Hematological and molecular analyses of the HbS allele among the Sudanese population

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Abstract

Objective: The purpose of this study was to perform hematological and molecular analyses of the HbS allele of the hemoglobin subunit beta gene in the Sudanese population.

Methods: This was a descriptive cross-sectional study. Hematological parameters and fetal hemoglobin (HbF) levels were assessed in all participants. Data were gathered through the use of questionnaires and laboratory investigations. The β^S-globin haplotypes, S allele distributions, and hematological parameters with HbF levels were investigated using PCR-restriction fragment length polymorphism, gel electrophoresis, and a Sysmex hematology analyzer, respectively.

Results: According to our findings, the Bantu (BA) haplotype was found in 10.8% of participants with homozygous uncontested haplotypes, followed by Benin (BA) and Sudan (SU), each in 9.8% of participants. This Sudanese group from Northern Kordofan lacked the Arab-Indian haplotype. Two heterozygous versions of undisputed haplotypes were found in 17.3% of participants: SU/BA in 10.8% and CA/BE in 6.5%.

Conclusion: As a result of sickle cell anemia, this investigation found changes in hematological parameters. In the Sudanese population, a new haplotype of the S gene was discovered.

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Introduction

A single point mutation in the human hemoglobin (Hb) subunit beta that substitutes the amino acid valine for glutamic acid in the sixth position of the globin chain causes sickle cell anemia (SCA). This allele is termed HbS, and HbSS is the homozygous variant of this defect; patients with this genotype have SCA. Unless subjected to severe conditions, heterozygous carriers of the variant (HbAS) are essentially healthy.[1] In comparison, the HbAA genotype (wild type Hb) encodes a tetramer with four globin chains in total, two alpha and two beta chains. Each chain has one group that reversibly binds oxygen.[2] Normal hemoglobin is soluble in both oxy and deoxy forms, whereas HbS is less soluble in deoxy forms, resulting in the sickle cell crisis.

SCA is one of the most common types of hemolytic anemia, especially in western Sudan, and notably in the Darfur and Kordofan regions,[3–6] where this study was conducted. This abnormality poses a significant public health concern to a variety of ethnic groups.[5]

Parents of children who have been affected are resigned to their children's fate and hospitalization expenditures. Although improving health education in general is crucial,[7] very little effort has been paid to this aspect of SCA management.[8] This study aimed to perform hematological and molecular analyses of the HbS allele in the Sudanese population.

Patients and methods

Study design

This was a descriptive cross-sectional study conducted at a hospital. The findings are a snapshot of the situation across a given time period. The HbS allele is widespread in the Sudanese population of the Northern Kordofan state.

Study population

Patients were chosen selectively. All patients who required follow-up at the Northern Kordofan State Sickle Cell Center (El-Obeid) between December 2018 and July 2020 were requested to participate in the study and formally consented if willing. All patients' details have been de-identified. Fellow researchers may reproduce the methodology of this study from the description given in this section.

Blood samples were taken from consecutive consenting patients (study participants) using the probability proportionate to size (PPS) random sampling method, of which 51.2% were SCA patients, 8.9% were carriers of the sickle cell trait (SCT) and 39.9% were healthy volunteers.

Full blood counts

All blood samples were processed within 2 hours of collection. Laboratory investigations were performed in accordance with normal procedures, with quality control checks performed on a regular basis.

An automated particle cell counter was used to examine hematological parameters (Sysmex XE 5000 Analyzer, Toa Medical Electronics, Kobe, Japan).

Capillary electrophoresis

The capillary system contained capillaries that work in parallel, allowing for simultaneous measurements of Hb (it has the same principle of capillary electrophoresis in free solution). A sample diluted with hemolysis solution was mixed and injected *via* aspiration at the capillary's anodic end. Hb was then directly detected at 415 nm at the cathodic end of the capillary after high voltage protein separation. Before every run, the capillaries were washed with wash solution and buffered in preparation for the next analysis. The electrophoretic mobility of charged molecules in the capillary system separated them in an alkaline buffer with a specified pH. Separation then occurred according to the electrolyte pH and electro osmotic flow (Capillary electrophoresis system Capel-205; Lumex Instruments, Mission, BC, Canada).

