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Original Research Paper

Hematology

SICKLE CELL DISEASE IN BIHAR: AN EXPERIENCE OF TERTIARY HEALTH INSTITUTES.

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ABSTRACT Sickle cell disease is commonly seen in rural population of western part of India. It is one of the common causes of recurrent hospitalization, morbidity and mortality in paediatric population. As there are limited studies addressing the pattern of sickle cell disease amongst paediatric population in **Bihar**, This study was taken up to evaluate the clinic-haematological profile of paediatric population with sickle cell in tertiary health centres. **METHODS:** This was a retrospective observational study. Data were retrieved from haemato-pathology departments of tertiary care centres of Bihar. Data of children diagnosed with sickle cell disease from January 2019 to January 2021 were collected and analyzed to assess the , clinical ,haematological and HPLC profile at the time of diagnosis. **RESULTS:** 10 patients were included in the study. Clinically, Vasoocclusive crisis was the most common presentation (43.93 %) followed by generalized body ache and joint pain (36.99 %) and acute febrile illness (26.39 %), while 3 (30%) patients presented with severe anemia. Haematological finding was suggestive of moderate anemia, low Mean corpuscular volume and low Mean hemoglobin concentration. **CONCLUSION:** At the time of diagnosis vasoocclusive crisis and generalized bodyache were the most common manifestations in patients with sickle cell disease while haematological picture was suggestive of microcytic hypochromic moderate anemia. All cases were confirmed with HPLC.There was a positive correlation between age at presentation and severity of anemia at the time of diagnosis.

KEYWORDS : Sickle cell disease, anaemia, vasoocclusive crisis, HPLC

INTRODUCTION:

Sickle cell disease are inherited disorders characterised by an abnormality in the structure of haemoglobin[1]. It is the most common hemoglobinopathy, accounting for about 70% of the world's major haemoglobinopathies [2]. It comprises sickle cell anaemia and other compound heterozygous state such as haemoglobin SC disease, Sβ-thalassaemia, and SD-Punjab. About 5% of the world's populations are carriers of genes responsible for haemoglobinopathies and about 300,000 children are born annually with haemoglobin disorders. Due to the high prevalence of sickle cell trait at 20 to 30%, the frequency of sickle cell anaemia in Nigeria is about 20 per 1000 births resulting in about 150,000 babies being born each year with the disorder [3]. This makes Nigeria the country with the largest burden of sickle cell disease globally with 4.1% of the population being affected [5].The prevalence of heterozygotes in India varies from 1-40%[7,8]. Current methods used by laboratories are high performance liquid chromatography (HPLC), capillary electrophoresis, globin chain electrophoresis and DNA analysis/protein analysis [4]. Evaluation of the peripheral blood smear, as well as correlation with the results of a complete blood count (CBC) are very important as many of the clinically significant haemoglobin disorders show characteristic peripheral blood findings, and are often co-inherited. This present study evaluated the high performance liquid chromatographic patterns and red cell indices of sickle cell disease patients to determine the co-inheritance of other haemoglobin (Hb) variants and β-thalassaemia traits in SCD patients in Bihar.

Sickle Cell anemia is an autosomal recessive inheritance disease. Its clinical severity varies from the milder sickle cell trait (heterozygous) to severe sickle cell anemia (homozygous). Variation in hemoglobin occurs due to substitution of glutamic acid by valine at position six of the B chain of hemoglobin. The clinical manifestation of sickle cell disease (SCD) arises as sickle haemoglobin (HbS or α2βs) tends to polymerize at reduced oxygen tension resulting in deformation of red cell into the characteristic rigid sickle shape. Such inflexible red cells cannot pass through microcirculation efficiently, resulting in destruction of the red cell and intermittent vaso-occlusion. It causes anemia, tissue damage and pain.[10] Clinical variations such as death in earlier childhood to a normal life span with few complications. Sickle cell anemia also happens in combination with HbC, HbE or α /β – thalassaemia. Haemoglobinopathies are wide spread all over the world due to migration of peoples from place to place.[12,13]

