ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Prevalence of Hemoglobinopathies in Rural Tertiary Care Hospital of West-Central Maharashtra.

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ABSTRACT

Hemoglobinopathies are common public health problems in the world and in India. These diseases possess a large burden on the health and financial resources of the country. As hemoglobinopathies shows geographic variation, it is important to know about the actual prevalence of this lifelong disorder by conducting various regional studies. The present study aims to observe the frequency of the various hemoglobinopathies in the region of West-Central Maharashtra, distribution of these hemoglobinopathies in different age groups and sex. Observational descriptive study of 7 years duration from January 2016 to December 2022. The study was carried out by retrieving cases, reports and data from archives. 1132 patients were evaluated by High Performance Liquid Chromatography (HPLC) for diagnosis of haemoglobinopathies. A total of 189 (16.69%) patients showed abnormal haemoglobins variants. Of these 102 (9.01%) were diagnosed to have beta–thalassaemia trait based on high level of Hb A2 (4 - 9%), 42 (3.71%) as thalassaemia major/intermedia, 15 (1.32 %) as sickle cell trait, 13 (1.15 %) patients as homozygous sickle cell anaemia and 17 (1.50 %) as sickle-β thalassaemia. The commonest hemoglobinopathy in western central Maharashtra region is thalassemia followed by sickle cell hemoglobinopathy. The present study conducted in present region using HPLC reflects the magnitude of thalassemia and other hemoglobinopathies in rural population of this region.

Keywords: Haemoglobinopathy, High-performance liquid chromatography, Thalassaemia, Sickle cell.

https://doi.org/10.33887/rjpbcs/2024.15.3.55

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ISSN: 0975-8585

INTRODUCTION

All the genetic disorders of haemoglobin are termed as hemoglobinopathies and are the most common inherited single gene red cell disorders globally. These hereditary disorders are major public health problem in many parts of the world including India [1]. Hemoglobinopathies possess a large burden on the health and financial resources of the country. As hemoglobinopathies shows geographic variation, it is important to know about the actual prevalence of this lifelong disorder by conducting various regional studies. The early precise and accurate diagnosis of these conditions is also very important because it prevents the occurrence of clinically overt hemoglobinopathies and thus lessens the economic and psychological burden of this lifelong health problem.

At present, approximately 250 million people constituting 4.5 % of the world population carry a potentially pathological haemoglobinopathy gene. Each year about 3 lakh infants are born with a major haemoglobinopathy. Beta (β)-thalassaemia and sickle cell anaemia (homozygous SS state) represents the most frequent haemoglobinopathies [2-5] Migration and consanguinity contributes to burden of this disease.

Kharche et al. [6] published a systematic review of Indian studies on hemoglobinopathies and showed that there is a regional predilection in the prevalence of these diseases. They showed that BTT remained the highest reported hemoglobinopathies in all four regions of India and sickle hemoglobinopathy constituent next common disease across the all the regions. Beta thalassemia major was fourth common haemoglobinopathy in their study and its distribution across the region was varied.

The prevalence of beta thalassemia trait in India ranges of 0-17% [7]. HbE is common in eastern India, sickle cell anaemia and thalassemia are common in central India while HbD Punjab is common in northern India [8]. The average frequency of HbS in Indian population is 4.3% [9]. The clinical spectrum of the disorders varies from asymptomatic conditions to serious disorders like Thalassaemia major and sickle cell anaemia that requires regular blood transfusions and extensive medical care [2]. Untreated thalassemia major is invariably fatal by 2-5 years of age. Sickle cell anaemia is a homozygous state, and is most severe form of disease. Sickle cell trait is heterozygous state, clinically they have normal growth, development and life expectancy.

Majority of the centres in India use conventional methods for diagnosis of haemoglobinopathies which includes clinical and family history, red cell indices, CBC, HbA2, HbF, HbS and Hb electrophoresis. The use of cation– exchange high performance liquid chromatography to separate and quantify various normal and abnormal haemoglobin (Hb) fractions has been increasing. It is highly sensitive, specific, and quick but more expensive method for diagnosis [5].

High Performance Liquid Chromatography (HPLC) has advantage that it can quantify HbA2 and HbF along with variant haemoglobin screening. Clinical history and findings of thorough haematological evaluation including complete blood count, reticulocyte count and red blood cell morphology are helpful to reach an accurate diagnosis [10]. In some cases, family studies are also required to detect a particular Hb variant.

