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Haemoglobin Patterns in Patients with Sickle Cell Haemoglobinopathies

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Abstract **Background:** Hemoglobinopathy is a group of inherited disorders characterized by structural variations of the hemoglobin molecule; and sickle cell disease constitutes one of the major genetic blood disorders in Sudan. The aim of this study was to determine the haemoglobin patterns of patients with sickle cell haemoglobinopathies. **Methods:** Blood samples were collected from 70 subjects diagnosed or suspected to have sickle cell disease. Blood samples were also taken from 30 control patients. Screening was done by sickling test and capillary electrophoresis was done. **Results:** 37 patients (52.9%) showed AS, 1 patient (1.4%) showed AS/C, 8 patients (11.4%) showed S/ßThalassaemia, 1 patient (1.4%) showed S/C, 3 patients (4.3%) showed S/D and 20 patients (28.6%) showed SS. The mean level of Hb showed lower level in patients group while HbA2 showed no significant change and HbF and HbS showed different levels according to the type of haemoglobinopathy. **Conclusion:** Different variants of sickle cell haemoglobinopathies were identified; AS, AS/C, S/ßThalassaemia, S/C, S/D and SS patterns were reported. Haemoglobin A2 have no significant difference in patients with sickle cell disease, while Hb F and Hb S show significant elevation.

Keywords: *Hemoglobinopathy, sickle cell disease, haemoglobin patterns*

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1. Introduction

Hemoglobinopathy is a group of inherited disorders characterized by structural variations of the hemoglobin molecule; result from either production of an abnormal haemoglobin chain, such as substitution of one amino acid, or underproduction of a given globin chain [1]. It may present as heterozygous or the homozygous form. More than 100 abnormal types of haemoglobin have been identified [2]; however, only hemoglobins S, C, and D are commonly seen [3].

Sickle cell disease (SCD), first described in the early twentieth century, is an inherited haemoglobinopathy resulting from a mutation mutation occurs in beta-globin gene, on chromosome 11 [4]. It is includes disorders affecting the structure, function or production of haemoglobin [5]; There is a substitution of glutamate with valine in position 6 of the beta globin resulting in the formation of haemoglobin S [6]. The disease is expressed when Haemoglobin S (HbSS) is inherited from both parents, the homozygous patient or HbSS suffers from sickle cell anaemia, while the heterozygous child or Haemoglobin AS (HbAS) is a carrier of a sickle cell trait [1]. HbSS is the most common pathological haemoglobin variant worldwide and majority of children born with SCA die before reaching five years of age [7]. Other sickle cell haemoglobinopathies like HbAS/C, HbS/C, HbS/D, HbS/ß Thalassaemia pattern has been reported [8].

Desaturation of HbSS results in the polymerization of haemoglobin, forming large aggregates called tactoids, which deform the red cells into the typical sickle shape. When compared to HbAS, HbSS begin to sickle at much higher oxygen saturation; hence, sickling with sickle cell trait is rarely a problem without concomitant stasis [1,5]. This study aimed to determine the haemoglobin patterns of patients with sickle cell haemoglobinopathies and to measure Hb A, HbA2 and Hb F levels in these patients.

2. Material and Methods

2.1. Study Design

Hospital based descriptive case control study design with well structured interviewer administered research questionnaire developed for this purpose, was used to collect data.

2.2. Study Location

This study was undertaken in a Military Hospital in Khartoum State, one of the biggest hospitals in Sudan.

2.3. The Study Population

Blood samples were collected from 70 subjects diagnosed or suspected to have sickle cell disease. Blood samples were also taken from 30 control patients case) were selected from patients attending for follow up and from the co-patients respectively.

2.4. Inclusion and Exclusion Criteria

Inclusion criteria: All patients who diagnosed or suspecting to have a sickle cell haemoglobinopathies and confirmed by positive sicklling test.

Exclusion criteria: Healthy people who suspected to have sickle cell haemoglobinopathies with negative sicklllling test.

