Original Article

DOI: 10.7860/IJNMR/2022/53469.2329

Paediatrics Section

Prevalence of Haemoglobin Variants and Haemoglobinopathies in a Single Paediatric Centre in Southern India: A Retrospective Cross-sectional Study

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ABSTRACT

Introduction: Haemoglobinopathies are qualitative disorders of Haemoglobin (Hb) resulting from structural defects in the amino acid sequence of one of the globin chains, whereas, thalassaemia results from quantitative defects in the synthesis of one or more of the globin chain subunits of the Hb tetramer. Cation Exchange-High Performance Liquid Chromatography (CE-HPLC) is one of the methods for initial screening of Hb variants like HbS, HbD, HbE etc and for quantification of HbF, HbA and HbA2 levels.

Aim: To find out the prevalence of haemoglobinopathies in patients of a Government paediatric tertiary care hospital in south India.

Materials and Methods: In this retrospective, cross-sectional study, all laboratory requests, in the period from August 2019 to July 2021, for Hb variant analysis by HPLC were collected, irrespective of provisional diagnosis. The Hb variant analysis was carried out by CE-HPLC on the Bio-Rad D-10 analyser. This study was conducted in a Government Paediatric tertiary care hospital in Southern India for patients who had any clinical or familial suspicion of haemoglobinopathies. The Statistical analysis was performed using Microsoft Excel 2010.

Results: Total data of 704 laboratory requests for Hb Variant analysis were obtained. Out of 704 laboratory request, 585 were from children younger than 12 years of age and 119 were parental screening. There were 164 abnormal chromatograms. Out of 164 patients, 91 were female patients and 73 were male patients. Out of 164 abnormal chromatograms, 97 (59.15%) were beta-thalassaemia trait, 18 (10.98%) were beta-thalassaemia major, 13 (7.93%) were sickle cell trait, 2 (1.22%) were sickle cell disease, 21 (12.80%) were HbE trait, 2 (1.22%) were homozygous HbE, 2 (1.22%) were HbD trait, 3 (1.83%) were Hereditary Persistence of Foetal Hb (HPFH)/delta beta-thalassaemia, 2 (1.22%) were HbJ trait, 1 (0.61%) was HbE beta-thalassaemia, 1 (0.61%) was sickle-beta-thalassaemia and 2 (1.22%) were alpha thalassaemia.

Conclusion: From this study, beta-thalassaemia trait and beta-thalassaemia major were found to be the first and second most prevalent haemoglobinopathies in children below 12 years of age. This data suggests the importance of premarital and antenatal screening procedures that can help in reducing the possibility of such haemoglobinopathies in the future generation, suffering and burden of disease to the family and society.

Keywords: Antenatal screening, High performance liquid chromatography, Sickle cell anaemia, Thalassaemia

INTRODUCTION

Haemoglobinopathies are qualitative disorders of Hb resulting from structural defect in the amino acid sequence of one of the globin chains, whereas, thalassaemia results from quantitative defects in the synthesis of one or more of the globin chain subunits of the Hb tetramer. Reduction in alpha chain synthesis is called alpha thalassaemia, while deficient beta chain synthesis is beta-thalassaemia. In beta-thalassaemia trait, the genetic mutation causes reduced synthesis of beta chains leading to ineffective erythropoiesis causing microcytic hypochromic red cell indices with elevated HbA2 in the range of 4-9% [1,2].

In beta-thalassaemia major, the genetic mutation in beta-globin gene causes absence or reduced chains synthesis of beta chains leading to severe anaemia, ineffective erythropoiesis and extramedullary haematopoiesis. Patients show severe, microcytic hypochromic anaemia with elevated HbF ranging from 10-90% and marked reduction in HbA. The HbA2 levels may be reduced, normal or elevated [3,4]. The "thalassaemia belt" extends along the shores of the Mediterranean region, Arabian peninsula, Turkey, Iran, India and Southeast Asia with the carrier prevalence of thalassaemia ranging from 2.5-15% [5-7]. A recent study in India by Mohanty D et al., showed that overall prevalence of beta-thalassaemia trait was 2.78% [8]. In different states in India, the prevalence of beta-thalassaemia trait ranges from 1.48-3.64% [8]. Other types like delta-beta-thalassaemia, Hb Lepore, HPFH are related conditions. Hb Lepore is composed of two alpha chains and two delta-beta chimeric chains.

