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# Sickle ß-globin haplotypes among patients with sickle cell anemia in Basra, Iraq: A cross-sectional study

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**Abstract:**

**BACKGROUND:** Sickle cell disease is a monogenic disease with heterogeneous clinical course. Many genetic factors such as inheritance of α-thalassemia trait and fetal hemoglobin (Hb) level, related to the presence of specific haplotypes, are among the factors that modify disease severity.

**OBJECTIVES:** To identify $\beta^S$ haplotypes of children with sickle cell anemia (SCA) in Basra and assess the association of clinical variables and hematological parameters with different $\beta^S$ haplotypes.

**PATIENTS AND METHODS:** This analytical cross-sectional study included 62 patients with SCA registered at Basra Center of Hereditary Blood Disease. In addition to clinical data, blood samples were obtained for complete blood count, lactate dehydrogenase and polymerase chain reaction, and Sanger sequencing analysis of HBB gene. Statistical analysis was done using SPSS program version (23) software.

**RESULTS:** The mean age of studied patients was 7.15 ± 3.81 years, with a male to female ratio of 1:1.7. The most common haplotype was the Arab Indian (AI) in 34 (54.8%) patients, followed by Benin and Senegal haplotypes in 12 (19.4%) patients for each, and an atypical haplotype in 4 (6.5%) patients. No significant differences were found in the mean age of diagnosis, the frequency of vaso-occlusive crises, blood transfusions and hospitalizations among patients with different $\beta^S$ haplotypes, $P > 0.05$. However, patients with AI haplotype have significantly higher Hb, red blood cell count, hematocrit and fetal Hb compared to other haplotypes, $P < 0.05$.

**CONCLUSIONS:** The AI is the most common haplotype among SCA patients from Basra and it was associated with significantly higher Hb, hematocrit, and fetal Hb levels.

**Keywords:**

Basra, haplotypes, sickle cell anemia

## Introduction

Sickle hemoglobin (HbS) is the most common pathological Hb mutation worldwide.[1] Sickle cell anemia (SCA) is caused by homozygosity of the beta-S (βS) allele (located on chromosome 11p15.5), which often leads to acute and chronic complications.[2,3]

The prevalence of the HbS is high throughout large areas of sub-Saharan Africa, the Mediterranean basin, the Middle East, and India.[4] SCA accounts for over 305,000 births annually, with millions of people are affected worldwide, with evidence that the global burden of SCA is increasing as a consequence of improved survival in high prevalence low- and middle-income countries and population migration to higher income countries.[5,6]

Patients with SCA are clustered in two geographical areas in Iraq, where they constitute a major health problem. The highest frequency is among the Arabs in the extreme south where 6.48% of the

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population of Basra are carriers for the sickle cell trait,[7] and the second most prevalent area is among the Kurdish population in the north, where 1.2% of individuals of Dohuk are carriers.[8]

The clinical presentation of SCA is highly variable. The disease often progresses as severe chronic hemolytic anemia, and multisystem organ damage. Inheritance of α-thalassemia trait and a high fetal Hb (HbF) are among the factors that can modulate many complications of SCA.[9,10] In addition, the degree of phenotypic variation in a disease caused by a single-base substitution illustrates the importance of other genetic loci in modifying disease severity.[10] Milder clinical symptoms have been described in patients with coinheritance of α and β thalassemia and high levels of HbF related to the presence of specific haplotypes.[11,12]

The sickle cell gene is associated with several DNA structures surrounding the β globin locus which are located on chromosome 11. These different DNA structures (haplotypes) were established using restriction fragment length polymorphism (RFLP) analysis across the β-globin cluster. The gene responsible for SCA [β6(A3) Glu→Val, GAG→GTG] has been found to be associated with five different restrictions haplotypes.[13,14]

Many SCA patients in the Arabian Peninsula have the Arab/Indian (AI) haplotype characterized by elevated HbF levels. They tend to have a mild phenotype, presenting with fewer pain episodes and mild anemia. Stroke, leg ulcers, acute chest syndrome (ACS), priapism, and early splenic dysfunction are uncommon.[12]

