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Original Article

Pathology Section

Spectrum of Sickle Cell Diseases in Patients Diagnosed at a Tertiary Care Centre in Karnataka with Special Emphasis on their Clinicohaematological Profile

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ABSTRACT

Introduction: Sickle cell disease is a monogenic disorder with considerable clinical diversity and Sickle haemoglobin is responsible for wide spectrum of disorders which vary with respect to severity of anaemia, frequency of crises and duration of survival. As they are confused with many other clinically aggressive disorders, precision in diagnosis is essential both to proper clinical management and subsequent genetic counselling. Hence, this study was taken up in order to diagnose these conditions and administer suitable counselling measures to minimise the incidence of sickle cell disease in the future.

Aim: The aim of this study was to identify the spectrum of all Sickle cell diseases diagnosed at a tertiary care centre in Bangalore, Karnataka, India who presented over a period of five years from 2009 to 2013 and also to screen the parents and siblings of the patients for their carrier status.

Materials and Methods: We reviewed 26 cases of Sickle Cell Disease (SCD) and also 38 parents & 10 siblings of these children for their carrier status. Haemoglobin electrophoreses was performed by using alkaline gel method, followed by High Performance Liquid Chromatography when needed.

Results: A total of 26 children diagnosed with SCD were enrolled in the study. Most common entity was Sickle Cell Anaemia (SCA), followed by sickle thalassaemia and Sickle Cell Trait (SCT). Commonest clinical presentation was fever and pallor. Amongst the parents and siblings, sickle cell trait was the most common entity followed by thalassaemia trait. One interesting case of HbSE disease was encountered, which is a rare entity in India.

Conclusion: This study brings out the total spectrum of SCDs in a tertiary care centre in Karnataka, with more emphasis on screening of the parents and siblings for their carrier status.

Keywords: Sickle cell anaemia, Family screening, Electrophoresis

INTRODUCTION

Sickle Cell Anaemia (SCA) is the most common heritable haematological disease affecting the humans. Because of their prevalence and worldwide distribution, disorders resulting from sickle haemoglobin are of enormous clinical importance. Sickle Cell Disease (SCD) occurs in Homozygous form, heterozygous form and doubly heterozygous forms [1]. SCA is a disease of the blood, which is caused by an inherited HbS gene. SCA affects all age groups globally with demographic variations [2]. It is caused by a point mutation in the 6th codon of the beta globin gene leading to the substitution of glutamic acid to the valine [3,4]. In South India, sickle haemoglobin was noted in the tribal population of Nilgiri hills for the first time in 1952 [5]. According to several studies, SCD has been a significant health burden in India over the last 60 years [6]. There are very few studies from Karnataka and South India. Hence, an attempt is made to study and identify the spectrum of all SCDs in a tertiary care centre of Bangalore in Karnataka, India.

MATERIALS AND METHODS

A total of 26 children diagnosed to have SCD over a period of five years (2009-2013) from inpatient and outpatient departments of Indira Gandhi Institute of Child Health, Bangalore were studied retrospectively. Thirty eight parents and 10 siblings of these children were also studied for their carrier status. Complete blood count was done by using Mindray BC 5200 5 part differential cell counter and biochemical investigations were done by using fully automated Beckman Coulter analyser. Haemoglobin electrophoresis was done by alkaline gel method. High performance liquid chromatography (HPLC) was performed for one case in which HbA2 level was more than 10%. Blood for sickling was examined by sealed preparation of Daland and Castle by using sodium metabisulphite.

RESULTS

A total of 26 children were enrolled in this study. The age of the children ranged from 1½ to 14 years as summarised in the table below [Table/Fig-1]. Sex ratio observed was 1.9:1 in males and females respectively.

CLINICAL FEATURES: Majority of the study population presented with symptoms of generalised weakness, irregular fever, abdominal /joint pain and pallor. Out of these, 24 children had splenomegaly, in 2 children spleen was not palpable. 3 children had icterus, 5 children had dactylitis and 12 children had pain abdomen [Table/Fig-2]. However, neurological signs were not observed in any of the children.

