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Research Article

Study of sickle cell anaemia with clinical and hematological correlation

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ABSTRACT

Background: Sickle cell anaemia is a hemoglobinopathy with the production of abnormal hemoglobin, HbS which when exposed to hypoxemia produces sickling of RBC. Severity of the disease depends on the percentage of HbS. Rise in the levels of Hb F is associated with less intense clinical course. Drugs targeted at increasing levels of HbF have prognostic significance. The aim of the study is to analyse the age, sex distribution, clinical, hematological and electrophoretic appearances and to compare with other studies.
Methods: 300 sickle cell positive patients, both male and females who attended OPD were studied with all the clinical findings and hematological parameters. 52 cases were subjected to electrophoresis.
Results: Males were 154 and females were 146. Degree of anaemia was severe in females when compared to males. Of 52 cases subjected for electrophoresis 36 were homozygous, 15 were heterozygous and 1 case Sickle thalassemia. 7 cases showed HbF above 5%.
Conclusions: Most of the patients were under 40 yrs suggesting decreased survival after that age. An increased level of HbF was associated with better prognosis suggesting the need to target at drugs which increase HbF.

Keywords: Sickle cell anaemia, Haematological parameters, Hb electrophoresis, HbF

INTRODUCTION

Sickle cell anaemia is a hereditary haemolytic disease, where there is production of abnormal haemoglobin Hb S, caused by the substitution of Valine for Glutamic acid in 6th position of β-globin chain (β6Glu ->Val).[1] When exposed to hypoxemia the abnormal Hemoglobin undergoes polymerization, to form needle like structures that give sickled RBC, which is responsible for the pathogenesis.[2] The highest prevalence of HbS is in tropical Africa and among blacks in countries that participated in the slave trade with a lower frequency in the Mediterranean basin, Saudi Arabia, and parts of India.[3] Red cells containing HbS acquire the sickle-shape deformity upon deoxygenation because of the intracellular polymerization of Haemoglobin. Both Haemoglobin polymerization and membrane injury contribute to the pathophysiology of the sickling syndromes and haemolysis. Painful crisis and acute chest syndrome are the most common sickle cell related events in homozygous sickle cell anaemia (ss), HbSC disease and SβThalassemia patients.[4] Two well-known genetic modifiers of disease severity are concomitant α, β-thalassemia and high red cell HbF content. Although no definite cure is available for Sickle cell disease it can be managed and prevented by family counselling .Drugs targeted at increasing levels of HbF have prognostic significance. In the present study hemoglobin of the patients suffering from sickle cell anaemia was analyzed by electrophoresis and the results were correlated with the clinical picture.

Aims and objectives

To study the age and sex distribution of sickle cell anaemia, to correlate with clinical findings and to compare the clinical, electrophoretic and haematological parameters with other studies.

METHODS

Total of 300 sickle cell positive patients from various departments of KGH were studied and analyzed for a period of 2yrs in the department of pathology, Andhra Medical College, Visakhapatnam. Out of these 52 were subjected to electrophoresis.

Patients were of all age groups ranging from 1-75 yrs. Clinical history, physical findings, duration of the complaints, history of transfusions and past history was recorded. Family history and history of consanguinity was taken. Patients were examined for jaundice, stunted growth, hepatomegaly, splenomegaly and other relevant features. Hemogram with various parameters and Sickle cell test was done in our department. Peripheral smears were prepared and stained routinely with Leishman's stain and studied under oil immersion. Smears were also stained with supravital stains for Reticulocytes. Sickling test was done by adding 2% Sodium metabisulphite and smears were examined for sickle cells after 12 hrs, 24 hrs and 48 hrs. Total of 52 cases were subjected to electrophoresis. Initially Cellulose Acetate Electrophoresis at Alkaline pH 8.6 was done which separates the major Hb variants S, D, G, C, and E from Hb A. This was followed by Citrate Agar Electrophoresis at pH 6.0. Citrate agar electrophoresis at an acidic pH provides ready separation of hemoglobin that migrates together on cellulose acetate: S from D and G, and C from E and O (Figure 1).[5]

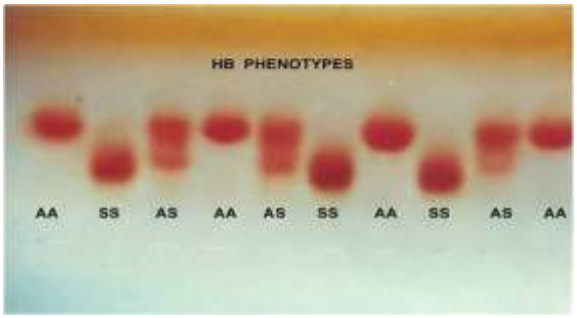

Figure 1: HB electrophoresis.