Restriction fragment length polymorphisms (RFLPs) for HbS haplotypes

RFLPs are a type of polymorphism that are determined by differences in DNA sequences following digestion by specific restriction enzymes. A RFLP probe is a tagged DNA sequence that hybridizes with one or more pieces among a digested DNA sample after gel electrophoresis, revealing a distinct blotting pattern characteristic of a certain genotype at a specific locus (Genetic Analyzer, Applied Biosystems, Foster City, CA, USA).

Data analysis

SPSS (IBM Corp., Armonk, NY, USA) was used to evaluate all data and to create tables and graphs. Some variables, such as abnormal Hb and hematological parameters, are given descriptive statistics. To find significant differences, the Student's t-test and ANOVA were used. The reporting of this study conforms to STROBE guidelines.[9]

Ethical approval

The Faculty of Medical Laboratory Science at the University of Kordofan provided ethical approval of the study protocol.

Results

Classification of the study population

Table 1 shows clinical characteristics of the 158 study participants, of whom 51% were SCA patients, 8.9% were SCT carriers, and 39.9% were healthy volunteers. The latter were used as the control group. Fetal hemoglobin (HbF) levels were found to be high in 7% of SCA patients.

Red blood cell (RBC) indices in individuals with the HbS allele and in the control group

The mean blood profiles for the control group (HbAA; $n = 63$ [39.9%]) were as follows: Hb, 13.9 g/dL; packed cell volume (PCV), 44.6%; mean corpuscular volume (MCV), 82.3 fL; mean corpuscular hemoglobin (MCH), 26.2 pg; mean corpuscular hemoglobin concentration (MCHC),

Table 1. Classification of study population according to genotype.

Hb type	Frequency (%)
HbSS	81 (51.2)
HbAS	14 (8.9)
HbAA	63 (39.9)
Total	158 (100)

Hb, hemoglobin.

34.5 g/dL; and RBCs, 4.4×10^6/cmm, all of which were within normal values. Regarding the SCA patients (HbSS; $n = 81$ [51.2%]) and SCT carriers (HbAS; $n = 14$ [8.9%]), these values were as follows: Hb, 7.2 and 12.6 g/dL; PCV, 22.3% and 37%; MCV, 81.2 and 80.6 fL; MCH, 25.6 and 26.7 pg; MCHC, 35.6 and 36.2 g/dL; and RBCs, 3.2×10^6 and 3.8×10^6/cmm, respectively (Table 2).

Differential white blood cell (WBC) counts in the study population

The differential WBC counts in the control group were 47% neutrophil, 33% lymphocyte, 8.0% monocyte, 3.4% eosinophil, and 0.9% basophil. Total WBC counts in the SCA and SCT groups were 12.7×10^3 and 6.9×10^3/cmm, respectively. The differential WBS counts in these groups were 62% and 51% neutrophil, 28% and 34% lymphocyte, 8.9% and 8.2% monocyte, 2.7% and 3.2% eosinophil, and 0.8% and 0.9% basophil, respectively (Table 3).

HbF levels among patients with SCA

Five groups of HbF levels were detected among the SCA patients. These were <5% (92.7%), between 5% and 10% (5.1%), between 10% and 15% (1%), between 15% and 20% (0.6%), and >20% (0.2%). Some SCA patients with high HbF levels are delineated in Table 4.

Platelet counts were significantly higher in participants with the HbSS genotype than in those with the HbAA or HbAS genotypes (p < 0.05). Platelet counts were also found to be significantly higher in the HbAS group compared with the HbAA group (Figure 1).

Haplotype analysis

The classification and prevalence of β^S-globin haplotypes (uncontested and

Table 2. Complete blood cell indices of sickle cell anemia patients, carriers, and the control group.

