

*Full Length Research Paper*

# Tribal distribution of haemoglobinopathies in a Sudanese patient population

Abozer Y. Elderderly<sup>1,2\*</sup>, Babiker A. Mohamed<sup>3</sup>, Alan J. Cooper<sup>1</sup>, Gavin Knight<sup>1</sup> and Jeremy Mills<sup>1</sup>

<sup>1</sup>School of Pharmacy and Biomedical Sciences, Portsmouth University, UK.

<sup>2</sup>Department of Pathology, Faculty of Medicine, University of El Imam El Mahdi, Sudan.

<sup>3</sup>Sudan Medical Specialization Board, Khartoum, Sudan.

Accepted 13 June, 2011

Haemoglobin (Hb) abnormalities are inherited disorders in the globin chains when the haem group is in the normal state. They are mostly autosomal recessive abnormalities and common worldwide, particularly within the malarial regions of Africa. This study aimed to identify abnormal Hb stratified tribe by tribe within a cohort of Sudanese patients attending the Khartoum Teaching Hospital between March and July 2005. Initially, cellulose acetate electrophoresis (CAE) and full blood count (FBC) were used to determine the Hb type. Cation-exchange-high performance liquid chromatography (CE-HPLC) was subsequently used to evaluate results. The following range was found: HbAA (93.1%), HbAS (5.1%), HbSS (1.0%), HbAC (0.6%) and HbCC (0.2%). Thus the S gene was the most common variant found (6.1%), and it was most prevalent in the western tribes of Sudan (12.5%). Other notable findings included three patients out of HbAA subjects (0.5%) with an increased Hb F. Results from CAE were validated with CE-HPLC and findings were identical. Additional quantitative data is available with CE-HPLC, but it is expensive in terms of operation and maintenance. CAE therefore remains the technique of choice in developing countries. CAE is recommended as a suitable technique for clinical use in the developing world. Rates of genetic disorder can be reduced with improved health management and further research is also recommended into the presence of Hb F, hereditary persistence of foetal Hb (HPFH) and  $\delta\beta$ -thalassaemia in HbAA.

**Key words:** Haemoglobin disorders, sickle cell anaemia, cation-exchange-high performance liquid chromatography (CE-HPLC), electrophoresis.

## INTRODUCTION

Haemoglobinopathies are the most common single gene disorder. The WHO estimated about 5 to 7% of the world's populations are susceptible to inherited haemoglobinopathies (Organization, 2006). More than 7% of Africans suffer from the inherited sickle cell disorder, (Makani et al., 2007) mainly as a result of

increasing migration throughout the continent. The high incidence of abnormal Hb among people who prefer to marry consanguineously is already very well documented (Pedersen, 2002) and high levels of abnormal Hb are already known to protect against diseases such as malaria (Aidoo et al. 2002; Oniyangi and Omari, 2006).

No major reports exist on Hb variants within the Sudanese peoples. This is due to the lack of technology to determine types of abnormal Hb for example, cation exchange-high performance liquid chromatography (CE-HPLC), iso-electric focusing (IEF) and molecular methods. This is primarily due to lack of funding and the necessary resources to identify blood disorders accurately. Cellulose acetate electrophoresis (CAE) is available in parts of the country, but it cannot distinguish certain variants for example, Hb S.

Hb S is known to be prevalent in Sudan and has been

\*Corresponding author. E-mail: Abozer.Elderderly@port.ac.uk.  
Tel: 0044 2392 845609. Fax: 0044 2392 843265.

**Abbreviations:** Hb, Haemoglobin; CAE, cellulose acetate electrophoresis; FBC, full blood count; CE-HPLC, cation-exchange-high performance liquid chromatography; SCA, sickle cell anaemia; EDTA, ethylenediaminetetraacetic acid; RTM, retention time marker; RFLP, restriction fragment length polymorphism; RBC, red blood cells.

suggested to be more common in populations from Kordofan and Darfur (western Sudan) (Attalla et al., 2006). The presence of Hb S is already well documented among the Albagara, an Afro-Arab constellation of tribes with a predominantly African descent (Taha et al., 1985). In a sub group of Albagara (Misseria) studies showed the prevalence of sickle cell disease (SCD) to be 30%, 16% among immigrants from the province of Blue Nile (Ahmed and Baker, 1986) and 18% among Nilotes in the south of Sudan (Kondi et al., 1954). Furthermore, haemoglobinopathies are also known to be prevalent in the Khartoum area (Omer et al., 1972).

