Original Article

Sickle Cell Disease in Central India

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Abstract. ***Objective :*** The incidence and the risk factors of sickle cell disease (SCD), vaccinated with Pneumococcal vaccine and on penicillin prophylaxis has not been previously reported in India. ***Methods :*** This prospective hospital based study followed 325 children on penicillin prophylaxis, of which 161 were vaccinated for pneumococci, over 146.84 person years to determine the incidence and determinants of crisis (SCC) and infections. The average age at presentation was 7.05 ± 3.26 years with male preponderance below 2 years. ***Results :*** The main causes for hospitalizations were for blood transfusion, SCC and infections. The incidence of SCC was 1.25 per patient per year and that of infection was 1.38 per person per year. The risk factors for SCC were Mahar caste (p = 0.007) non-compliance (p = 0.000) and protein energy malnutrition (PEM) (p = 0.0015) and for infection were also PEM (p = 0.023), Mahar caste (p = 0.021) and noncompliance (p = 0.001). ***Conclusion:*** Malnutrition and non-compliance with medication increased the patient's susceptibility to SCC and infections.
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Key words : *Penicillin prophylaxis; Protein energy malnutrition; Sickle cell anemia; Sickle cell crisis.*

Sickle cell disease (SCD) is highly prevalent in certain tribal and ethnic groups in India.[1,2] The clinical course of these patients is punctuated by episodes of "crisis" (SCC) and increased susceptibility to serious infections because of functional asplenia.[3,4,5] The clinical and hematological manifestations of patients with SCD vary according to the haplotypes associated with the β^s mutation in the different geo-ethnic groups.[6] The Asian/Indian haplotype is associated with higher Hb F levels and milder course than the three African haplotypes.[7] Within the same region also different patient characteristics may influence the clinical course of the illness.[8] We conducted a study to determine the incidence and the determinants of sickle cell crisis and infections in patients from Central India attending the sickle cell clinic in our hospital.

MATERIALS AND METHODS

This was a prospective study conducted in sickle cell patients attending two tertiary care hospitals from October 1996 to October 1999. The patients were between 6 months to 12 years who had HbSS pattern of hemoglobin on cellulose acetate electrophoresis and were completely immunized as per the national immunization program in India, which did not include *H. Influenzae*. All the children were on penicillin prophylaxis and some (N=163) had received pneumococcal vaccine. Since we wanted to assess the clinical course of only sickle cell anemia patients in this region, any child with co-morbidity such as a preexisting chronic illness other than SCD or major congenital anomaly was not included in the study. The purpose of the study was explained and consent for participation in the trial was obtained.

Baseline Assessment

The baseline data was collected as soon as the patients were diagnosed as homozygous sickle cell anemia (SCA). The following baseline data recorded was: age, gender, caste, education of the mother, annual per capita income, area of residence (urban or rural), the nutritional status, size of the spleen and their hemoglobin percentage. In children under two years of age the length was measured on an infantometer in the supine position. Patients whose weight for height was less than 2 standard deviations from normal were characterized as malnourished (PEM). All the information was recorded on a pre-designed and pre-tested form.

Follow-up Data Collection

All patients (N=325) were put on penicillin prophylaxis. Polyvalent Pneumococcal vaccination was administered to 163 patients. The patients were followed up for the type of crisis, time from onset of symptoms to reporting to clinic, the location of pain if in vaso-occlusive crisis (VOC), joint swellings if any, the hemoglobin percentage and clinical pallor during the crisis, presence of fever, the episodes of infection, the compliance with penicillin prophylaxis, any adverse reactions, and for mortality. For patients who needed hospitalization, information was recorded from the hospital records.

