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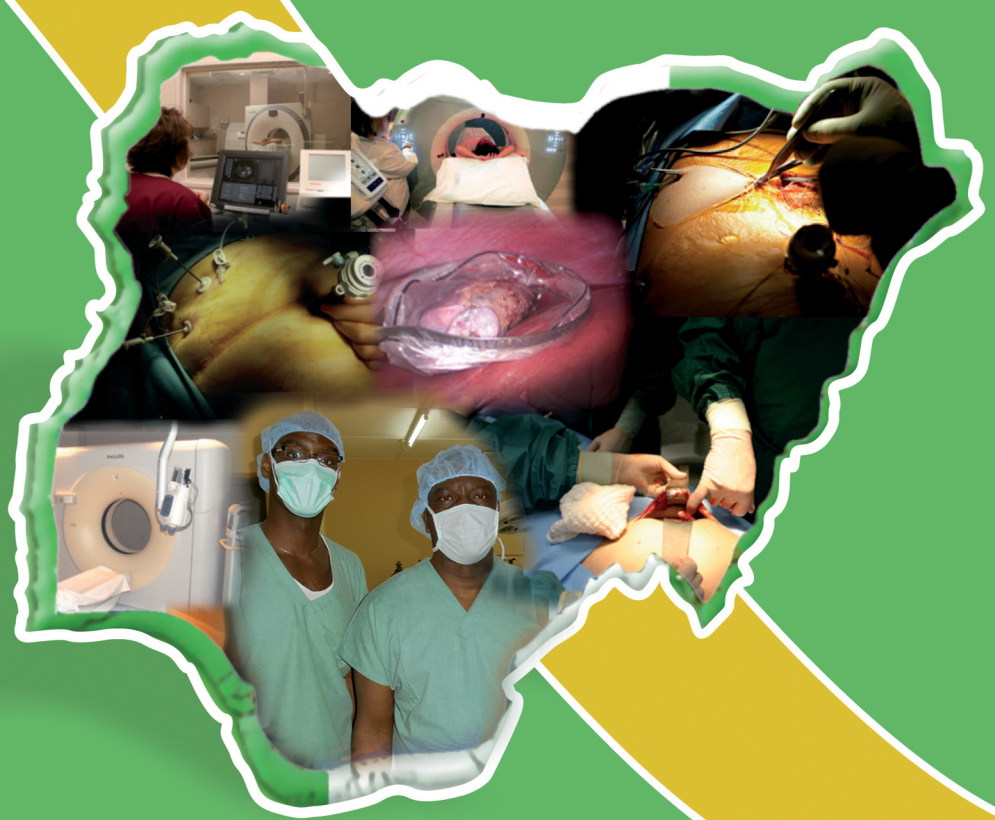

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Original Article

Co-Inheritance of α-Thalassemia Gene Mutation in Patients with Sickle Cell Disease: Impact on Clinical and Hematological Variables

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Abstract

Background: Sickle cell disease (SCD) is a monogenic, phenotypically highly variable disease with multisystem pathology. The phenotypic heterogeneity of SCD is attributed to environmental and genetic factors such as fetal hemoglobin and co-inheritance of α-thalassemia. **Objectives:** To look for different types of α-thalassemia gene mutations among SCD patients and evaluate the influence of the co-inheritance of α-thalassemia on clinical and hematological variables. **Methods:** This cross-sectional analytical study included 765 SCD patients, and 150 patients (with low mean corpuscular volume (MCV), low mean corpuscular hemoglobin (MCH) and normal serum ferritin levels) were tested for α-thalassemia gene mutations. Multiplex PCR and reverse hybridization and sequencing for both α genes using the Vienna Lab Strip Assay PCR study were performed using conventional PCR technology. **Results:** Out of 150 patients tested for α-thalassemia gene mutations, 141 patients were found to have one or more of the mutational types, representing 18.4% of all studied SCD patients. The most common mutations found were the $-^{3.7}$ deletion (76.6%), followed by the $-^{4.2}$ deletion (12.1%), mutant $\alpha2^{polyA-1}$ (Saudi type) (9.2%), and $--^{MED}$ double gene deletion (7.8%). Acute painful episodes did not differ significantly in sickle cell anemia (SCA) patients with or without α-thalassemia, although the co-inheritance of α-thalassemia has a protective role against many disease-related complications. However, this role was not observed with other types of SCD. The means of red blood cell count, hemoglobin, and hematocrit were significantly higher, while the MCV, MCH, reticulocyte count, and hemoglobin A2 percentage were significantly lower in patients with α-thalassemia gene mutations than in those without α-thalassemia gene mutations ($P < 0.05$). **Conclusions:** The co-inheritance of α-thalassemia and SCA confers protection against many disease-related complications and is associated with improved hematological indices. However, this protection was not noticed in patients with other types of SCD.