MATERIALS AND METHODS:

Samples of clinically suspected anaemic patients (with hemoglobin < 10 gm/dl.) and age ranging between 6-20 years (average age was 10.6 years) were randomly collected from the different area of Bihar. Retrospective study of 10 confirmed cases of sickle cell disease was done at Departments of Pathology of Government Medical Colleges of Bihar and Gardiner Superspeciality Hospital, Patna. The samples were collected in EDTA vials and subjected to complete blood count in full automatic haematology analyser and also peripheral blood smears (PBS) were made. Only the clinically suspected cases with low MCV and MCH were further screened by HB HPLC for estimation of various hemoglobin variants. The haematological parameters such as estimation of hemoglobin, MCV, MCH, Mean Corpuscular Hemoglobin Concentration (MCHC), packed cell volume (PCV), red blood cell distribution width RDW-CV and Total Red Blood Cell Count were measured.

Hemoglobin HPLC were conducted for refractory anemia cases with low MCV, MCH and hemoglobin to rule out or confirm sickle hemoglobinopathies. It was determined by using cation exchange HPLC(Biorad, D10). It is a rapid and easy method for qualitative and quantitative determination of various hemoglobin variants.[4] Red cell indices were evaluated using an automated haematology analyser (Mindray, BC-2800). Microcytic, hypochromic anaemia is

pivotal to diagnosis of -thalassaemia trait with MCV and Hb A_2 being significant diagnostic elements [6]. In this study, subjects with MCV<76fL and MCH<25pg, in the presence of elevated A_2 >4.0% on HPLC were presumed to have Sβ-thalassemia trait while those with A_2 <3.5% and borderline A_2 (<3.5-4.0%) with MCV<76fL and MCH<25pg are presumed to have either an iron deficiency or a co-existing α-thalassaemia [7].

Result: Male and female ratio was 2:1 and average age was 10.6 year, minimum age was 7 year and maximum age was 20 year. Cases with low MCV, MCH were subjected to HPLC to confirm different types of hemoglobinopathies. Majority of total 10 cases were noted carrying Sβ+ / Sβ0 (45.71%) trait and HbAS (20%). Table1 and 2 represented the HPLC and haematological values in 10 samples. General clinical symptoms and signs noted in the patients with various forms of hemoglobinopathies were pallor, weakness, fatigue, abdominal pain, joint pain, fever, jaundice, poor appetite and chest pain [Table 3]. Microcytosis, hypochromia and nucleated RBC's were detected in various haemoglobinopathies but in present study microcytosis seen maximum in S/β0, where as hyperchromasia and nucleated RBC's maximum in SS, S/β+ and S/β0 [Fig.10]. Hemoglobin HPLC of these cases revealed presence of the average HbS values 87.46% in SS followed by 70.45% in S/HPFH, 57.17% in S/β+, 49% in S/β0 and 23.4% in S/α. Similarly average HbF values were highest in S/HPFH (24.63%) and lowest in S/α (0.6%). Average value of HbA was highest in S/α (69.2%) and lowest in S/HPFH (1.56%). The value of HbA2 was highest in S/β+ (4.19%) followed by AS (3.32%), S/α (2.73%), S/β0 (2.6%) and SS (1.26%) respectively [Table 1 & 2]. In two patients ,HPLC findings were affected due to post transfusion sampling.The clinical symptoms were moderate to severe in SS, S/HPFH and S/β0 followed by mild to moderate in S/α and S/β+. The common clinical features were pallor, pain , splenomegaly and one patient presented with avascular necrosis of femoral heads.

