First recorded case of haemoglobin SC in sudan

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Abstract

A haemoglobin (Hb) disorder was the first polymorphism suggested to have a relationship with malaria, as Hb S and C offer a survival advantage in malarial endemic regions. Two schoolgirls aged 11 and 16 were admitted to the Military Hospital in Omdurman on the 2nd of April 2009. They had Hb (haemoglobin) SC and their parents were carriers of abnormal Hb; the mother was (HbAS) and father (HbAC). These girls were the first reported cases of HbSC in Sudan. Capillary Electrophoresis (CE) was initially used to determine the Hb type. CE-High Performance Liquid Chromatography (CE-HPLC) was used as a confirmatory test. A new type of abnormal Hb in Sudan is not unexpected, because is of its wide tribal diversity. Additionally, Sudan is a high malarial region and a new Hb variant is likely to be discovered.

Keywords: Hb S and C, haemoglobinopathies management and population

Introduction

Haemoglobin (Hb) S ($\alpha_2\beta_2^{6\text{glu} \rightarrow \text{val}}$) and Hb C ($\alpha_2\beta_2^{6\text{glu} \rightarrow \text{lys}}$) are autosomal recessive disorders with a mutation in the β globin gene. They have the same substitution at the 6th position of the β -globin chain, however, their amino acid types differ, as Hb S is an abnormal Hb with substitution of valine (Val) for glutamic acid (Glu), whereas Hb C is an abnormal Hb with substitution of (Lys) for Glu [1]. Despite being a small change, it can cause disastrous complications [2]. These abnormal variants may be found in a double heterozygous form HbSC and cause (HbSC disease), but at a lower incidence. Individuals with HbSC disease have atypical haemolytic anaemias and sickling also occurs. Individuals with HbSC have been found to be more at risk for renal papillary necrosis, retinopathy and thrombotic complications than patients with sickle cell trait (SCT) [3]. However, the complications in patients with HbSC are lower than those with HbSS. Internationally, approximately 4.5% of the world population carry Hb disorders [4], with most detected in parts of Africa, particularly Hb S which is found most frequently in equatorial Africa and in people of African descent [5]. In contrast, the highest prevalence of Hb C is in the West coast of Africa [6] where approximately 0.3% of children are heterozygous for HbAC and homozygous HbCC occurs in approximately 1 in 5,000 births in that population [7]. Hb C is either homozygous or heterozygous as in HbCC or HbAC, respectively, or it can be compounded with another Hb abnormality, such as HbSC [3].

The geographic coincidence of Hb S and C genes is well reported in

regions where malaria is endemic [8,9]. Geographically, Sudan is situated in the centre of Africa, with a high prevalence of malaria [10]. Hb variants and Malaria are also known to be prevalent in the Khartoum area [11,12], as it has variety in its populations accompanied by variation in Hb phenotypes [13,14]. Therefore, different types of Hb variants are not unexpected to emerge in Khartoum, because in 1967 a published study showed a new Hb like HbSO^{Arab} [15]. Therefore, a new type of abnormal Hb is anticipated among the Sudanese.

Electrophoresis, CE-HPLC, Iso Electric Focusing (IEF) and DNA analysis are the main methods for detecting haemoglobinopathies. Obtaining family history and full blood count (FBC) are also an important factor in gaining a fuller picture of the cases. Medical history within the family is particularly important in communities, where consanguineous marriage is preferred [16,17]. Awareness of these issues within communities will help to prevent carrier parents having children with the disease.

At present, no study exists on the effect 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 in the area. Therefore, this study illustrates the hypotheses that new types of Hb may be discovered in Sudan due to the ethnic diversity there.

Materials and methods

Ethical approval was obtained for the study and written informed consent was given by the family guardian. Initially, interviews

and questionnaires were used to collect demographics: age, sex, ethnicity (tribe) and family history. A total of 6 subjects representing a Sudanese family were recruited from Khartoum military Hospital.

5ml of venous blood with K₂-EDTA (anti coagulant) were collected from each participant and delivered to the haematology laboratory at the Military Hospital, Khartoum. Each sample was processed as follows:

Firstly, FBC and Capillary electrophoresis (CE) were used to diagnose all participants. FBC was performed in a fully automated machine (Sysmex NE-800 analyzer; Toa Medical Electronics, Kobe, Japan). CE was performed in completely automatic equipment (Cebia-Model 2007). Control samples were prepared using a mixture of Hbs A, F, S and C and included in each experiment alongside the test samples [18].