Keywords: *α-thalassemia, mutation, sickle cell disease*

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Introduction

Sickle cell disease (SCD) is a single-gene disorder causing a debilitating systemic syndrome characterized by chronic anemia, acute painful episodes, organ infarction, chronic organ damage, and by a significant reduction in life expectancy.[1]

Sickle cell disease is a common hematological disorder, affecting an estimated 30 million people and increasing the global burden of the disease worldwide.[2-4] It represents a major public health problem because of its associated morbidity and mortality.[4] Sickle cell anemia (SCA) is often

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neglected in public health policies, and excess mortality in SCA patients in many low- and middle-income countries is still high.[3] Despite this, the global mortality burden of SCD remains poorly understood.[2]

The term SCD refers to all the different genotypes that cause the characteristic clinical syndrome. SCA, the most common form of SCD, refers specifically to homozygosity for the βS allele (HBB; glu (E) 6val (A); GAG-GTG; rs334).[5]

Although SCD is monogenic, phenotypically, the disease is highly variable with multisystem pathology.[4,6] Multiple cellular and genetic factors may contribute to phenotypic heterogeneity. Fetal hemoglobin was identified as one of the determinants of disease severity, with high fetal hemoglobin (HbF) associated with mild disease. Other disease-ameliorating factors include the coinheritance of α-thalassemia, ß-thalassemia-specific ß-globin haplotypes, genetic determinants outside the ß-globin gene cluster, and the cMYB gene on chromosome 6p23, which are responsible for the variability in HbF levels.[4,5,7]

The α-thalassemia trait reduces the concentration of hemoglobin (Hb) in red blood cells (RBCs), decreasing the tendency of sickle hemoglobin to polymerize, which results in increased Hb concentrations and decreased rates of hemolysis.[4,8]

More than 30% of most populations with SCA carry one or more determinants for α-thalassemia. In people of African descent, this is usually heterozygosity or homozygosity for the − 3.7α-globin gene deletion.[8]

The co-inheritance of α-thalassemia was reported to protect against cerebral vasculopathy, cholelithiasis, leg ulcers, acute chest syndrome (ACS), and chronic kidney disease.[9-11] However, other studies reported that the interaction between α-thalassemia and SCA did not influence HbF concentrations or the number of clinical manifestations,[12,13] while others reported that the co-inheritance of α-thalassemia was associated with higher rates of bone pain crisis compared to those without α-thalassemia.[14,15]

The current study was carried out to look for the types of α-thalassemia gene mutations among SCD patients in Basra and for the influence of the co-inheritance of α-thalassemia on clinical and hematological variables of these patients.

Patients and Methods

Study design

This cross-sectional analytical study included 765 SCD patients registered at the Centre for Hereditary Blood Diseases in Basra from the 1st of April 2018 through June 2019.

Out of the total 765 patients, 150 patients were tested for α-thalassemia gene mutations. Selection depended on the following criteria: SCD with low MCV (<80.0 fL), low mean corpuscular hemoglobin (MCH) (<27.0 pg) and normal serum ferritin levels.[16,17]

The hemoglobin pattern for all patients was determined by high-performance liquid chromatography (HPLC) and capillary zone electrophoresis. Classification of the type of SCD was performed depending on the results of two different methods.[18-22]

Detailed explanation of the patients and/or one of the parents (for pediatric cases) of the aim of the study was provided. All the procedures were also explained to each patient or their parents. A written consent was obtained from all patients and/or one of the parents before enrolment in the study. The study was approved by the Institutional Review Board of the College of Medicine, Al-Nahrain University.

