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Double Heterozygosity for Hemoglobin S and D Punjab in Basra, Iraq: A Clinical and Hematological Study of 42 Patients

Zuhair Abd Ali Al-Barazanchi, Subeh Salim Abdulateef[1], Mea`ad Kadhum Hassan[2,3*], Rawshan Zuhair Jaber[3]

Abstract:

BACKGROUND: Patients with compound heterozygosity for sickle hemoglobin (HbS) and hemoglobin D ^{Punjab} (Hb SD ^{Punjab}) may present with a variable clinical course and may be indistinguishable from those with homozygous HbS.

OBJECTIVES: The objective was to identify Hb SD ^{Punjab} phenotypes and the association of fetal Hb with disease severity.

PATIENTS AND METHODS: This descriptive, cross-sectional study included 42 (17 males and 25 females) patients with double heterozygosity for HbS and D. In addition to full clinical data, the complete blood count and Hb quantitation were assessed. Statistical analyses were performed using SPSS software version 20.0.

RESULTS: The mean age at diagnosis was 4.6 ± 0.7 years. Pallor and acute painful episodes (>90%) were the most common presenting symptoms, followed by jaundice (76.2%). Six patients (14.3%) had acute splenic sequestration crises and five (11.9%) had cardiac complications. Of the 42 studied patients, 17 (40.5%) had nonsevere disease and 25 (59.5%) had severe disease. Patients with severe disease had statistically significantly more blood transfusions (47.4 ± 10.27) than those with nonsevere disease (17.14 ± 1.82, $P < 0.001$). No significant association was reported between HbF level and either the number of blood transfusions or acute painful episodes requiring hospitalization/year.

CONCLUSION: Double heterozygosity for Hb S and D ^{Punjab} in Basra results in SCD with a severe phenotype, and the Hb F level did not modulate the disease severity.

Keywords:

Basra, hemoglobin SD, Iraq

Department of Laboratory, Basra Children Specialty Hospital, [1]Department of Pathology and Forensic Medicine, Al-Nahrain Medical College, Baghdad, [2]Department of Pediatrics, College of Medicine, University of Basra, [3]Center for Hereditary Blood Diseases, Basra Health Directorate, Basra, Iraq

Address for correspondence:
Dr. Mea`ad Kadhum Hassan, Department of Pediatrics, College of Medicine, University of Basra, Basra, Iraq.
E-mail: alasfoor_mk@yahoo.com

Introduction

Hemoglobin (Hb) disorders present a significant health problem in 71% of countries worldwide, with over 330,000 affected infants born annually. These disorders account for approximately 3.4% of deaths in children younger than 5 years of age. Globally, approximately 7% of pregnant women carry β^+ or B^0 thalassemia or HbS, C, D Punjab, or E, and over 1% of couples are at risk.[1]

Hb variants usually result from single aminoacid substitutions caused by point mutations in the genes that encode globin chains, resulting in a tetramer with different chemical properties and physical effects ranging from insignificant to severe.[2]

Hb D Punjab, also known as Hb D Los Angeles, is a → -chain variant characterized

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by a GAA → CAA missense mutation in the → globin gene, leading to Glu → Gln substitution in the 121st amino acid of the → chain; and with electrophoretic mobility at an alkaline pH is similar to that of HbS (→ 6, Glu → Val).[3] Hb D Punjab is one of the most common Hb variants worldwide, after Hb S and Hb C. Hb D Punjab is prevalent in the Punjab region of India. The average frequency of Hb D in India is 0.86%.[4] Hb D Punjab is also prevalent in other countries such as Turkey,[5] Italy,[6] and Brazil.[7] In Basra, in southern part of Iraq, the frequencies of Hb S and Hb D are 6.48% and 0.19%, respectively.[8]

Of all the D or G Hbs, Hb D Punjab (D Los Angeles) alone interacts with Hb S to produce moderately severe hemolytic anemia in compound heterozygotes. Compound heterozygotes for Hb S and D may present a variable clinical course and may be indistinguishable from Hb S homozygotes.[9,10]