Measurement of Outcome Variables

Vaso-occlusive crisis was diagnosed clinically if patient

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presented with painful swelling of the hands and/or foot mostly accompanied by warmth, redness and fever or with acute abdominal pain or as "acute chest syndrome" diagnosed by a rapid respiratory rate, by chest radiology and by worsening of anemia or as stroke diagnosed by a neurological deficit. "Hyperhemolytic crisis" was diagnosed by worsening of anemia, unconjugated hyperbilirubinemia and reticulocytosis. "Acute splenic sequestration crisis" was diagnosed by worsening of anemia, splenomegaly and cardiovascular collapse. "Aplastic crisis" was diagnosed by a blood count showing a fall in hemoglobin, hematocrit levels and reticulocylopenia. Severe infections were diagnosed clinically if children presented with pneumonia as per WHO criteria or radiological evidence of pneumonia, fever more than 2 days with lethargy and difficulty in feeding or convulsions. Blood samples and appropriate tissue or body fluid analysis was done to identify the organisms and its serotypes. Compliance was assessed by pill count and a urine examination for penicillin levels by the Micrococcus lutea inhibition technique.[9] If the patient missed more than 2 days of the oral medicine on more than 2 occasions (detected on pill count and urine examination) or did not follow up for the consecutive injectable penicillin dose for more than 4 weeks on 2 occasions then he was defined as non-compliant. Drug adverse reactions were characterized as none, mild or severe. Drug reactions with local tenderness, pain or mild gastro-intestinal symptoms were characterized as mild and those associated with systemic symptoms or generalized skin allergies were characterized as severe.

The annual per person incidence of crisis and infections was calculated. Factors, which tended to increase the probability of crisis or infection on univariate analysis, were then analyzed using Poisson regression.

RESULTS

The authors studied 325 patients of SCD were studied over a cumulative 146.84 person a year. The baseline characteristics of the patients are shown in Table 1. The average age of diagnosis at the clinic was 7.05 ± 3.26 years. There was a male preponderance in children below 2 years of age (overall sex ratio = 0.72). SCD was found to be most common in the Mahar caste (38.94%). The overall prevalence of PEM was 40.6%, which decreased with age. The spleen was palpable in all the patients at diagnosis and ranged from 1 to 7 cm below subcostal margin. The mean hemoglobin increased with age and was 8.70 ± 0.76 gm %, 8.92 ± 1.09 gm %, and 9.26 ± 1.06 gm % respectively in children below 2 years, 2-5 years and above 5 years. None of the children had severe anemia, mild anemia was present in 45.80% and moderate in 54.20% of the patients.

During follow-up the incidence of SCC was 1.25 per person per year and vaso-occlusive crisis (VOC) was the most common (Table 2). The SCC were equally distributed between the genders but older children had a higher prevalence of SCC as compared to the younger children (70.27% in >5years, 20.5% in 2-5 years and 7.02% in < 2 years). The mean duration between onset of symptoms of SCC and the patient's visit to the clinic was 2.03 ± 1.15 days.

VOC presenting as abdominal pain was the commonest manifestation of SCC and was observed in 111(65.29%) of children with VOC, in 3 children of sequestration crisis and in 1 with hyper hemolytic crisis. VOC also presented as general body ache (1.76%), chest pain (26.47%), or pain in hand, feet or joints (38.23%). Joint swellings were observed in 8 (4.71%) children with VOC. Nutritional deficiency was present in half the patients with crisis and significantly increased the number of episodes of crisis in these patients ($p = 0.0015$ from the Poisson regression). Similarly other predictors of crisis were the Mahar caste ($p=0.007$), and non-compliance ($p = 0.000$).

Fever was observed in 63% and coexisting infection was seen in 12 % patients with SCC. The incidence of infection was 1.38 per person per year and the most common infection was upper respiratory infection. There were 5 cases of pneumonia, 2 of osteomyelitis, 2 of enteric fever, 2 of septicemia, 4 of urinary tract infection, 3 of hepatitis and 30 cases of viral fever. No organisms were grown in the blood culture in any of these episodes. The predictors of infection on Poisson regression were also PEM ($p=0.023$), Mahar caste ($p=0.021$) and non compliance ($p=0.001$).

Non-compliance with medications was seen in 75.13 % patients of SCC. Adverse drug reactions were observed in 12.62 % of all patients in form of transient pain and tenderness at the injection site. Skin rash was seen in 2.76% but an anaphylactic reaction was not observed in any patient.

The incidence of hospitalization was 0.44 per person per year. The most common cause of hospitalization was blood transfusion (21.31%), followed by SCC (13.44%) and severe infection (3.44%). Four patients died during the follow-up period: three patients due to acute splenic sequestration crisis and one due to sepsis. (Table 3). Three of these had PEM and all were females who were non-compliant with penicillin prophylaxis.

