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Fetal Hemoglobin Levels in Patients With Sickle Cell Disorders and its Correlation With Disease Severity

KEYWORDS: Sickle cell disease, Fetal hemoglobin, Vaso-occlusive crisis

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ABSTRACT *Background: Fetal haemoglobin (HbF) levels show a wide variation in sickle cell patients throughout world resulting in variable disease severity. Though the condition is prevalent in India, there are only few studies that deal with HbF levels in these patients*

Aim: To study HbF levels in patients with sickle cell disorders (sickle cell trait and sickle cell disease) and its correlation with disease severity.

Methods: Patients above 12-years of age with sickle cell disorders were included in the present study. A detailed demographic and clinical history was recorded. Quantitative estimation of HbF was done in each patient by alkali denaturation test. HbF levels were then correlated with vaso-occlusive episodes.

Results: 100 patients had sickle cell disease and 138 had sickle cell trait. The mean age of presentation was higher in AS patients (24.89± 7.1 years) as compared to SS patients (22.22±7.9 years). Total 87.39% patients presented with joint pains while 11.34% patients were asymptomatic. Complications of sickle cell disease were noted in 2.94% patients. Mean HbF levels were 1.88±3.27% in AS group and 9.22±6.12% in SS group. In AS group, when HbF levels were studied in relation to number of VOC per year i.e. up to 3 per year and >3 per year, the difference is found to be significant. Similar correlation in SS group found to be significant. In this group, a strong negative correlation was found between HbF levels and VOC on application of correlation coefficient.

Conclusions: The mean HbF levels were high in both AS and SS patients than in normal patients Also, there was a strong negative correlation between number of vaso-occlusive crises and HbF levels.

Introduction

Sickle cell disease is an inherited multisystem disorder characterized by chronic hemolytic anemia and recurrent painful episodes. The clinical problems encountered in this disorder relate to reduced life span of sickle cells and vaso-occlusion caused by polymerization of de-oxygenated hemoglobin S. The disorder consists of sickle cell trait (heterozygous state for hemoglobin S), sickle cell disease (homozygous state for hemoglobin S), and compound heterozygous state (together with hemoglobin C, D or other structural variants). These patients display a remarkable variation in severity of their clinical profile. While this variation is difficult to quantify, at one extreme are the patients with virtually no medical problems and at other, are patients incapacitated by repetitive bouts of crippling pain due to infarctive damage.

Factors modifying the severity of sickle cell disease have been the subject of investigations by many workers. High fetal hemoglobin (HbF), concurrent presence of α-thalassemia and alteration of membrane proteins are considered to ameliorate the severity of sickle cell disease. Over the years high HbF levels has gained significance as the most powerful modulator of the clinical and hematologic features of sickle cell anaemia. The epidemiological studies suggested that disease complications like acute painful episodes, acute chest syndromes and osteonecrosis were most closely linked to sickle vaso-occlusion and ultimately were strongly related to HbF concentration[1].

Fetal haemoglobin levels show a wide variation in sickle cell patients throughout world resulting in variable disease severity and complications. Though the condition is a major public health problem with high morbidity, mortality and reproductive wastage in certain parts of India, only few studies are available in this context. Study from Orissa quoted markedly elevated HbF levels in sickle cell disease patients with clinically mild disease while study from Madhya Pradesh failed to show significant correlation between HbF levels and clinical severity[2,3] . The prevalence of sickle cell disease is high in Central India. Recent study from Nagpur identified 5466 patients of sickle cell trait and 1010 patients of sickle cell disease in a community screening of 35636 high risk individuals.[4] However very little data is available in reference to HbF levels in sickle cell population in this area. Hence, the present prospective study was planned to study HbF levels in patients with sickle cell disorders and its correlation with disease severity.

