Original Article

DOI: 10.7860/JCDR/2017/31731.10947

Pathology Section

Spectrum of Haemoglobinopathies: A Hospital Based Study in Uttarakhand

SHIVANI NAYAR[1], SEEMA ACHARYA[2], RAJIV ACHARYA[3], SANJEEV KISHORE[4], BRIJESH THAKUR[5]

ABSTRACT

Introduction: Haemoglobinopathy is a worldwide inherited problem as recognized by WHO and care of affected patients incurs heavy expense on the limited resources of developing countries. There is a need for prevention of births of children with clinically significant haemoglobinopathies by population screening. Cation Exchange-High Performance Liquid Chromatography (CE-HPLC) has emerged to be a simple and precise method to quantify HbA_2, HbF and other variant haemoglobins with certain limitations. Most of the variant haemoglobins can be identified by their retention times, percentages and peak characteristics.

Aim: The present study was undertaken to assess the prevalence and spectrum of various haemoglobinopathies in patients reporting to a tertiary health care centre in Uttarakhand, India.

Materials and Methods: This was a prospective study conducted on 8144 samples. RBC indices were obtained by sysmex XP 100. CE-HPLC was performed on Biorad D10. The variant haemoglobins were identified on the basis of their percentages, retention times and peak characteristics. Peripheral blood film, reticulocyte count, HbH inclusion and sickling test were done in selected cases. Continuous variables were expressed as mean±SD. Categorical variables were expressed as percentages.

Results: Antenatal population formed the bulk of the 8144 cases enrolled in this study. Haemoglobinopathy was seen in 5.9% of the cases with β thalassaemia trait being the commonest abnormality (2.82% of cases). HbD (Punjab) trait was the commonest variant haemoglobin encountered in the study population. There was a significant difference in percentages of variant fractions between compound heterozygotes and variant traits.

A presumptive diagnosis of alpha thalassaemia trait was rendered based on RBC indices, iron profile and chromatogram study. Molecular studies were recommended in 81 cases with borderline increase in HbA_2 levels to rule out silent mutations.

Conclusion: A reasonably high frequency (5.9%) of haemoglobinopathies warrants a routine antenatal screening of total population. An accurate diagnosis can be made in majority of cases by haematological parameters, CE-HPLC chromatograms, cascade screening for haemoglobinopathies and spouses of antenatal cases positive for haemoglobinopathy.

Keywords: Beta thalassaemia trait, Haemoglobin variants, High performance liquid chromatography

INTRODUCTION

Haemoglobinopathies are hereditary disorders of haemoglobin occurring as a result of abnormal production or structure of the haemoglobin molecule [1].

Thalassaemia results from decreased synthesis of globin chains while haemoglobin variants are a result of abnormal structure of the same [2].

The World Health Organization estimates that about 7% of the world population are carriers of a globin gene mutation and that every year 60,000 thalassaemia babies are born all over the world [2].

India is a significant reservoir of β thalassaemia and various allelomorphs of HbβA with variable geographical distribution. The frequency of total haemoglobinopathies in India is reported to be 4.2% with an estimated 30 million carriers and 15,000 infants with major haemoglobinopathies [2].

A high prevalence of HbD has been reported from the North in the Punjabi population [3], HbE from eastern region of India [1] and HbS amongst people of Orissa [4].

With advances in molecular studies, more than 1200 mutant alleles have been identified with a variable geographical distribution of haemoglobin variants and thalassaemia mutations [5].

Many haemoglobinopathies, both transfusion dependent and independent require expert services for treatment and follow up for detection of long term complications. In low resource countries like ours, prevention of birth of such children is preferable. An assessment of carrier frequency helps in formulating regional strategies and sensitizing the medical fraternity. Antenatal screening is routinely being followed in most of the tertiary hospitals [1].