RESULTS

Out of the 300 cases studied, 154 were males (51.33%) of whom 97 were adults and 57 were children.146 were females (48.67%) of whom 109 were adults and 37 were children. Youngest age at presentation was 1yr 3months, oldest age was 75Yrs. Investigations of the patients revealed no hematological abnormalities in 18 cases. Out of the remaining 282 cases mild anaemia (9-11gms/dl) was observed in 74 cases (24.6%). Moderate anaemia(6-9gms/dl) in 118 cases(39.4%) and severe anaemia (≤6gms/dl) was observed in 90 cases (30%).Mean haemoglobin was 7.8gms/dl with standard deviation of 2.5. (Table- 1 showing cases with various degrees of anaemia) Packed cell volume ranged from 12%-54%.Mean value of PCV (Hct) was33.8% and standard deviation was 6.5. Mean corpuscular value (MCV) ranged from 65fl-82fl. with one case showing MCV of 102fl which showed megaloblastic bone marrow picture (Figure-4). Mean value of MCV was 77.4fl and standard deviation was 7.8. MCH ranged from23-29pg.Mean MCV was 24.4 pg. with standard deviation 2.5. MCHC ranged from 25-34 gms% with Mean MCHC 28.2gms% and standard deviation 2.4. RBC count ranged from 1.5 millions / cu.mm to 4.8 millions /cu.mm. Mean count of RBC was 3.1 mill/cu mm with standard deviation 0.8. Reticulocyte count was normal in 203 cases & increase in 97 cases. Leucocyte count ranged from 2,500-18,000 with mean value of leucocyte count 7,400/cu.mm and standard deviation 200. Peripheral smear showed normocytic hypochromic anaemia in 75 cases microcytic hypochromic anaemia in 125 cases, dimorphic anaemia in 30 cases, features of haemolytic anaemia with presence of sickle cells (Figure 3) in 52 cases and normal study in 18 cases. Of these one case showed Plasmodium Vivax infection (Figure 5).

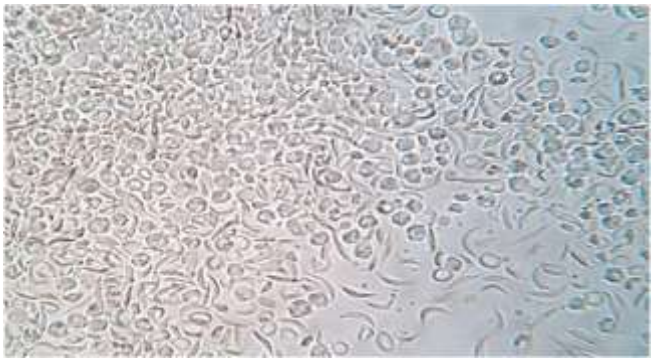

Figure 2: Sickle preparation smear.

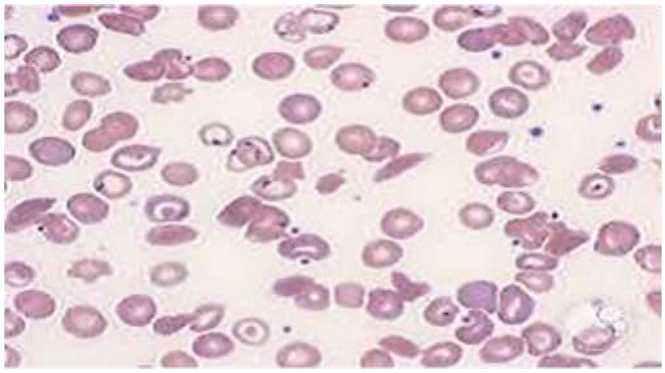

Figure 3: Smear showing marked anisopoikilocytosis with sickled RBC.

