Types of Haemoglobin in Patients Attending Khartoum Teaching Hospital – Sudan Yousif A<sup>1</sup>, Mohamed B A<sup>2</sup>, Karsani M<sup>2</sup>, Thoria A/Gadir<sup>2</sup>, A/Rahman A M<sup>2</sup>, Hind Siddig<sup>2</sup>, Knight G <sup>3</sup>, Ibrahim Z O<sup>4</sup>, Khalid K M<sup>4</sup>, F/Almola Y F<sup>4</sup>

## Abstract

Background: Abnormal haemoglobins lead to significant morbidity and mortality.

**Design:** This is a prospective study, evaluating types of haemoglobin and haematological parameters in blood samples of 631 Sudanese patients.

Materials and Methods: Participants were patients who attended different departments in Khartoum Teaching Hospital in the period from March 2005 through July 2005. Electrophoresis was used to determine the type of haemoglobin.

Results: 585 cases have normal haemoglobin (Hb A). Mean red cell indices were: Hb11.5 g/dl, PCV 34.9%, MCV 85.4 fl, MCH 32.1pg, MCHC 33.2 g/dl, RDW 37 fl and WBC 7013 /cm3. 46(7.3%) patients had abnormal Hb. The frequency of Hb types were AA: 92.7%, AS: 4.9%, SS: 0.8%, AC: 0.5% and AF: 0.5%.

**Conclusions**: The presence of abnormal haemoglobin such as haemoglobin S is not unexpected because we live in the centre of an affected area. The study showed low haematological parameters due to various causes including poor nutrition as well as infections and haemolytic processes.

Key words: Haemoglobinopathy, Sickle cell, Africa.

The inherited diseases of haemoglobin (Hb) are the commonest single gene disorders<sup>1</sup>. In several areas of Africa, the prevalence of sickle cell trait (heterozygote) is as high as 30%. These conditions are seen with increasing frequency in countries where they were not recognized before<sup>1</sup>. Haemoglobinopathies (HPs) are known to occur in Khartoum, in patients of Western Sudanese ancestry<sup>2</sup>. However, there are no large-scale reports on the effects of Hb variants on red cell indices of whole blood from Sudan due to local scarcity of advanced techniques to determine types of abnormal Hb.

Objectives: of this study is to detect the types of Hb and haematological parameters in Sudanese patients attending Khartoum Teaching Hospital.

### Methods

A number of 631 patients in various clinics at Khartoum Teaching Hospital were consented to donate venous blood sample for this study during the period March through July 2005. Each sample was assessed using full blood count (FBC) cellulose acetate electrophoresis in an attempt to correlate FBC indices with abnormal Hb expression. FBC was performed within 30 minutes to one hour on a Sysmex NE-8000 machine. The remainders of the samples were treated as haemolysates and refrigerated for electrophoresis within one month. Cellulose acetate Hb electrophoresis (pH 8.6) was performed. Initially the blood samples were centrifuged for 20 minutes<sup>3</sup>.

- 1. University of Imam Mahdi, Sudan
- 2. University of Juba, Sudan
- 3. University of Portsmouth, UK
- 4. Research student, University of Juba

The red cells were washed three times in isotonic solution PH 7.4. Haemolysates were prepared from the washed cells and haemoglobin electrophoresis was performed in TRIS-EDTA-Borate buffer pH 8.6 on cellulose acetate strips<sup>3</sup>. **Statistics:** 

The data were analysed using the Excel program with the Student's *t-test* for significance.

### Results

FBC indices and results of electrophoresis were depicted in the table below.

Hb level and PCV were found to be low in all the patients with abnormal Hb. MCHC is low in Hb SS and RDW is high in most cases particularly in HbSS and Hb AS. MCH and WBCs are not different between the groups except in HbSS and Hb AS groups. 30% of patients with abnormal Hb were from Western tribes, 20% from middle/Blue Nile tribes, 15% are Southern tribes, 20% are Northern/Eastern tribes and 15% are unknown tribes.

