DOI: https://doi.org/10.53350/pjmhs2115103080

**ORIGINAL ARTICLE**

# Sickle Cell Disease and B Globin Gene Haplotypes Among Selected Sudanese Population in North Darfur State

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## ABSTRACT

**Aim:** To identify β globin gene haplotypes and their frequencies in patients with SCD in North Darfur state, western Sudan

**Methods:** This is a cross-sectional prospective community-based study that was carried out between December 2017 and August 2018. The study took place in the North Darfur state which is located in western Sudan. The study included 666 individuals (369 females and 297 males). Participants were screened for haemoglobinopathies using haemoglobin electrophoresis, while β globin haplotypes analysis for patients with SCD was carried out using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP).

**Results:** Among the 666 participants, 579 (86.94%) had normal hemoglobin (AA), while 70 (10.51%), 13 (1.95%), and 4 (0.6%) had AS, SS, and AD respectively. Cameroon haplotype was found in 42.3% of the study group. Benin was 26.9%, Bantu was 23.1% and Senegal was 7.7%.

**Conclusion:** The Cameron haplotype was found to be most prominent in Sudanese patients, thereby confirming the findings of previous studies in the country.

**Keywords:** sickle cell disease; anemia; β-globin; haplotype; North Darfur; Sudan

## INTRODUCTION

Haemoglobinopathies, such as sickle cell anaemia and thalassaemia, are genetically inherited, widely distributed and one of the world's major health problems [1]. Sickle cell anaemia is inherited in an autosomal recessive manner, that is, dysfunctional beta-chains are formed by single amino acid substitution of glutamic acid to valine in the beta-chain; as such, both copies of the gene in each cell have mutants [2]. The conceptual formula for sickle disorder (Hb SS) is α2β26Glu-Val, and normal adult haemoglobin is made up of 2 alpha and 2 beta globin polypeptide chains (Hb A)(α2β2) [3]. In adults, HbA constitute approximately 97%, while the two other forms namely: Hb A2 (α2δ2) and Hb F (α2γ2) were represented by minimal concentrations of 1.5%–3.2% and < 1%, respectively [4]. In malaria-endemic environments, 30%–40% of individuals possess one of these significant mutant variants, and the prevalence of Hb abnormalities varies from 0.3 to 25 per 1000 live births [5]. Most people with haemoglobin Hb S live in Africa [6]. The haemoglobin S disorder in Africa follows a more severe clinical course than in other parts of the world because of the significant role of malaria in increasing the disease severity [7]. In Sudan, sickle cell anaemia is prevalent in many parts of the country, with residents of the western part having highest the prevalence among the Sudanese population. The first case of the disease in the country was reported in 1926 and was considered the first case of sickle cell anaemia in Africa [8]. In western Sudan, haemoglobin S is very well documented among the Albagara, an Afro-Arab community of tribes with a prevalent African ancestry [9]. A previous study conducted among a subgroup of Albagara (Misseria) reported a high prevalence of SCD (30%), with 16% of immigrants from the Blue Nile province suffering from the disease [10]. Moreover, HbS has been associated with the high prevalence of SCD in the White Nile [10] and Khartoum states [11].

All patients with SCD have the same mutation, but their clinical presentation varies widely. This phenomenon could be attributed to several factors, one of them is the β-globin gene haplotype influences the clinical severity of SCD in patients from different populations. Different haplotypes have been identified and named according to the geographic location or ethnic group in which they were originally identified, i.e. Cameroon [12], Benin, Senegal [13], Bantu or Central African Republic and Arab-Indian [14]. The β-globin gene cluster haplotype efficiently serves as a marker for the genetic background of patients with SCD and for predicting the disease severity. In this context, data regarding the β globin gene haplotype in Sudanese patients with SCD are scarce, and additional studies are required. Therefore, the present study aimed to determine β globin gene haplotypes in North Darfur community

## MATERIAL AND METHODS

**Study setting:** This study was carried out in North Darfur state, which is one of the five states in the western part of Sudan and has an area of 296,420 km$^2$.