Blood parameters	Control (Mean ± SD)	HbAS (Mean ± SD)	HbSS (Mean ± SD)
RBCs/cmm	4.4×10^6 (±0.5)	3.9×10^6 (±0.4)*	2.1×10^6 (±0.4)**
Hb g/dL	13.9 (±2.2)	12.6 (±1.1)*	7.2 (±1.2)**
MCH/pg	26.2 (±1.1)	26.1 (±1.5)	27.2 (±2.1)
MCHC g/dL	34.5 (±1.1)	32.2 (±1.6)	35.6 (±1.8)
PCV %	44.6 (±4.2)	37.0 (±5.6)*	22.3 (± 4.2)**
MCV fL	82.3 (±5.3)	80.6 (±6.5)*	81.2 (±7.9)*
RDW fL	42.1 (2.6)	43.7 (3.5)	83.8 (4.3)*

*P ≤ 0.05 is significant.
RBCs, red blood cells; Hb, hemoglobin; MCH, mean cell hemoglobin; MCHC, mean cell hemoglobin concentration; PCV, packed cell volume; MCV, mean cell volume; RDW, red cell distribution width.

Table 3. Differential white blood cell counts in the study population.

Genotype	Neut% ± SD	Lymph% ± SD	Mono% ± SD	Eosino% ± SD	Baso% ± SD
HbSS	62.0 ± 11.0*	28.0 ± 9.0	8.9 ± 2.3	2.7 ± 2.8	0.8 ± 0.5
HbAS	51.0 ± 15.0	34.0 ± 7.0	8.2 ± 2.4	3.2 ± 2.1	0.9 ± 0.7
HbAA	47.0 ± 11.0	33.0 ± 9.0	8.0 ± 3.7	3.4 ± 1.9	0.9 ± 1.2

*P ≤ 0.05 is significant.
Hb, hemoglobin; Neut, Neutrophil; Lymph, Lymphocyte; Mono, Monocyte; Eosino, Eosinophil; Baso, Basophil.

contested) is shown in Table 5. The haplotypes were constructed from the presence (+) and absence (−) of each restriction enzyme site in patients with HbSS in accordance with results from the Genetic Analyzer (Applied Biosystems).
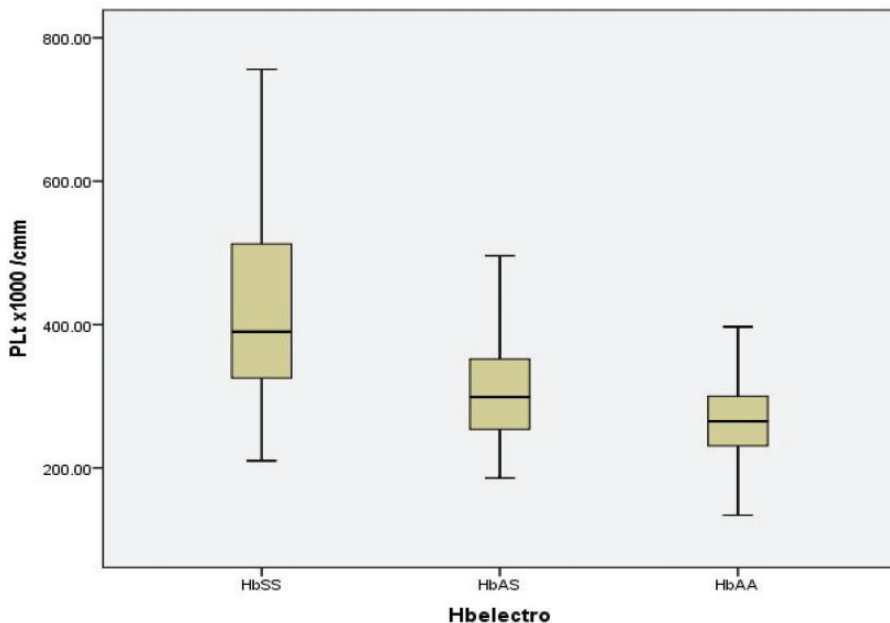
Among the studied samples, 93% and 100% of cases had a (−) allele for the ε-HindII and $^{G}\gamma$-XmnI RFLP sites, respectively. In contrast, the 5′ $\psi\beta$-AvaII site was (+) in most cases (97%), and the remainder were found to be either (+/−) or (+/+).