Of the Hb variants, HbS constitutes the most common variety worldwide. Sickle Cell trait (βSβA) is due to point mutation in one allele of beta gene (GAG to GTG, Glu to Val at β6). Valine is a hydrophobic amino acid and the substitution causes the clumping or polymerisation of HbS molecules. In Sickle cell trait (βSβA), HbS ranges from 30-40% and in sickle cell anaemia (βSβS), HbS is in the range of 70-90%, increased HbF of 5-25% [9]. In compound heterozygotes for HbS and beta-Thalassaemia, HbS is lesser than 50%, HbA of 5-10% with elevated HbA2 [9]. HbS, HbE, and HbD are prevalent in India with a cumulative gene frequency of 4.2% [10]. The prevalence of haemoglobinopathies varies with the geographic

locations and in ethnic groups. In a study by Balgir RS, the prevalence of sickle cell disorders was found to vary from 2.4-5.6% among the tribes of Orissa in eastern India [11]. The HbE is mostly restricted to the north eastern regions of India with gene frequency of 10.9% [12]. The HbE trait is due to point mutation in one allele of beta gene (GAG to AAG, Glu to Lys at 06) which generates an alternative splice site in Exon 1, resulting in a proportion of abnormally spliced mRNA. The HbE traits have about 30% HbE with normal HbF, whereas HbE homozygotes accounts for 85-95% HbE with normal or mild increase in HbF [12]. HbD Punjab trait is due to point mutation in one allele of beta gene (GAG to CAA, Glu to Gln at β121) with 30-45% HbD with normal HbF. The frequency of HbD in Uttar Pradesh by Agarwal S et al., study was shown as 0.5-3.1% [13].

The CE-HPLC is one of the methods for initial screening of Hb variants like HbS, HbD, HbE etc., and for quantification of HbF, HbA and HbA2 levels. In CE-HPLC, the Hb separates into various components as follows: HbA1a, HbA1b, HbF, LA1c/CHB-1, LA1c/CHB-2, HBA1c, P3 (HB d component), HbA0, and HbA2. The A1a, A1b, A1c and the P3 are minor Hb formed by post-translational modifications of the globin chains. The foetal HB composed of two alpha and two gamma chains in a normal adult is found to be less than 1%. The HbA2 composed of two alpha and two delta chains in normal adults is found to be less than 3.4%. The HbA2 values between 3.5-4% is reported with caution, keeping in mind the red cell indices and silent beta-thalassaemia [14].

This study was conducted in a Government Paediatric tertiary care hospital in Southern India who had clinical or familial suspicion of haemoglobinopathy as there is limited available data on large scale studies of haemoglobinopathies in Southern India. The detection of the haemoglobinopathies and thalassaemias was carried out by CE-HPLC on the Bio-Rad D-10 analyser.

MATERIALS AND METHODS

This retrospective cross-sectional study was conducted at the Institute of Child Health and Hospital for children, Chennai, Tamil Nadu, India. In the current study, authors focused only on re-analysing the available data. The data were retrieved from the archived samples. The data were collected from August 2019 to July 2021 and were analysed at a point in time during October 2021 for determining the prevalence of Hb variants and haemoglobinopathies in a single paediatric centre in Southern India. Informed consent was obtained from all participants included in the study.

Inclusion criteria: All patients for whom an Hb Variant analysis was requested, were included in this study.

Exclusion criteria: The patients who had received blood transfusion in the previous three months were excluded from the study.