Dried specimens were then prepared from the blood samples with Guthrie cards as per instructions (Whatman UK, whatmaninfo@ge.com); i.e., a drop of blood was positioned onto the card, allowed to soak through and left to dry. Samples on Guthrie cards were stored at -20 °C with dessicant material to keep specimens dry. CE-HPLC was used as a confirmatory test after Capillary electrophoresis [18]. Guthrie card samples were analyzed, using CE-HPLC [19]. The equipment used was the VARIANT nbs, Bio-Rad GDM System.

Results

(Figure 1) summarized the results of CE and CE-HPLC. The former showed three zones with the mobilities of Hb C, S and F in two sisters (A&B) of 11 and 16 years of age with HbSC; their brother (C) 7 years old and sister (D) aged 13 were heterozygous HbAS. Their parents yielded heterozygous forms, the mother of 38 years of age with HbAS and father of 52 with HbAC. (Figure 2) illustrates the findings using CE-HPLC chromatographs. The results were exactly the same as CE without overlap.

Among the family members the HbSC cases had high WBC and RDW, whereas their Hb, PCV and RBC count were low. They had lymphocytosis and their neutrophils were near the lower limit of the normal range (Table 1).

Hb F was found at levels between (4.4 to 4.6%) in those with HbSC, 0.2% in the mother but undetectable in the other heterozygous forms (Table 2).

Discussion

Hb S is known to be prevalent in Sudan and was suggested to be more common in populations from Western tribes [20,21]. On the other hand, Hb C is not as well documented as HbS in Sudan, but was recently reported in 2008 [12]. Interestingly, the current study showed two schoolgirls with HbSC, as their parents are carriers of HbAS and HbAC (Figures 1 and 2). Their family is currently living in the Khartoum area (Omdurman) but originate from Western Sudan. The parents come from different tribes; the father from the Darhamid tribe and the mother from the Taisha tribe.

This family had four children with abnormal Hb: two daughters with HbSC, a daughter with HbAS and a son with HbAS, as illustrated in the diagram of (Figure 3). Interestingly, half of their children are carriers, half with HbSC disease and none of them with normal Hb. This is the first finding of HbSC among the Sudanese and as with the discovery of Hb -O^{Arab} [15], further discoveries of new Hb abnormalities are expected to emerge, the supposition being that the variety in the population will be accompanied by variation in Hb types. Therefore, Screening and genetic counseling are required deter carrier parents from having children with abnormalities and so minimize incidence.

That no major reports exist on Hb variants within the Sudanese is due to the lack of technology to determine types of abnormal Hb e.g., CE-HPLC, IEF and molecular methods. This is due to lack of funding and the resources to identify blood disorders accurately. CE is available in parts of the country, which is accepted in conjunction with CE-HPLC or IEF as the most appropriate test for detecting Hb variants. The current study used the availability of CE at the Military Hospital, Omdurman, Sudan and CE-HPLC was used as a confirmatory test, using Guthrie cards in order to transport dry samples for analysis at the University of Portsmouth, UK.

The blood count parameters of SC patients in the current study were similar to sickle patients. They had high WBC and RDW and low Hb, PCV and RBC count. This is the same as reported for HbSC by Stevens and his colleagues [22]. In contrast, Hb, PCV and RBC levels were low in their mother, but her RDW and WBC were normal (Table 1). The haematological parameters of their father and brother were normal, but the sister with AS had low Hb and PCV.

The two sisters with HbSC had lymphocytosis and their neutrophils were at the lower limit of the normal range. However, studies reported that sickle cell patients have neutrophilia, as they are prone to the bacterial infections. Lymphocytosis is asymptomatic; it is diagnosed by blood test and caused by an illness or other problems such as viral infections or tuberculosis. In the current study the HbSC patients had not any history of tuberculosis so their lymphocytosis may be due to viral infection.

Samir K and coworkers reported that MCHC is higher in HbC patients than healthy individuals [23]. Similarly, the current data showed that SC subjects' erythrocytes were smaller than normal RBC with a higher MCHC value than other family members (Table 1). Other RBC indices were within the normal range for Sudanese individuals [12].