Before the birth, HbF is predominant and remains so up to 3 months of age, whereas HbA starts increasing shortly after birth. Quantification of various haemoglobin is helpful in diagnosis of various hemoglobinopathies. The knowledge of the common Haemoglobin variants encountered in a particular area is important for the formulation of specific diagnostic, preventive and therapeutic strategies and meet the future challenges.

The present study aims to observe the frequency of the various hemoglobinopathies in the region of West-Central Maharashtra using HPLC and distribution of these hemoglobinopathies in different age groups and sex.

MATERIAL AND METHODS

The present study was carried out in the department of pathology, Dr. Balasaheb Vikhe Patil Rural Medical College, Loni, Maharashtra, India. It is Observational descriptive study of 7 years duration from January 2016 to December 2022. All the anaemic patients suspected of having hemoglobinopathies

were subjected to HPLC testing after collecting 2 ml sample from patient in EDTA. All specimens were assessed by the Bio – Rad Variant HPLC system with the use of the Variant Beta – Thalassaemia Short Program. The study was carried out by retrieving cases, reports and data from archives.

The diagnosis of BTT was made if HbA2 was found in range of 4 -9%. If HbF was found to be predominant haemoglobin then diagnosis of beta thalassemia major / intermedia was made. The sickle cell hemoglobinopathy was considered when HbS fraction was noted, and further classified as sickle cell anaemia if HbS is more than 50% and as sickle cell trait if HbS is less than 50%. Raised HbA2 and HbF prompted diagnosis of sickle beta thalassemia. [11]

RESULTS

A total of 1,132 cases were studied. Abnormal haemoglobin fraction was noted in 189 (16.69%) cases while 827 (73.05 %) cases showed normal Haemoglobin fraction on HPLC. (Table I)

Table I: Distribution of various abnormal haemoglobin patterns (n = 1132).

Haemoglobinopathy		% of hemoglobinopathy	% of total cases
Thalassemia		144(76.19)	
	Thalassemia trait	102 (54 %)	9.01
	Thalassemia major/ intermedia	42 (22 %)	3.71
Sickle-Beta Thalassemia		17 (9 %)	1.50
Sickle cell		28 (14.81%).	
	Sickle cell trait	15 (7.9 %)	1.32
	Sickle cell anaemia	13 (6.8%)	1.15
Total		189 (100%)	1132

Of all hemoglobinopathies, thalassemia (BTT, Thal major/intermedia) was the commonest with 144 cases (76.19%) and sickle hemoglobinopathy (sickle trait and sickle anaemia) constituted 28 cases (14.81%). Sickle-Beta Thalassemia combined abnormalities were found in 17 (19%) cases.

Beta thalassemia trait was the commonest haemoglobin abnormality with 102 cases (54%). The mean Hb A2 value among BTT cases was 5.02%. In all these cases of BTT, Hb F was either normal or was elevated up to 15% in adults or more if patient is less than 1 year of age. Adult haemoglobin (Hb A) was in normal range in these cases. In thalassemia major/intermedia cases, Hb F was predominant with variable Hb A2. The mean Hb F in thalassemia major/intermedia was 81.4%. Hb A2 was normal, increased or decreased. 10 cases of thalassemia major/intermedia had lower level of Hb F. These patients had history of prior blood transfusion and family history of both parents being beta thalassemia trait. These were labelled as thalassemia major post transfusion.

In present study, Sickle cell anaemia (SS homozygous state) was seen in 13 cases (6.8%) and sickle cell trait (AS heterozygous state) was seen in15 cases (7.9%). In sickle cell trait, mean Hb S was 25.7%.

17 cases (9%) of Hb S-beta-thalassemia were also found. The compound heterozygous Hb S-Beta thalassemia was diagnosed in 2 cases (1.05%) where Hb S was less than 50%, Hb A2 was more than 4 %. Hb F was lower in these cases as compared with compound homozygous cases. Compound homozygous Hb S-Beta thalassemia was diagnosed in 15 cases (7.93%) where HbS was more than 50%, Hb A 2 was more than 4 % and Hb F was variably increased.

ISSN: 0975-8585

Table II: Distribution of various hemoglobinopathies according to age.

Age (years)	BTT	Thal.Major / intermedia	Sickle Trait	Sickle anaemia	Sickle – Beta thal.	Total
0-10	44	39	10	9	13	115 (60.84%)
11-20	13	2	0	3	1	19 (10.05%)
21-30	32	0	5	1	1	39 (20.63%)
31-40	11	1	0	0	2	14 (7.40%)
41-50	1	0	0	0	0	1 (0.52%)
51-60	1	0	0	0	0	1 (0.52%)
Total	102	42	15	13	17	189 (100%)

A total of 115 hemoglobinopathies were seen in first decade accounting for 60.84% of all hemoglobinopathies. BTT was the commonest hemoglobinopathy in all age groups. 92 % of hemoglobinopathies were seen in first 3 decades of age as shown in (Table II).