Data collection

Demographic and clinical data were obtained, including age, gender, residence, age at presentation, acute painful crises and their frequency/year, frequency of blood transfusions/year, pallor, jaundice, history of ACS, acute splenic sequestration crises (ASSC), stroke, avascular bone necrosis (AVN), retinal complications, renal complications, priapism, gallbladder stones, and history of cholecystectomy.

Severity of the disease was assessed on the basis of the following criteria: history of ≥ 3 VOC/year requiring hospitalization, history of ≥ 3 blood transfusions/year, history of at least one episode of priapism, ACS, stroke, or AVN of femoral or humeral heads.[23,24]

Thorough examination of the patients was performed, including body weight (Wt.), height (Ht.), general examination, and splenomegaly and/or hepatomegaly.

The body mass index (BMI) was calculated using the equation: BMI (kg/m^2) = body weight (kg)/the square of body height (in meters).

For adults, the WHO classification, which defines overweight as a BMI ≥ 25 and obesity as a BMI ≥ 30, was used.[25] For pediatric patients, Z-scores for BMI for age (BMIZ) were calculated, and accordingly, cases were classified as severely thin (<-3 SD), thin (<-2 SD), normal, overweight (> +2 SD), and obese (>+3 SD).[26,27]

Hematological study

For each patient, the laboratory workup included the following:

- Blood count: an automated hematology analyzer (Mindray BC 6800, seven parts, Shenzhen, China)

- Blood smear examination using Leishman-stained smears

- Screening sickling phenomenon and solubility testing

- Immunoscans for HbA, HbS, and HbC (Biomedomics, USA) were performed for all patients with an HbS level below the detection limit of the solubility test (20.0%), all patients with HbS/C genotype, HbS/S^{Oman}, and HbS/HOPE genotypes.[28]

- HPLC using the Bio-Rad:\ D-10 and/or the variant-II systems (Bio-Rad: D-10 Hemoglobin testing system, Bio-Rad Laboratories Inc. Hercules, USA, and Bio-Rad variant-II Hemoglobin Testing System, USA) with the Beta-thalassemia Short Program was performed on every newly diagnosed patient.

Genetic study

- DNA was extracted from whole EDTA blood samples using the QIAamp DNA Blood Mini Kit.[29] Samples were stored in a deep freezer (-70°C) until the time of hybridization.

- Extracted DNA was used for gap polymerase chain reaction (gap-PCR). Multiplex PCR (m-PCR) and reverse hybridization and sequencing for both α genes using the Vienna Lab Strip Assay PCR study (which detects 21 mutations covering > 90% of α-globin defects found in Mediterranean, Middle Eastern, and Southeast Asian countries), which detected the presence and type of α-thalassemia mutation, were performed using the conventional Bio-Rad PCR technology according to the manufacturer guidelines.[30]

Statistical analysis

Statistical analyses were performed using the Statistical Package for Social Science version 25. Data were expressed either as a number (N) and percentage (%) or as the mean ± standard deviation. Comparisons of proportions were performed using the Chi-square test and Fisher's exact test.

Intraclass differences in the means of the parameters of different samples were analyzed using the independent t-test. For all tests, a *P* value of < 0.05 was considered statistically significant.

Results

α- Thalassemia phenotypes and genotypes among patients with sickle cell disease

The study included 765 patients with different SCD genotypes; 150 patients were tested for α-thalassemia gene mutations. One hundred and forty-one of the examined patients were found to have one or more of the mutational types, representing 18.4% of all studied SCD patients.

Most of the α-thalassemia gene mutations were reported among patients with SCA, with 115 (81.6%), followed by HbS/β^{+} thalassemia 11 (7.8%), HbS/β^{0} thalassemia 8 (5.7%), HbS/S^{Oman}4 (2.8%), HbS/D 2 (1.4%), and HbS/HOPE disease 1 (0.7%).

Eleven α-thalassemia mutational determinants were found among SCD patients: three were deletional, seven were nondeletional, and 1 was a triplicate mutation. The majority of mutations (79.4%) were deletional in type, 8.5% were nondeletional, and 12.1% had both deletional and nondeletional mutations. Single-gene deletions/point mutations constituted 57.4% of cases. The most common mutations were $-^{3.7}$ deletion (76.6%), followed by $-^{4.2}$ deletion (12.1%), mutant $\alpha 2^{polyA-1}$ (Saudi type) (9.2%), and $--^{MED}$ double gene deletion (7.8%) [Table 1].