Material and methods

Patients above 12-years of age with sickle cell disorders (sickle cell trait and sickle cell disease) who presented in Department of Medicine at our institute over two years period were included in the present study. Patients with history of blood transfusions in last six months, patients with chronic infections, those with compound heterozygote states or coexisting thalassemia, patients with organ failures like congestive cardiac failure, chronic renal failure and patients taking medicines which modify HbF levels like hydroxyurea were excluded from the study. Hospital ethical committee approval was taken. A detailed demographic and clinical history was recorded. Special emphasis was given to history of vaso-occlusive episodes (VOC) which included age at which symptoms started, frequency, site, and episodes requiring hospitalization. In present study, VOC was defined as the occurrence of pain in the extremities, back, abdomen, chest or head that lasted for at least 2 hours, led to a clinic visit, and could not be explained

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except by sickle cell disease. The number of vocs >3 per year requiring hospitalization was taken as a measure of clinical severity in present study. Detailed history was also collected about blood transfusions. Complete blood count, peripheral smear, sickling test, hemoglobin electrophoresis, serum bilirubin levels were carried out in all patients. On the basis of hemoglobin electrophoresis, the patients were categorized into sickle cell trait (AS) and sickle cell disease (SS) groups. Quantitative estimation of HbF was done in each patient by alkali denaturation test as described by Singer[5]. Normal levels of HbF were taken as 1%[6]. Other investigations were done according to their clinical presentation. Statistical analysis was performed using Stata Software version 10.0.

Results:

Of 238 patients of sickle cell disorder enrolled in this study, 100 patients had sickle cell disease and 138 had sickle cell trait. Demographic data of these patients is given in Table1. The mean age of presentation was higher in AS patients (24.89± 7.1 years) as compared to SS patients (22.22±7.9 years). Maximum prevalence of sickle cell disease was found in Mahar caste (66.39%) followed by Kunbi and Teli caste. Total 87.39% patients presented with joint pains (81.88% in AS group and 95% in SS group).

Details of patients' symptoms are shown in figure 1. Complications of sickle cell disease are noted in 2.94% patients that included hemiplegia, avascular necrosis of femur and chronic leg ulcer. About 11.34% (17.39% in AS group and 3% in SS group) patients were asymptomatic for this condition and were incidentally diagnosed. Only 28.57% patients had single symptom (more common: joint pain), 27.52% (23.17% of AS group and 34% of SS group) had 2 symptoms and 26.04% (23.17% of AS group and 34% of SS group) had 3 symptoms at presentation. Typical hemolytic facies was seen in nearly one-third of patients while hepato-splenomegaly was present only in one-fifth of patients. Positive musculoskeletal findings like local tenderness, swelling and restricted mobility of joint was observed in 42.01%, 3.36% and 18.9% patients respectively.

Details of blood counts and HbF levels are given in Table 2 and Table 3 respectively. Nearly 70% patients of AS group had HbF levels less than 1% while only 3.6% had levels more than 10%. However, in SS group, 13% had HbF levels less than 1% while nearly half of them had levels more than 10%. Relationship between number of VOC and HbF levels is shown in scatter diagram (Figure 2a and 2b). In AS group, when HbF levels were studied in relation to number of VOC per year i.e. Up to 3 per year and >3 per year, the difference is found to be significant. [Pearson Chi2 (2) = 9.2646; p=0.010, Significant] However, the correlation between the two was weak (r=0.1152, p>0.05). Similar correlation in SS group was also found to be significant. [Pearson Chi2 (2) = 11.79771; p=0.003, Significant]. In this group, on application of correlation coefficient, a strong negative correlation was found between HbF levels and VOC (r = -0.4179, p<0.001, highly significant).

The mean HbF levels in patients who presented with complications i.e. Hemiplegia, avascular femoral necrosis and chronic leg ulcer were 0.33%, 2.0% and 4.8% respectively.

Discussion:

The role of HbF in sickle cell disease was first studied by Watson while studying infants with sickle cell disease[7]. She observed that their deoxygenated erythrocytes took long time to sickle compared to their sickle cell trait mother's cells. She attributed these observations to high HbF levels in infant blood. Since then the various studies have confirmed the protective role of HbF in sickle cell disease. It is due to the effect of HbF to hinder polymerization of sickle haemoglobin. Studies have shown that high HbF levels are related to reduced disease severity and mortality[8,9]. Also, HbF can ameliorate some complications of sickle cell disease like acute painful episodes, acute chest syndromes, leg ulcers, osteonecrosis. However, HbF levels had a weak or no clear association with complications like priapism, stroke and cerebral infarction, hypertension, and sickle vasculopathy[1]. The failure of HbF to uniformly modify all complications of sickle cell disease might be related to the pathophysiologic events that impact the likelihood of developing these complications. The complications due to vaso-occlusion and blood viscosity were strongly related to HbF concentration whereas complications associated with the hemolysis were less affected[1].