2.5. Determination of Haemoglobin Genotype

Blood sample was obtained by venepuncture of the antecubital vein and 5ml of blood was collected in ethylenediaminetetraacetic acid (EDTA) bottles for determination of haemoglobin genotype using the usual electrophoretic method (electrophoretic equipment model MUPID-EXU, Japan). A small quantity of blood haemolysate from each subject was placed on the cellulose acetate membrane and carefully introduced into the electrophoretic tank containing Tris-EDTA borate buffer at PH 8.9. The electrophoresis was allowed to run for 15 minutes at 160V. Haemolysates from blood samples of known genotypes (Hb A, HbA2 and Hb F,, HbAS, HbSS and HbSC) were run as reference standards. The results were read according to the migration pattern of the haemoglobin variant. The results were treated with utmost confidentiality [9]. Sickling test and haemoglobin electrophoresis were carried out using Capillartys2 Flex Piercing (SEBIA).

2.6. Ethical issues

This study conforms to the ethical principles of medical research developed by the World Medical Association Declaration of Helsinki [10]. Ethical clearance was given by the Research Committee in Al Neelain University Faculty of Medicine. Written consents were obtained from each participant before entry into the study.

2.7. Data Analysis

All data obtained with questionnaire and biochemical analysis were analyzed using the Statistical Package for the Social Sciences (SPSS) version 19. The chi square test was used to test distribution of categorical variables. The differences between test and control groups were assessed with the student's t test. Statistical significance was accepted when P value is ≤ 0.05.

3. Results

As illustrated in Table 1, 36 (51.4%) of patients were female and the remaining 34 (48.6%) were male, while in control group 10 (33.3%) were female and 20 (66.7%) were male. Moreover, Figure 1 demonstrated the age distribution among the study participants. The frequent of each sickle cell haemoglobinopathies were shown in Figure 2 as follow: 37 of patients (52.9%) showed AS pattern, 1 patient (1.4%) showed AS/C Pattern, 8 patients (11.4%) showed S/β-Thalassaemia pattern, 1 patient (1.4%) showed SC pattern, 3 patients (4.3%) showed S/D pattern and 20 patients (28.6%) showed SS pattern.

Table 1. The demographic characteristic of patients and control group according gender

Characteristic	Case	Frequency	Percent
Female	Patients	36	51.4%
	Control	10	33.3%
Male	Patients	34	48.6%
	Control	20	66.7%

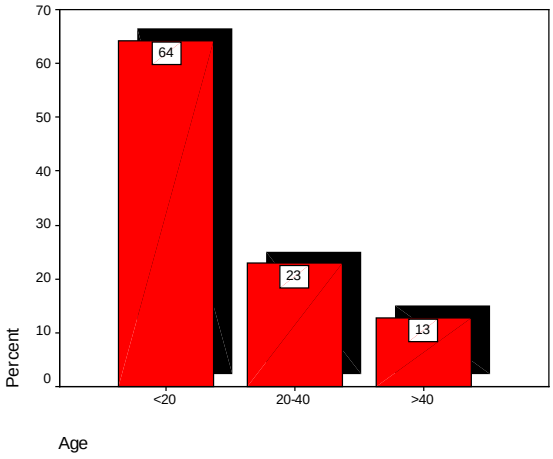

Figure 1. Age distribution in study group

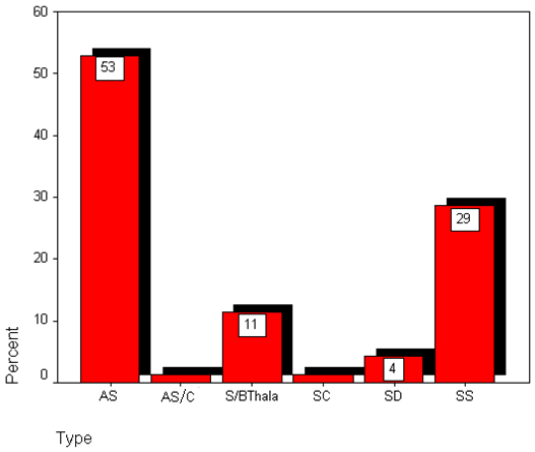

Figure 2. The frequency of variants sicklling haemoglobinopathies among patients group

Table 2. The mean level of Haemoglobin A, HbA2, HF and HbS level in AS patients compared with control group

Hb%		No.	Mean	SD	P. Value
Hb A%	Control	30	96.70%	0.579	0.000
	AS	38	55.40%	15.23	
Hb A2%	Control	30	2.61%	0.437	0.209
	AS	38	2.67%	0.502	
Hb F%	Control	30	0.70%	0.358	0.004
	AS	38	2.14%	2.645	
Hb S%	Control	30	0.00%	0.000	0.000
	AS	38	39.30%	13.840	

AS patient demonstrated a significant higher mean level of Hb F (2.14%) ± 2.7, and significant lower level of the mean of Hb A (55.4%) ±15.2 in comparison with control group, while Hb S was only show in patients group. while

Hb A2 show no significant different of mean level when compared with control group as show in (Table 2).

