Original article

A hospital based study of Hereditary Hemolytic Anaemias in Davanagere district of Karnataka, India

BP Preethi[1], K Monika[2], DS Maitreyee[3], K Rashmi[4]

Abstract

Background: Hereditary hemolytic anaemias constitute important cause of mortality and morbidity in developing countries next only to infection and malnutrition.These group of anaemias have various clinical presentations starting from their age of onset of symptoms, failure to thrive, anaemia, prostration, jaundice, splenomegaly, cholelithiasis, cardiomegaly, congestive cardiac failure, severe life threatening infections and chronic disabilities leading to distress in the families. ***Methodology:*** An analysis of 40 cases of hereditary hemolytic anaemia in the age group of 2 months to 12 years was done in the present study. On the basis of clinical presentations, physical findings, routine hematological investigations and hemoglobin electrophoresis pattern in hemoglobin defects were carried out to identify the type of hemolytic anaemias. ***Results:*** This clinoco-hematological study of hereditary hemolytic anaemia showed membrane defects-Hereditary spherocytosis in 4 cases (10%). The remaining 36 cases were having diseases affecting hemoglobin molecule which included Sickle cell anaemia-5 cases (12.5%), Sickle cell trait- 1 case (2.5%), Sickle cell/β thalassemia-1 case (2.5%), β thalassemia major- 23 cases (57.5%) and β thalassemia trait 6 cases(15%). Hereditary hemolytic anaemia with enzyme defects were not observed in this study. Majority of these cases presented with progressive pallor and hepatosplenomegaly. Peripheral blood smear examination showed microcytic hypochromic anaemia (87.5%) in majority of the cases. All cases were associated with reticulocytosis. Hemoglobin electrophoresis confirmed the diagnosis. ***Conclusion:*** Inspite of advanced diagnostic inestigations, the basic hematological investigation remains first panel or step towards the approach to diagnose hereditary hemolytic anaemia and hemoglobin electrophoresis will help in confirming the diagnosis.

Keywords: hereditary hemolytic anaemia, clinoco-hematological study, hemoglobin electrophoresis

Introduction

Hereditary hemolytic anaemias are group of disorders characterized by intrinsic defect in red blood cells associated with accelerated erythrocyte destruction, hyperbilirubinemia and erythroid hyperplasia. These group of disorders include defect in red cell membrane such as Hereditary spherocytosis, elliptocytosis, presence of abnormal hemoglobin synthesis-sickle cell anaemia, impaired globin synthesis-thalassemia and defect in erythrocytre enzymes such as G6PD, pyruvate kinase deficiency.[1]

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1. *Dr Preethi B.P, Assistant professor, MD Biochemistry,
2. Dr Monika, post graduate student, MD Biochemistry,
3. Dr Maitreyee D.S, post graduate student, MD Biochemistry,
4. Dr Rashmi K, post graduate student, MD Pathology,

J.J.M. Medical College, Davanagere, Karnataka, India.

Corresponds to:** Dr Preethi.B.P, Assistant Professor, Department Of Biochemistry, J.J.M. Medical College Hospital,Davanagere 577004, Karnataka, India. ***Tel: 09844316801. ***Email:*** Preethi_Chidan@Yahoo.Co.In.

onset of symptoms, failure to thrive anaemia, prostration, jaundice, splenomegaly, cholelithiasis, cardiomegaly, congestive cardiac failure, severe life threatening infections and chronic disabilities leading to distress in the families.[2]