Nineteen α-thalassemia genotypes were encountered among SCD patients. The five most common α-thalassemia mutation genotypes were $-\alpha^{3.7}/\alpha\alpha$ (41.8%), $-\alpha^{3.7}/-\alpha^{3.7}$ (19.9%), $-\alpha^{4.2}/\alpha\alpha$ (7.1%), $\alpha^{poly\ A-1}$ a/αα (5.7%), and $-^{MED}/\alpha\alpha$ (5.0%) [Figure 1].

α-Thalassemia gene mutation among patients with sickle cell anemia

Homozygous Hb SS disease comprised 514 (67.2%) of the total SCD studied patients. Of the 122 SCA cases with low MCV and MCH and normal serum ferritin selected for the

Table 1: Characteristics of α-thalassemia gene mutations among sickle cell disease patients

Variable	α gene mutation	*n* (%)
Type of mutation	Deletional	112 (79.4)
	Nondeletional	12 (8.5)
	Both	17 (12.1)
Number of genes mutated	Single	81 (57.4)
	Double	55 (39.0)
	Triple	5 (3.5)
α thalassemia mutation alleles	$-^{3.7}$ single gene deletion	108 (76.6)
	$-^{4.2}$ single gene deletion	17 (12.1)
	Mutant $\alpha 2^{poly\ A-1}$	13 (9.2)
	MED double gene deletion	11 (7.8)
	Mutant $\alpha 2^{IVS\ 1-5\ nt}$	6 (4.3)
	Mutant $\alpha 2^{cd\ 59(G>A)}$	5 (3.5)
	Mutant $\alpha 2^{cd19}$	2 (1.4)
	Anti 3.7 Triplication mutation	2 (1.4)
	Mutant $\alpha 2^{poly\ A-2}$	1 (0.7)
	Hb $^{Constant\ spring}$	1 (0.7)
	Hb ^{Icaria}	1 (0.7)
Total		141 (100.0)

α thalassemia Genotype	N.	%
$-\alpha^{3.7}/\alpha\alpha$	58	41.1
$-\alpha^{3.7}/-\alpha^{3.7}$	27	19.1
$-\alpha^{4.2}/\alpha\alpha$	10	7.1
$\alpha^{poly\ A1}\alpha/\alpha\alpha$	8	5.7
$--^{MED}/\alpha\alpha$	7	5.0
$-\alpha^{3.7}/\alpha^{IVS}\alpha$	5	3.5
$-\alpha^{3.7}/-\alpha^{4.2}$	5	3.5
$-\alpha^{3.7}/\alpha^{Poly\ A1}\alpha$	4	2.8
$-\alpha^{3.7}/\alpha^{cd\ 59}\alpha$	3	2.1
$-\alpha^{3.7}/--^{MED}$	3	2.1
$\alpha^{cd\ 19}\alpha/aa$	2	1.4
$-\alpha^{3.7,4.2}/\alpha^{Poly\ A1}\alpha$	1	0.7
$-\alpha^{3.7}/\alpha^{CS}\alpha$	1	0.7
$-\alpha^{3.7}/\alpha^{Poly\ A2}\alpha$	1	0.7
$-\alpha^{4.2}/-\alpha^{4.2}$	1	0.7
$--^{MED}/\alpha^{Icaria}\alpha$	1	0.7
$\alpha^{cd59}\alpha/\alpha\alpha$	1	0.7
$\alpha^{IVS}\alpha/\alpha\alpha$	1	0.7
$\alpha\alpha\alpha^{anti\ 3.7}/\alpha\alpha$	1	0.7
$\alpha\alpha\alpha^{anti\ 3.7}/\alpha^{cd59}\alpha$	1	0.7
Total	141	100