In addition, AS/C patient showed no significant different in Hb A2 and Hb F level when compared to control group, while a significant lower level of Hb A (47.8%) ±0.31was observed when compared with control group. Hb S as well as Hb C were only showed in AS patient group with mean level of (42.8%) ± 1.1 and (6.4%) ± 0.1 respectively as show in (Table 3). As demonstrated in (Table 4) Hb A2 in S/C patient show no significant different in mean level when compared with control group (2.1%) ± 2.5, Hb F shows significant higher mean level (4.4%) ± 2.54, but there is no level of Hb A, Hb S or Hb C (0.00%) ±0.0.

Table 3. The mean level of Haemoglobin A, HbA2, Hb F, Hb S and Hb C in AS/C patients compared with control group

Hb%		No.	Mean	SD	P. Value
Hb A%	Control	30	96.70%	0.579	0.000
	AS/C	1	47.80%	0.310	
Hb A2%	Control	30	2.61%	0.437	0.203
	AS/C	1	2.60%	0.420	
Hb F%	Control	30	0.70%	0.358	0.120
	AS/C	1	0.40%	0.100	
Hb S%	Control	30	0.00%	0.000	0.000
	AS/C	1	42.80%	1.100	
Hb C%	Control	30	0.00%	0.000	0.000
	AS/C	1	6.40%	0.100	

Table 4. The mean level of Haemoglobin A, Hb A2, Hb F, Hb S and Hb C level in S/C patients compared with control group

Hb%		No.	Mean	SD	P. Value
Hb A%	Control	30	96.70%	0.579	0.000
	S/C	1	00.00%	0.000	
Hb A2%	Control	30	2.61%	0.437	0.257
	S/C	1	2.10%	2.540	
Hb F%	Control	30	0.70%	0.358	0.000
	S/C	1	4.40%	2.540	
Hb S%	Control	30	0.00%	0.000	0.000
	S/C	1	47.50%	0.100	
Hb C%	Control	30	0.00%	0.000	0.002
	S/C	1	46.00%	0.210	

In patients with S/βThalassaemia Hb A2 show statistically significant higher mean levels when compared with control group (6.2%) ± 2.3, Hb F also showed significant higher mean level (4.4%) ± 2.54, Hb A level showed lower level than control group (42.58%) ± 28.3. While Hb S was only showed in S/β Thalassaemia patients with mean level of (45.33%) ± 23.9 as show in (Table 5).

Table 5. The mean level of Haemoglobin A, Hb A2, Hb F and Hb S level of S/βThalassaemia patients group compared with normal control group

Hb%		No.	Mean	SD	P. Value
Hb A%	Control	30	96.70%	0.579	0.000
	S/βThal	8	42.58%	28.26	
Hb A2%	Control	30	2.61%	0.437	0.002
	S/βThal	8	6.15%	2.266	
Hb F%	Control	30	0.70%	0.358	0.031
	S/βThal	8	6.18%	13.853	
Hb S%	Control	30	0.00%	0.000	0.000
	S/βThal	8	45.33%	23.93	

While (Table 6) for S/D patients Hb A2 have no significant different of mean level when compared with mean level in control group (2.5%) ± 0.44, Hb F showed slightly significant increase in mean level (1.9%) ± 2.3, but there is no level of Hb A (0.00%) ±0.0 SD. While Hb S as well as Hb D were only showed in S/D patient group with mean level (56.33%) ± 8.31 and (43.5%) ± 0.03 respectively.