The Hb concentration is usually between 5 and 10 g/dl, and the reticulocyte count is between 5% and 20% (occasionally higher). The mean corpuscular volume (MCV) is very variable, but macrocytosis is quite common, with some individuals having an MCV of 110–120 fl.[11]

The blood film shows anisocytosis, poikilocytosis, target cells, irreversibly sickled cells, boat-shaped cells, nucleated red cells, and sometimes macrocytes. The bone marrow shows erythroid hyperplasia and sickle cells. On cellulose acetate electrophoresis at an alkaline pH, Hbs S and D Punjab show the same electrophoretic mobility, but high-performance liquid chromatography (HPLC) and electrophoresis at an acidic pH separate these two Hbs from each other, with Hb D forming a somewhat higher proportion of total Hb than Hb S. Hb A_2 may be slightly elevated.[12,13] Many studies have reported elevated Hb F.[10,14]

Our objectives were to look for Hemoglobin D Punjab phenotypes and determine whether the fetal Hb level modulates disease severity in these patients.

Patients and Methods

Selection of patients

A total of 42 (17 males and 25 females) patients with double heterozygosity for Hb S and D who were registered at Basra Center for Hereditary Blood Diseases were included in this study during the period from April 1, 2018, until June 1, 2019.

Data collection

Full personal and demographic information was obtained, including date of birth, sex, residence, age at diagnosis, and duration of illness.

Complete clinical data were obtained regarding the presenting complaint(s), including pallor, jaundice, the number and frequency of blood transfusions, acute painful episodes, disease-related complications (pulmonary, cardiac, neurological, bone, retinal, and renal complications), gallbladder stones, cholecystectomy, and splenectomy.

All patients were thoroughly examined for pallor, jaundice, anthropometric measurements, hepatomegaly, and/or splenomegaly.

Severe disease was defined as frequent vaso-occlusive crisis requiring hospitalization ≥3 times/year, blood transfusion ≥3times/year,frequenthospitalization ≥3times/year, an episode of acute coronary syndrome (ACS), stroke, or avascular necrosis of bone.[15,16]

A detailed explanation of the aim of the study was provided to the patients or to their parents (for pediatric cases). Participation was strictly voluntary. A written consent was obtained from each patient. The study strictly obeyed the instructions of the Declaration of Helsinki for human rights.

For children and adolescents, Z-scores for body mass index (BMI) for age (BMIZ) were calculated, and accordingly, the patients were classified as severely thin (<−3 standard deviation [SD]), thin (<−2 SD), normal (+1SD to −1SD), overweight (> +2 SD) or obese (>+3 SD).[17,18]

Laboratory methods

For each patient, 5 mL of ethylenediaminetetraacetic acid blood samples was aspirated under strict aseptic techniques. The laboratory workup included the following:

1. Complete blood count: It was done using an automated (Mindray BC 6800 Shenzhen, China) 7-part hematology analyzer
2. Hb variants were studied using HPLC/beta-thalassemia short program with two systems: Bio-Rad: D-10 Hemoglobin testing system (Bio-Rad Laboratories Inc. Hercules, USA) and Bio-Rad variant-II Hemoglobin testing system (USA). Capillary zone electrophoresis using the Minicup Sebia Flex piercing system, Sebia, France, was performed as well in cases of doubt. The diagnosis was performed using two different systems [Figure 1][7]

   Due to the unavailability of molecular diagnostic tools to make an accurate decision, the decision regarding Hb D Punjab/Los Angeles for the Hb D peak was postulated for the following reasons:

   a. The clinically severe disease was observed in the double heterozygous Hb SD disease, knowing that other Hb D variants, such as Hb D Ibadan, are unlike the Hb S/Hb D Los Angeles [β121(GH4) Glu-->Gln]

