



ORIGINAL ARTICLE

The Influence of Fetal Hemoglobin on Clinical and Hematological Variables of Children and Adolescents with Sickle Cell Anemia in Basra, Southern Iraq

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ABSTRACT

Background: There are many parameters that modulate the severity of sickle cell anemia. Fetal hemoglobin (Hb F) is one of these major variables. However, its effect is clinically inconsistent. We conducted a descriptive study to assess the influence of Hb F on clinical events and hematological variables in patients with sickle cell anemia.

Methods: 151 patients with sickle cell anemia with a stable condition, aged 1-18 years, were recruited from March through November 2010. The results of complete blood count and Hb F level and various clinical variables were recorded.

Results: Of the 151 patients, the Hb F was more than 20%, 10-20%, and less than 10% in 77 (51%), 60 (39.7%), and 14 (9.3%) patients. A significant negative association was reported between Hb F level and frequency of painful crisis (95% CI=0.05-0.96, OR=0.22), acute chest syndrome (95% CI=0.01-0.43, OR=0.07) and frequency of hospitalizations (95% CI=0.03-0.85, OR=0.11). There was a significant positive association between hemoglobin level (95% CI=2.14-27.17, OR=7.63) and splenomegaly (95% CI=1.37-57.4, OR=12.88) with Hb F level.

Conclusion: In children and adolescents with sickle cell anemia, the higher the Hb F levels, the lesser clinical complications of the disease would be. Therefore, patients with low Hb F need close follow-up and monitoring since early age to detect complications as early as possible and consider use of disease modifying agents.

Introduction

Hemoglobinopathies, mainly sickle cell disease (SCD), are challenging health problems in the population of Basra, Southern Iraq where around 6.48% of the population are carriers of sickle cell gene with a gene frequency of 0.0324%.¹ Individuals with sickle cell anemia (SCA) are characteristically asymptomatic until the second half of the first year of life. This lack of clinical expression of the hemoglobin (Hb) SS genotype during early postnatal life can be explained by production of a sufficient quantity of fetal hemoglobin (Hb F) that limits clinically important sickling process.²

Properties of Hb F which help in attenuating the severity of SCA are due to the lack of participation of

Hb F molecules in polymerization of Hb S. As a result higher amount of Hb F in a cell causes a lower Hb S concentration.³ The preventive effects of Hb F on Hb S polymerization appear to be concentration dependent.⁴ The effect of Hb F on Hb S may affect other red blood cells' characteristics directly or indirectly as Hb F level affects the RBC adhesive properties of patients with SCA.⁵ High levels of Hb F may reflect increased synthesis resulting in mild disease, or greater amplification through accelerated destruction of cells containing no Hb F, a manifestation of more severe disease.² Levels of Hb F influence the age at which symptoms develop and partially determine the risk of acute splenic sequestration crises (ASSC), stroke, acute chest syndrome (ACS), leg ulcers,

pain crises, loss of spleen function, and mortality.^{2,6,7}

It was suggested that some complications of SCD such as vaso-occlusion and blood viscosity are strongly associated with Hb F level; whereas, complications related to the degree of hemolysis are dependent on Hb F to a lesser degree.⁸

Although many studies have considered Hb F level $\geq 10\%$ as high Hb-F,⁹⁻¹¹ other studies have defined levels of more than 5.4% as high.¹² The impact of Hb F on clinical course of the disease is variable and not consistent as Hb F percentage reaching 20% may be found in patients with severe disease.¹³

We aimed to study the level of Hb F among children and adolescents with SCA and the association between Hb F level and clinical variables and hematological parameters reported in these patients.

Patients and Methods

This descriptive study was done on children and adolescents with SCA (homozygous Hb S) over a 9-month period (from the first of March until the end of November 2010). 151 children and adolescents with SCA registered at the Center for Hereditary Blood Diseases, which is the only center caring for these patients in Basra were the study was done. Patients were in a stable condition with an age range of 1-18 years, consisting of 84 boys and 67 girls. Patients with sickle cell/ β -thalassemia and patients on Hydroxyurea (HU) were excluded from the study.

Baseline steady state was defined as a steady hemoglobin and hematocrit level reported during 2-3 clinical visits with an interval of 4-6 weeks and a condition of wellbeing with no symptom or sign suggestive of crisis, infection, and other diseases confirmed by thorough history and physical examination.^{14,15} All Information including socio-demographic and clinical data were taken from patients and/or their parents or other caregivers in addition to patients' records.

