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Original Article

Pathology Section

Haemoglobinopathies: A Retrospective Study from a Tertiary Care Centre, Southern India

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ABSTRACT

Introduction: Thalassaemia and other structural haemoglobinopathies are the major genetic disorders that cause significant morbidity in children. Haemoglobinopathies need to be diagnosed at the earliest in order to offer suitable treatment, carrier identification and counselling which will help to decrease the incidence of haemoglobinopathies.

Aim: To study the clinicohaematological spectrum of children with thalassaemia and other haemoglobinopathies, followed by family screening and counselling.

Materials and Methods: This retrospective cross-sectional study was taken up at Department of Pathology, Indira Gandhi Institute of Child Health, Bangalore, Karnataka, India from January 2018 to December 2020, during which records of 235 cases were studied, out of which 100 were the children visiting Institute and 135 were the parents and siblings of these children. Diagnosis of these cases was followed by counselling for these families. Results were calculated by data analysis. Percentages were calculated from the frequency of the variables.

Results: Amongst the records of 235 cases studied, β-thalassaemia major was the most common haemoglobinopathy found in 75 patients, followed by sickle cell anaemia in 10 patients. β-thalassaemia trait was the most common haemoglobinopathy among the parents of these children, which was found in 125 individuals.

Conclusion: β-thalassaemia major is the most common disorder amongst the children with haemoglobinopathy, followed by sickle cell anaemia. β-thalassaemia trait is a common entity amongst the carriers of haemoglobinopathy.

Keywords: Clinicohaematological profile, Family screening, Haemoglobin electrophoresis, Thalassaemia

INTRODUCTION

Thalassaemia and other structural haemoglobinopathies are the major red cell genetic disorders prevalent in various parts of the world, including India. Defects in genes of haemoglobin can produce abnormal haemoglobin, which gives rise to anaemia and the condition is termed as haemoglobinopathy. Decreased synthesis of one or more globin subunits of normal haemoglobin gives rise to thalassaemia and abnormal globin chain synthesis leads to sickle cell disease [1-3]. The β-thalassaemia is a heterogenous group of genetic disorders of haemoglobin synthesis characterised by variable reduction (β^+ thalassaemia) or complete absence of β globin (β^0 thalassaemia) chains of haemoglobin which results in an imbalanced chain synthesis with excess of α chains which leads to premature destruction of red cell precursors in the bone marrow and extramedullary sites which is the hallmark of β-thalassaemia [4].

Sickle cell disease is caused by an inherited haemoglobin S gene which is characterised by point mutation in the 6th codon of the β-globin gene leading to the substitution of glutamic acid to valine [5]. According to World Health Organisation (WHO), 5% of the world population is a carrier of haemoglobin disorder [6]. The frequency of β-thalassaemia in India is between 3.5-15% [7]. In India, every year, over 10,000 thalassaemic children are born [8]. Most of the studies are reported from northern, western and eastern India [9,10]. Very few studies are reported from southern India [10,11].

Since the study involves paediatric tertiary care Institute having a thalassaemia nodal centre catering to a large chunk of patients with thalassaemia and related haemoglobinopathies, an attempt has been made to study and identify the spectrum of haemoglobinopathies along with their varied clinical presentation, which is the first of its kind in the state. This would help to formulate appropriate preventive measures and implement the same, in the entire state.

MATERIALS AND METHODS

A retrospective cross-sectional study was carried out in the Department of Pathology, Indira Gandhi Institute of Child Health, Bangalore, Karnataka, India from January 2018 to December 2020, was collected from the case records, which included 100 children with haemoglobinopathy and 135 siblings and parents of these children. The collected data was analysed from January 2021 to March 2021. Prior to the initiation of the study, clearance was taken from the Institutional Ethics Committee (IGICH/ACA/EC-II/P5/12/2020-21).

Inclusion criteria: Records of 235 cases with thalassaemia syndrome and haemoglobinopathies diagnosed by haemoglobin electrophoresis from both Inpatient and Outpatient Departments of this Institute were included in the study.

Exclusion criteria: Children with history of transfusion in the last one month, were excluded from the study.

