Heterogeneity and variability of clinical and haematological expression of haemoglobin S in Saudi Arabs, Acta Haematol. 1992; 88: 67-71.
6. Fleming AF: Sickle cell anaemia in the Non-Pregnant over twelve years: In: Sickle Cell Disease-A handbook for General Clinicians, Fleming AF ed. Churhill Livingstone 1982; pp 23-33.

7. Powars D, Chan LS, Schroeder WA: The Variability of Sickle Cell Disease is Genetically Determined. Seminars in Haematology 1990; 27: 360-376.
8. Powars DR: b^S-Gene Cluster Haplotypes in Sickle Cell Anemia. Hematology/Oncology Clinics of North America 1991;5: 475-493.
9. Falusi AG and Esan GJF: Hpa I polymorphism and the sickle gene in Nigerians. Gene Geography 1988; 2: 9-14.
10. Stevens MCG, Hayes RJ, Valdya S, Serjeant GR: Fetal hemoglobin and clinical severity of homozygous sickle cell disease in early childhood. The Journal of Pediatrics 1981; 98: 37-41.
11. Odenheimer DJ, Sarnaik SA, Whitten CF, Rucknagel DL, Sing CF: The Relationship Between Fetal Hemoglobin and Disease Severity in Children With Sickle Cell Anemia. Am J Med Genet 1987; 27: 525-535.
12. Anionwu E, Walford D, Brozovic M and Kirkwood B: Sickle –cell disease in a British urban community. Brit. Med. J. 1981; 282: 283-286.
13. Balarajan R and Raleigh VS: The ethnic populations of England and Wales: the 1991 Census. Health Trends 1992; 24: 113-116.
14. Flint J, Harding RM, Boyce AJ and Clegg JB: The popu lation genetics of the haemoglobinopathies. Clinical Haematology 1993; 6: 215-262.