Table 4. Fetal hemoglobin levels in sickle cell anemia patients.
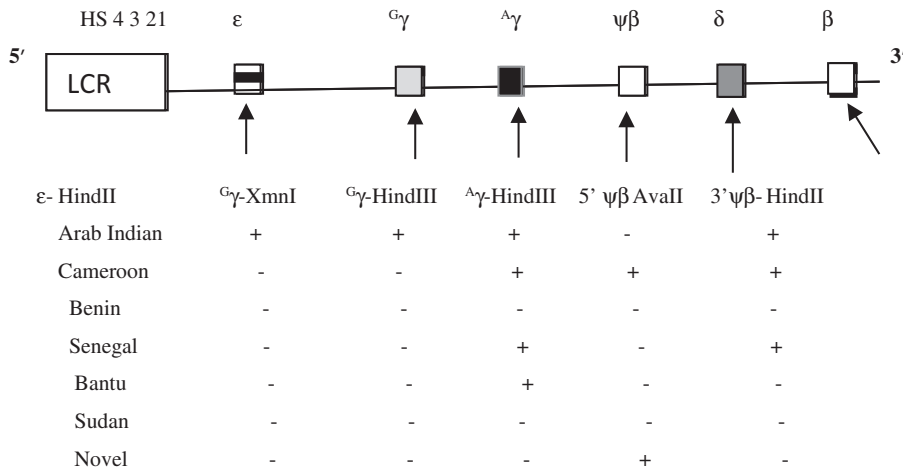
Group	Number (%)
< 5%	24 (15.1)
5%–10%	32 (20.3)
10%–15%	22 (13.9)
15%–20%	9 (5.7)
> 20%	8 (5.1)
Total	158

To identify the five known β^{S}-globin haplotypes, the $^{G}\gamma$-XmnI and 5′$\psi\beta$-AvaII results were not used, as the Senegal, Benin, Central African Republic (Bantu), Cameroon, and Arab-Indian haplotypes can be differentiated using only the ε-HindII, $^{G}\gamma$-HindIII, $^{A}\gamma$-HindIII, and 3′$\psi\beta$- HindII RFLP results. Distinguishing the β^{S}-globin haplotypes from the RFLP results was interpreted in the following way: only the Arab-Indian haplotype has (+) ε-Hind II, while the others have (−); Benin (BE) has (−) for $^{G}\gamma$-HindIII and all others are (+); Bantu (BA) has (−) for 3′$\psi\beta$-HindII and all others are (+); Cameroon (CAR) has (+) for $^{A}\gamma$-HindIII and all others are (−) (Figure 2).

β^{S}-globin haplotypes could be identified in patients homozygous for each RFLP allele (−/− or +/+), and for those with just one heterozygous RFLP site (−/+). These are designated as uncontested. For patients with heterozygous results for two or more of the four key RFLPs, the

Figure 1. Platelet counts among the study participants.

	ε-HindII	Gγ-XmnI	Gγ-HindIII	Aγ-HindIII	5' ψβ AvaII	3'ψβ-HindII	
Arab Indian	+	+	+	-	+	+	+
Cameroon	-	-	+	+	+	+	+
Benin	-	-	-	-	-	-	+
Senegal	-	-	+	-	+	+	+
Bantu	-	-	+	-	-	-	+
Sudan	-	-	-	-	-	-	+
Novel	-	-	-	-	+	-	+

Figure 2. Location of the six polymorphic restriction enzyme sites within the β-globin gene cluster used to construct the β^S-globin haplotypes. Results for all β^S-globin gene haplotypes including the novel one are displayed.

haplotypes cannot be identified because there are more than two possible haplotypes, or they could be only partially characterized in some cases; thus, these results are listed as contested.