Study Procedure

Complete blood count which was performed in fully automated 5-part differential cell counter (Mindray BC 5200) was taken into consideration. As per hospital protocol, haemoglobin electrophoresis was done by capillary electrophoresis method (Sebia minicap flex-piercing instrument). Blood for sickling was examined by sealed preparation of Daland and Castle by using freshly prepared reducing agent (2% sodium metabisulphite) in suspected cases of sickle cell disease. Alkali denaturation test of Betke was done for visualisation of distribution of Haemoglobin Foetal (HbF) among red cells (serum ferritin kit; Roche Diagnostics).

STATISTICAL ANALYSIS

Gender and age distribution in the study population was calculated by using descriptive statistics. Percentages were calculated from the frequency of the variables.

RESULTS

Of the 235 cases with haemoglobinopathies, 100 children presented to the Institute with various symptoms and the rest 135 were the parents and siblings of these children. Out of these 100 children, 75 were less than five years of age and the rest were in the age group of 5-15 years. The spectrum of haemoglobinopathies, over a period of 36 months amongst both the groups, is depicted separately in [Table/Fig-1,2]. Family screening revealed two rare cases; one each of δβ thalassaemia and Hereditary Persistence of Foetal Haemoglobin (HPFH) in the parents and they were asymptomatic. Among sickle cell disease, sickle cell anaemia was more common which was found in 10 cases, followed by sickle β-thalassaemia in six cases and β-thalassaemia intermedia in five cases.

Haemoglobinopathies	Total number	Percentage (%)	Male	Female
β-thalassaemia major	75	75	48	27
β-thalassaemia intermedia	05	5	04	01
HbE disease	01	1	01	0
HbE β-thalassaemia	02	2	01	01
Sickle cell anaemia	10	10	06	04
Sickle cell trait	01	1	01	0
Sickle β-thalassaemia	06	6	04	02
Total	100	100	65 (65.00%)	35 (35%)

[Table/Fig-1]: Spectrum of haemoglobinopathies amongst patients along with sex-wise distribution.
β: Beta; Hb: Haemoglobin; N=100

Haemoglobinopathies	Total number	Percentage (%)	Male	Female
β-thalassaemia trait	125	92.59	72	53
δβ thalassaemia	01	0.74	01	0
HPFH	01	0.74	01	0
HbE trait	01	0.74	0	01
Sickle cell trait	07	5.19	04	03
Total	135	100	78 (57.78%)	57 (42.22%)

[Table/Fig-2]: Spectrum of haemoglobinopathies amongst carriers along with sex-wise distribution.
HPFH: Hereditary persistence of foetal haemoglobin; N=135

It was found, that, 56 children with β-thalassaemia major presented early (before one year of age) and most of the children presented before five years of age. Only seven children with β-thalassaemia major presented later in life, after five years of age. The earliest age of onset was two and half months. Age distribution of the 100 children presented to the Institute is depicted in [Table/Fig-3].

Haemoglobinopathies	<5 years	Percentage (%)	5-15 years	Percentage (%)
β-thalassaemia major	68	90.67	07	28
β-thalassaemia intermedia	0	0	05	20
HbE disease	0	0	01	4
HbE β-thalassaemia	1	1.33	01	4
Sickle cell anaemia	3	4	07	28
Sickle cell trait	0	0	01	4
Sickle β-thalassaemia	03	4.00	03	12
Total	75	100.00	25	100.00

[Table/Fig-3]: Age-wise distribution of the patients in the study.

Haematological profile of all these cases was analysed and it was found that, the mean haemoglobin was lowest in β-thalassaemia major. Red Blood Cell (RBC) count was highest in β-thalassaemia trait. The red cell indices Mean Corpuscular Volume (MCV) and Mean Corpuscular Haemoglobin (MCH) were lower in β-thalassaemia than in sickle cell disease. Red Cell Distribution Width (RDW) was significantly higher in β-thalassaemia major. Analysis of electrophoresis of all the haemoglobinopathies was done and is depicted in [Table/Fig-4] along with the haematological parameters. Average HbF level in patients with β-thalassaemia major was found to be 78.6% and severity of clinical manifestation was found to be the same irrespective of HbF level. HbA2 levels of more than 4.0% were noted in most of the cases with β-thalassaemia trait. Three cases with borderline HbA2 level (3.5-4.0%) were also identified among the parents of children with β-thalassaemia major. But, all the three were found to have associated iron deficiency. In capillary electrophoresis, HbE fraction was found to be 95%, 24% and 50% in HbE disease, HbE trait and HbE β-thalassaemia, respectively.