The World Health Organization (WHO) has suggested that about 5% of the world population are carriers for different inherited disorders of hemoglobin.[3] WHO reports also state that about 370,000 severely affected homozygotes or compound heterozygotes of thalassemia are born every year. The UNICEF in 1996 estimated that there were 29.7 million carriers of beta thalassemia trait in India and about 10,000 infants with homozygous beta thalassemia born every year[4]. The general incidence of thalassemia trait and sickle cell hemoglobinopathies in India varies between 3-17% and 1-44% respectively. [5 6 7] It is estimated that there are about 65,000-67,000 beta-thalassemia patients in India with around 9,000-10,000 cases being added every year. The carrier rate for beta-thalassemia gene varies from 1 to 3% in Southern India to 3 to 15% in Northern India.[8 9 10] In developing countries, in which there is high mortality from infections and malnutrition in the first year of life, many of the hemoglobinopathies are unrecognized. Sickle cell anaemia and thalassemia major can cause life-threatening situation and chronic ill-health. They pose economical and psychological burden on the affected individual and his/her family, and the society as a whole. Hence, the population needs to be screened for hemoglobin.

Materials and methods

Clinico-hematological study of hereditary hemolytic anaemias was conducted in department of Biochemistry and hematology section of Pathology department of J. J. M. Medical College for a period of two years from Jan 2008-Jan 2010.

Inclusion criteria: Patients of hereditary hemolytic anaemia in the age group of 2 months to 12 years attending pediatric out-patient department of Bapuji Child Health Institute and Research Centre, Chigateri District Hospital and Women and Children Hospital Davangere were included in the study.

Exclusion criteria: Infants less than 2 months and children more than 12 years, cases of acquired hemolytic anaemia and Coomb's positive hemolytic anaemia were excluded from the present study.

40 patients of hereditary hemolytic anaemias were included in the study after obtaining written informed consent from the parent/guardian. All the subjects underwent detailed clinical examination. Routine urine analysis and complete blood count using automated hematology analyzer (ABX microx ot18 abx haematologie montpellier Cedex 04) were done in all patients.

Additional investigations done were:

- Blood grouping and Rh typing
- Erythrocyte sedimentation rate by Wintrobes method,
- Peripheral smear examination using Leishman's stain,
- Bone marrow aspiration using Leishman's stain and estimation of iron stores.
- Reticulocyte count using Supravital stain,
- Direct Coomb's test using polyspecific antihuman globulin reagent,
- Sickle cell test by Sodium metabisulfite method,
- Hemoglobin F by Kleihauer acid elution method,
- Osmotic fragility test by Sanford method,
- Hemoglobin separation and quantification by automated hemoglobin electrophoresis (GENIO Co)

- Biochemical estimations include serum total bilirubin, direct and indirect bilirubin (Jendrassic and Groff method) by Roche Cobas Integra 400 plus autoanalyzer.

Results

Clinico hematological study of Hereditary hemolytic anaemias was done on 40 patients during the period of Jan 2008-Jan 2010. Salient features observed in this study were:

1. *Incidence:* 40 patients in this study formed 0.2% of the total number of 18000 pediatric patients attending the out-patient clinic.
2. *Age:* Age of these patients ranged from 4 months to 12 years. Majority of the patients were between 3-5 years followed by age group of 5-10 years. (Table 1)
3. *Sex:* Sex distribution showed Hereditary hemolytic anaemias affecting predominantly males with male to female ratio of 5:3. (Table 2)
4. *Religion:* In this study 37 patients (92.5%) belonged to Hindu religion and remaining were Muslims. (Table 3)
5. Caste wise distribution showed majority of the patients belonged to Lingayat community followed by Kuruba, Gowda and Lambani. (Table 4)
6. *Family history:* History of consanguineous marriage of parents were present in 22 cases (55%) which included second degree in 19 cases and third degree in 3 cases. Other members of the family were not affected by either similar or any other illness. (Table 5 & 6)
7. *Blood group:* Blood group analysis of these patients revealed majority of these patients belonged to B group followed by A, O and only one case of AB group. Rh typing showed 37 patients belonged to Rh positive and 3 patients were Rh negative. (Table 7 & 8)
8. *Types of Hereditary hemolytic anaemia:* In this study, diseases associated with membrane defects- Hereditary spherocytosis were observed in 4 cases (10%). The remaining 36 cases were having diseases affecting hemoglobin molecule which included Sickle cell anaemia-5 cases, Sickle cell trait- 1 case, Sickle cell/β thalassemia- 1 case, β thalassemia major- 23 cases and β thalassemia trait 6 cases. Hereditary hemolytic anaemia with enzyme defects was not observed in this study. (Table 9)