An algorithmic approach helps in achieving laboratory diagnosis of thalassaemia syndromes and variant haemoglobins. These include detailed family history, CBC with RBC morphology and protein analytic methods like CE-HPLC. Molecular studies and family studies are required in certain problematic cases.

This study was undertaken to see the spectrum of haemoglobinopathies diagnosed at our centre in Uttarakhand, India.

MATERIALS AND METHODS

This prospective study was carried out in the section of haematology, Department of Pathology of Shri Guru Ram Rai Institute of Medical and Health Sciences, Dehradun from October 2014 to October 2016. The study was vetted and approved by Institutional Ethics Committee.

The study included all antenatal cases screened for haemoglobinopathies, cases investigated for anaemia, spouses of antenatal cases positive for haemoglobinopathies, cascade screening of family members of index cases and abnormal chromatograms found during HbA1c estimation. Written consent was taken from all included subjects.

There were no absolute exclusion criteria, but patients with history of recent transfusion were deferred for 4-6 weeks. In patients requiring more frequent blood transfusions, samples were taken just before the next transfusion.

RBC indices were obtained by Sysmex XP100 and CE-HPLC was performed on Biorad D10. The variant haemoglobins were identified on the basis of their percentage, retention times and peak characteristics. PBF, reticulocyte count, HbH inclusion and sickling test were done in selected cases. Variant haemoglobins were not confirmed by an alternative method though the same was recommended.

Patients with HbA_2 levels of 4%-9% were diagnosed as β thalassaemia trait while further evaluation was recommended for borderline cases (3.6%- 3.9%).

Spouse screening for HbA_2 levels/variant haemoglobins was advised in all antenatal cases positive for haemoglobinopathy and those with borderline HbA_2 (3.5%-3.9%).

STATISTICAL ANALYSIS

T-test was applied using SPSS software version 20.0 for significance of association between continuous variables. The p-value<0.01 was considered as statistically significant.

RESULTS

A total of 8144 cases were evaluated during the study period. All the relevant data was recorded on the performa and routine haematological investigations were analysed. Out of total 8144 cases, 7106 cases (87.25%) were females and 1038 cases (12.75%) were males constituting female to male ratio of 6.8:1. There were 7663 cases (94.1%) with normal HPLC pattern while 481 cases (5.9%) had abnormal chromatograms.

Distribution pattern of various haemoglobinopathies and mean fraction values of variant haemoglobins in cases with abnormal chromatograms are presented in [Table/Fig-1,2] respectively. Compound heterozygous states for HbE/β thalassaemia and HbD/β thalassaemia were found in this study. Therefore, difference between percentages of variant haemoglobin fractions of compound heterozygotes and traits was studied and found to be statistically significant (p<0.01) [Table/Fig-3].

Percentages of Hb fractions become significant during analysis of variant haemoglobins especially when they coelute.

CE-HPLC results can further be fine tuned by taking retention times and peak characteristics into consideration during analysis. However, parental study becomes imperative in differentiation.

Type of Haemoglobinopathy	Number	Percentage
β Thalassaemia Trait	230	2.82
Homozygous thalassaemia	12	0.15
HbH Disease	2	0.02
HbD (Punjab) Trait	45	0.55
Homozygous HbE	5	0.06
HbE Trait	34	0.42
HbS Trait	12	0.15
HPFH Homozygous	3	0.03
HPFH Trait	4	0.05
HbQ India	2	0.02
HbD Iran (Trait)	3	0.03
HbJ Meerut	1	0.01
Compound heterozygous HbE/β Thalassaemia	5	0.06
Compound Heterozygous HbD/β Thalassaemia	4	0.05
Borderline HbA_2	81	0.99
Presumed Alpha Thalassaemia Traits	38/66	57.5

[Table/Fig-1]: Distribution of cases according to the type of haemoglobinopathy.