The children with HbE, β-thalassemia had clinical finding similar to that of β-thalassemia major as they presented with severe pallor (Hb-3.2 g/dL) and hepatosplenomegaly. The child with HbE disease had milder clinical findings in the form of mild pallor (Hb-10.0 g/dL) and good activity. HbE trait was diagnosed in one of the parents, who was asymptomatic. It was observed from the records that, pallor was the most common presenting complaint among the study population, followed by delay in growth and development. Clinical examination findings included splenomegaly, hepatomegaly, haemolytic facies and bone pain. Dactylitis and leg ulcer, were found to be the least common complaints; which were observed only in children with sickle cell anaemia [Table/Fig-5]. In sickle cell anaemia, on comparing HbF level with the severity of clinical manifestations, the child with HbF level of 31.20%, presented only with pallor and joint pains. In order to analyse, the correlation between HbF value and the clinical severity of the disease at presentation, the correlation coefficient was found to be -0.1223. On the other hand, the child

Parameters	β-thalassaemia major	β-thalassaemia intermedia	β-thalassaemia trait	Sickle cell anaemia	Sickle cell trait	HbS/β-thalassaemia	HbE disease	HbE trait	HbE β-thalassaemia	δβ thalassaemia	HPFH
Hb (g/dL)	2.1-5.80	3.5-6.90	9.8-12	2.5-12.1	9-13.9	7.9-8.1	9.1	14.1	5.7-6.8	11.2	12.6
RBC count (x106/uL)	0.61-3.5	1.71-3.39	4.9-6.1	1.51-5.5	3.5-5.5	2.71-3.96	4.62	5.69	3.50-4.28	4.75	4.80
MCV (fl)	45.7-70.0	63-77.1	55.0-69.0	58.0-85.0	70-85	60.1- 68.2	62.3	79.3	56.3-57.8	78.8	88.2
MCH (pg)	13.7-25.5	17.8-21.3	17.7-21.9	22.0- 29.0	24.1-30.2	20.1-23.2	19.7	27.9	15.5-15.9	27.5	29.5
RDW (%)	19.4-40.1	20.1-33.8	13.9-16.3	13.42-30.1	12.1-23.2	18.5-20.7	14.5	12.9	18.5-19.8	14.2	14.0
Electrophoresis											
Hb A2	2.9-3.3	2.4-5.8	4.4-7.0	1.5-5.0	3.0-4.6	4.7-5.1	2.0	4.0	5.0	1.5	-
Hb F	18.3-98.5	21-97	0.5-3.0	4.5-31.20	0.5-3.0	13.2-23.3	0.5	1.0	42.0	88.0	99.0
Hb A	0.4-78.8	0.5-66.9	74-90	0-2.2	40.1-66.6	1.5-19	2.5	71.0	3.0	10.5	1.0
Hb S	-	-	-	38.1-80.5	20.3-47.5	-	-	-	-	-	-
Hb E	-	-	-	-	-	-	95	24.0	50.0	-	-

[Table/Fig-4]: Haematological profile and electrophoretic pattern of all haemoglobinopathies in the present study.
Hb: Haemoglobin; RBC: Red blood cell; MCV:Mean corpuscular volume; MCH: Mean corpuscular haemoglobin; MCHC: Mean corpuscular haemoglobin concentration; RDW: Red cell distribution width; Thal: Thalassaemia; HPFH: Hereditary persistence of foetal haemoglobin; Hb S: Haemoglobin sickle cell trait; HbF: Haemoglobin foetal; HbA: Haemoglobin adult; Hb A2: Haemoglobin alpha-globin

Clinical signs of patients	Number
Pallor	100
Delay in growth and development	82
Splenomegaly	80
Hepatomegaly	60
Haemolytic facies	40
Bone pain	26
Dactylitis	04
Leg ulcer	01

[Table/Fig-5]: Clinical profile of the patients with haemoglobinopathy.

with HbF level of 4.5%, presented in sickle cell crisis with severe clinical manifestations.