The Benin (BEN) haplotype is the most prevalent globally, with highest frequency in West Africa, the Mediterranean region, America, and Europe. The Bantu/CAR haplotype is most prevalent in central, southern, and East Africa, and in relatively high proportion in Central and South America as compared to North America. The Senegal (SEN) haplotype is restricted to the far west regions of Africa, with the exception of Sudan and had a relatively low frequency in America. The AI haplotype is almost completely restricted to parts of India and the Middle East.[15,16]

In Iraq, one study was done by RFLP analysis method, for the detection of the β-globin gene haplotypes on patients from the North of Iraq, and it revealed that the most frequent was the BEN haplotype in 69.5%, followed by AI haplotype in 12.5% and the Bantu A1 haplotype in 7.8%.[17]

Carriers of the HbS gene on SEN or Arab-India haplotype usually have the highest HbF level and the mildest clinical course, while individuals with Bantu haplotypes have the lowest HbF level and the most severe clinical course. Carriers of the BEN and Cameroon haplotypes have intermediate features.[10]

The current study was carried out to identify $\beta^S$ haplotypes of SCA patients in Basra and assess the association of selected clinical variables and hematological parameters with different $\beta^S$ haplotypes.

## Patients and Methods

### Study population

This analytical cross-sectional study included 62 patients with SCA (32 females and 30 males), their ages ranged from 1 to 15 years. The study was conducted during the period between November 01, 2018 and June 2019, at Basra Center for Hereditary Blood Disease and the Bayan Group for advanced Lab Diagnostics at Basra province, Southern Iraq.

Data included date of birth, gender, residence, age at presentation, and age at diagnosis. Clinical data included acute painful episodes (age at first attack, frequency of the attacks, and number of crises requiring hospitalization in last year), stroke, ACS, acute splenic sequestration crises (ASSC), priapism, avascular bone necrosis (AVN), previous operation, and indication, frequency, and number of blood transfusion (BT) during the last year.

Severe disease was defined as frequent vaso-occlusive crises (VOC) requiring hospitalization ≥3/year, BT ≥3/year, frequent hospitalization ≥3/year, an episode of ACS, stroke, or avascular necrosis of bone.[18,19]

### Inclusion criteria

Patients from Basrah with SCA registered at the Center for Hereditary blood diseases, who were 1 year to 18 years of age and in steady state were recruited.

The patients in steady state have to fill the following criteria; no history of an acute painful episode that required treatment in the emergency department or in the hospital for at least 4 consecutive weeks after a previous painful crisis, and no history of BT during the previous 3 months.[20,21]

Patients who have received BT within 3 months, patients on HU treatment and those with history of intercurrent illness such as infection during the previous 4 weeks were excluded.

The study was approved by the Institutional Review Board of the College of Medicine, Al-Nahrain University. An informed consent was obtained from all patients and their guardians before recruitment in the study.

## Methods

Two ml of venous blood were drawn from SCA patients (62), the following tests were done;

- Complete blood count using fully automated hematology auto-analyzer, and reticulocyte percentage
- Serum lactate dehydrogenase (LDH) measurement using ARCHITECT PLUS machine, and total serum bilirubin (TSB) as markers of hemolysis
- DNA extraction was done using Promega kit.

## Protocol of polymerase chain reaction

In this study, a qualitative polymerase chain reaction (PCR) protocol was carried out to amplify DNA template prior to Sanger sequencing analysis of HBB gene. The extracted genomic DNA was used to amplify the fragment from HBB gene implementing a set of specific primers.[22,23] Preparation of primers and PCR mixture were done according to the manufacture instructions of the kit.

The PCR product was electrophoresed on 1% of agarose gel, then demonstrated using 336 nm UV source, the samples were transported to Korea by FedEx transportation to Macrogen Lab for doing Sanger sequencing.