HbF at 4.4 to 4.6% in the HbSC cases was higher than levels reported in Sudanese patients with HbSS [24]. Of the HbAS cases, HbF was very low in the sister and her brother, but it was 0.2% in the mother. This is a lower percentage than in father (Table 2).

The prevalence of abnormal Hbs can be reduced by the premarital diagnostic screening of carriers with hereditary disorders. At present, the majority of people in Sudan have no recorded medical history and there are currently no

Figure 1. Capillary Zone Electrophoresis. (A) and (B) S homozygous form (C), (D) and (E) S heterozygous form.

screening facilities. One outcome from the data reported here is that following birth, all babies undergo either CE or CE-HPLC screening for Hb variants, and have their medical history recorded.

CC individuals have mild chronic haemolytic anaemia with frequent splenomegaly and abnormal blood smears containing microspherocytes and target cells [25]. A pathogenetic correlate of these findings is the increased mean corpuscular haemoglobin concentration (MCHC) of CC cells, which leads to intracellular crystallization of HbC [26,27]. Although AC individuals do not show these changes, both AC and CC cells are significantly more rigid than their AA counterparts [26,28]. Because AC and CC cells can support high parasitemias *in vivo*,

the malaria protective mechanism of HbC may involve elements of both perturbed RBC physiology and non sterile immunity. In conclusion, two courses of action involving health education and screening are suggested as ways of avoiding the risk of Hb abnormality. Health education is recommended for all known carriers of Hb abnormalities and patients suitable for screening programmes should include pregnant women and their newly born children. Screening should also include the genetic counselling of carriers [29,30].

SCA and HbC disease are also continuing problems in Sudan, and this not unexpected because Sudanese patients with SCA were previously seen more in paediatric clinics than in adult clinics [12].

Table 1. FBC for six members of family C. The high ()↑ and low ()↓ level marked with and arrow to each parameter respectively.

Patients	Hb g/dl	PCV%	RBC 10 ⁹ /L	WBC10 ⁹ /L	PLt10 ⁹ /L	MCVfL	MCH pg	MCHC g/dl	RDW fL
A	10 ↓	28.5 ↓	3.2 ↓	17 ↑	358 ↑	87	30.8	35.1	65.8 ↑
B	7.6 ↓	22.4 ↓	2.5 ↓	18.3 ↑	187	87	29.6	33.9	79.6 ↑
C	11.4	34	3.9	7.1	265	82	28.7		
D	9.6 ↓	26.5 ↓	3.0 ↓	16.7 ↑	339 ↑	84	30.1	34.2	67.9 ↑
E	11.3 ↓	33.2 ↓	3.8 ↓	14.3 ↑	448	86.9	29.6	34	53
F	--	--	--	--	--	--	--	--	--

Two sisters family C had high WBC and RDW, whereas their Hb, PCV and RBC count were low. Similarly, Hb, PCV and RBC were low in their mother but her RDW and WBC was normal.

Table 2. Level of haemoglobin F.

Patients	Hb A	HbS	HbC	HbA2	HbF	Others
A	?† 4♣	47.5† 42.5♣	48.1† 45.3♣	?† 1.4♣	4.4† 3.9♣	?† 2.9♣
B	?† 3.2♣	46.9† 42.4♣	49.1† 44.8†	?† 1.3♣	4.2† 3.9♣	?† 4.4♣
C	53.9† 50.7♣	42.6† 41.4♣	?† ?♣	3.5† 3.1♣	?† ?♣	?† 4.8♣
D	55.5† 51.5♣	42.4† 41.3♣	?† ?♣	2.1† 2.6♣	?† 0.7♣	?† 3.9♣
E	53.9† 50.5♣	42.8† 41.8♣	?† ?♣	3.5† 2.9♣	0.2† 0.5♣	?† 4.2♣
F	---† 47.5♣	---† ?♣	---† 45.2	---† 1.7♣	---† 2.6♣	---† 3♣

†= Capillary Electrophoresis result; ♣= CE-High Performance Electrophoresis result; others= unknown Hb variants.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

Authors' contributions	AYE	BAM	SHALM	JM
Research concept and design	--	--	✓	✓
Collection and/or assembly of data	✓	--	--	--
Data analysis and interpretation	✓	✓	--	--
Writing the article	✓	--	--	--
Critical revision of the article	--	--	--	✓
Final approval of article	--	✓	--	--
Statistical analysis	✓	--	✓	--

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