Variables	Mean±SD (in percentage)
Haemoglobin E Fraction In HbE Trait	31.7±3.54
Haemoglobin D Fraction In HbD (Punjab) Trait	33.3±3.24
Haemoglobin S Fraction In HbS Trait	32.8±4.59
Haemoglobin Q Fraction In HbQ India	13.5±1.69
Haemoglobin J Fraction In HbJ Meerut	16.8
Haemoglobin E Fraction In HbE/β Thalassaemia	64.4±3.43
Haemoglobin D Fraction In HbD/β Thalassaemia	56.35±3.05

[Table/Fig-2]: Mean of haemoglobin fraction in HbE trait, HbD Trait, HbS Trait, HbQ India, HbJ Meerut and compound heterozygotes.

% Variant Haemoglobin Fraction	Traits	Compound Heterozygous (Trait/β Thal)	p-value
HbD Fraction	33.3%±3.24	56.35%±3.05	p<0.01
HbE Fraction	31.7%±3.54	64.4%±3.43	p<0.01

[Table/Fig-3]: Comparison of haemoglobin fraction of compound heterozygotes vs traits.

DISCUSSION

Haemoglobinopathies and thalassaemias are a major health burden in our country. Many haemoglobin variants are present in our population. The incidence of haemoglobin variants vary with geographical distribution [1,3,4]. The present study included individuals predominantly from Uttarakhand followed by Western Uttar Pradesh.

High Performance Liquid Chromatography (HPLC) is a rapid method of screening for haemoglobin variants and depends upon the interchange of charged groups on the ion exchange material with charged groups on the haemoglobin molecule [6]. CE-HPLC is highly sensitive and specific and helps in identifying haemoglobins which have the same mobility on electrophoresis [7]. It can detect 0.1% of total haemoglobin in 0.5 µl of whole blood [7]. It gives a precise estimation of haemoglobin fractions like HbA, HbF, HbA_2, HbS, HbD and HbC [7]. CE-HPLC has certain disadvantages like change in retention time with change in temperature of the column and pH of buffers [7]. Moreover, some different haemoglobins coelute with similar retention times [7].

In studies by Rao S et al., and Khera R et al., it was established that CE-HPLC is a rapid and reliable method of evaluation of thalassaemia syndromes and variant haemoglobins. A definitive diagnosis could be offered by CE-HPLC alone in the majority of cases using retention times, proportion of total haemoglobin and peak characteristics. Difficult cases warranted family studies and Hb electrophoresis [8,9]. In our experience too, family studies were required in a few cases especially compound heterozygotes.

A total of 87.25% of the total 8144 cases were women attending antenatal clinic and hence the preponderance of female subjects of reproductive age group in this study. Studies conducted in India by Rao S et al., and Dolai TK et al., also show similar female majority [8,10].

There were 5.9% of cases of haemoglobinopathy while 94.1% of cases had normal HPLC pattern. β thalassaemia trait was the commonest abnormality found in our study (2.82%). β thalassaemia trait has been reported in almost all population groups. Madan N et al., reported prevalence of β thalassaemia trait in Northern and Western India to be 4.05%. Various other studies from different regions have found prevalence of β thalassaemia trait ranging from 2.78% to 8.9% [9,11-13].

The incidence of variant haemoglobins is different in different parts of India. HbD is prevalent in Northern part of India. Sachdev R et al., in their study on 2600 patients from north India found β thalassaemia trait to be the commonest abnormality and HbD trait in 0.5% of the cases [12]. We found the incidence of HbD trait to be 0.55%. Khera R et al., in their study also found an incidence of HbD to be 0.5% [9].