Table III: Distribution of various hemoglobinopathies according to gender.

Total haemoglobinopathies	Male	Female	M: F
189	100	89	1.12:1

Out of 189 cases of haemoglobinopathy studied, 100 (53 %) were male and 89 (47%) were female. The male to female ratio was approximately 1.12:1. (Table III)

In present study, neonatal screening was done in 49 cases, out of which 7 were found to have thalassemia major with Hb F > 80% and absent Hb A. These patients on follow up did not show rise in Hb A and developed symptoms of severe haemolytic anaemia. 3 cases had Hb A2 elevated > 4%. These were suspected for thalassemia trait and advised to repeat testing after 1 year of age for confirmation of diagnosis. This is because Hb A2 is not sufficiently expressed to be a diagnostic parameter until one year of age. It was found in present study that, in 10 married couples both partners had BTT and in 2 married couples, both partners had sickle cell trait making them a high-risk couple.

Other haemoglobin variants like Hb E, Hb D Punjab, Hb D Iran, Hb C, Hb J Meerut, Hb Q India and HPHF were not observed in present study.

DISCUSSION

Present study is based on 1132 patients residing in West-Central part of Maharashtra while Gupta et al [15] studied 955 cases, Singh et al [14] studies 2698 cases and Srivastav et al [12] studied 7261 cases in the same region. (Table IV)

The frequency of Thalassemia trait was about 9.01% in present study which matches with study of Gupta et al (9.52%) and Biswas et al [16] (8.03%). The frequency of BTT in present study was less than what observed by Srivastav et al [12] (11.55%) and Singh et al [14] (14.27%).

Table IV: Comparison of HPLC study results with different authors.

Abnormal haemoglobin	Present study (%) n = 1132	Srivastav et al [12] n = 7261	Dangar et al [13] n = 400	Singh et al [14] n = 2698	Gupta et al [15] n = 955
Thalassemia trait	102 (9.01%)	839 (11.55%)	18 (4.5%)	385 (14.27%)	91 (9.52%)
Thalassemia major/ intermedia	42 (3.71 %)	308 (4.24%)	2 (0.5%)	NA	5 (0.52%)
Sickle-Beta Thalassemia	17 (1.5%)	52 (0.72%)	6 (1.5%)	11 (0.4%)	2 (0.2%)
Sickle cell trait	15 (1.32 %)	214 (2.95%)	109 (27.25%)	36 (1.33 %)	15 (1.57%)
Sickle cell anaemia	13 (1.14%)	85 (1.17%)	79 (19.75%)	22 (0.82%)	3 (0.2%)
Total hemoglobinopathies	189(16.69)	1615(22.24)	220(55)	543(20.12)	137(14.34)

Frequency of Thalassemia major/intermedia was higher in present study than study of Gupta et al. Similar difference was seen with study by Jain et al [17] in Gujrat.

The frequency of Thalassemia major/intermedia was 3.71% in present study which is closer to what Srivastav et al observed (4.24%). The study by Gupta et al (0.52%) and Dangar et al (0.5%) observed lesser cases of thalassemia major/intermedia. This is probably due to difference in the population which is being studied (Rural/Urban).

The frequency of sickle cell trait cases in present study was 1.32% which closely matches with observations from Gupta et al [15] (1.57%), Singh et al [14] (1.33%) and Biswas et al [16] (1.33%). Dangar et al [13] noted 27.25% cases of sickle cell trait which is higher than findings of present study. Cambell et al, Jain et al and Dangar et al observed that sickle cell trait is most common hemoglobinopathy in their study [13, 18]. This is probably due to regional variation.

1.13% of cases of sickle anaemia in present study closely matches with study by Srivastav et al [11] (1.21%). Dangar et al [13] noted 19.75% cases of sickle cell anaemia which is higher as compared to present study. Gupta et al [15] noted 0.2 % cases of sickle cell anaemia which matches with present study.

Sickle -beta thalassemia was observed in 1.5 % cases in present study which is higher as compared to other studies like Shrivastav et al (0.72%), Singh et al (0.4%) and Gupta et al (0.2%).

Among other hemoglobinopathies, Gupta et al found 5 cases (3.6%) of Hb E trait, 8 cases (5.9%) of Hb E disease, 7 cases (5.1%) of Hb D trait and 1 case (0.7%) of Hb Q India. Jain et al found 2 cases of Hb D and 1 case of Hb E. Singh et al also documented similar findings. The present study did not reveal these abnormal variants as these are less prevalent in this area.