Table 5 shows the frequencies of the uncontested and contested β^S-globin haplotypes. Among the homozygous uncontested haplotypes, the Bantu (BA) haplotype was the most frequently detected (10.8%), followed by Benin (BA) and Sudan (SU) (9.8% each). The Arab-Indian haplotype was not represented in this Sudanese population from Northern Kordofan. Two heterozygous forms of uncontested haplotypes were detected in 17.3% cases: SU/BA in 10.8% and CAR/BE in 6.5%.

The remaining haplotypes were divided into two categories: 30.8% were partially characterized and 14.8% were novel. The former were those who were classified as miscellaneous: BA or BA/?, SU or SU/?, CAR, SE or BE/?, BE, SE or CAR/?, and BE or SE/? haplotype combinations. The miscellaneous forms were found in 30.8% of samples. These were cases with two or more heterozygous sites, and therefore haplotype identification was impossible without further family studies and deduction by linkage analysis.

An atypical (contested) haplotype was defined as any haplotype not represented by the five main haplotype designations. Five forms of non-characterized haplotypes were observed, to be specific, 14% (−/−, −/−, −/−, +/+, −/−, +/+), 3.3% (−/−, −/−, −/−, +/+, +/−, +/+), 2,2% (+/−, −/−, −/−, −/−, −/−, +/+), 3.3% (+/−, −/−, −/−, +/−, −/−, +/+) and 2.2% (+/−, −/−, −/−, +/−, −/−, +/−) (εHindII, Gγ-XmnI, Gγ-HindIII, Aγ-HindIII, 3'ψβ-HindII, 5'ψβ-AvaII, respectively). The former is a new Sudan haplotype that is novel in the Northern Kordofan area. Its name is suggested to be the Northern Kordofan (NK) haplotype.

Discussion

Previous research has found that SCA is more prevalent among the central Sudanese population, particularly in the Northern Kordofan region,[10] than in eastern Sudan, the Khartoum region,[2,3] or the

Table 5. Prevalence of typical and atypical β^S-globin haplotypes.

Parameter	Number (%)	Haplotype	1. ε-HindII	2. Gγ-XmnI	3. Gγ-HindIII	4. Aγ-HindIII	5. 3′ψβ-HindII	6. 5′ψβ-AvaII
Uncontested								
Homozygous	10 (12.4)	BA/BA	−/−	−/−	+/+	−/−	−/−	+/+
Homozygous	9 (11.1)	BE/BE	−	−	−	−	+/+	+/+
Homozygous	9 (11.1)	SU/SU	−	−	−	−	−	−
Heterozygous	10 (12.4)	SU/BA	−/−	−/−	−/+	−/−	−/−	−/−
Heterozygous	6 (7.4)	CAR/BE	−/−	−/−	+/−	+/−	+/+	+/+
Contested								
Partially Characterized	25 (30.8)	BA, SU, CAR	−/−	−/−	−/−	−/−	−/−	−/+
		SE or BE/?	−/−	−/−	+/−	+/−	+/−	+/+
		BE, SE or	−/−	−/−	+/−	+/−	+/+	+/+
		CAR/? BE or SE/?	−/−	−/−	+/−	−/−	+/+	+/+
Novel	12 (14.8)	New – Sudan	−/−	−/−	−/−	+/+	−/−	+/+

BA, Bantu; BE, Benin; CAR, Cameroon; SE, Senegal; SU, Sudan.

Blue Nile province.[1] In this study, we screened 158 participants, among which 95 (60.1%) had the HbS allele. Among these, 81 (51.3%) had the HbSS (SCA) genotype, whereas the remaining 47 (8.9%) were carriers (SCT).