Data Collection

A complete blood count, peripheral smear examination for red blood cell morphology, Naked Eye Single Tube Redcell Osmotic Fragility Test (NESTROFT test) and Hb variant analysis were studied for all the patients [15]. Complete blood count and peripheral smear findings were considered for the interpretation of HPLC graphs.

The Hb variant analysis was carried out by CE-HPLC on the Bio-Rad D-10 analyser [14]. The Bio-Rad D-10 analyser is an automated instrument which operates on the principle of HPLC and the column comprises of a small cation exchange catridge.

The abnormal chromatogram identified both quantitative haemoglobinopathy (alpha, beta and delta-beta-thalassaemia) and qualitative haemoglobinopathy (homozygygous and heterozygous HbS, HbE, HbD trait and HbJ trait). This procedure was performed after collecting the sample and only data of these tests were collected.

STATISTICAL ANALYSIS

The statistical analysis was performed using Microsoft Excel 2010. The descriptive data is presented as mean and the percentage.

RESULTS

Total 704 laboratory requests for Hb Variant analysis data were obtained in the period from August 2019 to July 2021. Out of 704 laboratory request, 585 was from children <12 years of age and 119 was from parental screening. There were one 164 abnormal chromatograms (85 from children less than 12 years of age and 79 were from parenteral screening). Of these, 91 (55.5%) were females and 73 (44.5%) were males. Out of 164 abnormal chromatograms, 97 (59.15%) were beta-thalassaemia trait, 18 (10.98%) were beta-thalassaemia major, 13 (7.93%) were sickle cell trait, 2 (1.22%) were sickle cell disease, 21 (12.80%) were HbE trait, 2 (1.22%) were homozygous HbE, 2 (1.22%) were HbD trait, 3 (1.83%) were HPFH/Delta beta-thalassaemia, 2 (1.22%) were HbJ trait, 1 (0.61%) were HbE beta-thalassaemia, 1 (0.61%) were sickle-beta-thalassaemia and 2 (1.22%) were alpha thalassaemia. [Table/Fig-1] shows the distribution of the haemoglobin (Hb) disorders in our study subjects.

Disorders	Total	Percentage (%)	(Children <12 years) Percentage (%)	(Subjects >12 years) Percentage (%)
Beta-thalassaemia trait	97	59.15	42 (25.61%)	55 (33.54%)
Beta-thalassaemia major	18	10.98	18 (10.98%)	-
Sickle cell trait	13	7.93	5 (3.05%)	8 (4.88%)
Sickle cell disease	2	1.22	2 (1.22%)	-
HbE trait	21	12.80	9 (5.49%)	12 (7.32%)
Homozygous HbE	2	1.22	1 (0.61%)	1 (0.61%)
HbD trait	2	1.22	1 (0.61%)	1 (0.61%)
HPFH/Delta beta-thalassaemia trait	3	1.83	2 (1.22%)	1 (0.61%)
HbJ trait	2	1.22	1 (0.61%)	1 (0.61%)
HbE beta-thalassaemia	1	0.61	1 (0.61%)	-
Sickle-beta-thalassaemia	1	0.61	1 (0.61%)	-
Alpha thalassaemia	2	1.22	2 (1.22%)	-

[Table/Fig-1]: Distribution of haemoglobin disorders in study subjects (N=164).

The lowest average Hb of 5.9 g% was seen in beta-thalassaemia major and the highest average Hb of 12.6 g% was seen in HbJ trait. The lowest average MCV of 65.9 was seen in beta-thalassaemia trait and highest average MCV of 84.7 was seen in HbD trait. The lowest average of MCH and MCHC was observed in alpha thalassaemia. The lowest average RBC count of 2.5 was seen in beta-thalassaemia major and the highest average RBC count of 5.6 was seen in beta-thalassaemia trait. The highest percentage of HbF of 69.1% is noted in beta-thalassaemia major and lowest average HbF of 0.9 % was noted in alpha thalassaemia. The highest average HbA of 81.5% was noted in beta-thalassaemia trait and lowest average HbA of 3%