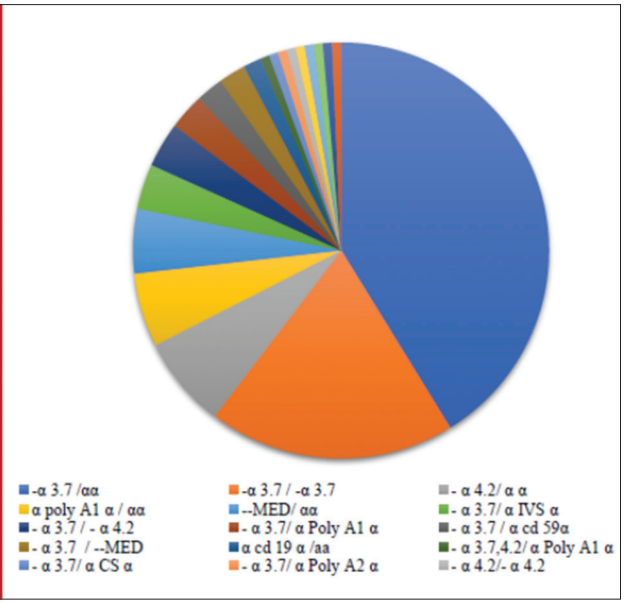

Figure 1: α-thalassemia genotypes reported among sickle cell disease patients

Table 2: Selected clinical variables of sickle cell anemia patients in relation to co-inheritance of α-thalassemia gene mutation

Variable	With α-thalassemia (Total 115) *n* (%)	Without α-thalassemia (Total 399) *n* (%)	*P*
Painful crises	103 (89.6)	364 (91.2)	0.586*
Pallor	41 (35.7)	370 (92.7)	<0.001
Jaundice	48 (41.7)	263 (65.9)	<0.001*
ASSC	12 (10.4)	100 (25.1)	<0.001*
ACS	2 (1.7)	51 (12.8)	<0.001†
AVN	3 (2.6)	45 (11.3)	0.004†
Gall stones	18 (15.7)	124 (31.1)	<0.001*
Renal complications	6 (5.2)	29 (7.3)	0.442*
Cardiac complications	5 (4.3)	54 (13.5)	0.006*
Stroke	0 (0)	18 (4.5)	0.020†
Retinal complications	2 (1.7)	8 (2.0)	0.856†
Priapism	0 (0)	4 (1.0)	0.318†
Hepatomegaly	14 (12.2)	51 (12.8)	0.863*
Splenomegaly	56 (48.7)	183 (45.9)	0.592*
Splenectomy	5 (4.3)	47 (11.8)	0.020*
Cholecystectomy	8 (7.0)	55 (13.8)	0.049*
Age at presentation			
Mean±SD	7.27±6.61	4.52±6.03	<0.001‡
BMI Classification			
Mean±SD	20.15±4.93	17.99±4.29	<0.001‡
Under Wt.	16 (13.9)	98 (24.6)	0.009*
Normal Wt.	76 (66.1)	250 (62.7)	
Over Wt.	23 (20.0)	51 (12.8)	
Frequency of blood transfusions/year			
None	58 (50.4)	110 (27.6)	<0.001†
<3	57 (49.6)	160 (40.1)	
≥3	0 (0)	129 (332.3)	
Frequency of painful crises/year			
None	12 (10.4)	35 (8.8)	<0.001*
<3	85 (73.9)	199 (49.9)	
≥3	18 (15.7)	165 (41.4)	

*Chi- square test was used, †fisher's exact test was used, ‡*t*-test was used. ACS: acute chest syndrome; ASSC: acute splenic sequestration crises; AVN: avascular bone necrosis; BMI: body mass index

Table 3: Hematological variables of sickle cell anemia patients in relation to co-inheritance of α-thalassemia gene mutation

Variable	Mean±SD		*P**
	With α-thalassemia Total *n*=115	Without α-thalassemia Total *n*=399	
RBC (x10^{12}/L)	4.86±0.49	3.04±0.62	<0.001
Hb (g/dl)	10.81±0.58	8.45±1.36	<0.001
HCT (%)	32.86±2.50	25.75±4.26	<0.001
MCV (fL)	68.06±5.40	85.72±8.92	<0.001
MCH (pg)	22.44±1.88	28.20±3.55	<0.001
MCHC (g/dl)	33.00±1.73	32.87±2.20	0.552
RDW-CV (%)	18.30±3.59	19.45±4.04	0.006
Retics (x10^{9}/L)	140.19±91.44	208.20±119.78	<0.001
N-RBC (/100 WBCs)	0.49±1.60	2.33±13.74	0.152
Hb A2 (%)	2.73±0.77	3.16±1.14	<0.001
Hb F (%)	19.66±6.25	19.29±8.24	0.653
Hb S (%)	77.56±6.22	77.55±7.85	0.985