Table 1: age wise distribution of hereditary hemolytic anaemias

Age group	Number of cases	Percentage
2-5 months	1	2.5
6-11 months	1	2.5
1-2 years	3	7.5
3-5 years	15	37.5
5-10 years	12	30.0
11-12 years	8	20.0

Table 2: sex wise distribution of hereditary hemolytic anaemias

Sex	Number of cases	Percentage
Male	25	62.5
Female	15	37.5

Table 3: religion wise distribution of hereditary hemolytic anaemias

Caste	Number of cases	Percentage
Hindu	37	92.5
Muslims	3	7.5

Table 4: caste wise distribution of hereditary hemolytic anaemias

Subcaste	Number of cases
Lingayat	11
Lambani	6
Kuruba	7
Gowda	5
Akkasaliga	2
Lohana	1
Jain	1
Brahmin	1
Muslim	3
Marathas	1
Adikarnataka	2
Total	**40**

Table 5: number of patients born out of consanguineous marriage

Consanguinity	Number of cases	Percentage
Present	22	55
Absent	18	45

Table 6: type of consanguinity between parents

Type of consanguinity	Number of cases
First degree	0
Second degree	19
Third degree	3

Table 7: blood group wise distribution of hereditary hemolytic anaemias

Blood group	Number of cases	Percentage
A	11	27.5
B	18	45
O	10	25
AB	1	2.5

Table 8: rh wise distribution of hereditary hemolytic anaemias

Rh	Number of cases	Percentage
Positive	37	92.5
Negative	3	7.5

Table 9: distribution of various causes of hereditary hemolytic anaemias

Cause	Number of cases	Percentage
Hereditary spherocytosis	4	10
Sickle cell anaemia	5	12.5
Sickle cell trait	1	2.5
Sickle / β-thalassemia	1	2.5
β-thalassemia major	22	57.5
β-thalassemia trait	6	15

Discussion

Incidence of Hereditary hemolytic anaemia in India is 0.1 to 0.2%. [5] Even in the present study also the incidence of hereditary hemolytic anaemia is 0.2%. The present study included patients' age ranging from 2 months to 12 years. Maximum cases were between 3-5 years (37.5%) (Table 1). Less than 2 months cases were not included as to exclude non hereditary hemolytic anaemia caused by immune mediated mechanism such as Rh mediated hemolytic disease of the newborn. The hereditary hemolytic anaemia was seen more commonly in males in the present study (Table 2) and this is in agreement with studies conducted by Juwah et al[12], Shivashankara et al[13] and RS Balgir.[14]

Hereditary hemolytic anaemia being genetic disorder is commonly seen in particular communities where inbreeding is common. There are various community based studies conducted throughout India showing increased preponderance of particular community to hemolytic anaemia. In the present study also majority of cases (27.5%) were seen in Lingayat community (Table 4) which is in agreement with literature and this finding may in part be related to population distribution in this part of Karnataka.[11] In the present study, neither other members of the family were affected by similar nor any other illness, but history of consanguineous marriage was seen in 55% of cases and in turn it was second degree consanguinity which was more commonly observed (Table 6). This suggests the possibility of individual spontaneous mutations and requirement of further genetic studies for evaluation of the same.

Blood group analysis of these 40 patients revealed majority of patients belonged to B group (27.5%) and Rh positive (92.5%) (Table 7 & 8). In the present study, 10% had hereditary sperocytosis, 12.5% had Sicklecell anaemia, 2.5% had Sickle cell trait, 2.5% had Sickle cell/β thalassemia, 57.5% of children were suffering from β thalassemia major and 15% from β

thalassemia trait. Most common hereditary hemolytic anaemia in India is β thalassemia major [11] and this is reflected in present study also.