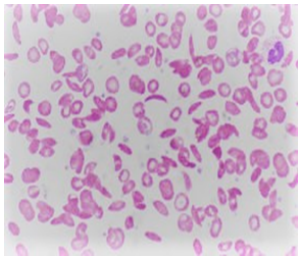

Fig. 1 Sickle cells in PBS

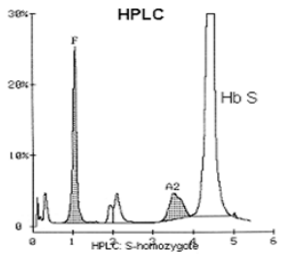

Fig. 2 HPLC pattern in sickle cell anemia

Table: 1 HB HPLC

CASE NO	HBA0	HBA2	HBF	HBS	HB	MCV	MCH	AGE	GENDER
1	49.44	3.32	1.25	45.32	8.90	73.4	30	15	M
2	17.1	1.26	10.53	87.46	7.90	66.56	28	10	M
3	1.56	2.40	24.63	70.45	8.10	70.83	26	7	F
4	69.2	2.73	0.60	23.40	8.90	68.6	29	20	M
5	13.0	4.19	15.53	57.17	9.90	68.64	31	11	M
6	31.0	2.6	16.0	49.0	8.30	73.5	25	8	F
8	33.6	2.0	14.25	51.00	9.00	67.64	28	6	M
9	19.5	1.50	3.25	75.00	6.90	66.64	32	18	M
10	26.0	2.6	16.20	39.00	7.80	65.64	27	11	F

Table: 2 Hemoglobin parameters in sickle cell disease

HPLC	AS	SS	AS/HPFH	AS/A	AS/B+	AS/B0
HBS	45.32	87.46	70.45	23.4	57.17	49.0
HBF	1.25	10.53	24.63	0.6	15.52	16.9
HBA0	49.44	17.1	1.56	69.2	13.01	31.25
HBA2	3.52	1.26	2.4	27.3	4.19	2.60
CLINICAL	ASYMPTOMATIC	SEVERE	MODERATE	MILD	MILD	MODERATE
SICKLE CELLS	+	++	++	–	–	+

Table: 3 Distribution of study population on basis of clinical manifestationS

Vasoocclusive crisis	(43.93%)
Febrile illness	(23.69%)
Bodyache and Joint Pain	(30.99%)
Severe anemia	(26.01%)
Hepatomegaly	(8.09%)
Splenomegaly	(15.02%)
Hepato-splenomegaly	(6.93%)
Jaundice	(20.80%)
Avascular necrosis of femoral head	(6.00)

DISCUSSION:

Bihar state is surrounded by Jharkhand and West Bengal .District Purnea is situated in the north eastern India having assorted population where Muslims, and tribal population are the major carrier of sickle cell gene. In present retrospective study, so far 10 patients were studied out. This coincides with the earlier study conducted, indicating high prevalence rate.[5] Our study concludes that most of the cases belong to sickle anaemia with B thalassaemia (45.71%) closely following the sickle cell trait (20%).As supported in earlier study this could be due the presence of mixed population and high prevalence of Beta Thalassemia in that area..[5] Male / female ratio was almost double which can be accounted for the prevalent socio – cultural condition in our country where more males seek medical attention than females as noted earlier too.[7, 8], weakness, fatigue, abdominal pain, joint pains jaundice, poor appetite and chest pain.From the present study it is concluded that HB electrophoresis is an efficient method for primary screening and diagnosis of hemoglobinopathies at mass scale, along with estimation of various haematological analysis. Further screening with HPLC verifies the result and help in precise diagnosis. The ratio of affected subjects with sickle cell anaemia trait is 7% among screened population. The more symptoms were observed in SS followed by S/HPFH and S/β0 [10,11].Avascular necrosis of femoral head is also common complication of Sickle cell disease .The symptoms were subjective varying with age, sex, personal hygiene, socio-economic condition and other associated diseases.

CONCLUSION:

Sickle cell disease is of two type: double heterozygous and homozygous. Heterozygous sickle cell trait with Beta-Thalassemia trait is common disease in certain geographical area of Bihar. Sickle cell anemia homozygous is uncommon but important entity for with early diagnosis and treatment is needed to prevent serious complications. Thus awareness for early diagnosis is essential for diagnosis and management.

Conflict of interest: None

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