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Original Article

Pathology Section

Incidence of Thalassaemia in Jammu and Kashmir, India

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ABSTRACT

Introduction: Haemoglobinopathies are common genetic disorders of haemoglobin which occur due to abnormal production or structure of the haemoglobin molecule. They pose a considerable health problem in India and contribute significantly to morbidity and mortality. Cation Exchange-High Performance Liquid Chromatography (CE-HPLC) is a very simple and a precise method for quantifying HbA2, HbF and other variant haemoglobins.

Aim: The present study was undertaken to study the spectrum of haemoglobinopathies in patients reporting to a Tertiary Health Care Centre in Jammu, India.

Materials and Methods: Present study was a prospective study carried on 2356 samples from August 01, 2019-January 31, 2020. Sysmex XP 100 was used to obtain RBC indices and Biorad D10 was used to perform CE-HPLC. To identify the variant haemoglobins their retention times, percentages and peak characteristics are taken into consideration. Continuous variables were expressed as mean±SD. Categorical variables were expressed as percentages.

Results: A total of 2356 cases were evaluated during the study period. Haemoglobinopathy was observed in 5.89% of the cases with β-thalassaemia trait was the most common abnormality (3% of cases). The most common variant haemoglobin encountered in the study population was HbD (Punjab) trait. Molecular analysis was advised in 14 cases with borderline increase in HbA2 levels to rule out silent mutations.

Conclusion: A routine antenatal screening of total population is mandatory to detect the high frequency (5.89%) of haemoglobinopathies. An accurate diagnosis can be made in most of the cases by haematological parameters, CE-HPLC chromatograms, cascade screening for haemoglobinopathies and spouses of antenatal cases positive for haemoglobinopathy.

Keywords: Anaemia, β-thalassaemia, Haemoglobinopathies, High performance liquid chromatography

INTRODUCTION

Haemoglobinopathies are common genetic disorders of haemoglobin which occur due to abnormal production or structure of the haemoglobin molecule [1]. They pose a considerable health problem in India and contribute significantly to morbidity and mortality. According to World Health Organisation (WHO) about 7% population all around the world are carriers of a globin gene mutation and around 60,000 thalassemia babies are born all over the world every year [2]. Around 60-80 million people in the world are β-thalassaemia trait carriers. On an average frequency of β-thalassaemia carriers in the Indian population is 3-4%, which leads to an overall estimate of 30-40 million carriers among a population of over a billion people [2-4]. The prevalence of HbD is high amongst the people of North India i.e. punjabi population [4], whereas HbE is more prevalent in eastern region of India [1] and in Orissa, HbS is prevalent. Most of the haemoglobinopathies require expert services for the treatment and follow-up and for detection and prevention of the long term complications. An overall assessment of the carrier frequency helps in forming the strategies for the region and also in sensitising the medical fraternity. Most of the tertiary care centres do antenatal screening for hemoglobinopathies in routine practice [1]. An algorithmic approach helps in formulating the laboratory diagnosis of thalassemia syndromes and various variant haemoglobins. These include a detailed family history, Complete Blood Count (CBC) along with RBC morphology and methods for protein analysis like CE-HPLC. To diagonose problematic cases, aid of molecular and family studies was undertaken. This study was done to assess the spectrum of haemoglobinopathies in Jammu & Kashmir, India.

MATERIALS AND METHODS

The present prospective study was conducted in the Department of Pathology of Government Medical College, Jammu, Jammu and Kashmir, India for a period of six months from August 01, 2019-January 31, 2020. A total number of 2356 patients referred by various practitioners for the diagnosis of haemoglobinopathies were included in the study. The inclusion criterias were as follows: all antenatal cases which were screened for hemoglobinopathies, investigation for anemia, hemoglobinopathies positive spouses of antenatal cases, family members of index cases were screened and chromatograms abnormalities encountered during HbA1c estimation. No absolute exclusion criterias were there, but, patients having history of recent blood transfusion were deferred for four-six weeks.

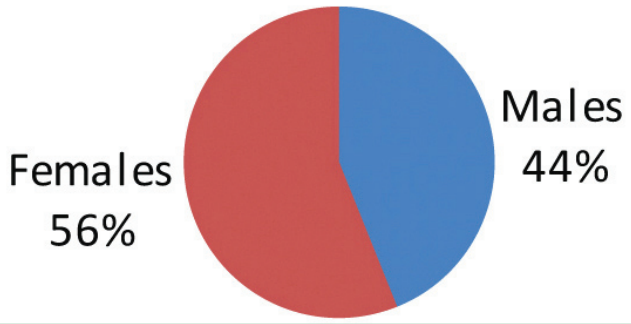
Intravenous blood samples were collected by disposable syringes in lavender top vacutainer which contains Ethylene Diamine Tetra Acetic Acid (EDTA) as anticoagulant. Samples of the patients requiring frequent blood transfusions were taken prior to the next transfusion. Sysmex XP100 was used to obtain RBC indices and Biorad D10 was used for performing CE-HPLC. Area percentage, retention time and peak characteristics was used to identify the variant hemoglobin. In selected number of cases peripheral blood film examination, reticulocyte count and sickling test were done. β-thalassemia trait is detected if area percentage of HbA2 levels amount to 4%-9% while further evaluation was recommended for borderline cases (3.6%-3.9%). In all the antenatal cases which were positive for haemoglobinopathy and those with borderline HbA2 (3.5%-3.9%) screening of spouse for HbA2 levels/variant haemoglobins was advised.