This investigation discovered changes in hematological parameters, primarily among SCA patients, due to Hb abnormalities, recurrent infection-induced hemolytic processes, and coagulopathy.[11] MCV and RDW were both significantly increased in SCA patients compared with the national norm. MCV was higher in the majority of patients with the HbSS and HbAS genotypes, which could be attributable to enhanced hemolysis. RDW was also elevated in these groups, which was ascribed to anisocytosis.[4,5]

WBCs, particularly neutrophils, were considerably increased in those with HbSS compared with those with HbAS and HbAA. This leukocytosis could be the result of a recurring bacterial infection.[5,8] Platelets were also considerably increased in HbSS cases compared with HbAS and HbAA instances. Thrombocytosis can also be produced by hemolysis-induced bone marrow hyperplasia.[5,12]

HbF levels in HbSS cases were comparable with HbAA and HbAS instances but showed substantial fluctuation ($p<0.05$). Clinically, sickle cell disease with high HbF levels has been linked with moderate symptoms.[13,14] This could be attributable to innate genetic make-up, and it shows that the disease may manifest as mild clinical symptoms in patients with high HbF levels. As a result, S haplotypes were also determined in the research population.

In this study, we classified the HbF levels in SCA patients into three groups: >10% (68.4%), between 5% and 10% (19.6%), and <5% (12%). The average HbF level in HbSS samples (12.2%) was greater than in control and HbAS samples. HbF is present at low levels in African haplotypes, with the exception of the Senegal haplotype.[15,16] Senegal has the same mutation as the Arab-Indian haplotype (C—T), and Xmn1 has been found to be positive in both. Clinical symptoms are also less severe than in other African haplotypes.[15,17]

Population-level data of SCA haplotypes are useful for monitoring the migration and spread of the sickle cell allele and assessing the clinical severity of the disease. Given the large disparities in clinical and hematological findings amongst different groups, it is critical to identify haplotypes and understand the clinical and behavioral characteristics of this aggressive disease among the different haplotypes. The presence of four haplotypes was discovered in this population by haplotype analysis.[16] Although our study included fewer SCA patients than prior studies, given the diversity of the Sudanese people, our findings should be regarded as preliminary, and replications of this haplotype analysis should be undertaken in much larger samples from various regions of the North Kordofan State.

According to their geographical distribution, the main haplotypes present in Sudan are classified as Benin (BEN), Bantu or Central African Republic (BAN or CAR), Senegal (SEN), Cameroon (CAM), and Arab-Indian (ARB). Different haplotypes are associated with various clinical symptoms and HbF levels.

According to the findings of this study, among the homozygous uncontested haplotypes, the Bantu (BA) haplotype was found in 10.8% of patients, followed by Benin (BA) and Sudan (SU) in 9.8% each. This Sudanese group of Northern Kordofan lacked the Arab-Indian haplotype. Two heterozygous versions of undisputed haplotypes were found in 17.3% of cases: SU/BA in 10.8% and CA/BE in 6.5%. The remaining haplotypes were separated into two groups: those that had been somewhat defined (27.1%) and those that had not been characterized (24%). A similar study was conducted at Khartoum Teaching Hospital, and the haplotypes connected with the HbS allele revealed that the most abundant haplotypes were the Cameroon, Benin, Bantu, and Senegal haplotypes, in that order. That study also found no link between haplotypes and hematological parameters.[18]

Another study conducted in Cameron discovered that the haplotype of the study population suggested that Benin (74%) and CAR (19%) were the most prevalent haplotypes observed among Cameroonian patients. There was no link found between Hb haplotypes and clinical events, anthropometric measurements, hematological parameters, or HbF levels. When HbSS patients with Senegal, Benin, and CAR haplotypes are compared, the lifetime severity of sickness increases from Sen to Ben to CAR.[19]

One drawback of this study is the small sample size, which is due to some patients' refusal to participate in the study and provide their samples and data due to local traditions and customs.

Conclusion

This study detected a new haplotype of the HbS allele in the Sudanese population. Accordingly, haplotype status may need to be considered in both clinical and public health settings. This has long been a subjective assessment of the situation. Haplotype analysis is necessary to reduce morbidity and mortality in SCA patients; therefore, pre-marital examinations, neonatal diagnostic screening for SCA, and timely family health education are required.

Declaration of conflicting interest

The authors declared that there is no conflict of interest in this research

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Author contributions

All authors had equal roles in the preparation, writing, and revision of the manuscript.

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