We selected seven single nucleotide polymorphisms (rs968857, rs3834466, rs10837631, rs10768683, rs7482144, rs2070972, and rs28440105) which define all of the haplotypes spanning the β-globin gene cluster, according to the pattern of nucleotides at this site.[14,22]

## Statistical analysis

Statistical analysis was done using SPSS program version 23 software (IBM Corp., Armonk, NY, USA). Data were expressed by number (N.) and percentages (%) or means ± standard deviation.

Comparisons of proportions were performed by cross tab using Chi-square test and the Fisher's exact test were used.

Independent *t*-test was used to assess the significant association between means of different studied variables, while one-way analysis of variance was used for the quantitative comparison of more than two means of different samples.

Statistical tests with probability values <0.05 were considered statistically significant.

# Results

A total of 62 patients with SCA were recruited in the study, all of them were Arabs. Most of the studied patients were 5–10 years old with a mean age was 7.15 ± 3.81 years and male to female ratio of 1:1.7. In addition, higher number of patients were from the center of Basra (59.7%) and were the products of consanguineous marriage (69.4%) [Table 1].

The main presentation was acute painful episodes (85.5%), followed by ASSC (16.1%) and ACS (11.3%). Patients with severe disease were those who had ≥3 VOC requiring hospitalization/last year (8.1%), history of ACS (11.3%) and ≥3 hospitalizations (for causes other than VOC) during last year (4.8%) [Table 2].

The most common haplotype reported in this study was the AI in 34 (54.8%) patients, followed by BEN and SEN haplotypes in 12 (19.4%) patients for each, and an atypical haplotype in 4 (6.5%) patients [Figure 1].

No significant differences were found in the mean age of diagnosis, and in the frequency of VOC, BTs and hospitalizations among patients with different $\beta^S$ haplotypes, $P > 0.05$. However, AVN was reported in

**Table 1: Selected sociodemographic and clinical characteristics of sickle cell anemia patients**

| Variable | SCA group (*n*=62), *n* (%) |
|---|---|
| Age groups (years) | |
| <5 | 18 (29.0) |
| 5-10 | 29 (46.8) |
| >10 | 15 (24.2) |
| Mean age (years) | 7.15±3.81 |
| Sex | |
| Female | 32 (51.6) |
| Male | 30 (48.4) |
| Residence | |
| Center | 25 (40.3) |
| Periphery | 37 (59.7) |
| Parental consanguinity | |
| 1st cousins | 28 (45.2) |
| Beyond 1st cousins | 15 (24.2) |
| Not relatives | 19 (30.6) |
| Age at time of diagnosis (months) | |
| Range | 7-156 |
| Age at diagnosis (months) | |
| ≤23 | 17 (35.5) |
| 24-47 | 21 (30.6) |
| ≥48 | 24 (33.9) |
| Acute painful episodes | 53 (85.5) |
| ASSC | 10 (16.1) |
| ACS | 7 (11.3) |
| CVA | 1 (1.6) |
| AVN | 2 (3.2) |
| Splenomegaly | 6 (9.7) |
| Splenectomy | 6 (9.7) |
| Cholecystectomy | 2 (3.2) |

ACS=Acute chest syndrome, ASSC=Acute splenic sequestration crises, CVA=Cerebro-vascular accident, AVN=Avascular bone necrosis, SCA=Sickle cell anemia

1 (25%) patient with atypical $\beta^S$ haplotype and 1 (8.3%) patient with SEN $\beta^S$ haplotype, $P < 0.05$ [Table 3].

The study also revealed that patients with AI haplotype have significantly higher Hb, red blood cell (RBC) count, hematocrit and HbF compared to other haplotypes, $P < 0.05$ [Table 4].

Markers of hemolysis include absolute reticulocyte count, TSB and LDH, in addition to HbF. Patients with atypical haplotype have the lowest HbF level (14.63 ± 6.85), followed by BEN haplotype (18.63 ± 6.15), SEN haplotype (18.98 ± 5.10) and AI haplotype (24.45 ± 7.00), $P < 0.05$. Other markers (absolute retics count, TSB, and LDH) were not significantly different among patients with different haplotypes, $P > 0.05$ [Table 4].