Khartoum the capital city of Sudan is situated in the centre of the country; it is multiethnic, with a blend of almost all the Sudanese tribes. This has various implications. Most of its original citizens are from the tribes of northern Sudan. Additionally, there are immigrants from the southern and western Sudanese tribes who moved as a result of drought and desertification in the 1970s and 1980s. The supposition is that this variety in the population will be accompanied by variation in Hb types (Ali et al., 1972; Lauden, 1990). At present, no study exists on the profile of Hb variants within the Sudanese population, as a result of migrating populations into Khartoum. This is because there is no multidisciplinary approach to managing haemoglobinopathy. Inter-marriage is common throughout Sudan and is a significant cause of the spread of Hb disorders. Sickle cell anaemia (SCA) is also a continuing problem; its prevalence mainly arising as a result of sufferers visiting paediatric clinics.

Crucially, the Sudanese health service requires public health physicians to establish a health programme involving medical records for the population, screening and health education programmes. This study also examines the hypothesis that haemoglobinopathies are a major cause of anaemia. Moreover, certain types of abnormal Hbs have been suggested to be more prevalent in the Sudanese population.

Despite their apparent genetic simplicity, it has long been appreciated that both  $\beta$  thalassemia SCD display a remarkable diversity in the severity of associated disease. High levels of Hb F have been shown to have a beneficial effect in both of these disorders, although the mechanism for the ameliorating effect differs (Best et al., 2006). Hb F levels vary considerably; it is found with normal Hb and also in cases with  $\beta$ -globin disorder. Studies have also shown that erythrocytes contain Hb F in varying degrees within the healthy population. Furthermore, levels of Hb F in the erythrocyte can vary considerably (Menzel et al., 2009).

This study is intended to compare the performance of CAE against CE-HPLC. Samples were first checked using CAE in Khartoum Teaching Hospital and then checked under CE-HPLC in University of Portsmouth, UK, with the differing results evaluated. The majority of adults display Hb F levels of less than 0.6% of total Hb,

but up to 15% may have levels between 0.8 and 5%. This is due to over-expression in the  $\gamma$ -globin chains (Doneanu et al., 2007). Hb F is also unevenly distributed in patients with hereditary persistence of foetal Hb (HPFH) (Doneanu et al., 2007). Some individuals have a genetic predisposition to unusually high levels (Menzel et al., 2009). Additionally, extremely high levels of Hb F have been detected in sufferers of SCD (Pembrey et al., 1980; Fabry et al., 1985).

HPFH is only found in a few individuals with SCD. Levels vary considerably, however, among sickle patients without HPFH (Kosteas, Palena et al. 1997). Hb types were initially investigated with CAE and FBC. Family history, age, ethnicity and clinical symptoms were also considered. CAE is considered a robust intermediate technology for identifying Hb variants and it was used in this study (D'Souza et al., 2002). This study has mapped the regional prevalence of haemoglobinopathies within the Khartoum area by also undertaking blood counts and considering the medical history for each sample.

Results from CAE are taken from patterns of protein migration on cellulose paper. Patternation is affected by pH in the electrophoresis buffer. It identifies protein separation in areas where structural Hb gives differing electrical charges (Lewis, 1995; Kutlar, 2007). CAE analysis is rapid and reproducible, but is incapable of identifying certain Hb variants such as HbS and HbC with similar migratory paths.

CE-HPLC obtains results from column chromatography, which separates samples by differences in polarity and the partitioning of Hb between the mobile (solvent) and stationary (column) phases. Fractionation arises in elution when each sample is measured in the stationary phase. Eluted Hb is classified according to its "retention time" relative to a standard. Concentrations of the eluate are also identified by the area under the peak (Hadzi-Nesic et al., 2004; Joutovsky and Nardi, 2004).

Under alkaline electrophoresis, certain Hbs display similar migratory path, which can affect Hb identification. For example, Hb variants D Punjab and G demonstrate the same separation characteristics as Hb S. Migration paths of Hb E and O Arab are also similar to Hb C. Therefore, electrophoresis is accepted in conjunction with FBC as the most common test for detecting haemoglobinopathies (El-Hazmi and Warsy, 2001; D'Souza et al., 2002).

CE-HPLC, however can distinguish between these Hbs despite the similarities in protein migration (Joutovsky and Nardi, 2004; Ridley et al., 2005). CE-HPLC is currently regarded as one of the most reliable methods for Hb analysis and it can also diagnose patients with thalassaemias (Joutovsky and Nardi, 2004; Kutlar, 2007).