was noted in HbE beta-thalassaemia. The highest average HbA2 of 82.3% was noted in homozygous HbE because HbE co-elutes with HbA2 and lowest average HbA2 of 1.4% was noted in alpha thalassaemia. The highest average HbA1a of 9.5% was seen in α-thalassaemias and highest average HBA1b of 11.5% was noted in beta-thalassaemia major. The HBJ trait had an average P3 peak of 24.9% because HbJ elutes in P3 peak. The abnormal Hb such as HbS and HbD are seen as S-Window and D-window respectively. The highest percentage of abnormal Hb (HBS-79%) was seen in sickle cell disease. [Table/Fig-2] shows the average haematological parameters and quantitative Hb in study subjects.

Disorder	No. of cases	Hb (g%)	MCV (fl)	MCH (pg)	MCHC (g/dL)	RDW %	RBC count	HbF %	HbA %	HbA2 %	Abnormal Hb %
Beta-thalassaemia trait	97	11.4	65.9	21.1	31.4	16.8	5.6	1.2	81.5	4.9	-
Beta-thalassaemia major	18	5.9	67.9	25.3	33.6	33.5	2.5	69.1	7.8	3.4	A1b- 11.5
Sickle cell trait	13	11.2	79.4	29.5	34.0	13.3	4.7	1.5	55.0	3.2	31.0 (S-window)
Sickle cell disease	2	7.4	75	22.3	30.3	23.6	3.6	11.3	3.9	2.0	79.0 (S-window)
HbE trait	21	10.9	70.1	21.4	32.8	16.4	4.4	1.1	60.5	26.1	-
Homozygous HbE	2	9.2	68.4	20.8	30.7	17.5	4.5	4.1	3.8	82.3	-
HbD trait	2	10.7	84.7	25.8	34.0	16.4	3.9	1.0	59.2	2.9	33.7 (D window)
HPFH/Delta beta-thalassaemia trait	3	11.6	72.3	23.9	34.9	18.2	4.9	20.7	62.2	2.0	-
Hb J trait	2	12.6	83.4	29.7	35.1	12.1	4.2	1.0	67.8	2.2	24.9 (P3)
HbE beta-thalassaemia	1	7.1	69.8	21.4	31.4	25.6	3.9	35.9	3.0	55.4	-
Sickle-beta-thalassaemia	1	8.8	75.8	21.6	31.7	17.4	4.0	15.3	7.6	4.8	63.5 (S-window)
Alpha thalassaemia	2	8.6	68.4	20.1	29.6	20.3	4.1	0.9	72.1	1.4	A1a- 9.5 A1b- 7.3

[Table/Fig-2]: RBC indices and haemoglobin fractions in various haemoglobinopathies (N=164).
Values presented as average values

DISCUSSION

In this study, Beta-thalassaemia trait was the commonest abnormality in both children <12 years as well as adults. This is in concordance with other studies which reported beta-thalassaemia trait to be the commonest disorder [16]. A cut-off of HbA2 over 3.9% was taken for diagnosis of beta-thalassaemia trait. Characteristic haematological findings in a typical case of beta-thalassaemia trait include microcytosis with raised Red Blood Cells (RBC) counts. The mean HbA2 was 4.9% in beta-thalassaemia traits which is similar to other studies [17,18]. In comparison to this study, the number of Beta-thalassaemia trait cases was found to be less in the studies carried out by Sachdev R et al., (number of beta-thalassaemia trait cases=8.9%) and Bhalodia JN et al., (number of beta-thalassaemia trait cases=5.2%) on the Indian population [16,19]. Beta-thalassaemia major is the second commonest abnormality in children less than twelve years of age but HbE trait is the second commonest in adults. In beta-thalassaemia major, the average Hb was found to be 5.9 g% with average HBF of 69.1%. In our study, sickle cell trait showed average HbS of 31% as compared to 63.5% in sickle beta-thalassaemia but homozygous HbS reported average HbS of 79%.