**Study design and population:** This cross-sectional, prospective community-based study was conducted in North Darfur state between December 2017 and August 2018. Six hundred and sixty-six inhabitants from all parts of North Darfur were randomly enrolled in this study, and all of the major North Darfur tribal groups were represented.

**Sample collection and laboratory investigation:** A total of 5 ml of venous blood samples were collected from each participant for determination of haemoglobin type. DNA from the specimens identified as Hb S was extracted and used for determination of βS globin gene haplotypes. Direct structural interviewing and informed consent questionnaire were used to collect individual socio-demographics data.

**Hemoglobin Electrophoresis:** Capillary zone electrophoresis Minicap Flex Piercing (CZE) was used in this study to separate the Hb variants in whole blood [15,16].

**DNA extraction and β globin haplotyping assay:** DNA extraction was carried out using the Chelex matrix (Bio-Rad, USA) according to the manufacturer's instruction. The isolated DNA was stored at -20 °C until use in subsequent analyses. β-globin haplotyping assay was carried out for six specific loci of the β globin gene cluster by using polymerase chain reaction (PCR) followed by restriction fragment length polymorphisms (RFLP) as described previously [17]. The six loci were amplified using specific sets of primers to generate PCR fragments of 760 bp, 650 bp, 323 bp, 635 bp, 914 bp and 328 bp (Supplementary Table 1). The PCR reaction was prepared in final volume of 20 µl containing 4 µl of 5x HOT FIREPol Blend Master Mix, 1.5 µl from each primer and 2 µl of DNA template. The amplification was carried out under the following conditions: initial denaturation at 94 °C for 6 min, followed by 35 cycles of amplification at 94 °C for 1 min, annealing at 58 °C for 1 min, extension cycle at 72 °C for 1 min and final extension cycle at 72 °C for 8 min.

The amplified fragments containing the six restriction sites were digested by the appropriate restriction enzyme (Supplementary Table 1). The restriction digestion reaction was performed in a total volume of 20 µl containing 10 µl of the amplified products of the β globin gene and 5 units of appropriate enzyme. The mixture was then incubated at 37 °C overnight. β Globin haplotypes were determined through absence (-) and/or presence (+) of each of the six restriction enzyme sites in individuals with Hb SS.

**Visualisation of β-haplotype fragments:** In brief, 15 µl of the digested products were run on 2% agarose gel dissolved in 1X TBE buffer and electrophoresed for 1 h at 80 V. The fragments were visualised on UV trans-illuminator to interpret and identify the haplotypes (Table 1).

**Ethics declarations:** Prior to be recruited, all the participants were briefed about the objectives of the study. A written informed consent was obtained from all adult participants and from a parent or guardian for child participants aged less than 18 years prior to obtaining the blood sample. The study protocol was reviewed and approved by the State Ministry of Health ethics committee (NO: 23 at 13.8.2017).

## RESULTS

A total of 666 residents comprising 369 (55%) females and 297 (45%) males of the North Darfur state were enrolled in the current study. The mean ages of the female and male participants were 21.7± 18.3 years and 20.9 ±15.4 years, respectively. Table 2 shows the average values of haematological parameters in all study population (total), normal group (Hb AA), hemoglobin D (Hb AD), sickle trait (Hb AS) and sickle cell disease (Hb SS) groups. The respondents came from 59 tribes, which included the entirety of the North Darfur community as well as all of the state's localities (Table 3). All the 666 samples were successfully genotyped by capillary electrophoresis. The predominant genotype detected was the Hb AA type, with a prevalence of 86.94%, followed by heterozygous Hb AS (10.51%), Hb SS (1.95%) and Hb AD 0.6%.