## MATERIALS AND METHODS

Between March and July 2005, 632 random samples were analysed; 5 ml of venous blood in K<sub>2</sub>- ethylenediaminetetraacetic

acid (EDTA) (anti-coagulant) for each sample. Interviews and questionnaires were used to collect demographic and clinical data. Initially, FBC and CAE were used to diagnose all participants; FBC was performed in a fully automated machine (Sysmex NE-800 analyzer; Toa Medical Electronics, Kobe, Japan).

All samples were converted to haemolysates as described below and refrigerated for alkaline electrophoresis within 28 days. Samples were then centrifuged for 20 min and the resultant red blood cells (RBC) then washed three times in an isotonic solution (pH 7.4). Electrophoresis was then used to separate the Hb variants (Dacie and Lewis, 1995). Control samples were prepared using a mixture of Hbs A, F, S and C and included in each experiment alongside the test samples, as illustrated in Figure 1.

Dried specimens were prepared from the blood samples with Guthrie cards as per instructions (Whatman UK, whatmaninfo@ge.com), that is, a drop of blood was positioned onto the card, allowed to soak through and left to dry for fifteen minutes and stored at -20°C with desiccant material.

CE- HPLC was performed using the BioRadVnbs CE-HPLC system. Eluate absorbance was measured by the visible wavelength detector. The cation exchanger can alter the electrical charge for Hb separation at particular pH values (Surve et al., 2007; Kutlar, 2007). The resultant Hb eluted from the column was then classified according to its "retention time", relative to a standard retention time marker (RTM). Positively charged Hb S and Hb C thus have a longer retention time than Hb AA and Hb F molecules. Sample concentrations can also be detected by the area under the peak from the spectrophotometric evaluation of the eluate (Ridley et al., 2005). Data was analysed using SPSS version-15.

## RESULTS

The phenotypes of Hb encountered are summarized in Table 1. Hb AA (adult Hb or normal Hb) was predominant (93.1%). Four abnormal Hb phenotypes were detected including Hb AS (5%), Hb SS (1.0%), Hb AC (0.6%) and Hb CC (0.2%). Additionally, three patients with HbAA had an increased F band. A single patient with Hb SS also had an increased F band. All of the Sudanese tribes were represented in the cohort of patients analysed (Table 2). Tribal distribution was representative, except that subjects from central tribes were over doubly represented compared to other tribes.

Subjects with S or SS Hb are summarized in Table 2. Of the six tribes studied presence was highest in the Western tribes and Central and Blue Nile tribes (12.5% ~6%, respectively). Consequently, these tribes are more susceptible to SCA.

CAE results were validated by CE- HPLC. HbAA, HbAS, HbSS, HbAF, HbAC and HbCC were all detected within the sample. No discrepancies were found between the two tests results using the two methods. An example typifying this consistency of results is found in Figure 1. The selection encompasses all the Hb variants found.

## DISCUSSION

HbSS and HbAS were present in 12.5% of patients from the Western tribes and 6.1% of subjects of the entire study group of patients attending Khartoum Teaching

hospital. SCA in this study was represented in all tribes (Table 2 and Figure 1). Certain types of Hb are reported to prevail in certain tribes (Ali et al., 1972; Lauden, 1990).