Physical examination was performed for all patients. Body weight and height were measured and body mass index (BMI) was calculated for all patients. According to BMI Classification of Children and Adolescents; the patients were considered underweight when BMI was $< 5^{\text{th}}$ percentile, normal weight with a BMI between 5^{th} - 84^{th} percentile, at risk for overweight with a BMI of 85^{th} - 94^{th} percentile, and overweight when BMI was $\geq 95^{\text{th}}$ percentile.¹⁶ Moreover, follow-up of these patients and their complications were recorded during the study period. An informed consent was obtained from one of the parents/caregivers before enrollment in the study. The study was approved by the Research Ethics Committee of Basra Medical College.

Disease severity was assessed so that severe disease was defined as patients requiring hospitalizations for SCD-related complications ≥ 3 times/year, acute painful crises requiring hospitalization of ≥ 3 times/year, frequent blood transfusion ≥ 3 times/year, history of ACS, ASSC or avascular bone necrosis.^{2,14}

The diagnosis of SCA was confirmed by High Performance Liquid Chromatography (HPLC), (VARIANT™, β - Short Programs; Bio-Rad Laboratories, Hercules, CA, USA).

The relationship between Hb F level and clinical events was assessed by dividing patients into three groups based on Hb F level; $< 10\%$, $10\text{-}20\%$ and $> 20\%$.

Estimation of hemoglobin, white blood cell (total and differential), platelets counts, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were done by an Automated Hematology Analyzer CBC+3 part diff. Symex KX-21N.

Other investigations including echocardiography, electrocardiography, chest x-ray, urinalysis, urine culture and ultrasonography were done when indicated.

Statistical analysis were carried out using the Statistical Packages for Social Sciences (SPSS Inc., Chicago, IL, USA) software version 17.0. Data were expressed as mean \pm standard deviation (SD). Comparisons of proportions were performed by crosstab using Chi-square test. Comparisons between groups were assessed using one-way analysis of variance (ANOVA). Logistic regression analysis was also done for the analysis of different variables, the 95% confidence interval (CI) were assessed. For all quantitative variables $P < 0.05$ was considered as statistically significant.

Results

In this study, 151 children and adolescents with SCA were evaluated with a mean \pm SD age of 7.6 ± 4.94 years for boys and 8.4 ± 4.04 years for girls. The mean \pm SD Hb-F level was 19.65 ± 7.42 with no significant difference between boys (19.97 ± 7.50) and girls (19.26 ± 7.30) ($P > 0.05$). The mean \pm SD Hb S was 70.76 ± 7.39 . The Hb F level was more than 20%, 10-20%, and less than 10% in 77 (51%), 60 (39.7%), and 14 (9.3%) patients.

Pallor, jaundice, acute painful episodes, ACS, and history of hospitalizations were significantly higher among patients with Hb F $< 10\%$ ($P < 0.05$). While splenomegaly was reported in a significantly higher number of patients with Hb F more than 20% (table 1). Persistent splenomegaly was found in 30.10% of patients beyond 6 years of age. Aplastic crisis, ASSC, priapism and stroke were not reported among patients enrolled in this study.

The mean frequency of painful episodes, total blood transfusions, frequency of hospitalizations/year and total hospitalizations were significantly higher among patients with Hb F less than 10% ($P < 0.01$). There was no significant difference between age of presentation and number of blood transfusions/year among patients with different Hb F levels ($P > 0.05$, table 2).

Concerning hematological parameters, the mean \pm SD Hb level for the studied patients was 8.9 ± 6.83 g/dL and it was significantly higher among patients with Hb F levels more than 20% ($P < 0.01$). Other parameters were not statistically significant among patients with different Hb F levels ($P > 0.05$, table 3).

History of blood transfusion was positive in 62 (41%) patients. Blood transfusions were significantly more frequent among patients with Hb F $< 10\%$ ($P < 0.05$).