**t*-test was used. RBC: red blood cells; Hb: hemoglobin; HCT: hematocrit; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; RDW: red cell distribution width; Retics: reticulocytes; Hb F: fetal hemoglobin; Hb S: sickle hemoglobin

Table 4: Selected clinical variables of patients with double heterozygosity sickle cell disease in relation to co-inheritance of α-thalassemia gene mutation

Variable	With α-thalassemia (Total 26) *n* (%)	Without α-thalassemia (Total 251) *n* (%)	*P*
Painful crises	21 (80.8)	194 (86.2)	0.453*
Jaundice	12 (46.2)	125 (55.6)	0.362*
ASSC	5 (19.2)	56 (24.9)	0.524*
ACS	1 (3.8)	21 (9.3)	0.349†
AVN	1 (3.8)	25 (11.1)	0.825†
Gall stones	6 (23.1)	57 (25.3)	0.802*
Renal complications	1 (3.8)	11 (4.9)	0.813†
Cardiac complications	1 (3.8)	23 (10.2)	0.295†
Stroke	1 (3.8)	6 (2.7)	0.729†
Retinal complications	2 (7.7)	3 (1.3)	0.028†
Leg ulcers	0 (0)	1 (0.4)	0.733†
Priapism	0 (0)	3 (1.3)	0.241†
Hepatomegaly	5 (19.1)	36 (16.0)	0.673*
Splenomegaly	15 (57.7)	152 (67.6)	0.313*
Splenectomy	2 (7.7)	35 (15.6)	0.284†
Cholecystectomy	3 (11.5)	26 (11.6)	0.998†
Age at presentation			
Mean±SD	7.69±10.12	6.05±6.56	0.259‡
BMI Classification			
Mean±SD	19.77±4.52	18.89±4.68	0.363‡
Under Weight	3 (11.5)	51 (22.7)	0.297†
Normal Weight	17 (65.4)	141 (62.7)	
Over Weight	6 (23.1)	33 (14.7)	
Frequency of blood transfusions/year			
None	12 (46.2)	59 (26.2)	0.101*
<3	9 (34.6)	110 (48.9)	
≥3	5 (19.2)	56 (24.9)	
Frequency of painful crises/year			
None	5 (19.2)	31 (13.8)	0.241*
<3	16 (61.5)	114 (50.7)	
≥3	5 (19.2)	80 (35.6)	

*Chi-square test was used, †fisher's exact test was used, ‡*t*-test was used. ACS: acute chest syndrome; ASSC: acute splenic sequestration crises; AVN: avascular bone necrosis; BMI: body mass index

Table 5: Hematological variables of patients with double heterozygosity sickle cell disease in relation to co-inheritance of α-thalassemia gene mutation

Variable	Mean±SD		*P**
	With α-thalassemia Total *n*=26	Without α-thalassemia Total *n*=225	
RBC (x10^{12}/L)	4.82±1.02	3.84±0.79	<0.001
Hb (g/dL)	10.60±1.81	8.70±1.59	<0.001
HCT (%)	32.31±4.78	27.02±4.65	<0.001
MCV (fL)	69.28±13.79	71.29±8.34	0.283
MCH (pg)	22.69±4.62	22.93±3.17	0.734
MCHC (g/dL)	32.70±2.09	32.15±2.10	0.208
RDW-CV (%)	19.29±4.01	20.75±4.30	0.101
Reticulocytes (x10^{9}/L)	169.54±124.76	208.13±138.22	0.175
N- RBCs (/100 WBCs)	0.47±0.76	2.82±3.75	0.125
Hb A2 (%)	3.58±1.29	4.75±1.44	<0.001
Hb F (%)	15.18±7.28	18.55±7.11	0.023
Hb S (%)	58.26±21.14	65.06±15.62	0.044

**t*-test was used. RBC: red blood cells; Hb: hemoglobin; HCT: hematocrit; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; RDW: red cell distribution width; Retics: reticulocytes; Hb F: fetal hemoglobin; Hb S: sickle hemoglobin

α-thalassemia mutational study, 115 were positive for the α-thalassemia gene mutation. Thus, in the Hb SS patients, 22.4% had documented concomitant α-thalassemia.