A study by Mondal SK and Mandal S on 119336 cases from Eastern India found haemoglobinopathies in 12.17% of cases while 87.63% of cases showed normal HPLC pattern. Antenatal cases comprised 55.6% of their study population and β thalassaemia trait was found to be the commonest abnormality. Since their study was conducted in Eastern India, they found HbE trait in 3.02% and HbE disease in 0.13% of total cases respectively [14]. We found HbE trait in only 0.40% of cases. Two thirds of HbE traits in our study were immigrants from West Bengal. Another study by Jain BB et al., conducted in Eastern India also found β thalassaemia trait to be the commonest abnormality followed by HbE trait. Studies by Baruah MK et al., on 9000 patients in upper Assam region and Ghosh N et al., on an Eastern Indian population found HbE trait to be the commonest abnormality followed by HbE disease [15,16]. However, a lower incidence of HbE trait and HbE disease was reported by studies conducted in North Western India [9,12].

The highest frequency of sickle cell gene in India is reported in Orissa followed by Assam, Madhya Pradesh, Uttar Pradesh, Tamil Nadu and Gujarat [17]. HbS trait was found in 0.15% of total cases in our study. Four of the six cases of HbS trait in this study belonged to Uttarakhand. Similar prevalence has been reported by Sachdev R et al., (0.2%, including one case of SS and two cases of HbS/β thalassaemia) and Khera R et al., (0.2%, including one case each of SS, S trait and HbS/β thalassaemia) [9,12].

Balgir RS in his study reported that sickle cell disorder was present in 3-4 million in Orissa of which one-fourth belonged to scheduled tribes. He found that 19.32% people in Orissa suffered from haemoglobinopathies. Of these 13.2% had sickle cell disorders (sickle cell trait-8%, sickle cell disease- 4%, sickle cell thalassaemia-1.2% [18].

Sickle cell disorder is also found among the tribal groups in Gujarat. Bhukhanvale DS et al., in their study reported a prevalence of 14% in the Dhodia Patel community. This is the third largest tribal group in Gujarat [19].

Urade BP in his study has reported the incidence of HbS among different communities in Eastern Maharashtra [20]. A high frequency was found among the Bais, the Pardeshi, the Pardhan and the Marar communities. Sickle cell trait was the most common haemoglobinopathy found followed by sickle cell disease in these people.

Three cases were proposed to be HbD Iran (trait) with the variant haemoglobin coeluting with A_2 and comprising 46%, 45.4% and 45.1% of total haemoglobin respectively. These were provisionally diagnosed by clinicohaematological parameters, haemoglobin fraction percentage and elution time as was done in other studies [8,9].

A variant Hb was detected incidentally during HbA1C evaluation in P3 window which comprised 16.8% of total haemoglobin. This was suggested to be HbJ Meerut with a recommendation for confirmation by alkaline electrophoresis. Baruah MK et al., and Srinivas U et al., too have reported almost similar incidence [15,21].

Two cases showed variant haemoglobin eluting at 4.43 and 4.44 minutes comprising 14.7% and 12.3% of total haemoglobin respectively. These had normal RBC indices and were diagnosed as HbQ India (a prevalence of 0.02% in this study). Rao S et al., have reported prevalence of HbQ India to be 0.3% [8].

Five cases which presented as thalassaemia intermedia were diagnosed as compound heterozygotes for HbE/β thalassaemia by HPLC evaluation of parents. Two of these cases were siblings. All the cases were from Uttarakhand. Similar prevalence has been reported by Sachdev R et al., (0.2%). Khera R et al., reported a prevalence of 0.5% (8 cases out of 1500) [9,12].

Compound heterozygotes for HbD/β thalassaemia had mild microcytic hypochromic anaemia with a higher percentage of HbD and a raised HbA_2 level. Homozygotes for Hereditary Persistence of Foetal Haemoglobin (HPFH) could easily be differentiated from β thalassaemia major on evaluation of clinical history and RBC indices and represented 0.03% of our study population. Mondal SK and Mandal S in their study found a prevalence of 0.12% [14].

Serum ferritin was assayed in 139 cases only randomly. Of these evaluated cases, 66 cases had normal HPLC pattern, 38 of which had normal iron stores with microcytosis and hypochromia. Having excluded sideroblastic anaemia and anaemia of chronic disease, these were presumptively diagnosed as α thalassaemia trait. None of these were however confirmed by further molecular assays. There were 81 cases with borderline HbA_2 levels which could not be evaluated further for associated nutritional anaemia, silent mutations or triple alpha gene.