In this study, β globin haplotypes were determined by RFLP using five restriction sites in the β globin gene cluster. Four major haplotypes and seven haplotype combinations were identified among the chromosomes of the 13 Sudanese patients with homozygote Hb S in the North Darfur state. Cameroon haplotype was the most frequent (42.3%), followed by Benin and Bantu haplotypes, with frequencies of 26.9% and 23.1%, respectively (Table 1). Only two chromosomes (7.7%) had the Senegal haplotype, while the Arab haplotype was not observed in this population (Table 4). The heterozygote Cameron/Benin β globin gene haplotype was the most common haplotype detected in four of the studied patients (4/13, 30.8%) followed by heterozygous Cameron/Bantu in 3/13 (23.1%) patients and homozygous Cameron/Cameron in 2/13 (15.3%) patients. The following haplotype combinations, namely, Senegal/Benin, Benin/Benin, Senegal/Bantu and Bantu/Bantu, were identified in each of the four remaining patients. The haematological parameters of homozygote Hb S patients as well as their physical signs and clinical features are presented in Supplementary Table 2, 3 and 4, respectively.

Table 1: Interpretation of haplotypes,

| Haplotype | AvaII | HindII | HindIII Ay | HindIIIGy | XmnIGy | HindII |
|---|---|---|---|---|---|---|
| Benin | + | + | _ | _ | _ | _ |
| Arab-Indian | + | + | _ | + | + | + |
| Cameron | + | + | + | + | - | - |
| Senegal | + | + | _ | + | + | _ |
| Bantu | + | _ | _ | + | _ | _ |
| Bantu A1 | + | _ | _ | _ | _ | _ |
| Bantu A2 | + | _ | _ | _ | _ | + |
| Bantu A4 | _ | + | _ | + | + | _ |
| Bantu A6 | + | _ | + | + | _ | _ |

Table 2: Mean haematological parameters among study group

| Blood parameters | Total (n=668) | AA (n=579) | AD (n=4) | AS (n=70) | SS (n=13) |
|---|---|---|---|---|---|
| Hb (g/dl) | 12.6±2.19 | 12.9±2.4 | 12.4±2.9 | 11.8±2.0 | 7 |
| PCV% | 38.5 | 39.2 | 38.9 | 35.2 | 22.1 |
| RBCs ( $10^{12}$/L ) | 4.7±0.8 | 4.7±0.9 | 4.7±0.6 | 4.3±0.8 | 2.2±0.5 |
| MCV (fl) | 84.9 | 85.2 | 83.3 | 81.3 | 92.3 |
| MCH (pg) | 27.9 | 27.9 | 26.3 | 27.2 | 29.1 |
| MCHC(g/dl) | 32.8 | 32.7 | 31.6 | 33.2 | 31.5 |
| TWBCs ($10^9$/L) | 6.9±2.2 | 6.7±9.9 | 8.6±8.0 | 6.7±4.5 | 18.5±15.0 |
| PLT ($10^9$/L) | 263.1±116.7 | 259.9±114.2 | 180.3±86.3 | 278.4±110.7 | 392.4±178.1 |