The frequencies of various clinical variables of SCA patients in relation to co-inheritance of α-thalassemia gene mutations were evaluated. The frequency of acute painful episodes did not differ between SCA patients with α-thalassemia compared to those without α-thalassemia gene mutation, although those with α-thalassemia have significantly less frequent VOCs/year. Furthermore, SCA patients with α-thalassemia gene mutations tended to have significantly lower incidences of pallor, jaundice, gall stones, splenectomy, ASSC, AVN and cardiac complications, and ACS compared to those without α-thalassemia gene mutations. In addition, patients with α-thalassemia gene mutations tended to have significantly delayed disease onset, higher BMI, and lower frequencies of blood transfusions than those without α-thalassemia gene mutations (*P* < 0.05, Table 2).

The study also reveals that the means of RBC count, Hb concentration, and hematocrit are significantly higher, while those of MCV, MCH, and RDW-CV, reticulocyte count and Hb A2 percentage are significantly lower in patients with α-thalassemia gene mutation than those without it [Table 3].

α-Thalassemia gene mutation among patients with double heterozygous states of sickle cell disease

A total of 251 (32.8%) patients with double heterozygosity for sickle hemoglobin and β thalassemia or other structural Hb variants were included in this study; 28 of whom were tested for α-thalassemia gene mutations. Twenty-six (10.4%) of them were positive for α-thalassemia gene mutations.

The study did not find significant differences between patients with and without α-thalassemia gene mutations concerning various clinical variables and disease-related complications except for pallor [Table 4]. Furthermore, patients with α-thalassemia gene mutations had significantly higher mean RBC count, Hb concentration, and hematocrit [Table 5].

Discussion

Arabs constitute the main ethnic group in Basra, the third largest of the Iraq provinces with a population of approximately 3 million. Sickle cell disease is an important health problem in Basra, whose population has a high carrier rate for HbS (6.48%) and an estimated birth incidence of homozygotes of 0.52/1000.[31]

Sickle cell disease is characterized by phenotypic heterogeneity. Patients usually present with different rates of hemolysis/vasculopathy and viscosity/vaso-occlusion-related complications.[32] The high clinical diversity of SCD can be attributed, in part, to significant genetic disease modifiers, including the coinheritance of α-thalassemia (the α-3.7 globin gene deletion) and the presence of HbF-inducing genotypes at the three major quantitative trait loci for HbF persistence.[33,34]

The current study evaluated the impact of co-inheritance of α-thalassemia on clinical and hematological variables of patients with SCD, and it revealed that the co-inheritance of α-thalassemia was documented in 18.4% of SCD patients (22.4% in homozygous HbS and 10.4% in those with double heterozygosity of HbS and other hemoglobinopathies) and that it has a protective role against selected disease-related complications, especially in patients

with homozygous HbS, when compared with other types of SCD.

The majority of α-thalassemia gene mutations were deletional in type (79.4%), only 12% were nondeletional, and the rest had both types of mutations. The most common α genotype found was a 3.7 kb α-globin gene deletion (76.7%).

Other mutations reported in this study were 4.2 kb α-globin deletion, mutant α2 poly A-1, MED double gene deletion, mutant α2 IVS1-5nt, and mutant α2 cd59(G > A).