Table 1: Age, sex distribution and degree of anaemia.

Grading	Sex	1-10yrs	11-20yrs	21-30	31-40	41-50	51-60	61-70	71-80	Total
Mild	M	3	8	11	3	4	3	0	1	33
	F	3	8	20	6	2	2	0	0	41
Moderate	M	23	14	16	4	2	3	1	1	64
	F	4	19	21	5	1	3	1	0	54
Severe	M	14	11	8	5	3	1	0	O	42
	F	12	10	15	3	6	1	1	0	48
Normal	M	0	1	5	5	3	0	0	0	14
	F	0	1	0	1	0	2	0	0	4
Total		59	72	96	32	21	15	3	2	300

Table 2: Pattern of Hb electrophoresis in Sickle cell disease (SS).

Age group (Yrs)	No of cases	HbA≤50%	HbS≥80%	HbA2 1-1.5%	HbF≥ 5%
1-10	10	10	10	10	4
11-20	14	14	14	14	3
21-30	6	6	6	6	0
31-40	3	3	3	3	0
Total	33	33	33	33	7

Table 3: Pattern of Hb electrophoresis in Sickle cell trait HbAS.

Age group	No of cases	HbA ≥50%	HbS	HbA2 ≥3.5%	HbF
1-10	5	≥50%	30-35%	0	≤1%
11-20	7	≥50%	30-35%	0	≤1%
21-30	3	≥50%	30-35%	0	≤1%

Table 4: Comparative study of haematological parameters.

Studies	Hb mean gms/dl±sd		RBC /cu mm Mean ± sd		Hct% Mean ± sd		MCV fl Mean ± sd		MCH pg Mean ± sd		MCHC gms/dl Mean ± sd		WBC/cumm Mean ± sd	
Kar et al HbSS	8.7	1.7	3.3	0.8	0	0	83.6	9.4	26.6	3.6	31.5	2.7	0	0
Roy et al HbAS	11.5	1.8	4.5	0.6	33.7	5.8	73.6	9.9	25	4	33.8	1.8	8700	3000
Roy et al HbSS	8.1	1.4	3	0.6	27.1	6.3	78.6	9	27	3.7	33.1	5.8	13700	5600
Present study HbSS	7.8	2.5	3.1	0.7	27.5	5.6	72.5	8.1	23.4	2.5	27.6	2	7500	2300
Present study HbAS	9.4	1.8	3.8	0.8	33.8	6.5	77.4	7.8	26.8	3.7	30	2.3	6700	3400

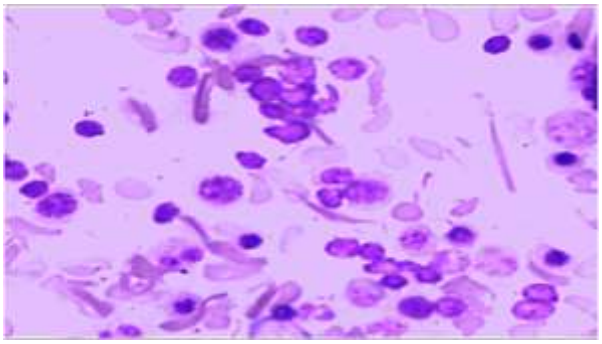

Figure 4: Aspiration smears of bone marrow showing megaloblastic anemia with sickled RBC

WBC count was normal in 180 cases, leukocytosis in 77 cases and mild leucopenia in 43 cases. Platelets were normal in 249 cases. 3 cases showed thrombocytosis and 48 cases showed thrombocytopenia. Reticulocyte count was normal in 203 cases and increased in 97 cases. Sickling test showed positivity (Figure 2) in all cases. 80%of cases showed sickling of RBC within 24 hrs. Remaining 20% cases showed positivity after 48 hrs.

Hb Electrophoresis (Figure 1) was done in 52 cases of which 45 cases were of 1-30yrs age group, the remaining 7 were above 40 yrs. out of 52 cases 36 cases were homozygous (Table 2),15 heterozygous (Table 3) and one case of Sickle β Thalassemia; HbF was increased in 29 cases and HbA2 in 17 cases. The study was compared with the studies of Kar et al and Roy et al (Table 4).