#### Discussion

Sickle cell disease affects an estimated 50,000 Americans<sup>4, 5</sup> who belong to different ethnic backgrounds. Among infants born in the U.S.A sickle cell disease occurs in one in every 375 African Americans, one in 3,000 Native Americans, one in 20,000 Hispanics, and one in 60,000 whites<sup>6</sup>. As compared to African American in the general population, the average life expectancy of patients with sickle cell anaemia is less by 25-30 years<sup>7</sup>. In this study 46 patients were found to have abnormal haemoglobin. 35 of them were heterozygotes. The common abnormal Hb phenotypes as HbSS, Hb AS Hb CC, Hb SC, Hb AF and Hb SF. Hb AS.

A secondary test, such as gel electrophoresis at acidic pH is required to identify bands isolated by alkaline electrophoresis. On the other hand Isoelectric Focusing (IEF) gives the best

resolution of abnormal haemoglobins by Hb electrophoresis but is expensive<sup>8</sup>. High Performance Liquid chromatography (HPLC) is

Table: The FBC in cases with normal Hb versus cases with abnormal Hb

	Hb	No	Hb mg/dl	WBC	МСН	мснс	PCV	RDW
Means	Α	585	11.5	7013	32.92	33.2	34.9	37.0
SDs	Α		2.88	5952	114.79	9.78	7.71	16.59
Mean	AS	32	7.975 <sup>(a)</sup>	5731	26.20	31,1	25.01 <sup>(a)</sup>	50.66 <sup>(a)</sup>
SD	AS		1.53	3688	4.38	2.94	5.26	13.10
Mean	SS	5	7.06 <sup>(a)</sup>	8080	25.74	26.06 <sup>(c)</sup>	25.82 <sup>(b)</sup>	70.60 <sup>(a,d)</sup>
SD	SS		1.67	4115	5.93	5.19	6.38	18.80
Mean	AC	4	9.53 <sup>(e,g)</sup>	5850	22.88	40.50 <sup>(c,f)</sup>	28.15	38,50 <sup>(g)</sup>
SD	AC		0.411	624	2.59	2.380	1.48	11.36
Mean	AF	3	9.17	7033.3	23.3	28.0	28.3	32.3
	CC	1	8.5	3600	22	27	25	24
	SF	1	7.8	9200	27.1	32.9	23.7	55

SD-standard deviation (+/-1)

Comparing with Hb A (a) <0.001; (b) <0.01.

Comparing with Hb SS (f) <0.001; (g) <0.05.

Comparing with Hb AS (c) < 0.001; (d) < 0.01; (e) < 0.05.

Figures without superscript letters are deemed not statistically significant.

accurate, fast and quantitative. HPLC or IEF have been recommended for the initial screening test and using further precise techniques for the positive results<sup>8</sup>. However, DNA analysis gives a precise identification of the variant.

The tribal distribution of Hbs in our findings denote that these mutations are ethnic-specific. Our results are very much comparable with previous reports from Sudan that showed prevalence of sickle cell disease (SCD) to be 30% among the Misseria tribes in the west of the country<sup>9</sup>, 16% among immigrants from the Blue Nile province<sup>10</sup> and 18% among Nilotics in the south of Sudan<sup>11</sup>.

The majority of Hb mutants are not associated with clinical manifestations. Many were discovered during the course of large scale surveys and this reflects the importance of such studies. Obviously, HbA was dominant in Khartoum city. The presence of 4.9% of Hb AS and 0.8 % of Hb SS was expected. Patients with

abnormal Hb suffer much when an infectious disease is contracted.

The "classical" diagnostic technique for the detection of new Hb mutants is zone electrophoresis, which separates proteins differing in electrical charge. However, many mutant Hbs have normal electrophoretic mobility, and must be studied by other methods, such as high performance liquid chromatography. A number of more sophisticated techniques have also been applied to the detection of mutant Hbs, including mass spectrometry and sequencing of DNA fragments generated by the polymerase chain reaction<sup>12</sup>.

Malnutrition and malaria are common health problems in Sudan. Low blood count findings in our study are probably attributed to the effects of such diseases as well as haemlysis. This is reflected in our observed low blood counts which are in keeping with others<sup>8</sup>.

Consanguinity is one of the causes of the spread of the disease. Tests for detection of abnormal haemoglobins are advised before marriage. Also, public education over time may reduce incidence of Hbs<sup>2</sup>.

#### Conclusion

abnormal haemoglobin Detecting is important as it has socioeconomic impact especially in our community. The majority of abnormal haemoglobins can be prevented by good education and avoidance of consanguinity marriages. The study showed low also haematological parameters probably due to multifactorial reasons.

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