LIMITATION

a) Thalassaemia and haemoglobinopathies can be found in males as well as females. But due to female preponderance in this study, it is gender biased (as most of the subjects were women attending antenatal clinics).

b) Patients with borderline HbA_2 levels were not screened for nutritional deficiency or silent mutations.

c) Diagnosis of α thalassaemia was presumptive.

In future, studies that compensate for these shortcomings may be undertaken to obtain more credible inferences.

CONCLUSION

A reasonably high frequency (5.9%) of haemoglobinopathies warrants a routine antenatal screening of total population. An accurate diagnosis can be made in majority of cases by haematological parameters, CE-HPLC chromatograms, cascade screening for haemoglobinopathies and spouses of antenatal cases positive for haemoglobinopathy.

Thalassaemia is a common genetic disorder in our country. Antenatal screening is helpful in the detection of high risk couples and prevents the birth of thalassaemia major babies.

Antenatal screening plays a vital role in the identification of thalassaemia and other haemoglobinopathies. Antenatal screening helps to reduce morbidity and mortality in later life. Prenatal diagnosis is done when both the prospective parents are identified as thalassaemic carriers. CE-HPLC is a reliable tool in the diagnosis and management of haemoglobinopathies and thalassaemias. CE-HPLC was found to be a reliable methodology to diagnose haemoglobinopathies with a few cases like compound heterozygotes who required parental screening.

REFERENCES

[1] Jain BB, Roy RN, Ghosh S, Ghosh T, Banerjee U, Bhattacharya SK. Screening for thalassaemia and other haemoglobinopathies in a tertiary care hospital of West Bengal: Implications for population screening. Indian J Public Health. 2012;56:297-300.

[2] Chandran P, Laxmi MS, Yadagiri B, Noorjahan M, Rao MN. Biochemical characterization of spectrum of haemoglobinopathies and thalassaemia syndromes- Experience with 689 cases in a tertiary care hospital in South India. Int J Pharm Bio Sci. 2013;4(4):1234-42.

[3] Lukens Jn. The abnormal haemoglobins: General principles. In: Lee GR, Foerster J, Lukens J, Paraskevas F, Greer JP, Rodgers GM, editors. Wintrobe's Clinical Haematology. 10th ed. Baltimore: Lippincott Williams and Wilkins; 1998. Pp. 1329-45.

[4] Balgir RS. Spectrum of haemoglobinopathies in the state of Orissa, India: A ten year cohort study. J Assoc Physicians India. 2005;53:1021-26.

[5] Colaco S, Surve R, Sawant P, Nadkarni A, Ghosh K, Colah R. HbD Punjab/HbQ India compound heterozygosity: An unusual association. Mediterr J Haematol Infect Dis. 2014;6(1):1-47.

[6] Wild BJ, Bain BJ. Investigation of abnormal haemoglobin and thalassaemia. In: Lewis SM, Bain BJ, Bates I, editors. Practical haematology. 11th ed. Philadelphia, PA: Elsevier; 2012. Pp. 301-332.

[7] Tyagi S. High – Performance liquid chromatography in haematology. In: Saxena R, Pati HP, Mahapatra M, editors. De Gruchy's Clinical Haematology in Medical Practice. 6th ed. New Delhi, India: Wiley India; 2013. Pp. 481-90.

[8] Rao S, Kar R, Gupta SK, Chopra A, Saxena R. Spectrum of haemoglobinopathies diagnosed by cation exchange –HPLC and modulating effects of nutritional deficiency anaemias from north India. Ind J Med Res. 2010;132(5):513-19.