After subjecting various variables to regression analysis, there was a significant positive association between Hb

Table 1: Distribution of SCA patients according to the level of fetal hemoglobin and clinical events

Clinical events	Hb F level			Total No %	P value
	<10% (No. 14) %	10-20% (No. 60) %	>20% (No. 77) %		
Pallor	10 (71.4)	27 (45)	26 (33.8)	63 41.72	0.005
Acute painful episodes	14 (100)	56 (93.3)	63 (81.9)	133 88.07	0.001
Jaundice	6 (42.9)	12 (20)	11 (14.3)	29 19.20	0.000
ACS	6 (42.9)	13 (21.7)	6 (7.8)	25 16.55	0.007
Infections	5 (35.7)	13 (21.7)	21 (27.3)	39 25.82	0.121
Heart failure	1 (7.1)	4 (6.7)	4 (5.2)	9 5.96	0.981
Splenomegaly	2 (14.3)	16 (26.7)	33 (42.9)	51 33.77	0.001
Hepatomegaly	1 (7.1)	7 (11.7)	7 (9.1)	15 9.93	0.721
Gall stone	1 (7.1)	7 (11.7)	2 (2.6)	10 6.62	0.096
Splenectomy	0 (0.0)	2 (3.3)	0 (0.0)	2 1.32	
Bone necrosis	0 (0.0)	2 (3.3)	0 (0.0)	2 1.32	
History of hospitalization	12 (85.7)	39 (65)	40 (51.9)	91 60.26	0.021
BMI (<5 th percentile)	4 (28.57)	21 (35)	21 (27.27)	46 (30.46)	0.942

Table 2: Selected clinical variables in relation to fetal hemoglobin level

Variables*	Hb-F level			Total (151)	P value
	<10% (No. 14)	10-20% (No. 60)	>20% (No. 77)		
Age at first presentation(years)	3.40±1.65	3.91±2.05	3.60±1.76	3.73±1.87	0.138
Acute painful episodes /year	8.52±5.72	7.64±6.2	4.80±4.3	6.31±5.45	0.005
BT/year	1.85±2.68	1.13±1.81	0.94±1.91	1.10±1.96	0.314
Total BT	20.71±24.54	7.03±9.49	5.29±11.47	7.41±13.16	0.001
Hospitalization/year	3.15±4.92	0.69±1.07	0.31±0.41	0.72±1.80	0.029
Total hospitalization	23.10±4.92	5.63±8.47	2.24±3.54	5.52±11.22	0.000

*Variables are presented as mean±SD; ANOVA test was used

Table 3: Distribution of SCA patients according to the level of fetal hemoglobin and hematological variables

Hematological Variables *	Hb-F level			P value
	<10 (N. 14)	10-20 (N. 60)	>20 (N. 77)	
Hb(g/dl)	7.85±1.25	8.17±1.25	8.69±1.40	0.000
PCV	23.64±3.71	24.23±3.75	25.55±4.50	0.120
MCV(fl)	80.42±5.72	78.69±5.62	77.81±5.36	0.180
MCHC(Hb/l RBC)	316±28.45	307±33.28	303±39.31	0.785
Retic(%)	6.52±2.42	6.63±2.38	6.16±2.70	0.653
MCH	26.29±3.22	25.35±3.05	24.61±2.62	0.783
WBC(×10 ⁹)	11.007±3.07	9.778±3.69	9.825±3.38	0.265
Platelets(×10 ⁹)	336.357±126.94	300.083±126.49	279.93±106.90	0.132

*Variables are presented as mean±SD; ANOVA test was used

F level with splenomegaly and total Hb level (table 4). Moreover, a significant negative association was found between pallor, jaundice, acute painful episodes, ACS and history of hospitalization with Hb F levels.

Discussion

Hemoglobinopathies are common hematologic disorders throughout the Arab world due to high level (25-60%) of consanguineous marriages.¹⁷ SCA is the most common hemoglobinopathy in Basra, followed by β -thalassemia.¹

A high mean Hb F was reported among patients with SCA in Basra, and more than half of them had Hb F level more than 20%. This percent is lower than that reported by Chopra et al. in Eastern Province of Saudi Arabia, where 75% of patients with SCA had Hb F level above 20%.¹⁸ In the United Arab of Emirates, those with Arab-Indian haplotypes had a mean Hb F level of 27% and a

milder clinical course compared to those with the African haplotypes, Bantu and Benin (mean Hb-F level of 11.3%) who had a severe clinical presentation.¹⁹ Mpalampa and colleagues in Uganda reported that only 37% of children aged 1-18 years had Hb F levels of more than 10% (mean level 9%).⁹