## Discussion

SCA is a highly heterogeneous disease, some patients present with a mild phenotype and others being severely affected. Many of the contributory factors that lead to such heterogeneity are genetically determined ($\beta^S$ haplotypes, concomitant α-thalassemia, and HbF levels) while others are influenced by environmental or socioeconomic conditions.[24]

In this study, the AI $\beta^S$ haplotype is the most common haplotype in Basra, southern of Iraq (54.8%), followed by BEN and SEN haplotypes (19.4% for each). These findings are in contrast to that found in the north of Iraq, where the Arab Indian $\beta^S$ haplotype was found in only 12.5% of patients with SCA.[17] However, when compared with other neighboring countries, the AI $\beta^S$ haplotype is the predominant the βS haplotype in Kuwait (80.8%),[25] Saudi Arabia (94%),[26] Southwestern Iran (69.1%),[27] and in the United Arab Emirates (68%).[28]

In India, the AI $\beta^S$ haplotype is reported in 78%–91.5% of SCA patients.[29,30] It is believed that the AI haplotype had originated in the Indus Valley on the Indian subcontinent, from which it spread through the trade routes to the Eastern Arabian Peninsula, Iran and most likely our locality.[31]

The BEN and SEN haplotypes have been detected in equal frequency in Basra after the AI haplotype. This

**Table 2: Clinical indicators of disease severity**

| Clinical parameter | Total (*n*=62), *n* (%) |
|---|---|
| VOC (requiring hospitalization)/last year | |
| No VOC | 40 (64.5) |
| 1-2 | 17 (27.4) |
| ≥3 | 5 (8.1) |
| BT/last year | |
| No transfusion | 49 (79) |
| Once | 11 (17.7) |
| Twice | 2 (3.2) |
| Hospital admission/last year | |
| No admission | 44 (71.0) |
| 1-2 | 15 (24.2) |
| ≥3 | 3 (4.8) |
| ACS | 7 (11.3) |
| CVA | 1 (1.6) |
| AVN | 2 (3.2) |

ACS=Acute chest syndrome, CVA=Cerebrovascular accident, AVN=Avascular bone necrosis, VOC=Vaso-occlusive crises, BT=Blood transfusion