Table 3: Distribution of hemoglobin variant according to localities

| Locality | Total | Hemoglobin variant | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | AA | | AS | | SS | | AD | |
| | | Fre | % | Fre | % | Fre | % | Fre | % |
| Alfasher | 195 | 160 | 82.1 | 26 | 13.3 | 6 | 3.1 | 3 | 1.54 |
| Sarf-omr | 15 | 13 | 86.7 | 2 | 13.3 | 0 | 0 | 0 | 0 |
| Kalmendo | 15 | 13 | 86.7 | 2 | 13.3 | 0 | 0 | 0 | 0 |
| Almalha | 17 | 17 | 100 | 0 | 0 | 0 | 0 | 0 | 0 |
| Alkoma | 19 | 14 | 73.7 | 3 | 15.7 | 1 | 5.3 | 1 | 5.3 |
| Ombaro | 26 | 25 | 96.2 | 1 | 3.8 | 0 | 0 | 0 | 0 |
| Altewsha | 12 | 8 | 66.7 | 3 | 25 | 1 | 8.3 | 0 | 0 |
| Alwaha | 33 | 30 | 90.9 | 3 | 9.1 | 0 | 0 | 0 | 0 |
| Um-kadad | 14 | 14 | 100 | 0 | 0 | 0 | 0 | 0 | 0 |
| Allayeed | 33 | 33 | 100 | 0 | 0 | 0 | 0 | 0 | 0 |
| Alseraf | 21 | 20 | 95.2 | 1 | 4.8 | 0 | 0 | 0 | 0 |
| Dar-alslam | 41 | 27 | 65.9 | 11 | 26.8 | 3 | 7.3 | 0 | 0 |
| Tawela | 56 | 49 | 87.5 | 7 | 12.5 | 0 | 0 | 0 | 0 |
| Kutom | 40 | 38 | 95 | 2 | 5 | 0 | 0 | 0 | 0 |
| Maleet | 17 | 17 | 100 | 0 | 0 | 0 | 0 | 0 | 0 |
| Korma | 52 | 47 | 90.4 | 5 | 9.6 | 0 | 0 | 0 | 0 |
| Kabkabya | 10 | 9 | 90 | 1 | 10 | 0 | 0 | 0 | 0 |
| Altena | 33 | 30 | 90.9 | 3 | 9.1 | 0 | 0 | 0 | 0 |
| Karnoy | 17 | 15 | 88 | 0 | 0 | 2 | 12 | 0 | 0 |
| Total | 666 | 579 | 86.9 | 70 | 10.5 | 13 | 2 | 4 | 0.6 |

Table 4: Frequency of β globin gene haplotypes in studied SCD patients

| | | Count | % |
|---|---|---|---|
| Haplotype | | | |
| | Cameron | 11 | 42.3 |
| | Benin | 7 | 26.9 |
| | Senegal | 2 | 7.7 |
| | Bantu | 6 | 23.1 |
| | Total | 26 | 100 |
| Genotype | | | |
| | Cameron/Benin | 4 | 30.8 |
| | Cameron/Cameron | 2 | 15.3 |
| | Cameron/ Bantu | 3 | 23.1 |
| | Senegal/Benin | 1 | 7.7 |
| | Benin/Benin | 1 | 7.7 |
| | Senegal/Bantu | 1 | 7.7 |
| | Bantu/Bantu | 1 | 7.7 |
| | Total | 13 | 100 |

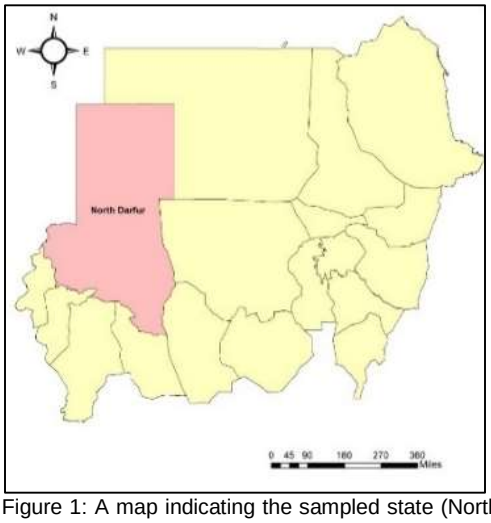


Figure 1: A map indicating the sampled state (North Darfur) within the Sudan borders

## DISCUSSION

In this study, heterozygote Hb AS and homozygous Hb SS types were found in 83 of the 666 respondents, with an overall prevalence of 12.5%. Of these participants, 70 (10.51%) were patients with sickle cell trait (Hb AS) and 13 (1.9 5%) were patients with sickle cell disease (Hb SS). These values were lower than the 11.3% of Hb AS and 3.5% of Hb SS observed in another study conducted in the same area [18] and the 44.06% among the Beja tribes in eastern Sudan [19]. In contrast, it is less than the prevalence of SCT (52%) and SCD (14%) in Meseria tribes in the south west of Kordofan [20]. However, the prevalence was higher than that in Khartoum area [21,22] and Blue Nile states [10]. The considerable variation in the disease prevalence could be due to variations in sample size and diagnostic methods. Most of these studies reported the high prevalence of Hb S among patients who are originally from west Sudan. The discrepancy in the prevalence of Hb SS and Hb AS was also reported in other African and Arab countries. A study conducted in Uganda revealed that the overall prevalence of sickle cell trait was 13.3% and that of the disease was 0·7% [23]. Although Hb AS sickle cell trait was reported in 18.8% of Nigerian children, none of them had Hb SS [24]. The existence of HbSS has been established in Arabic countries of variable extents; for example, in Saudi Arabia, the prevalence of Hb AS was 4.20%, while 0.26% of the participants in the same study had Hb SS [25]. Moreover, 20% of Egyptian primary school children were found to have sickle cell disorder [26].