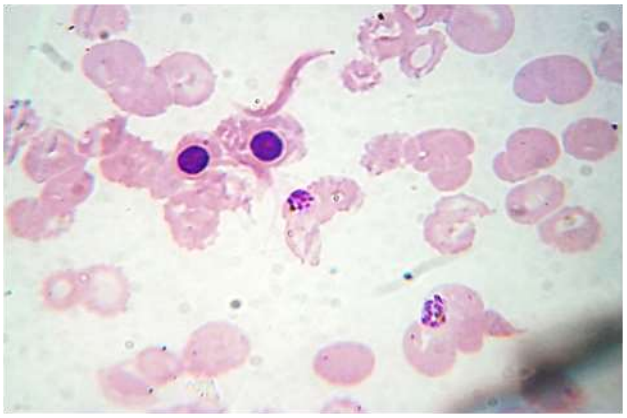

Figure 5: Case of sickle cell anemia showing *Plasmodium vivax* ring forms and Schizonts.

DISCUSSION

The sickle cell anaemia is a very common genetic disorder, 50% of the world population affected by sickle cell anaemia reside in India. High prevalence of sickle cell gene has been demonstrated in various tribal communities of Gujarat, Orissa and AndhraPradesh.[6] This study was conducted in department of pathology, Andhra Medical College, in King George Hospital, Visakhapatnam on patients who had attended various departments of KGH and found to be sickle positive on routine hemogram.

Of all the 300 cases studied 86% of the cases (258 cases) were of ≤40 yrs age where as only 14% were of 40yrs age (42 cases).The reason for recording few cases in this age group might be that most of the patients did not survive beyond this age.[7] Considering the distribution of cases in both the sexes male to female ratio is 1.08:1 showing no sex preponderance. Contrary to the studies we have registered equal distribution of the disease among both the sexes. In the age group of above 40yrs males outnumbered females, where as in the age group 20-40yrs the reverse was seen. Regarding the symptoms of the patients most frequently noted symptom was painful crisis of the bones and joints with Hand-Foot syndrome in children. This was seen in almost 2/3rdsof the patients, followed by fever, abdominal symptoms, chest symptoms, nephrological and neurological symptoms. Hand-Foot syndrome and acute anaemia episodes occur frequently in SS and $S\beta^0$Thalassemia.[8] In our study also it was observed in SS patients. The risk of painful crisis and acute chest syndrome begins in the first year of life and increases steadily.

In the adult patients who were having bone pains 2 patients had avascular necrosis of bone, one in the femoral head in a female of 56yrs, the other in the fibula of right leg in a male patient of 45yrs. Both dactylitis and painful crisis may be because of avascular necrosis of bone marrow which is due to vascular occlusion and decreased oxygen supply.[9] Studies showed significant relation between the levels of HbF and avascular necrosis of bone marrow.[10] There was no rise of Hb F in these cases. Among the cases suffering from fever 4 cases showed *Plasmodium vivax* infection and 1 case showed tuberculosis. Loss of protective function of spleen could be the reason in these cases.

Acute chest syndrome was observed in 19 cases (6.7%), of which 13 cases were recorded in the age group1-20 yrs showing almost equal preponderance. Studies of Marsenic, Couloures and Wiley's suggest that renal damage occur very early in Sickle cell disease.[11] 10 cases (3%) showed nephrological symptoms of these 4 patients had moderate proteinuria and generalized oedema. Neurological symptoms were observed in a male of 20yrs (0.3%) who presented with severe head ache, irritability and restlessness and on CT scanning he was found to be having an infarct in the cerebellar hemisphere. Transient ischaemic attacks or stroke due to cerebral infarction or haemorrhage occur in 25% of patients with Sickle cell disease. The risk of stroke is increased with lower baseline haemoglobin, low HbF level, high leucocyte count or high systolic blood pressure. Ohene-Frempong et al reported that approximately 11% Sickle cell disease patients have apparent strokes before aged 20yrs with a higher risk in the first decade of life.[12] Low level of HbF (1.8%) in the present case correlated well with the clinical picture.