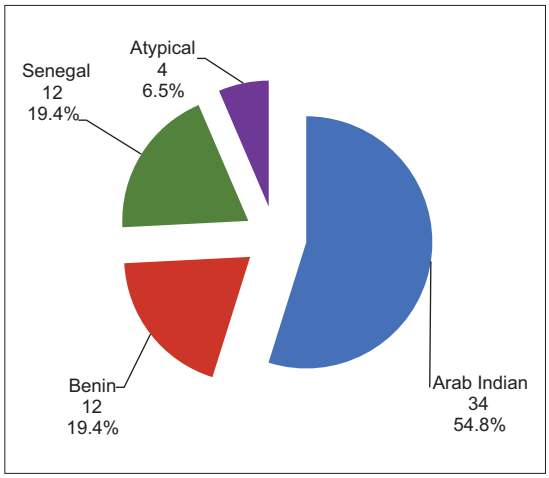


**Figure 1:** The distribution of sickle cell anemia patients according to $\beta^S$ haplotypes

**Table 3: Selected clinical variables in relation to sickle cell disease haplotype**

| Clinical variable | AI (*n*=34) | BEN (*n*=12) | SEN (*n*=12) | ATP (*n*=4) | *P* |
|---|---|---|---|---|---|
| Mean age at diagnosis (months) | 45.85±31.90 | 34.25±22.78 | 46.67±27.69 | 42.75±41.72 | 0.692‡ |
| History of VOC*, *n* (%) | 28 (82.4) | 11 (91.7) | 11 (91.7) | 3 (75.0) | 0.714† |
| Number of VOC in last year** | 0.88±0.33 | 0.5±0.23 | 0.42±0.14 | 0.25±0.25 | 0.685‡ |
| History of transfusion*, *n* (%) | 21 (61.8) | 9 (75.0) | 7 (58.3) | 2 (50.0) | 0.762† |
| Number of transfusion in last year** | 0.18±0.07 | 0.25±0.18 | 0.33±0.19 | 0.5±0.29 | 0.575‡ |
| Number of hospitalization in last year** | 0.53±0.15 | 0.33±0.19 | 0.58±0.42 | 0±0.0 | 0.675‡ |
| ASSC*, *n* (%) | 5 (14.7) | 2 (16.7) | 3 (25.0) | 0 (0.0) | 0.677§ |
| ACS*, *n* (%) | 4 (11.8) | 1 (8.3) | 1 (8.3) | 1 (25.0) | 0.809§ |
| CVA*, *n* (%) | 1 (2.9) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0.841§ |
| AVN*, *n* (%) | 0 (0.0) | 0 (0.0) | 1 (8.3) | 1 (25.0) | 0.035§ |

*Values are expressed as *n* (%), **Values are expressed as mean±SE, †Chi square test was used, ‡ANOVA test was used, §Fisher's exact test was used to assess *P* value. AI=Arab Indian, BEN=Benin, SEN=Senegal, ATP=Atypical, ACS=Acute chest syndrome, ASSC=Acute splenic sequestration crises, CVA=Cerebrovascular accident, AVN=Avascular bone necrosis, VOC=Vaso-occlusive crises, SE=Standard error, ANOVA=Analysis of variance

**Table 4: Selected hematological and biochemical variables in relation to sickle cell anemia haplotype**

| Variable | AI (*n*=34) | BEN (*n*=12) | SEN (*n*=12) | ATP (*n*=4) | *P* |
|---|---|---|---|---|---|
| Hb (g/dl) | 9.10±1.47 | 7.77±1.02 | 8.21±0.92 | 7.95±0.48 | 0.009 |
| Erythrocytes (×10$^9$/l) | 3.42±0.60 | 2.80±0.42 | 3.16±0.68 | 2.81±0.42 | 0.009 |
| Hematocrit (%) | 27.24±3.89 | 23.29±2.80 | 24.90±2.90 | 23.43±1.44 | 0.004 |
| MCV (fl) | 80.47±7.55 | 83.58±6.59 | 80.27±10.19 | 84.25±9.12 | 0.563 |
| MCHC (g/dl) | 33.34±1.84 | 33.34±1.58 | 33.03±2.46 | 33.95±0.86 | 0.867 |
| Reticulocytes (%) | 5.81±1.86 | 7.25±3.16 | 5.53±1.60 | 6.30±3.80 | 0.226 |
| Absolute reticulocytes count | 192.91±53.00 | 198.36±82.13 | 167.91±37.24 | 165.51±85.60 | 0.473 |
| WBC (×10$^9$/l) | 10.48±3.30 | 12.01±2.94 | 11.57±3.65 | 14.84±2.32 | 0.068 |
| Lymphocytes (×10$^9$/l) | 4.21±1.50 | 5.09±2.23 | 4.22±1.72 | 6.10±2.38 | 0.127 |
| Platelets (×10$^9$/l) | 427.72±159.00 | 536.74±215.48 | 476.85±307.46 | 547.00±162.50 | 0.365 |
| Hb F | 24.45±7.00 | 18.63±6.15 | 18.98±5.10 | 14.63±6.85 | 0.003 |
| Hb S | 68.68±5.80 | 74.43±5.82 | 71.78±7.39 | 68.23±15.19 | 0.083 |
| TSB* | 3.66±3.22 | 3.50±1.75 | 3.94±4.03 | 4.73±2.57 | 0.938 |
| LDH** | 503.03±254.20 | 563.17±243.21 | 476.55±113.30 | 715.67±309.22 | 0.395 |