HbF levels are variable among patients with sickle cell disease and are highly heritable[10,11]. Studies from Saudi Arabia show that sickle cell disease patients with Arab-India haplotype had higher mean HbF levels [average 17%, range 4-32%][12] than those with African origin[13]. TheHBS genes which are indigenous to the Middle East and India are associated with high HbF levels, and carriers of these haplotypes can have milder disease[14, 15, 16]. The present study is one of the few studies from India that has studied HbF levels in sickle cell patients. The mean HbF level was 1.88±3.77% in our AS patients and 9.22±6.12% was in SS patients. We observed higher HbF levels in AS patients than HbF in normal population (normal levels <1%). Since in this study, combined sickle cell-beta thalassemic patients were not included, high HbF levels due to associated thalassemia was ruled out. Brittenham reported mean HbF of 20% (range 8-36%) amongst 15 sickle cell disease patients in South India[17] while Kar found mean HbF of 16.44±5.42% amongst 131 patients of Orissa[3]. However, Sharma reported mean HbF levels of 5.3% in 64 patients of sickle cell disease in Bhilai, Madhya Pradesh[2].

In the present study, when the number of vaso-occlusive crises per year was studied in relation to HbF levels in SS patients, a significant negative correlation was found between the two. 42% SS patients who had vocs less than 3 per year, had HbF > 5%. These patients were also found to have higher haemoglobin levels, less number of reticulocytes, less number of peripheral smears suggestive of hemolysis and also required less hospitalisations than patients having HbF levels <5%. This suggests that SS patients with elevated HbF levels tend to have more benign course. The various other studies have also corroborated this finding.

In present study, nearly one-fourth of SS patients who have HbF levels more than 5%, presented with more than 3 vocs per year. Reasons for such varied findings are unclear as genetic studies were not done. However, we think that environmental and socio-economic factor play an important role in final presentation in 'borderline cases.' The environmental factors like hot climate, occupation of hard labour, endemicity of malaria and socioeconomic factors like poor economic strata which is responsible for delay in hospital care, delay in management of infections and lack of periodic health check-up could modify the course of disease.

The present study is important as it provides the insight

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not only in pattern of presentation of patient with sickle cell disorders but also their HbF levels in central India (Vidarbha and Madhya Pradesh). It also correlates HbF levels with disease severity (measured in terms of number of vaso-occlusive crises). In addition, it underlines the need to use the pharmacological therapy (e.g. Hydroxyurea) to increase HbF levels in symptomatic sickle cell disease patients. However, all these patients were tested only once for HbF levels, so it is unclear, whether the haematological indices measured represent the steady state for the individuals. Also, we have not studied the genetic patterns of the disease.

To conclude, the mean HbF levels were high in both AS and SS patients. However, HbF levels were significantly higher in SS patients than in AS patients. Also, there was a strong negative correlation between number of vaso-occlusive crises and HbF levels. Thus, the pharmacological manipulations to increase the HbF levels will definitely benefit these patients.

Legends for Illustrations:
Table 1: Demographic Characteristics of patients
Table 2: Hematological parameters
Table 3: Hemoglobin F levels
Figure 1: Details of patients' symptoms
Figure 2a & 2b: Scatter diagram showing relationship between number of VOC and HbF levels