DISCUSSION

The clinical manifestation and severity in sickle cell anemia are dependent not only on the haplotype of that region but are also influenced by other patient characteristic.[6] The authors have found several interesting features in this study population, such as older age of presentation, absence of severe anemia, male preponderance, persistence of palpable spleen, presence of PEM, and an alarming percentage of non-compliance with medication. Each of these deserve to be discussed. The main predictors of crisis or infection were the

Table 1. Baseline Characteristics of the Population

Variable		Age ≤ 2 years N = 31 (9.54)	Age 2 yr – 5 yr N = 70 (21.54)	Age ≥ 5 yr N = 224 (68.92)
Sex	1. Male	15 (48.38)	40 (58.57)	132 (58.92)
	2. Female	16 (51.62)	29 (41.43)	92 (41.08)
Caste		N = 18	N = 55	N = 171
	1. Mahar	7 (38.88)	27 (49.09)	91 (53.21)
	2. Teli	3 (16.66)	11 (20.0)	36 (21.05)
	3. Neo-Buddhist	6 (33.33)	11 (20.0)	30 (17.54)
	4. Sutar	1 (5.55)	2 (3.6)	12 (7.01)
	5. Kunbhi	0	1 (1.8)	1 (0.5)
	6. Others	1 (5.55)	3 (5.4)	1 (0.5)
PEM		N = 31	N = 69	N = 220
	Present	15 (48.38)	24 (34.78)	91 (41.36)
	Absent	16 (51.62)	45 (65.21)	129 (58.36)
Residence			N = 68	N = 220
	Rural	10 (32.25)	29 (42.64)	70 (31.38)
	Urban	21 (67.75)	39 (57.36)	150 (68.19)
Income		Rs. 4827.87 ± 641.50	Rs. 4509.41 ± 538.14	Rs. 4204.19 ± 178.46
Previous child health		N = 31	N = 69	N = 220
	No	18 (58.06)	53 (76.81)	143 (65.00)
	Yes	13 (41.93)	16 (23.17)	77 (35.00)
Mothers Education		N = 31	N = 69	N = 220
Iv standard or less		12 (38.70)	29 (42.02)	95 (43.17)
V to X standard		17 (54.83)	34 (49.26)	111 (50.44)
College or degree		2 (11.10)	6 (9.6)	14 (6.35)
Size of spleen*		1.48 ± 0.56 cm	1.67 ± 1.13 cm	1.79 ± 1.06 cm
HB		8.70 ± 0.76 gm %	8.92 ± 1.09 gm %	9.26 ± 1.06 gm %

* Palpable below sub-costal margin
•Figures in the parenthesis are percentage

Table 2. Clinical Features of Sickle Cell Crisis

Variable	Vaso-occlusive crisis	Acute splenic Sequestration crisis	Hyperhemolytic crisis	Aplastic
No. of episodes	170	4	6	5
Age < 2 years	13 (7.64)	0	0	0
Age 2-5 years	36 (21.17)	0	2 (33.33)	0
Age > 5 years	121 (71.17)	4 (100)	4 (66.66)	5 (100)
Sex				
1. Male	88 (51.76)	2 (50)	3 (50)	3 (60)
2. Female	82 (48.24)	2 (50)	3 (50)	2 (40)
PEM Yes	87 (51.18)	2 (50)	4 (66.66)	5 (100)
No				
Compliant	43 (25.29)	0 (0)	1 (16.66)	2 (40)
Non-compliant	127 (74.21)	4 (100)	5 (83.33)	3 (60)
Time from onset of Symptoms to reporting to clinic	2.41 ± 1.56 days	1.75 ± 0.5 days	1.33 ± 1.03 days	2.6 ± 1.51 days
HB%	8.69 ± 1.32 gm %	5.9 ± 1.96 gm %	5.8 ± 1.93 gm %	5 ± 1.48 gm%
Site of pain				
1. No pain	5 (2.94)	0	0	0
2. Chest	45 (26.47)	0	1	0
3. Hand, feet or joints	65 (38.23)	0	0	0
4. Abdomen	111 (65.29)	3 (75)	1	0
5. General body ache	3 (1.76)	0	0	0
Fever	106 (62.35)	4 (100)	4 (66.66)	3 (60)
Pallor	72 (42.35)	4 (100)	5 (83.33)	5 (100)
Decreased activity	44 (31.77)	3 (75)	4 (66.66)	3 (60)
Joint swelling	8 (4.71)	0	0	0
Co-existing infection	18 (10.58)	1 (25)	1 (16.66)	2 (40)

•Figures in the parenthesis are percentage

presence of PEM, the Mahar community and non-compliance with medication. Because the episodes of pneumonia were small it precluded a valid assessment of the protective effect of the vaccination.