It was found in present study that, in 10 married couples both partners had BTT and in 2 married couples, both partners had sickle cell trait making them a high-risk couple. Hence there is a need to set a special program for premarital and antenatal screening particularly in rural population to prevent birth of thalassemia major in the off spring.

As this study was a retrospective in nature, comparison with haematological parameters could not be done. Genetic studies were not done. Family screening could not be obtained in all cases. Prospective study with large samples can be done to strengthen the findings of present study. This type of study can definitely help to increase awareness among both health care givers and general population.

CONCLUSION

The commonest hemoglobinopathy in present region is thalassemia followed by sickle cell hemoglobinopathy. The present study conducted in this region using HPLC reflects the magnitude of thalassemia and Sickle haemoglobinopathy in rural population.

REFERENCES

[1] Higgins T, Schnabl K, Savoy M, Rowe P, Flamini M, Bananda S. A novel double heterozygous, HbD Punjab/HbQ India, hemoglobinopathy. Clin Biochem2012; 45:264-6.
[2] Angastiniotis M, Modell B, Englezos P, Boulyjenkov V. Prevention and control of haemoglobinopathies. Bull World Health Organ 1995; 73:375–386
[3] Riou J, Godart C, Hurtrel D, Mathis M, Bimet C, et al. Cation – exchange HPLC evaluated for presumptive identification of hemoglobin variants Clin Chem 1997; 43: 34-9.
[4] Joutovsky A, Hadzi-Nesic J, Nardi MA. HPLC retention time as a diagnostic tool for hemoglobin variants and hemoglobinopathies: a study of 60,000 samples in a clinical diagnostic laboratory. Clin Chem 2004; 50: 1736-47.
[5] Waters HM, Howarth JE, Hyde K, Goldstone S, Cinkotai KI, et al. An evaluation of the Bio – Rad Variant Haemoglobin Testing System for the detection of haemoglobinopathies. Clin Lab Haematol 1998; 20: 31-40.
[6] Kharche K, Bhake A. Hemoglobin Variants in Patients with Microcytic Hypochromic Anemia: A Review of Indian Studies. Cureus 2023; 15(4): e38357.

[7] Kumar M, Devisri Y. Detection of hemoglobinopathies in patients of anaemia using high performance liquid chromatography (HPLC) – A hospital based prospective study. Trop J Pathol Microbiol 2019; 5:51-7.
[8] Mondal SK, Mandal S. Prevalence of thalassemia and hemoglobinopathy in eastern India: A 10-year high-performance liquid chromatography study of 119,336 cases. Asian J Transfus Sci 2016; 10:105-10.
[9] Balbir RS. Genetic epidemiology of the three predominant abnormal hemoglobins in India. J Assoc Physicians India 1996;44(1):25-28.
[10] Fucharoen S, Winichagoon P, Wisedpancihkji R. Prenatal and postnatal diagnosis of thalassemias and haemoglobinopathies by HPLC. Clin Chem 1998; 44: 740-8.
[11] Tejindar singh Atlas and text of hematology. singh. (2018). Atlas and text of hematology (4th ed., Vol. 1). Arya publishing company.
[12] Shrivastav et al. Study of hemoglobinopathies and Hb variants in population of Western India using HPLC: A report of 7,000 cases. J Appl Hemato l2013; 4:104-9.
[13] Dangar et al. A Study of high-performance liquid chromatography in the patients presenting with anaemia. Asian J Microbiol Biotech Env Sci 2023;25(3):469-473.
[14] Singh et al. Prevalence of hemoglobinopathies using high-performance liquid chromatography as diagnostic tool in anemic patients of tertiary care center of Western India. Asian J Transfus Sci 0; 0:0.
[15] Gupta et al. Cation exchange high performance liquid chromatography for diagnosis of hemoglobinopathies. MJAFI 2009; 65(1).
[16] Biswas AK, Philip J. Incidence of hemoglobinopathies and hemoglobin variants using high performance liquid chromatography (HPLC) in a teaching hospital of Odisha. J Appl Res 2016 6:214-8.
[17] Jain R, Saxena S: Study of abnormal haemoglobin variants in patients of anaemia using high performance liquidchromatography (HPLC)in Gujarat. India J Trop 2019,5:2456-9887.
[18] Chandrashekar V, Soni M: Haemoglobin disorders in South India. ISRN Hematol 2011; 2011:748939.