Table 1: Demographic Characteristics

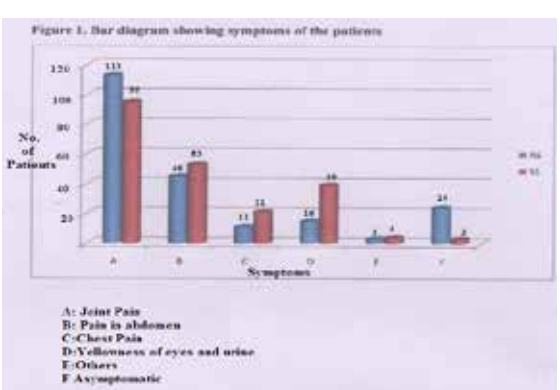
Parameter	AS		SS	
	Male	Female	Male	Female
Age (in years)	24.30± 6.98	25.50± 7.34	22.74± 9.23	21.61± 6.24
	Mean: 24.89±7.16		Mean: 22.22±7.97	
Gender (in %)	29.83	28.16	22.69	19.32
Caste (in %)				
Mahar	16.80	18.90	17.22	13.44
Kunbi/ Maratha	4.20	2.94	2.52	3.37
Teli	2.52	1.69	0.84	0.84
Gond	1.26	1.26	0.42	0.42
Muslim	1.69	0.84	0.42	0
Others	3.37	2.52	1.26	1.26

Table 2: Hematological parameters

Parameter	AS		SS		Comments
	Male	Female	Male	Female	
Hemoglobin (gm%) Mean+SD	8.91± 1.94	7.98± 1.47	7.51± 1.6	6.93± 1.57	Hb AS *vs.SS; p=0.00001* Hb AS(M) *vs.AS(F); p=0.0019* Hb SS (M) *vs.SS(F); p=0.0715*
Reticulocute count (%)	3.49±2.48		5.68±3.66		AS vs SS ; p=0.00001
WBC count (/mm^3)	7145.87±2265.43		7333.24±2154.12		--
Platelet Count (lacs/mm^3)	2.013±0.436		1.924±0.599		--
Peripheral smear (% of patients)					
Hemolytic	39.49		38.23		--
Normochromic- Normocytic	15.97		2.94		--

Table 3: Hemoglobin F levels

	AS		SS		Comments
	Male	Female	Male	Female	
HbF levels (%)	2.07±3.67 (Mean ± SD) Range 0-15.1	1.68±2.79 (Mean ± SD) Range 0-15.3	9.76±6.12 (Mean ± SD) Range 0-25.4	8.58±6.13 (Mean ± SD) Range: 0-18.5	For HbF: AS(M) *vs.AS(F): p=0.4853* For HbF: SS(M) *vs.SS(F); p=0.3393*
Mean HbF (%)	1.88±3.27 Range: 0-15.3		9.22±6.12 Range: 0-25.4		HbF levels AS vs. SS: p=0.00001 (S)



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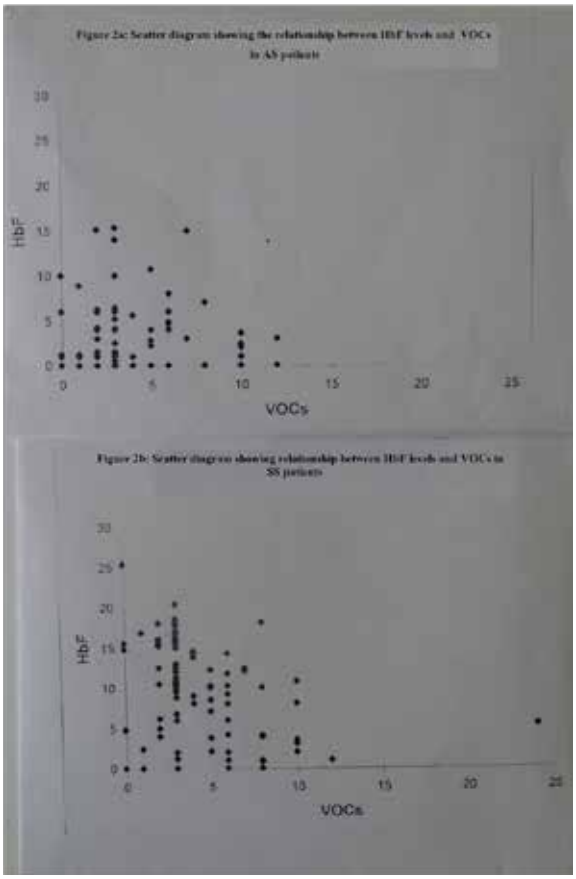

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