Frequency of hospitalizations was significantly associated with increased Hb F levels. Mpalampa and co-workers reported a significant negative correlation between Hb F level and the total number of blood transfusions, all cause hospitalizations and severe pain episodes in the past year.⁹ In addition, it has been reported that in India patients with SCD with Hb F more than 20% had a significantly lower morbidity mainly in terms of hospitalization and painful episodes.²⁰ However, Darbari and colleagues in the USA did not report a significant impact of Hb F on vaso-occlusive pain crises.²¹

Table 4: Logistic regression analysis of selected variables with fetal hemoglobin

Variables	95% CI	OR	P value
Pallor	0.05-0.71	0.20	0.032
Jaundice	0.03-0.92	0.18	0.044
VOC	0.05-0.96	0.22	0.029
ACS	0.01-0.43	0.07	0.049
Infections	0.46-12.7	2.42	0.098
Heart failure	0.08-18.1	3.78	0.125
Splenomegaly	1.37-57.4	12.88	0.004
Hepatomegaly	0.21-25.6	2.24	0.095
Gall stones	0.02-6.56	0.37	0.651
Splenectomy	0.19-2.19	0.75	0.787
Bone necrosis	0.21-2.21	0.51	0.921
History of hospitalizations	0.03-0.85	0.18	0.025
Hb level	2.14-27.17	7.63	0.004

We found a significant association between Hb F level and ACS. Increased levels of Hb F decrease intracellular polymerization of Hb S which increases total Hb and oxygen saturation and decreases chest pain and severity of ACS. This is in agreement with a previous report which showed that high Hb F level decreased the attacks of ACS.²²

Another finding was the positive association between higher Hb F level and splenomegaly. In Italy, researchers found that splenomegaly was present in 28% of adult patients with SCA due to lower degree of tissue infarction in Italian patients.²³ While others found that splenomegaly was prevalent in 28.9% of patients over 6 years of age with SCA in Lebanon²⁴ which was comparable with our study (30.1%). Parmar and Likhari in India reported higher Hb F levels among patients with SCD and splenomegaly at different age groups compared with patients with no splenomegaly and concluded that Hb F is one of the important etiological causes of persistence of splenomegaly.²⁵ This probably suggests that higher Hb F levels may have an ameliorating effect on sickling of RBCs and lower frequency of splenic infarctions.^{18,26}

We found that blood transfusions were less frequent as Hb F levels increased, although logistic regression analysis did not reveal such a significant association. Mpalampa and colleagues have reported a significant association between Hb F level and frequency of blood transfusions.⁹ Higher Hb F among patients with SCD is a well-known factor to decrease overall hemolysis and hence increased survival of RBCs containing relatively more Hb F is related to degree of polymer contents in RBCs.²⁷

Attaining weight and growth was not significantly different among patients with SCA of different age groups in relation with Hb F level. However, another study showed that high Hb F maintains growth with a significant positive association between Hb F level and BMI; as more frequent VOC decreases appetite and retards growth. The researchers postulated that low Hb F increases hemolysis and metabolic requirements for erythropoiesis that increases the risk of poor growth.²⁸

Although Hb F modulates the phenotype of SCA, Steinberg and colleagues have reported that the concentration of Hb F within the individual RBC is more important than the level of Hb F in the blood. Moreover, distribution Hb F within the cell is another important

variable that affects Hb S polymerization and as a result may affect disease severity.²⁹

A significant association between Hb F level with total Hb was reported in this study. The sickled RBCs become less deformable, leading to increased hemolytic breakdown particularly in the small arterial capillary bed, which results in local increase in ionized and non-ionized calcium concentration. This forms a greater population of RBCs with reduced flexibility causing a fall in Hb level; whereas, increased Hb F inhibits the aggregation of Hb S that can be clinically significant. However, other studies could not find a significant association between hematological parameters and Hb F level.^{30,31}

A few limitations need be addressed for the current study. First, the sample size was relatively small. Secondly haplotype testing was not available which is important in assessing severity of the disease. Despite these limitations, the results of this study are still comparable to published data.

Conclusion

It can be concluded that children and adolescents with SCA in Basra have a high Hb F level that significantly decreases many important clinical complications of the disease, although the influence on hematological variables was less prominent. Therefore, we recommend that children with low Hb F should have closer follow-up and monitoring since early age to detect complications earlier and consider use of disease modifying agents such as hydroxyurea.

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Conflict of Interest: None declared.

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