Table 6. The mean level of Haemoglobin A, Hb A2, Hb F, Hb S and Hb D in S/D patients compared with control group

Hb%		No.	Mean	SD	P. Value
Hb A%	Control	30	96.70%	0.579	0.000
	S/D	3	0.00%	0.000	
Hb A2%	Control	30	2.61%	0.437	0. 671
	S/D	3	2.50%	0.436	
Hb F%	Control	30	0.70%	0.358	0.002
	S/D	3	1.90%	2.330	
Hb S%	Control	30	0.00%	0.000	0.000
	S/D	3	56.33%	8.311	
Hb D%	Control	30	0.00%	0.000	0.000
	S/D	3	43.50%	0.032	

In SS patient group Hb A2 show slightly increased mean level when compared with group (3.6%) ± 0.66, Hb F shows statistically significant increase in the mean level (12.9%) ± 9.5, but there is no level of Hb A (0.00%) ±0.0 SD. While Hb S was only showed in SS patients group with mean level of (84.1%) ± 8.96 as show in (Table 7).

Table 7. The mean level of Haemoglobin A, Hb A2, Hb F and Hb S in SS patients compared with control group

Hb%		No.	Mean	SD	P. Value
Hb A%	Control	30	96.70%	0.579	0.000
	SS	38	0.00%	0.000	
Hb A2%	Control	30	2.61%	0.437	0.060
	SS	38	3.60%	0.660	
Hb F%	Control	30	0.70%	0.358	0.000
	SS	38	12.84%	9.49	
Hb S%	Control	30	0.00%	0.000	0.000
	SS	38	84.10%	8.964	

4. Discussion

In this study, Capillary Hb electrophoresis results obtained from normal healthy control group revealed that the means of HbA, HbA2 and HbF were consistent with study in New York USA which defined the mean of HbA A (α2β2) of the normal healthy adults was 95% with small amounts (<3.5%) of Hb A2 (α2δ2) and Hb F (α2γ2) present [11].

Considerable researches were done to determine the different variants of sickle cell haemoglobinopathies [1,12,13,14]. The current work revealed that (52.9%) of patients were AS pattern which a very high rate when compared to previous reports [9]. Nnaji and other in Southeastern Nigeria reported that the majority (72.6%) of the respondents had HbAA, only 26.4% were HbAS, while 0.94% were HbSS did not find any other variants of haemoglobinopathies [9]. Moreover, similar finding exposed in Turkey by Altay et al [15]. Several reports agreed that other variants of haemoglobinopathies were very rare when compared to the Mediterranean region [8,16]. This dispute may reflect different ethnic mixes in Sudanese population.

The results of the current study are further supported by a study in USA, which revealed in simple cases of HbS trait, the percentage of HbS is always greater than the percentage of HbA, also individuals with two copies of HbS develop sickle cell disease their capillary electrophoretic patterns results typically show 90 to 95% HbS, no HbA, and often slightly elevated HbF in the 5 to 10% range [17]. Moreover, the study also divulged that the mean of haemoglobin A pattern in patient group were statistically significant lower than means of control group (P value < 0.05) which also consistence with previous mentioned study at US [17].

The study reflect that patients with sickle cell haemoglobinopathies (with exception S/βThalassaemia) have no significant differences of haemoglobin A2 in comparison with control group, while Hb F and Hb S show significant elevation respectively in comparison with control group (P. value < 0.05), this is consistent with study in Belgium was obtained [Hb A2 CZE (%) = 1.233 ; Hb FCZE (%) = 1.118 Hb], and new reference values had to be determined (Hb A2 2.7–3.8%; Hb F <1.2%). The quantification of Hb A2 was not influenced by Hb S [18].

It is apparent from the current study; that hemoglobinopathies and sickle cell anaemia are important health problems in Sudan; that emphasis the important of premarital screening programmes and their value in such country where these diseases are endemic[14,19,20]. The use of such programmes must critically evaluated before implementation, including recent experiences in Saudi Arabia, followed by discussion of the outcomes of such programmes. The success of these programmes is highly depends on adequate religious support, government policy, education and counseling [21,22,23].

5. Conclusion

The study concluded that the distribution rate of sicklle cell haemoglobinopathies was among all age groups, affected both sexes equally. Haemoglobin A2 have no significant in pateints with sickle cell haemoglobinopathies in comparison with control group, while Hb F and Hb S show significant elevation respectively in comparison with control group.

Competing Interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

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