[9] Khera R, Singh AT, Khanna N, Gupta N, Dubey AP. HPLC in characterization of haemoglobin profile in thalassaemia syndromes and Haemoglobinopathies: A clinicohaematological correlation. Indian J Haematol Blood Transfus. 2015;31(1):110-15.
[10] Dolai TK, Nataraj KS, Sinha N, Mishra S, Bhattacharya M, Ghosh MK. Prevalence of iron deficiency in thalassaemia minor. Indian J Haematol Blood Transfus. 2012;28(1):7-9.
[11] Madan N, Sharma S, Sood SK, Colah R, Bhatia HM. Frequency of beta thalassaemia trait and other haemoglobinopathies in northern and western India. Indian J of Human Genetics. 2010;16(1):16-25.
[12] Sachdev R, Dam AR, Tyagi G. Detection of haemoglobin variants and haemoglobinopathies in Indian population using HPLC: Report of 2600 cases. Indian J Pathol Microbiol. 2010;53(1):57-62.
[13] Baxi A, Manila K, Kadhi P, Heena B. Carrier screening for beta thalassaemia in pregnant Indian women: Experience at a single centre in Madhya Pradesh. Indian J Haematol Blood Transfusion. 2013;29(2):71-74.
[14] Mondal SK, Mandal S. Prevalence of thalassaemia and haemoglobinopathy in eastern India: A 10-year high performance liquid chromatography study of 119,336 cases. Asian J of Transfusion Medicine. 2016;10(1):105-10.
[15] Baruah MK, Saikia M, Baruah A. Pattern of haemoglobinopathies in upper Assam region of North Eastern India: High performance liquid chromatography studies in 9000 patients. Indian Journal of Pathology and Microbiology. 2014;57(2): 236-43.
[16] Ghosh N, Chakrabart I, Chakraborty M, Goswami B. A community based pilot study of haemoglobinopathies among the antenatal women in a rural area of Darjeeling district, West Bengal. Int J med Public Health. 2013;3(2):107-10.
[17] Balgir RS. Genetic epidemiology of the three predominant abnormal haemoglobins in India. JAPI. 1996;44:25-28.
[18] Balgir R. Epidemiology, population health genetics and phenotypic diversity of sickle cell disease in India. The Internet Journal of Biological Anthropology. 2007;1(2).
[19] Bhukhanvale DS, Sorathiya SM, Shah AP, Patel AG, Gupte SC. Prevalence and haematological profile of beta thalassaemia and sickle cell anaemia in four communities of Surat city. Indian J Hum Genet. 2012;18(2):167-71.
[20] Urade BP. Haemoglobin S and β^{Thal}: Their distribution in Maharashtra, India. Int J of Biomed Sci. 2013;9(2):75-81.
[21] Srinivas U, Mahapatra M, Pati HP. HbJ Meerut, a fast moving haemoglobin – A study of seven cases from India and A review of Literature. Am J of Haematol. 2007;82:666-67.

PARTICULARS OF CONTRIBUTORS:
1. Junior Resident, Department of Pathology, SGRRIM and HS, Dehradun, Uttarakhand, India.
2. Professor, Department of Pathology, SGRRIM and HS, Dehradun, Uttarakhand, India.
3. Professor, Department of Obstetrics and Gynaecology, SGRRIM and HS, Dehradun, Uttarakhand, India.
4. Professor and Head, Department of Pathology, AIIMS, Rishikesh, Uttarakhand, India.
5. Associate Professor, Department of Pathology, SGRRIM and HS, Dehradun, Uttarakhand, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Shivani Nayar,
H. No. 1012, Sector 11C, Chandigarh-160011, Union Territory, India.
E-mail: drshivaninayar@yahoo.co.in

FINANCIAL OR OTHER COMPETING INTERESTS: None.

Date of Submission: **Jul 19, 2017**
Date of Peer Review: **Sep 01, 2017**
Date of Acceptance: **Oct 14, 2017**
Date of Publishing: **Dec 01, 2017**