Majority of children in our study presented with fever and pallor and only five children presented with dactylitis, which is one of the prognostic marker for the severity of the disease later in life. Splenomegaly was detected in 24 children and the spleen was not

Age	No of children
0-5yrs	08
5-10yrs	12
10-15yrs	06

[Table/Fig-1]: Age distribution of children in the study; n=3.

Clinical Features	No of children
Fever and pallor	26
Icterus	3
Dactylitis	5
Splenomegaly	24
Pain abdomen	12
Acute illness	9
Haemolytic crises	2
Aplastic crises	1

[Table/Fig-2]: Clinical features of the children in the study group.

Parameter	Lower limit	Upper limit
Haemoglobin	2.0 g/dl	10.7 g/dl
Haematocrit	6.2%	32.0%
Mean Cell Volume (MCV)	48.0 fL	115.4 fL
Mean Cell Corpuscular Haemoglobin Concentration (MCHC)	28.1 g/dL	35.0 g/dL
Reticulocyte count	0.2%	>25%
RBC count	0.72 x 10^6/uL	5.8 x 10^6/uL
Serum ferritin	<10 ng/mL	2182 ng/mL
Total WBC Count	1.0 x 10^3/uL	15.6 x 10^3/uL
Platelet Count	12 x 10^3/uL	656 x 10^3/uL
Serum Bilirubin	0.5 mg/dL	6.5 mg/dL

[Table/Fig-3]: Haematological parameters of the children in the study group.

Diagnosis	No of children	Parents	Siblings
Sickle cell anaemia	11	0	0
Sickle thalassaemia	8	0	0
Sickle cell trait	6	28	8
HbSE disease	1	0	0
Thalassaemia trait	0	8	2
Electrophoretically normal	0	1	0
HbE trait	0	1	0
Total	26	38	10

[Table/Fig-4]: Diagnosis after Haemoglobin Electrophoresis of patients, their parents and siblings

palpable in two children aged 12 years and 14 years respectively, which can be attributed to splenic fibrosis. Overall it was found that the severity of clinical manifestation was milder in those with Hb S Beta+ thalassaemia when compared with those in SCA.

LABORATORY FINDINGS: Among laboratory parameters, Haemoglobin ranged from 2.0 g/dL to 10.7 g/dL with mean Haemoglobin of 7.2 g/dL. The child with Haemoglobin of 2.0 g/dL was found to be in aplastic crisis with absence of polychromatophilia and NRBCs in the peripheral smear. Two children were found to be in haemolytic crises, with Haemoglobin of 2.8 g/dL and 3.0 g/dL respectively with polychromatophilia and increased NRBCs in the peripheral smear. Leucocytosis was observed in two of the children, which is a predictor of severity of the disease. One of the children with sickle cell trait had a low serum ferritin level which can be attributed to associated iron deficiency. It was also found that MCV and MCHC were lower in children with Hb SBeta+ thalassaemia compared to those with SCA and sickle cell trait. HbF value on electrophoresis ranged from 15.0% to 50.0% and the average HbF being 34.0%. Haematological parameters are summarised as above [Table/Fig-3].

The peripheral smear of all the children studied showed anisocytosis, polychromatophilia, with target cells observed in most of the cases and sickle cells were evident in 8 cases. Sickling test was positive in all the cases. Osmotic fragility test was decreased in all the cases. Bilirubin levels was analysed in the study population with indirect bilirubin levels being elevated in 22 children with SCD.

11 out of 26 children were diagnosed with SCA, 8 were diagnosed with sickle thalassaemia, 6 were diagnosed with sickle cell trait and one case was HbSE disease. Among the parents and siblings, 36 were found to have sickle cell trait, 10 were found to have thalassaemia trait, one parent was HbE trait and one was electrophoretically normal [Table/Fig-4].

DISCUSSION

The three common beta globin gene variants of haemoglobin HbS, HbE and Hb D Punjab are commonly seen in India, with HbS having a high prevalence in the central belt and some parts of western, eastern and southern India. HbE is prevalent in the eastern and north eastern region, whereas Hb DPunjab is mostly seen in the north western part of India. These haemoglobin variants have been reported in different population groups. Rare cases of HbSDPunjab and HbSE disease are noted in the other parts of India due to migration and intermixing of people from different geographic regions [7]. Sickle cell haemoglobinopathies involve SCA, SCT, Sickle/beta thalassaemia and HbSE. SCA and SCT are most common forms of Hb defect (HbS gene), which are inherited by children from parents [8,9]. Since, sickle cell haemoglobin is one of the common health problems globally and in India, where the gene frequency of this disease is highly prevalent, we focussed on study of spectrum of SCD in patients diagnosed at our institute with special emphasis on clinicohaematological profile of patients.