In this study, HbE trait showed average HbA2 of 26.1% as compared to 55.4% in HBE beta-thalassaemia but homozygous HbE reported average HbA2 of 82.3%. By our method, HbE elutes in the HbA2 window and hence the levels of HbA2 could not be evaluated. The HbA2 can also be elevated in other disorders like beta-thalassaemia trait, Hb Lepore and HbD Iran as explained by Dass J et al., [20]. Before labelling a patient with HbE other causes are ruled out based on the amount of abnormal Hb and HbF levels [20].

On comparing compound heterozygous for HbS beta-thalassaemia and HbE beta-thalassaemia disorders, it is noted that the percentage of abnormal/variant Hb was higher in HbS beta-thalassaemia than HbE beta-thalassaemia. But on comparing homozygous HbE and sickle cell disease, the same showed a higher abnormal/variant Hb in homozygous HbE (82.3%) when compared to sickle cell disease where it was 79%. This is in concordance with Chandrashekar V and Soni M, who reported a higher abnormal Hb in homozygous HbE (90.8%) when compared to homozygous HbS where it was 79.9% [21].

The A1a, A1b, A1c and the P3 are minor Hb formed by post-translational modifications of the globin chains. In our study, the highest average HbA1a of 9.5% was seen in alpha-thalassaemias where golf ball inclusions were identified by supravital stains. The highest average HbA1b of 11.5% was noted in beta-thalassaemia major along with increased HbF of 69.1% is noted. If the sample contains greater than 16.5% HbF, the HbF may elute in HbA1b or LA1c/CHb window. The peak within the P3 retention time window is usually due to the number of alpha and/or beta chain variants. The HBJ variant elutes in P3 window. In our study, HBJ trait was identified in two cases who had an average P3 peak of 24.9%.

The HbF contains two alpha and two gamma globin chains (α2 γ2). The HbF levels decline to less than 1% a few months after birth. However, persistence of high levels of HbF in adults is seen in conditions like delta-beta-thalassaemia and HPFH [22]. Delta-beta-thalassaemia is relatively a rare form of thalassaemia due to decrease in both beta and delta globin chain production. The HPFH trait is a benign condition in which the foetal Hb production continues into adulthood. HPFH is characterised by normal red cell morphology normal MCV and MCH levels whereas in delta-beta-thalassaemia trait, the red cell morphology is abnormal, MCV and MCH levels are reduced. The red cell morphology of all the three cases reported in our study showed anisopoikilocytosis with mild microcytic hypochromic picture along with decreased MCV and decreased MCH pointing towards heterozygous delta-beta-thalassaemia rather than HPFH but definitive diagnosis requires genetic analysis [23].

Limitation(s)

The study has certain limitations. The confirmation of Hb variants and haemoglobinopathies by genetic analysis was not done. The study does not reflect the exact prevalence of haemoglobinopathies in Southern India since this is hospital based study.

CONCLUSION(S)

From the present study, beta-thalassaemia trait and beta-thalassaemia major were found to be the first and second most prevalent haemoglobinopathies in children below 12 years. This data suggests the importance of premarital and antenatal screening procedures that can help in reducing the possibility of such haemoglobinopathies in the future generation, suffering and burden of disease to the family and society.

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PLAGIARISM CHECKING METHODS: [Jain H et al.]

- Plagiarism X-checker: Nov 30, 2021
- Manual Googling: Jan 27, 2022
- iThenticate Software: Feb 15, 2022 (20%)

ETYMOLOGY: Author Origin

AUTHOR DECLARATION:

- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? Yes
- For any images presented appropriate consent has been obtained from the subjects. Yes

Date of Submission: **Nov 27, 2021**
Date of Peer Review: **Jan 06, 2022**
Date of Acceptance: **Feb 01, 2022**
Date of Publishing: **Mar 31, 2022**