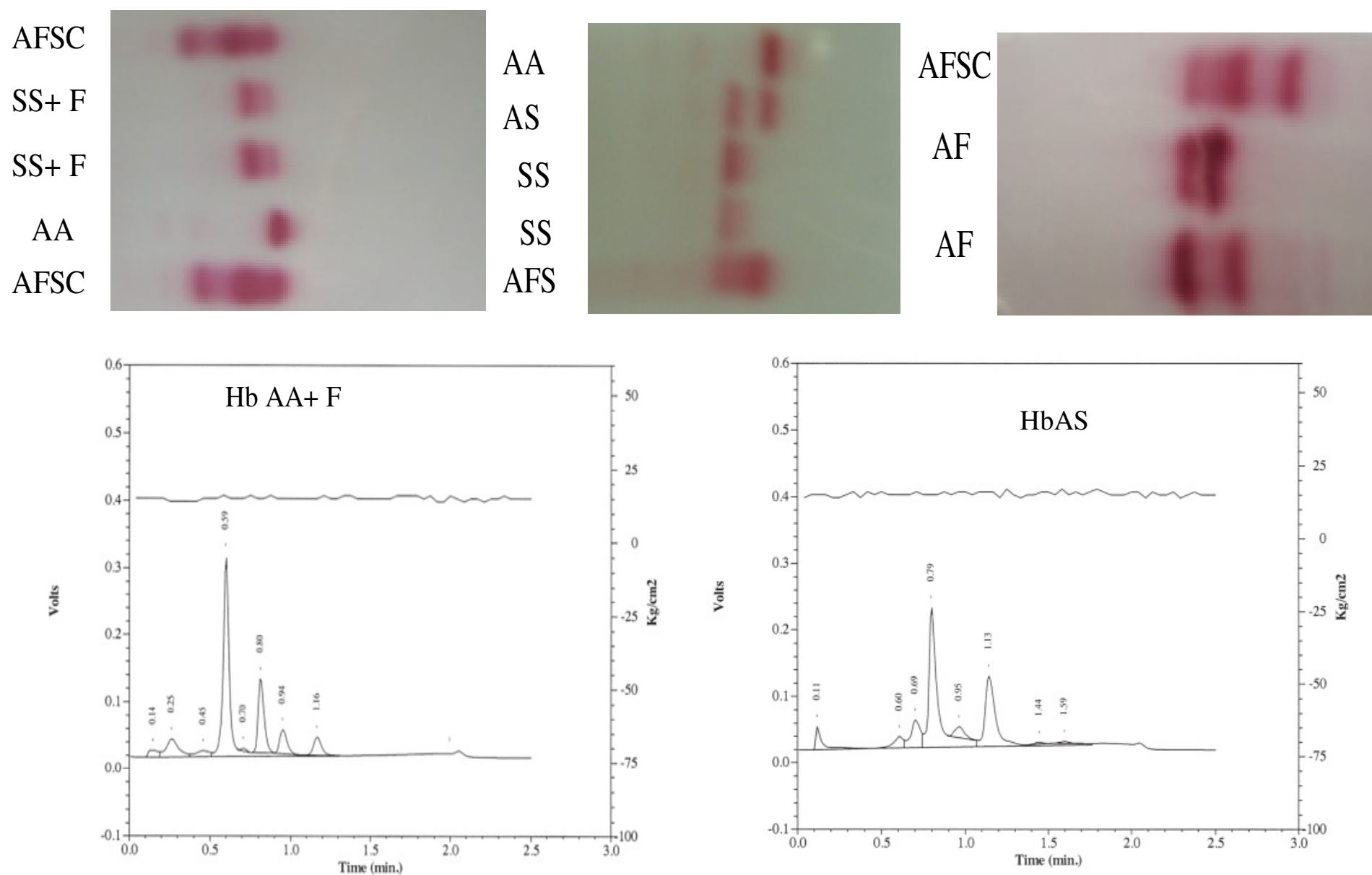
Table 2 also lists, by tribe all those participants either homozygous or heterozygous for S type Hb. Interestingly, low levels of AS and SS were found among subjects representing southern tribes of Sudan (4.4%). Incidence in participants from Blue Nile tribes was slightly higher (6.0%). Overwhelmingly, the high prevalence of AS and SS was confined to the western tribes (12.5%).

Khartoum was chosen as the study area because of its central location and high incidence of malaria (Kojal et al., 2006). The Khartoum teaching hospital, in the city centre treats patients of all ages and from all Sudanese races. As discussed, the high level of migration into Khartoum permitted the samples to represent a wide tribal diversity.

Consanguineous marriages have also been linked with the numerous abnormal Hb subtypes and this type of relationship is common in Sudan. Consequently, there is also the increased risk of continuing Hb abnormalities to future generation (Hussain, 1999). This report recommends a health education program regarding consanguineous marriage for people who are carriers of abnormal Hb and their families. In addition, the screening of pregnant women and children would help to identify carriers and minimize haemoglobinopathies.

The northern and southern Sudanese differ anthropologically; the former are primarily of Arab African extraction and the latter, African. Hb S is common among some of the southern tribes (Kondi et al., 1954), but it is mainly absent in the northern tribes (Kondi et al., 1954). In Khartoum of the 9,100 examined, there was also a minimal presence of Hb S (2%) (Taha et al., 1985; Attalla et al., 2006).