In our study, the electrophoretic pattern of one patient from west Bengal revealed HbA:0, HbF:10%, HbS:55% and HbA2:35% by alkaline gel method. The limitation of alkaline gel electrophoresis is that the HbC, HbE and HbO will merge with HbA2 band. Hence, whenever the HbA2 value exceeds 10 %, the bands of Haemoglobin have to be separated using other sophisticated technologies like HPLC and Capillary Electrophoresis. When HPLC was performed, the diagnosis was confirmed as HbSE disease with the proportions being, HbA-0, HbF-10%, HbS-55%, HbE-34% and HbA2-1%. This child had mild clinical presentation similar to the first case of HbSE disease reported from India [10].

In addition, we also tried to screen the parents and siblings of patients for their carrier status. We focussed predominantly on clinical findings and haematological parameters in addition to the age and gender of patients. Sickle cell disease is found to be more common among males which are consistent with the prevalence in Saudi Arabia [11].

Substitution of lysine for glutamic acid at position 26 of the beta chain results in HbE disease and is characterised by a mild beta thalassaemia phenotype. Patients with HbSE may have mild anaemia and microcytosis along with approximately 30% of haemoglobin E, but, blood smears look relatively normal (except for target cells) and patients are usually asymptomatic [12,13].

Clinical presentation in this study was comparable to those in Orissa [14]. On the other hand, number of patients with painful crises was less than those in the non tribal regions of Maharashtra and Gujarat [15]. Overall, clinical presentation in our study is found to be milder when compared to that in Central India. Taking into consideration, the entire spectrum of sickle cell diseases, SCT accounted for 56% of all the cases.

It was also noted that the severity of SCD in India is milder when compared to that in tropical countries. We have also correlated the severity of clinical symptoms with the level of HbF. The clinical manifestation of SCA in India seems to be milder than in Africa and Jamaica, which may be attributed to higher HbF levels in our study [16]. Mean HbF level in our study was found to be 34%. The severity of symptoms was inversely proportional to the levels of HbF. Haematological and clinical presentation of SCD are influenced by HbF levels, since polymerisation of sickle haemoglobin during

deoxygenation is inhibited by HbF. Foetal Hb seems to protect the patients from disease severity and crisis. The level of HbF in the blood circulation is very essential as they seem to protect the red blood cells from sickling and hence prevent them from blocking the blood flow, especially in small capillaries. However, some studies have shown that the patients who have higher HbF levels have very variable clinical presentation [17,18]. In fact, more than the amount of HbF or total number of F cells, the ratio of HbF/F cells is important and the severity of the disease is influenced by the proportion of F cells that have adequate HbF to inhibit the polymerisation of HbS [19].

In view of high prevalence and major public health problem, we recommend family screening of SCD patients to identify sickle cell carriers and the screening should be extended to all areas, which have the high frequency of HbS and premarital investigation should be considered as a routine investigation. The parents and the carriers can be counselled about the option of prenatal diagnosis. It is important to do newborn screening and include all the SCD cases into a comprehensive care programme which includes penicillin prophylaxis if required and pneumococcal vaccination. The parents can be educated on home care and recognising the symptoms of serious manifestation.

This study revealed the spectrum of SCD in patients attending the tertiary care centre with the added advantage of family screening which was followed up with counselling. Considering a smaller size of the sample in this study conducted at a tertiary care centre, a larger population based study is suggested in order to obtain the prevalence rate of SCD in the community.

CONCLUSION

SCA was the most common SCD in the patients followed by sickle cell thalassaemia, sickle cell trait and then HbSE disease. Sickle cell trait was the most common entity among the parents and siblings, followed by thalassaemia trait and then HbE trait. Clinical manifestations were milder in children with higher HbF levels. One case of HbSE disease was detected, which is relatively uncommon. To conclude, SCD should be suspected in a child presenting with fever, pallor and pain abdomen.

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