*$n$=40, **$n$=58. Values are expressed as mean±SD, ANOVA test was used to assess *P* value. AI=Arab Indian, BEN=Benin, SEN=Senegal, ATP=Atypical, MCV=Mean corpuscular volume, MCHC=Mean corpuscular hemoglobin concentration, Hb F=Fetal hemoglobin, Hb S=Sickle hemoglobin, TSB=Total serum bilirubin, LDH=Lactate dehydrogenase, SD=Standard deviation, WBC=White blood cell, ANOVA=Analysis of variance, Hb=Hemoglobin

finding differs from studies done in North of Iraq,[17] Jordan,[26] Lebanon,[32] Oman[33] and Egypt[34] where the BEN haplotype was found to be the predominant haplotype; (69.5%), (80%), (73%), (52.1%) and (80.8%) in these countries respectively. A possible explanation for the distribution of the BEN haplotype is that it had originated in Central West Africa and then spread vertically via population movement by trans-Saharan migration to North Africa and thence across the Mediterranean Sea to Southern Europe, Turkey, and probably to the north of Iraq, close to Southern Turkey.[35]

The SEN haplotype is present mainly in Mauritania (77.8%)[36] and SEN (100%).[37]

In Basra, the genetic population structure is Arab. However, the HbS gene in Basra might have be originated in India/Saudi Arabia and Africa and reached our locality many generations ago.

The presence of atypical haplotypes is not unexpected in any population and has been attributed to several mechanisms including isolated changes in one of the polymorphic sites, simple and double crossovers between two typical β haplotypes or between a typical $\beta^S$ haplotype and a different $\beta^A$ associated haplotype, and gene conversions.[38] The atypical haplotype was found in (6.5%) of patients in the current study. Leal *et al*. in Brazil reported atypical haplotype in (12.3%) of their patients.[39] This reflects the various genetic mechanisms of association with the sickle gene, i.e., the atypical haplotype differs from the five common haplotypes observed worldwide, confirms the hypothesis that these different structures are generated by recombination, specific replacement and/or nonreciprocal transfers between common preexisting haplotypes instead of new mutations in the β-globin genes.[40]

The study did not reveal significant differences in the mean age of diagnosis, and in the frequencies of VOC, BTs, and hospitalizations among patients with different $\beta^S$ haplotypes. The AI haplotype is reported to be associated with milder disease although vaso-occlusive events do occur.[26,41]

Leal *et al*.[39] in Brazil, and Bitoungui *et al*.[15] in Cameroon, reported also that BTs and ASSC were not significantly different between the different haplotypes. In addition, Loggetto in Brazil reported that painful crises, hemolytic crises, ACS and infections were not related to the $\beta^S$ globin haplotype in another retrospective study of under 6-year-old children.[42]

Nevertheless, despite the high mean HbF level, the lack of significant differences in the frequency of clinical events among different haplotypes could be due to the number of heterozygous individuals, the miscegenation of the population, and the multiplicity of the clinical expression in SCA.

Keikhaei *et al*. in Iran reported that the AI haplotype has been related with a more benign clinical course of sickle cell disease having less anemia and organ damage because of higher HbF level, which does not participate in HbS polymerization, thus decreasing the risk of sickling, leading to a lower degree of hemolysis and less severe clinical phenotypes.[43]

Patients with AI haplotype have significantly higher Hb, RBC count, hematocrit, and HbF followed by SEN haplotype; similar findings were reported in Iran by Rahimi *et al*.[27] However, Keikhaei *et al*. in Iran also did not find significant correlation between haplotypes and hematological characteristics including HbF.[43]

Both LDH and TSB were high in all haplotypes although highest in patients with atypical haplotype. HbF is best considered a determinant of hemolysis (by reducing sickling) rather than a marker of hemolysis, but there are no significant association with all haplotypes, a similar result was found by Quinn *et al*.[44]

Although this is the first study exploring the $\beta^S$ haplotypes in Basra, the study has many limitations. The first limitation is the small number of studied patients due to the cost of the tests; the other limitation is that the homozygous and heterozygous state of the haplotypes was not studied.

## Conclusions

From this study, it can be concluded that the AI haplotype is the most common genotype in SCA patients, and patients with AI haplotype have significantly higher Hb, RBC count, hematocrit and HbF compared to other haplotypes.

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### Conflicts of interest

There are no conflicts of interest.

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