$\alpha^{3.7}$ was reported as the most common α^{+}-thalassemia deletion worldwide.[35,36]

In northern Iraq, Nasir and colleagues reported a total of nine α-thal mutations, including four deletional mutations in the Kurdish population: $-\alpha^{3.7}$, $--^{MED-I}$, $-(\alpha)^{20.5}$, and $-\alpha^{4.2}$ and five nondeletional mutations: $A^{polyA1}\alpha$, $\alpha\alpha^{Adana}$, $\alpha^{-5\ nt}\alpha$, $\alpha^{CS}\alpha$, and $\alpha^{polyA2}\alpha$; the most frequent of which was $-\alpha^{3.7}/\alpha\alpha$ (59.6%), followed by $--^{MED-1}$ and αα (23.8%).[37] However, among SCA patients, Nasir *et al*. reported that the $\alpha^{3.7}$ deletion was documented in 10.0% of Hb SS patients and was the only α-thalassemia mutation detected.[17]

In comparison with neighboring other Arab countries, the frequency of α-thalassemia was 40% in Kuwait, 31.5% among SS patients, and 47% among Hb AS individuals. The most common mutations were the $-\alpha^{3.7}$ deletion (27.5%), the $\alpha2^{PA-1}$ mutation (10.2%), and the $\alpha2^{IVS\ I\ -5\ nt}$ deletion (3.3%).[38] In Bahrain, α-thalassemia defects were observed in 23% of adult patients with SCD. Heterozygous and homozygous $-\alpha^{3.7}$ defects constituted 41 (89%) of α-thalassemia genotypes, followed by $-\alpha^{4.2}$.[39]

In Oman, the gene frequency of α-thalassemia among Hb SS patients was confirmed to be very high. Homozygosity or compound heterozygosity (- α/- α) was found in 44% of SCA patients; 98.2% of these α-thalassemia mutations had $-\alpha^{3.7}/-\alpha^{3.7}$.[40]

In India, Pandy *et al*. reported α-thalassemia in 30% of SCA patients,[41] while Singh *et al.* reported an overall prevalence of α-thalassemia in 41.3% of patients with SCA.[42] The most frequent mutation in both studies was $\alpha^{3.7,}$ followed by $-\alpha^{4.2}$ deletion.[41,42]

The higher frequency of α-thalassemia among patients with SCA compared to those with double heterozygosity of HbS and other hemoglobinopathies is in agreement with that of Singh *et al*. in India, although the prevalence was higher (41.3% in SCA and 35.0% in S/ β-thalassemia).[42]

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The frequency of acute painful episodes did not differ significantly in SCA patients with or without α-thalassemia, although the co-inheritance of α-thalassemia has a protective role against many disease-related complications among patients with homozygous HbS in the current study. However, this role was not observed in patients with other types of SCD (except for the pallor). Furthermore, patients with SCA and α-thalassemia had significantly higher RBC count, Hb, and HCT and lower MCV, reticulocyte count and HbA2 compared to those without α-thalassemia. These findings are in agreement with those of Pandey *et al*. in India, who reported that patients with co-existing α-thalassemia and SCD had a mild phenotype, significantly improved hematological parameters (higher Hb, MCV, MCH, and MCHC), and fewer blood transfusions than patients with SCA without co-existing α-deletions.[41] Furthermore, Rumaney and colleagues in Cameroon reported that the co-inheritance of α-thalassemia was associated with a lower consultation rate in SCA patients ($P = 0.038$), although VOC frequency and BMI were not significantly different.[43]

Other studies did not confirm the protective effect of α-thalassemia on the clinical manifestations of SCA.[12,40,44] Few studies have reported more frequent bone pain crises with α-thalassemia co-inheritance.[14,39]

The benefits and liabilities afforded by the presence or absence of α-thalassemia in SCA are very likely due to its effects on hemolysis (lowers the intracellular concentration of HbS, which is an important determinant of sickling). Co-inheritance of α-thalassemia was reported to have protective effects and decreased the risk of organ failure, including stroke, leg ulcers, cholelithiasis, glomerulopathy, and priapism, in addition to the protective effects on heart and splenic function.[45,46] Furthermore, RBC deformability and aggregation were found to be significantly higher in patients with α-thalassemia compared to SCA without α-thalassemia.[44]

The study has many limitations. The first limitation is that disease-related complications and hematological variables were not analyzed in relation to the number of α gene mutations (heterozygous vs. homozygous). The other limitation is that due to financial reasons, it was not possible to screen all SCD patients for α-thalassemia gene mutations.

From this study, it can be concluded that the co-inheritance of α-thalassemia and SCA confers

protection against many disease-related complications and is associated with improved hematological indices. However, these favorable features were not noticed in patients with other types of SCD.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form, the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Conflicts of interest

There are no conflicts of interest.

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