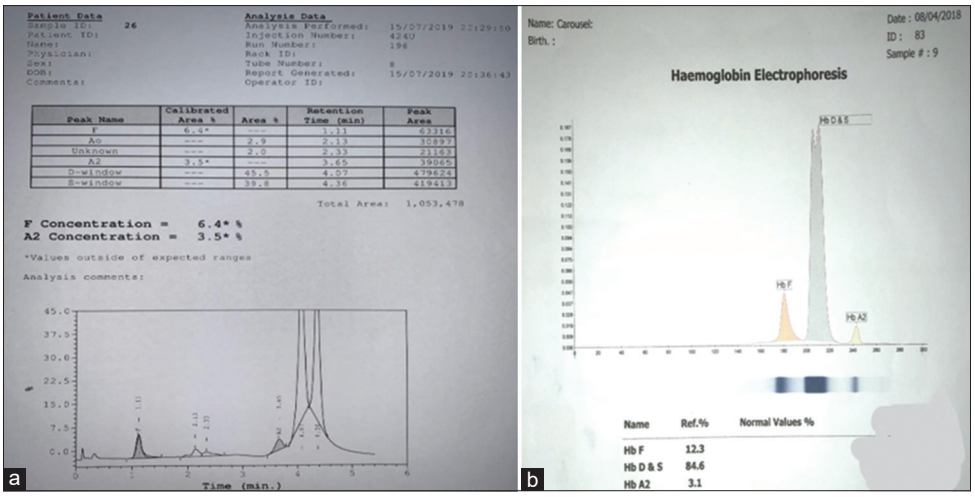

Figure 1: (a) A high-performance liquid chromatography chromatogram, using Variant II/BioRad, showing hemoglobin D and S of one of the cases in the study. (b) An electropherogram of another case using the Sebia capillary zone system, showing the intermingled peak of both hemoglobin D and S

combination, its compound heterozygosity with Hb S does not result in a sickling disorder.[19]

3. The retention time of Hb D Punjab is 3.9–4.3 min,[20] whereas the retention time of Hb D Iran, another Hb D variant found in the central part of Iran, is lower than the standard retention time of Hb A_2 (3.6 min).[21] For all cases, the confirmation of the presence of Hb S was achieved by the sickle scan using monoclonal anti-Hb A and anti-Hb S antibodies (BioMedomics Inc. Cedar Fork, North Carolina, USA).

Statistical analysis

All statistical analyses were performed using the Statistical Package for the Social Sciences (IBM SPSS Corp., Armonk, NY, USA) software version 20.0. Data were expressed either as a number (*N*) and percentage (%) or as mean ± SD. However, standard error was used if there was great variation in variables away from the mean or presence of extreme values in the sample data. 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.

Correlation coefficient analysis (Spearman's rho correlation test) was used to assess the association between fetal Hb and the number of blood transfusions and acute painful crises. For all tests, $P < 0.05$ was considered statistically significant.

Results

This descriptive, cross-sectional study included 42 patients with compound heterozygosity for Hb S and D (17 males and 25 females). Their ages ranged from 8 months to 37 years, with a mean age of 12.7 ± 7.5 years (interquartile range [IQR]: 7.75–17.25) and a median age of 12.5 years [Table 1].

The mean age at diagnosis was 4.6 ± 0.7 years (IQR: 1–6 years), and the median age was 5 years. Approximately two-thirds of the patients were born to nonrelative parents.

Pallor and acute painful episodes (>90%) were the most common presenting symptoms, followed by jaundice. Pain mainly occurred in the extremities and back. Six patients (14.3%) had acute splenic sequestration crises (ASSC), five (11.9%) had cardiac complications, four had left ventricular dilatation, and one had tricuspid regurgitation. The most important findings upon examination were splenomegaly in 20 patients (47.6%) and underweight in 11 patients (26.2%) [Table 2].

The Hb levels of the studied patients ranged from 4.8 to 10.8 g/dl, with a mean of 8.19 ± 1.47, whereas the MCV ranged from 61.70 to 98.90, with a mean of 80.05 ± 9.55. Sixteen patients had low MCV (38.1%) [Table 3].

Concerning male and female patients, no significant differences were found in red blood cell indices, white blood cell indices, platelet counts, or Hb D and Hb F levels.