STATISTICAL ANALYSIS

The collected data were entered and analysed using Microsoft excel. Continuous variables were expressed as mean±SD. Categorical variables were expressed as percentages (%).

RESULTS

During the study period, 2356 cases were analysed. Data was recorded and routine hematological investigations were carried out. Out of total 2356 cases, 1321 cases (56.06%) were females and 1035 cases (43.93%) were males [Table/Fig-1]. Female to male ratio is 1.27:1. There were 2217 cases (94.1%) with normal High Performance Liquid Chromatography (HPLC) pattern while 139 cases (5.89%) had one or the other form of haemoglobinopathies.



[Table/Fig-1]: Sex distribution of the study subjects.

[Table/Fig-2] represents the spectrum of haemoglobinopathies encountered during the study. β-thalassaemia minor was the most common form of haemoglobinopathy (3%), followed by α-thalassaemia trait (0.84%), HbD Punjab trait and borderline HbA2 (0.59%), HbE trait (0.25%), β-thalassaemia major (0.21%), HbS trait (0.12%) and HPFH trait, HbQ India, compound heterozygous β-thalassaemia/HbD Punjab 0.08% each.

Type of manifestations	No. of patients	Percentage of patients
Normal	2217	94.1
β-thalassaemia trait	72	3.05
β-thalassaemia major	4	0.21
HbD Punjab trait	14	0.59
HbE trait	6	0.25
HPFH trait	2	0.08
HbS trait	3	0.12
HbQ India	2	0.08
Compound heterozygous β-thalassaemia/ HbD Punjab	2	0.08
Borderline HbA2	14	0.59
α-thalassaemia trait	20	0.84

[Table/Fig-2]: Spectrum of haemoglobinopathies.
HPFH: Hereditary persistence of foetal haemoglobin

[Table/Fig-3] depicts the haematological parameters in different groups of haemoglobinopathies. Hb value was very low in case of β-thalassaemia major (4.6±1.6). In cases of β-thalassaemia trait RBC count was raised.

DISCUSSION

Haemoglobinopathies and thalassemias are one of the major health problem of our country. In Indians, many haemoglobin variants are present and their incidence varies with geographical distribution [1,4,5]. The present study includes individuals predominantly from Jammu & Kashmir followed by Punjab. HPLC is a rapid method for screening of haemoglobin variants and is based upon interchange of charged groups on the ion exchange material with charged groups on the haemoglobin molecule [6]. CE-HPLC has a high sensitivity and specificity and helps in identification of haemoglobins which have the similar mobility on electrophoresis [7]. It can even detect 0.1% of total haemoglobin in 0.5 µl of whole blood [7]. It gives a precise estimate of haemoglobin fractions like HbA, HbF, HbA2, HbS, HbD and HbC [7].

There were 5.89% of cases of haemoglobinopathy while 94.1% of study subjects had normal pattern of HPLC. The commonest abnormality found in our study was β-thalassemia trait (3.05%). β-thalassaemia trait has been reported in almost all population groups. Nayyar S et al., reported prevalence of β-thalassaemia trait in Uttarakhand to be 2.82% [8]. Madan N et al., reported 4.05% cases of β-thalassemia trait in Northern and Western India [9]. Many other studies from different regions have found that the prevalence of β-thalassemia trait ranges from 2.78% to 8.9% [10,11] [Table/Fig-4]. In different parts of India, incidence of haemoglobin variants varies based on geographic distribution. For example, HbD is most common in Northern part of India. Sachdev R et al., reported incidence of HbD trait in 0.5% of the cases in their study [10]. In present study, the incidence of HbD trait is 0.59%. Nayyar S et al., reported 0.55% cases of HbD trait [8]. Mondal SK and Mandal S, reported 0.09% HbD trait cases from eastern India [Table/Fig-4] [12].

In India, it is seen that the incidence of HbE trait is higher in people of eastern India as compared to the other parts of India. A study done by Mondal SK and Mandal S on 119336 cases from Eastern part of India observed HbE trait in 3.02% and HbE disease in 0.13% of total cases respectively [12]. We found HbE trait in only 0.25% of cases. Out of six cases of HbE traits in our study 4 of them were immigrants from east India. Another study done by Jain BB et al. conducted in Eastern India also found β-thalassemia trait to be one of the commonest abnormality followed by HbE trait [1]. Study done by Baruah MK et al. on 9000 patients in upper Assam region and Ghosh N et al. on Eastern Indian population found HbE trait to be the most common hemoglobinopathy followed by HbE disease [13,14] [Table/Fig4]. The incidence of sickle cell gene was highest in Orissa followed by Assam, Madhya Pradesh, Uttar Pradesh, Tamil Nadu and Gujarat [15]. HbS trait was found in 0.12% of total cases in our study. Nayyar S et al., reported similar prevalence of HbS trait i.e., 0.15% [8]. Raina K et al., reported the spectrum of haemoglobinopathies prevalent in descending order as 13.99% β-thalassaemic trait, followed by 6.26% α-thalassaemic trait, 4.6% elevated foetal haemoglobin, 2.57% false elevation of haemoglobin A2 etc., they further added that high prevalence of