Four patients had hereditary spherocytosis in this study and were in the age group of 6 months to 12 years. All patients had progressive pallor and mild to moderate splenomegaly. Peripheral smear showed mild anisopoikilocytosis with spherocytes forming 40 to 60% of red cells in all cases. Osmotic fragility test in all four cases showed increased hemolysis beginning at 0.50% and ending at 0.46%. Normal values for control started at 0.44% and ended at 0.32% of normal saline. Direct antiglobin test was negative in all cases. Features of hypersplenism were not observed in this study. Hemoglobin ranged from 5-10gm/dL, MCV 72-84 μm^3, MCH 25-31pg MCHC 37-41 gm/dL, Reticulocyte count 2.5-10%. These findings were in accordance with studies of Delhommeau et al [15], Kart et al [16] and Mariagabriella.[17]

Sickle cell anaemia was noted in 5 out of 40 cases (12.5%) (Table9). All five cases were seen in Lambani communities. Most common clinical presentation was pallor followed by cough associated fever. All cases had splenomegaly peripheral smear showed diagnostic sickle cells along with occasional target cells and mild normoblasts. No crisis was observed. Hemoglobin ranged from 8-9gm/dL, MCV 50-86μm^3, MCH 20-26pg, MCHC 25-31gm/dL, Reticulocyte count 5-26%. Hemoglobin electrophoresis revealed Hb A 0-10.2%, Hb A_2 3-31%, Hb F 4.4-12% and Hb S 68-97%. All the parameters were comparable to other previous similar studies of Juwah et al [12] and RS Balgir [14].

In the present study, sickle cell trait was identified in one patient. This child presented with microcytic hypochromic anaemia and peripheral smear did not show any evidence of sickle cells. However sickle cells were seen on sickling test. This patient had hemoglobin 9gm/dL, MCV 84μm^3, MCH 21.7pg, MCHC 29.2gm/dL, Reticulocyte count 3.5%. Hemoglobin electrophoresis revealed Hb A 90%, Hb A_2 4.4%, Hb F 3.0% and Hb S 3.6%. Hemoglobin electrophoretic pattern is similar to the pattern described in literature.[11]

In the present study β thalassemia major accounted for 57.5% (Table9) of cases. All patients had features of progressive pallor. 22.7% of these cases were within six months of age. Hemoglobin ranged from 3-11gm/dL, hematocrit 9-33%, MCV 50-75μm^3, MCH 16-22pg, MCHC 15-27gm/dL, Reticulocyte count 5-15%. Bone marrow examination revealed erythroid hyperplasia and megaloblastic change in ten cases indicating compensatory erythroid hyperplasia. Hemoglobin electrophoresis revealed Hb A 1.5-94%, Hb A_2 1.4-4.5%, Hb F 10-95%. These results are in accordance with study conducted by RS Balgir [14], Seema Tyagi et al [18] and Antanio Cao.[19]

Six patients in this study were diagnosed as β thalassemia trait. Their age ranged from three to five years. All these patients had mild pallor. Most common presentation next to pallor was cough with fever. Hemoglobin ranged from 6-10gm/dL, hematocrit 16-25%, MCV 54-73μm^3, MCH 15-32pg, MCHC 28-34gm/dL, Reticulocyte count 2.5-10%. Hemoglobin electrophoresis revealed Hb A 57-87.9%, Hb A_2 5.2-9.9%, Hb F 2.2-38%. These results are in accordance with study conducted by RS Balgir [14], Seema Tyagi et al [18] and Antanio Cao [19].

There were no cases of enzyme defects such as G6PD and pyruvate kinase which are described in some studies as screening tests for detecting enzyme defects were not done.

Conclusion

Differentiation of hereditary hemolytic anaemias using hematological investigations although useful, hemoglobin electrophoresis establishes the disease in hemoglobinopathies and osmotic fragility test in hereditary spherocytosis. Despite some recent encouraging initiative in the underdeveloped countries, the control of hereditary hemolytic anaemias is generally not given the importance it deserves.

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