Out of the 42 studied patients, 17 (40.5%) had nonsevere and 25 (59.5%) had severe disease. The mean number of blood transfusions was 29.05 ± 6.99 (IQR: 1–42). Patients with severe disease had a statistically significantly higher number of blood transfusions (47.4 ± 10.27) than those with nonsevere disease (17.14 ± 1.82, $P < 0.001$). Six out of the 17 patients (35.3%) with nonsevere disease did not require a blood transfusion compared to only 2 (8%) with severe disease.

Patients with severe disease had a significantly higher mean corpuscular Hb concentration and lower absolute reticulocyte count than those with milder disease ($P < 0.05$). Other hematological variables were not significantly different between the groups [Table 4].

No significant association was reported between HbF level and either the number of blood transfusions or acute painful episodes requiring hospitalization/year [Figures 2 and 3, respectively].

Table 1: Selected demographic characteristics of patients with compound heterozygosity for sickle hemoglobin and D (*n*=42)

Variable	Total, *n* (%)
Age (years)	
<5	5 (11.9)
5-10	13 (31.0)
>10-15	9 (21.4)
>15-20	10 (23.8)
>20	5 (11.9)
Mean age (years)	12.76±7.49
Median age (years)	12.5
Sex	
Male	17 (40.5)
Female	25 (59.5)
Residence	
Center	24 (57.1)
Periphery	18 (42.9)
Parental consanguinity	
Relatives	10 (23.8)
Not relatives	28 (66.7)
Missing	4 (9.5)

Table 2: Clinical data of the studied patients

Clinical variable	*n* (%)
Pallor	41 (97.6)
Acute painful episodes	38 (90.5)
Jaundice	32 (76.2)
ASSC	6 (14.3)
Stroke	3 (7.1)
Gall stones	7 (16.7)
Cardiac complications	5 (11.9)
ACS	4 (9.5)
AVN	4 (9.5)
Splenectomy	3 (7.1)
Cholecystectomy	1 (2.4)
Priapism	1 (2.4)
Organomegaly	
Splenomegaly	20 (47.6)
Hepatomegaly	0 (0.00)
BMIZ score	
Obese	0 (0.00)
Overweight	2 (4.8)
Normal	29 (69)
Thin	10 (23.8)
Severely thin	1 (2.4)

BMIZ score=Body mass index Z score; ASSC=Acute splenic sequestration crises; ACS=Acute chest syndrome; AVN=Avascular necrosis

Discussion

Sickle cell disease is an important health problem in many parts of Iraq. The frequency of sickle cell trait carriers ranges from 0.27% to 6.48%.[8,22,23] This study described patients with compound heterozygosity for Hb S and D registered at the Center for Hereditary Blood diseases in Basra, where the gene frequency for the sickle Hb is high.

The study revealed that most patients with Hb SD disease had a severe disease type, although there was a great variation in the disease phenotype. In addition, the level of Hb F was not found to be associated with disease severity variables, such as the frequency of blood transfusions and acute painful episodes requiring hospitalization.

Sickle cell disease, one of the most common genetic diseases, comprises a group of disorders characterized by the presence of at least one Hb S allele (p. Glu6Val in *HBB*) and a second *HBB* pathogenic variant. The most common form of SCD occurs in individuals homozygous for the HbS allele (SCA). Other forms of SCD result from the inheritance of β^S in combination with other *HBB* mutations. The two most common include coinheritance with Hb C or β-thalassemia. Coinheritance with other β-globin gene variants, such as D-Punjab, E, and O Arab, constitutes much rarer form of SCD.[24,25]

The median age of the study participants was 12.5 years, with 59.5% of them being females, and the median age at diagnosis was 5 years. These findings differ from those reported by Patel *et al*. The average age of the patients with Hb SD-Punjab in India was 22 years, with 62% of