The children in this study population were predominantly from the Mahar community, which reflected the distribution of this disease in the community.[2] Their average age at diagnosis was 7.05 years and none of the children had severe anemia. This differs from the early and more severe presentation in African patients[10,11] who have lower levels of fetal hemoglobin as compared to the Asian haplotypes.[12] The mean hemoglobin levels were higher in older children as the younger ones may have coexisting iron deficiency.[13] The preponderance of boys in the age group of more than 2 years could be either due to a cultural bias reflecting a favorable parental attention towards boys or due to greater severity of disease in boys. A study in Nigeria has reported decreasing severity of the disease in adult females as compared to adult males'.[14] This could be due to higher fetal hemoglobin levels in females[15] caused by X-linked dominant inheritance of the gene coding for Hb F.[16] The other interesting feature in these children was the failure of the spleen size to regress with age, which can be attributed to coexisting malarial infection in endemic regions.[17]

VOC was the commonest form of crisis and manifested with similar frequencies in either gender. As expected children with crisis had lower hemoglobin but clinical pallor was detected in only 42% which undermined the reliability to clinical estimation of anemia in these children. Abdominal pain as a manifestation of crisis predominated in this study population, unlike the traditional belief of hand foot syndrome as the commonest manifestation of SCC.[18] Only a fraction (4.7%) of patients with pain in joints (38.23%) had joint swellings. Fever was also frequently present in these patients without clinical or laboratory evidence of infection. This has often been reported in the literature and can be attributed to the release of interleukin-1 and tumor necrosis factor during crisis.[19,20] This fever usually subsides with the crisis and antimicrobials should be used with caution as development of antimicrobial resistance can disadvantage an already immunocompromised patient.

The prevalence of nutritional deficiency in these children was also considerably higher (40.6%) than in the general population (15-20% by National Family Health Survey Data 1998-99). Nutritional intake is compromised in any chronic illness; however recent studies have also reported that basal metabolic rate (BMR) is higher in children with sickle cell disease as compared to healthy children.[21,22] PEM can enhance the susceptibility to infections and infections are known to precipitate crisis.[23,24,25] However, in our patients this mechanism played a trivial role as coexisting infection was observed only in 22 PEM patients with crisis. Therefore other mechanisms, which may increase susceptibility to crisis, were explored. During VOC progressive obstruction of the microcirculation by sickle cells involves selective sequestration of the dense cells formed due to activation of the Gardos channel resulting in leakage of potassium.[26] The Gardos channel is responsible for CDND-induced dense sickle cell formation.[27] *In vitro* studies have demonstrated the importance of the anti-oxidant Glutathione in preventing activation of the Gardos channel and subsequent formation of dense erythrocyte.[28] Red cell Glutathione levels are significantly lower in malnourished children as compared to healthy controls.[29,30] Hence we hypothesize that the greater frequency of SCC in malnourished patients is perhaps due to reduced RBC Glutathione and subsequent increased susceptibility of the Gardos channel to oxidant or free radical injury. This hypothesis needed to be studied.

Patients who were compliant with their medications were less likely to experience SCC or infection. Studies on penicillin compliance have shown similar results of poor adherence to daily oral penicillin.[31] All patients in sequestration crisis were non-compliant and three out of

Table 3. Clinical Features of Patients Who Died During Follow-up

Variable	Patient 1	Patient 2	Patient 3	Patient 4
Age	6.58 years	3.91 years	11 years	2 years
Sex	F	F	F	F
Compliance	Non-compliant	Non-compliant	Compliant	Non-compliant
PEM	Present	Absent	Absent	Present
Time delay in presentation	2 days	1 day	10 days	5 days
Fever	Present	Present	Present	Present
Pain	Acute abdomen	Chest, hand, feet and joints, abdomen	Chest	No pain
Activity	Decreased	Decreased	Decreased	Decreased
Pallor	Present	Present	Present	Present
HB%	8.2 gm%	3 gm%	4.6 gm%	3 gm%
Crisis	Acute splenic sequestration crsis	Hyperhemolytic and VOC	Acute splenic sequestration crisis	Acute splenic sequestration crisis
Infection	Septicemia	None	None	None

the four deaths observed in this study were due to sequestration crisis. Also, the mean time between start of symptoms and patient contacting the health care provider was 2.5 days. This long delay could be fatal.

In conclusion, although the Asian sickle cell patients have a less clinically severe disease, the prevalence of malnutrition and non-compliance to medications increased their susceptibility to infection and crisis. This study suggests that improved nutrition and efforts on the part of the health care provider in educating and motivating the patients to increase compliance would further reduce morbidity and mortality.

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