SCA continues to provide a diagnostic challenge, particularly in identification of the Hb S gene. This study was designed to give a representative picture of the prevalence of haemoglobinopathies among Sudanese tribes. The study was focused on Khartoum because its multiethnic mix is representative of the wider population of Sudan. Three patients of Hb AA variant were detected with high level of HbF. The inheritance of genes determining high levels of Hb F is not well understood, but a very low level of Hb A and a high level of Hb F may indicate a more severe form of  $\delta\beta$ -thalassemia or HPFH (Clegg et al., 1977; Doneanu et al., 2007). High levels of Hb F have been reported in HPFH, a rare (Best et al., 2006; Doneanu et al., 2007; Menzel et al., 2009) condition believed to be hereditary and related to a high prevalence of SCD. The association between Hb F and HPFH, and the thalassaemias are discussed in the literature review (Best et al., 2006). Results of this study were unable to distinguish these HbAF diseases, so further studies would be necessary to detect HPFH and  $\delta\beta$ -thalassaemia patients from normal samples. At alkaline pH Hb D and G have the same separation



**Figure 1.** Detection of Hb variants by CAE and CE-HPLC shows six representative results to illustrate the consistencies found in the samples under both CAE and CE-HPLC. They are selected for having Hb AA+F, AS, SS+F band, SS AA and HbAC. Comparable results were also obtained for each variant wherever they occurred among these participants.