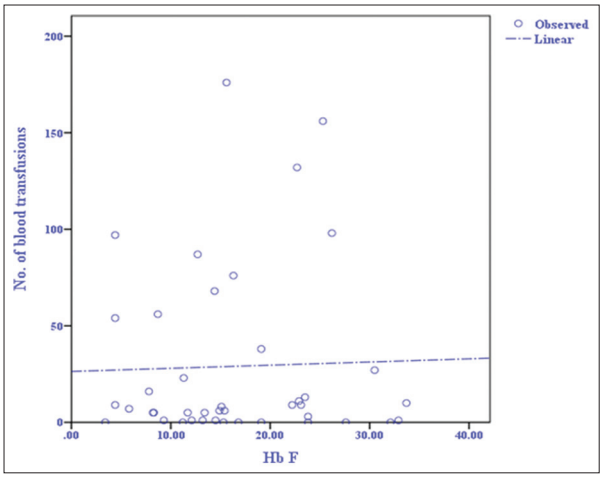

Figure 2: Correlation of fetal hemoglobin level and the number of blood transfusions. Spearman's rho correlation coefficient, -0.007-; $P = 0.967$

Table 3: Hematological data of patients of both sexes with compound heterozygosity for sickle hemoglobin and D

Variables	Mean±SD			*P*
	Male (*n*=17)	Female (*n*=25)	Total (*n*=42)	
Hb (g/dl)	7.71±0.21	8.51±1.57	8.19±1.47	0.084
RBC count (×10^{12}/l)	3.13±0.75	3.21±0.61	3.18±0.66	0.700
MCV (fl)	78.98±8.52	80.77±10.30	80.05±9.55	0.559
MCH (pg)	26.30±3.82	26.50±3.64	26.42±3.67	0.869
MCHC (g/dl)	33.25±2.83	32.34±3.82	32.71±3.44	0.408
WBC (×10^{9}/l)	11.67±4.76	14.65±6.55	13.44±6.01	0.117
PLTs* (×10^{9}/l)	324.94±34.05*	395.20±30.58*	366.76±23.19*	0.139
Absolute retics (×10^{12}/l)*	0.16±0.02*	0.15±0.01*	0.16±0.01*	0.794
Hb S (%)	31.93±6.40	34.90±6.17	33.70±6.36	0.140
Hb D (%)	42.97±4.27	43.24±4.59	43.13±4.41	0.849
Hb F (%)	19.26±7.48	14.78±8.46	16.59±8.29	0.086

Values are expressed as mean±SD. *Values are expressed as mean±SE. An independent *t*-test was used to determine the *P* value. Hb=Hemoglobin; RBC=Red blood cell; MCV=Mean corpuscular volume; MCH=Mean corpuscular Hb; MCHC=MCH concentration; WBC=White blood cell; PLTs=Platelet count; Hb S=Sickle Hb; Hb D=Hemoglobin D; Hb F=Fetal Hb; SD=Standard deviation; SE=Standard error

Table 4: Selected hematological variables in relation to disease severity

Variables	Mean±SD			*P*
	Nonsevere disease (*n*=17)	Severe disease (*n*=25)	Total (*n*=42)	
Hb (g/dl)	8.16±1.56	8.21±1.43	8.19±1.47	0.925
RBC count (×10^{12}/l)	3.20±0.64	3.16±0.69	3.18±0.66	0.837
MCV (fl)	79.02±10.96	80.74±8.62	80.05±9.55	0.572
MCH (pg)	25.41±3.97	27.10±3.36	26.42±3.67	0.144
MCHC (g/dl)	31.42±3.85	33.58±2.90	32.71±3.44	0.045
Absolute retics (×10^{12}/l)*	0.20±0.02*	0.12±0.01*	0.16±0.01*	0.003
WBC (×10^{9}/l)	15.19±6.32	12.25±5.62	13.44±6.01	0.122
PLTs (×10^{9}/l)	376.00±29.42*	360.00±23.87*	366.76±23.19*	0.747
Hb S (%)	33.76±5.74	33.66±6.87	33.70±6.36	0.959
Hb D (%)	44.24±3.72	42.37±4.75	43.13±4.41	0.180
Hb F (%)	17.14±7.53	16.22±8.90	16.59±8.29	0.782