The values of haematological parameters are affected by a number of factors even in apparently healthy populations. These factors include age, sex, and ethnic background, body build, social and nutritional. Complete blood components investigation revealed that sickle cell triat (SCT) in the study area, like other SCT individuals, were normal and not suffering or compiling from blood disorder, and if anaemia is present, it would be attributed to causes (such as iron deficiency) other than sickling state.

All the mean of haematological parameters were found within the normal range. The study shows a different pattern in the profile of hemoglobin values, this is because the study population was included AS trait, SS, AD and AA. The difference in mean hemoglobin values between AA and AS, as well as AD was statically not significant. (SS) patients they have extremely low Hb concentration.

The β globin gene cluster haplotypes are useful markers for identifying and tracing common genetic features in a population. The specific association of these haplotypes with the clinical severity of SCD and geographically distinct populations has provided valuable information on predicting disease severity and history and spreading of the sickle cell mutation. In the present study, the analysis of β-globin haplotypes showed the presence of four major haplotypes and seven haplotype combinations in the chromosomes of Sudanese patients with homozygote Hb S in the north Darfur state. In the present study, the Cameroon haplotype was the most common, accounting for 42.3% of all the haplotypes, followed by Benin, Bantu and Senegal haplotypes, with frequencies of 26.9%, 23.1% and 7.7%, respectively. This finding is consistent to previous reports from Sudan [27,28], thereby validating the predominance of Cameroon haplotype in Sudanese patients. The high frequency of Cameroon haplotype in this region of the country could be attributed to the gene flow from the surrounding regions; the Cameroon haplotype is common among ethnic groups of west Africa [29]. The second prevalent detected haplotype was Benin haplotype, with a frequency of 26.9%. This haplotype is the most predominant in many parts of the world, particularly African continent and Middle East countries [13,17]. The frequency of the Benin haplotype in the current study is consistent with earlier reports from Sudan and Brazil [27,30]. However, the frequency is higher than those reported in US and Brazil [31,32] and lower than those in Panama and Palestine [12,33]. Furthermore, Bantu and Senegal haplotypes were found in appreciable frequencies. The presence of both haplotypes was also reported earlier in India, Brazil and Panama [12,34,35].

Although Sudanese are a mix of African and Arab descent, only African haplotypes were observed and the Arab-Indian haplotype was not observed [27]. The present study was conducted in only one state in the western part of Sudan, which is close to the west Africa, where African haplotypes originated. Therefore, the haplotype patterns do not reflect the patterns in entire country. Additional studies are needed to further explore the presence of the Arab-Indian haplotype among Sudanese population.

## CONCLUSION

In conclusion, the main mutational event causing SCD in North Darfur, western Sudan was the mutation of the β globin gene on the Cameron haplotype, thereby confirming the findings of previous studies in the country. However, similar studies in other regions are highly recommended to further explore the presence of other haplotypes. Comprehensive investigations on different haematological parameters coupled with clinical outcomes could highlight the role β globin haplotype diversity in predicting clinical severity among patients with homozygous Hb S.

**Acknowledgments:** Our best regards and thanks to the staff member of hematology department in (SUST), Alneelain University- Faculty of Medicine, Medical Research Centre, and to the Tropical Medicine Research Institute, Alafia medical complex and El Fasher University for their co-operation and acceptance to do the research there.

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