Haemoglobinopathies	Hb	RBC count	PCV	MCV	MCH	RDW
β-thalassaemia trait	10.5±2.0	5.1±0.9	34.4±7.7	66.1±8.8	19.9±2.7	16.7±3.7
β-thalassaemia major	4.6±1.6	1.9±0.8	13.8±5.9	72.8±2.3	23.3±3.2	36.6±5.2
HbD punjab trait	11.8±1.6	4.1±1.6	35.5±4.2	75.3±7.6	25±5.4	12.5±2.0
HbE trait	9.4±5.4	3.5±2.5	30.1±18.3	89.9±13.4	28.8±5.7	17.05±4.1
HbQ India	12.6±1.7	4.4±0.6	37.4±4.4	84.7±6.0	28.7±2.7	12.5±2.5
HbS trait	7.3±1.9	2.6±0.7	23.6±6.5	91.2±5.07	28.3±2.0	22.1±1.8
Compound heterozygous β-thalassaemia/HbD Punjab	6.9±1.3	3.46±1.6	20.6±3.0	69.6±1.6	16.9±4.3	18.9±2.4
Borderline HbA2	10.9±3.8	4.3±1.5	35.1±12.6	81.0±3.0	25.4±1.0	15.8±5.9
Alpha thalassaemia trait	6.4±1.7	4.04±0.8	23.3±5.6	57.4±13.7	16.1±3.5	22.1±5.1

[Table/Fig-3]: Haematological parameters in different group of haemoglobinopathies.
Hb: Haemoglobin; RBC: Red blood cell; PCV: Packed cell volume; MCV: Mean corpuscular volume; MCH: Mean corpuscular hemoglobin; RDW: Red cell distribution width

Study	Year of publication	No. of study subjects	No. of cases of β-thalassaemia trait (%)	No. of cases of HbS trait (%)	No. of cases of HbD Punjab trait (%)	No. of cases of HbE trait (%)
Madan N et al., [9]	2010	11090	4.05%	0.1%	0.9%	0.02%
Sachdev R et al., [10]	2010	2600	8.9%	0.1%	0.5%	0.26%
Mondal SK and Mandal S [12]	2016	119336	4.60%	0.38%	0.09%	3.02%
Raina K et al., [16]	2017	543	13.99%	0.18%	0.18%	0.37%
Nayar S et al., [8]	2017	8144	2.82%	0.15%	0.55%	0.42%
Present study	2020	2356	3.05%	0.12%	0.59%	0.25%

[Table/Fig-4]: Comparison of previous studies with the present study [8-10,12,16].

haemoglobinopathies in Jammu division makes the disease a major public health problem in their population [16]. In present study, the prevalence of Hereditary Persistence of Foetal Haemoglobin (HPFH) trait was 0.08%. Mondal SK and Mandal S in their study found prevalence of HPFH 0.12% [12].

HPLC is a sensitive, specific, and accurate technique for the identification and quantification of different haemoglobin fractions. However, HPLC has its own limitations as it is unable to detect α thalassaemia and normal HbA2 β-thalassaemia. While interpreting chromatograms, nutritional anaemias should always be considered vital. Iron deficiency anaemia may sway a low level of HbA2, thus camouflaging β-thalassaemia trait. Similarly, cobalamin or folate deficiency may elevate HbA2 level, which can tip to a false diagnosis of thalassaemia trait [10]. HPLC must always be followed by molecular studies, such as Polymerase Chain Reaction (PCR), Amplification Refractory Mutation System (ARMS), and other similar tests to determine specific mutations responsible for the haemoglobin disorder.

Limitation(s)

Patients having borderline HbA2 (3.5%-3.9%) were not screened further to rule out nutritional deficiency or silent mutations. Diagnosis of α thalassaemia was presumptive. In future, studies that would compensate for these shortcomings may be undertaken to obtain more credible inferences.

CONCLUSION(S)

This study can be foreseen as representative of overall prevalence of haemoglobinopathies and thalassaemia in the union territory of Jammu and Kashmir, India as the study subjects represented different parts of the Union Territory (UT). A reasonably high frequency (5.89%) of haemoglobinopathies warrants a routine antenatal screening of total population. An effective strategy must be employed for preventing the progression of the disease. CE-HPLC is a reliable tool in the diagnosis and further management of haemoglobinopathies and thalassemias. Studies such as present study are important for better understanding the social, demographic and medical attributes of thalassaemia in this region so as to develop better health management strategies for the future.

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