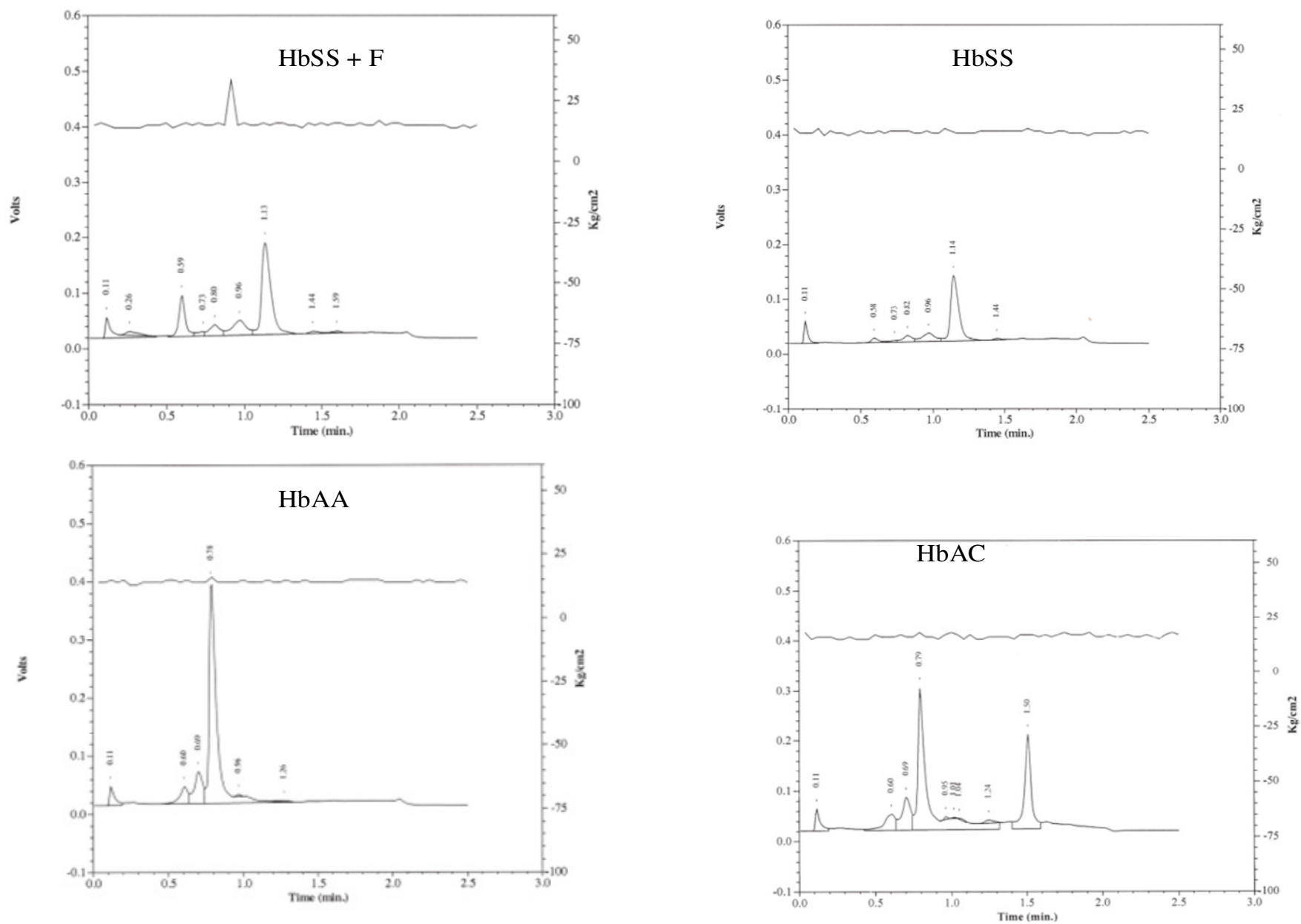


Figure 1. Continued.

**Table 1.** Overall frequency of the different haemoglobin phenotypes.

Hb variants	CAE frequency (%)	HPLC frequency (%)
Hb AA (normal)	589 (93.1)	44 (36)
Hb AS	32 - (5.1)	32 - (5.1)
Hb SS	6 - (1.0)	6 - (1.0)
Hb AC	4 - (0.6)	4 - (0.6)
Hb CC	1 - (0.2)	1 - (0.2)
Total	632 - (100)	632 - (100)

**Table 2.** Distribution of participants according to ethnicity and subjects with S or SS haemoglobin.

Ethnic origin	Number (male; female)	%- S or SS
C <sup>1</sup>	166 (90; 76)	6.02 (10)
N <sup>2</sup>	86 (40; 46)	4.65 (4)
S <sup>3</sup>	68 (31; 37)	4.4(3)
W <sup>4</sup>	80 (43; 37)	12.5 (11)
E <sup>5</sup>	78 (45; 33)	5.0 (4)
BN <sup>6</sup>	67 (39; 28)	5.9 (3)
?? <sup>7</sup>	87 (46; 41)	-
Total	632	

1 = Central tribes, 2 = North tribes, 3 = West tribes, 4 = East tribes, 5 = South tribes, 6 = Blue Nile tribe, 7 = unspecified tribes (Included both immigrants and people who did not provide tribal background).

characteristics as Hb S. Hb C also migrates as Hb E and Hb O Arab and they can be resolved from each other by CE-HPLC (Joutovsky and Nardi, 2004; Ridley et al., 2005). Therefore, the abnormal samples were collected into Guthrie cards and analysed by CE-HPLC. CE-HPLC and CAE analysis identified Hb SS, Hb AS, Hb AF, Hb AC, Hb CC and Hb AA accurately and without overlap (Table 1 and Figures 1b). HPLC is more informative, however because it evaluates Hb quantitatively (Godart et al., 1997; Surve et al., 2007; Kutlar, 2007). Both techniques are easy in day-to-day operation and have a high specificity and sensitivity in screening Hb types. However, CAE requires additional tests such as FBC and a sickling test for sickle patients to be fully informative, and additionally patient family history is also a vital consideration (D'Souza et al., 2002; Kutlar, 2007).

CAE is easy to implement, cost efficient, sensitive, and specific (D'Souza et al., 2002). CE-HPLC is more suitable in quantifying and differentiating Hb types, but is both expensive in terms of operation and maintenance (Ridley et al., 2005). CAE therefore remains the technique of choice in developing countries.

In the data reported here, CE-HPLC was used to quantify Hb F levels among the sample patients and controls in order to detect any relationship between Hb F level and the severity of SCA (Godart et al., 1997; Wild and Stephens, 1997).

## Conclusion

HbSS and AS were relatively common among the immigrant patients from the western Sudanese tribes. HbAC was found to be more common among immigrant patients from the Blue Nile Sudanese tribes. Health management needs significant improvement. A network of health centres is recommended, with screening programme and health education for the population. Advanced diagnostic technology is also required if such recommendations are to succeed. For example, restriction fragment length polymorphism (RFLP) with Hb F levels will be needed to evaluate  $\beta^s$  Haplotypes among patients with SCA and to identify thalassaemic syndromes.

## ACKNOWLEDGEMENTS

This study was supported by a Ministry of Higher Education to Faculty of Medicine and health Sciences, University of El Imam El Mahadi for research development in collaboration with School of Pharmacy and Biomedical Sciences, University of Portsmouth. We are grateful for support from all these institutes. Thanks also to the staff in Khartoum Teaching Hospital Research and Laboratory Unit.

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