Investigations revealed anaemias of various degrees in 282 patients and normal studies in 18 patients. Studies showed that patients with more severe anaemia at baseline have a greater probability of developing stroke and renal dysfunction. On the other hand, a higher haemoglobin level is associated with higher incidence of painful episodes, avascular necrosis and acute chest syndrome.[13] In our study cases which showed higher incidence of Hand-Foot syndrome and acute chest syndrome were mostly of younger age group and their haematological profile showed only mild anaemia. According to literature boys are slightly more anaemic than girls in the first decade, whereas adult men have higher haemoglobin values than women.[14] In our study severe degree of anaemia was prevalent in 20-30yrs age group with a slight female preponderance. The reason could be menstrual blood loss, blood loss due to parturition and nutritional deprivation.

HB electrophoresis was done in 52 cases out of which 36 cases were homozygous, 15 heterozygous and one case of Sickleβ Thalassemia ; HbF was increased in 29cases , of which 7 cases showed more than 5% HbF. HbA2 was increased in 17 cases with more than 3.5 % was observed in one case.

The 36 homozygous cases (Sickle cell disease) showed Hb SS 90-95% , Hb A2 1-1.5% and 5-10% of Hb F. HbF was raised in 29 cases of which 7 cases showed value of more than 5%. Those patients who showed increased levels of HbF were associated with mild clinical course. They did not show features of painful crisis or dactylitis. Jamaican studies showed that low concentrations of HbF were significantly related to the appearance of splenomegaly and a greater incidence of dactylitis and splenic sequestration with study of Baily et al showed cases associated with acute chest syndrome had low levels of HbF.[14] Haemolytic crisis, sickle cell crisis and aplastic crisis occur in the clinical course of these patients, when exposed to stimuli that aggravate the disease process. In our study haemolytic crisis was observed in 5 cases. Sickle cell crisis was observed in 8 and aplastic crisis was observed in 2 cases.

15 cases were heterozygous (Sickle cell trait-AS) showing 60-65% Hb A, 30-35% Hb S, 3-5% Hb A2 and ≤1%HbF. These patients had mild anaemia or no anaemia. Most of the patients were asymptomatic.[15] Hb S trait is a completely benign condition without clinical symptoms or hematologic abnormalities. Added risk associated with sickle cell trait is minimal, at the most, and only in very rare circumstances.[16] Slightly increased incidence of hematuria, impaired ability to concentrate urine and bacteriuria in women, have been reported.[17] No such complications were noted in our cases. Sickle cell trait confers protection to children from the lethal effects of falciparum malaria, which accounts for the major distribution of Hb S in central Africa.[18]

Out of the 52 cases studied on electrophoresis one case showed Sickle β Thalassemia. HbS was 56%, HbA was 30%, Hb A2 was 7.5% and HbF 6.5%.This patient was a male of 28 yrs who had mild anaemia and splenomegaly. Though these individuals clinically may resemble those with sickle trait (Hb AS), in S/β+-thalassemia the amount of Hb S always exceeds Hb A, while in Hb AS, Hb A always exceeds Hb S.

CONCLUSION

300 cases were studied, 154 were males (51.33%) of whom 97 adults and 57 were children.146 were females (48.67%) of whom 109 were adults and 37 were children. male to female ratio was 1.08:1. Youngest age at presentation was 1yr 3months; oldest age was 75Yrs. Common clinical presentation was hand-foot syndrome in the pediatric age group. Severe degree anaemia was observed in 20-30yr females. HB electrophoresis was done in 52 cases out of which 36 cases were homozygous, 15 heterozygous and one case of Sickleβ Thalassemia. HbF was increased in 29 cases of which 7 cases showed above 5%. Homozygous condition was observed predominantly in less than 30yrs age group. Complications were not observed in cases with Hb F more than 5%. Haemolytic crisis was observed in 5 cases. Sickle cell crisis was observed in 8 cases and aplastic crisis in 2 cases. Age and sex distribution, clinical symptoms and hematological parameters were comparable with other studies. Electrophoretic findings showed correlation of the clinical severity and complications with increased amount of HbS. Increased level of HbF was associated with less severe disease and better prognosis, suggesting the need to target at drugs which increase HbF.

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Ethical approval: The study was approved by the Institutional Ethics Committee

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