DISCUSSION

A large number of haemoglobinopathies prevalent in the population causing morbidity and mortality make them a major important public health problem in India [12]. β-thalassaemia is the commonest inherited haemoglobinopathy all over the world and is the most common single gene disorder in our country [7]. About 1.5-3% of world population carries the β-thalassaemia gene [13,14]. The most common haemoglobinopathy in present study was β-thalassaemia trait followed by β-thalassaemia major which is in agreement with the findings of Khera R et al., who reported 62 cases of β-thalassaemia trait out of 110 cases [13]. The studies done by Preethi BP et al., and Patel J et al., also noted similar findings [15,16]. However, the percentage of β-thalassaemia trait was not as high as in present study. The higher proportion of β-thalassaemia trait in present study could be due to the family screening of known β-thalassaemia major children, which cannot be regarded as representative of a community or population. The prevalence of β-thalassaemia trait varies from 3.5-14.9% in various parts of India [17]. Since the β-thalassaemia gene is not distributed uniformly in the Indian subcontinent, it has varying frequencies in different regions [17].

β-thalassaemia major accounted for 75 cases. Among them, 56 cases were diagnosed before one year of the age, similar to the findings of Preethi BP et al., and Anusha R et al., [15,18]. The earliest age of onset in the present study was two and a half months. In β-thalassaemia, there was no significant correlation between levels of HbF and clinical severity of the disease or frequency of blood transfusion. There were five cases of β-thalassaemia intermedia in present study, who presented after five years of age. These children will have more complications than β-thalassaemia major. Early splenectomy is helpful in β-thalassaemia intermedia [16].

Two rare cases, one each of δβ thalassaemia and HPFH were reported among parents in present study. They both had high levels of HbF and a mild clinical phenotype [19]. The only way to differentiate between the two was by alkali denaturation test of Betke, to see the HbF distribution among the red cells. It was distributed heterogeneously among the red cells δβ thalassaemia patient whereas parent with HPFH showed pancellular distribution [20].

HbE disease is most common in eastern and far eastern states of India [21,22]. HbE is not common in Karnataka. The three cases, one each of HbE disease, HbE trait and HbE β-thalassaemia were seen in the patients from West Bengal. The child with HbE β-thalassaemia had clinical finding similar to that of β-thalassaemia major whereas the child with HbE disease had milder clinical findings. HbE trait was diagnosed in one of the parents, who was asymptomatic.

The frequency of sickle cell disease in India is 4.3% [23]. Sickle cell disease includes sickle cell anaemia, sickle cell trait, sickle β-thalassaemia and HbSE. In present study, sickle cell anaemia and sickle cell trait were more common. Majority of the children presented with fever and pallor. Four children had dactylitis which determines the prognosis of the disease later in life. Clinical manifestations were milder in children with HbS β-thalassaemia when compared with sickle cell anaemia. In the present study, severity of clinical symptoms were correlated with the levels of HbF. Those children with higher haemoglobin F had milder symptoms. In sickle cell disease, the severity of symptoms was inversely proportional to the levels of HbF. The clinical manifestation of sickle cell anaemia in India, seems to be milder than in Africa and Jamaica, which may be attributed to the high HbF levels in Indian population [24]. Sicklers from India are protected from sickle cell crisis because of high HbF levels. The 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 reported variable clinical presentation [24,25]. 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 [26,27].

Limitation(s)

Since, the present study was conducted in a tertiary care centre, actual prevalence of thalassaemia and other haemoglobinopathies could not be evaluated. A population study will be more appropriate for the same.

CONCLUSION(S)

The present study revealed the spectrum of haemoglobinopathies attending the tertiary care centre, with the added advantage of family screening which was followed up with counselling. By far, β-thalassaemia major is the most common haemoglobinopathy amongst symptomatic children followed by sickle cell anaemia. Rare haemoglobinopathies such as HbS β-thalassaemia, δβ thalassaemia and HPFH were also encountered in the study.

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