Values are expressed as mean±SD. *Values are expressed as mean±SE. An independent *t*-test was used to determine the *P* value. Hb=Hemoglobin; RBC=Red blood cell; MCV=Mean corpuscular volume; MCH=Mean corpuscular Hb; MCHC=MCH concentration; WBC=White blood cell; PLTs=Platelet count; Hb S=Sickle Hb; Hb D=Hemoglobin D; Hb F=Fetal Hb; SD = Standard deviation; SE=Standard error

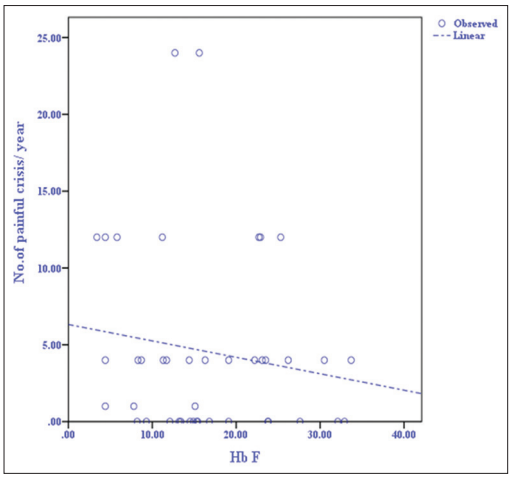

Figure 3: Correlation between fetal hemoglobin level and the number of acute painful crises/year. Spearman's rho correlation coefficient, -0.160-; *P* = 0.321

them being males, and the median age at presentation was 6 years.[14] Oberoi *et al*. in India also reported that the median age at diagnosis was 3.5 years among ten patients with Hb SD-Punjab.[26]

Although the overall rate of consanguineous marriage in Iraq ranges from 47% to 60%,[27] only 23.8% of patients in the current study were the product of consanguineous marriage.

The clinical presentation was very variable from very mild disease to a severe presentation requiring frequent blood transfusions and hospitalization and being associated with severe life-threatening complications such as stroke, ASSC, and ACS. Almost all patients had pallor (41/42, 97.6%). Painful crises were present in 90.5% of the registered patients, followed by jaundice (76.2%) and ASSC (14.3%).

Most researchers have also reported great variation in Hb SD-Punjab phenotypes, ranging from asymptomatic conditions to severe disease-related events.[14,26,28]

The severity of anemia was variable, and it was normochromic normocytic in most cases. Bonini-Domingos reported that 38.46% of individuals

with Hb SD required blood transfusions and presented microcytosis and hypochromia.[28]

The mean Hb D-Punjab was 43.13 ± 4.41, which is comparable to that identified in the study by Torres *et al*. in Brazil (44.8 ± 2.3).[29] Rezende *et al*. reported a mean Hb level of 8.6 g/dl and a mean Hb D of 38.9 among 11 children with Hb SD Punjab.[10]

The mean Hb F was 16.59 ± 8.29, with no significant difference between those with severe and nonsevere diseases. In addition, there was no significant correlation between Hb F and the frequency of blood transfusions and acute painful episodes. This finding is in agreement with Adekile *et al*., who reported that Hb F does not ameliorate the clinical phenotype in Hb SD patients.[30] However, Patel *et al*. reported a mean Hb F of 17.81 ± 7.65 and that Hb F reduced the frequency of painful episodes but had no influence on hemolytic markers in Hb SD individuals.[14]

In comparison with patients with homozygous Hb S, a previous study in Basra found that the mean Hb F level was 19.65 ± 7.42, and there was a significant positive association between Hb F and total Hb and a significant negative association with disease severity markers (pallor, acute painful episodes, ACS, and hospitalization).[31]

Although this is the first report of this type of hemoglobinopathy in Iraq, the study has many limitations. The first limitation is that we were not able to look for the β^S haplotype. The second limitation is that other disease-modifying factors, such as coinheritance of α thalassemia or the use of hydroxyurea, were not evaluated.

Conclusion

From this study, it can be concluded that double heterozygosity for Hb S and D ^{Punjab} results in SCD with a severe phenotype and that the Hb F level did not modulate disease severity. Further studies are required to identify other genetic and environmental modifiers that influence the clinical and hematological profile of patients with this disease.

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Conflicts of interest
There are no conflicts of interest.

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