Original Article
MVP Journal of Medical Sciences, *Vol 7(2), 240-245, July-December 2020*
ISSN (Print) : 2348–263X
ISSN (Online) : 2348-2648
DOI: 10.18311/mvpjms/2020/v7i2/24503

Spectrum of Hemoglobinopathies Diagnosed by High Performance Liquid Chromatography at a Tertiary Care Centre: An Observational Study

Preeti Bajaj[1], Aakash Gupta[2]*

[1]Professor and Head, Department of Pathology, Dr. Vasantrao Pawar Medical College, Hospital and Research Centre, Nashik – 422003, Maharashtra, India
[2]Former PG Resident, Department of Pathology, Dr. Vasantrao Pawar Medical College, Hospital and Research Centre, Nashik – 422003, Maharashtra, India; aakash1111gupta@gmail.com

Abstract

Introduction: Thalassemia and other hemoglobinopathies are the most common routinely encountered congenital anemias in India. Sickle cell anemia is very common, so a study to rule out all the hemoglobinopathies is essential. **Aims and Objectives:** The present study was conducted to diagnose various hemoglobinopathies among clinically suspected cases., the present study was conducted to diagnose various hemoglobinopathies among clinically suspected cases. **Materials and Methods:** A total of 212 cases cases were undertaken in this study after they met the inclusion and exclusion criteria. HPLC was performed on the samples received in the Central Clinical Laboratory of Dr. Vasantrao Pawar Medical College, Hospital and Research Centre, Nashik, Maharashtra. **Results:** Out of 212 cases, 42 cases showed positivity for thalassemia or presence of abnormal hemoglobin, with 8% cases of Beta thalassemia trait, 1.88% cases of Beta thalassemia major, 4.7% cases of Sickle cell trait, 4.24% cases of Sickle cell disease and 0.94% cases of Sickle-thalassemia. **Conclusion:** HPLC is a fast, reliable, and cost-effective method to diagnose any suspected case of hemoglobinopathy.

Keywords: HPLC, Thalassemia, Hemoglobinopathies

1. Introduction

The thalassemias are a group of congenital anemias that have deficient synthesis of one or more of the globin subunits in the normal human hemoglobins. The primary defect is usually quantitative, consisting of reduced or absent synthesis of normal globin chains, but there are mutations resulting in structural variants produced at reduced rate[1].

Detailed investigation of anemia keeping in mind the possibilities of detecting abnormal hemoglobin is very helpful in finding out more carriers of different hemoglobinopathies. HPLC (High Performance Liquid Chromatography) is a rapid, accurate and reproducible tool for early detection and proper management of hemoglobinopathies and its variants[2].

Mass screening and continuous awareness programs of the population in childbearing age and children going to school can help in early detection of heterozygous states. HPLC also helped us in detecting various heterozygous states. Though these abnormal variants have less clinical significance, they can give rise to severe disease if combined with other variants[3].

Sickle-thalassemia combination hemoglobinopathy is the most commonly encountered type in some areas. High index of suspicion in anemic subjects especially between 6–15 years of age is the most important factor for curbing this condition by early detection followed by genetic counselling and prenatal diagnosis in subsequent pregnancies. Larger studies spread over a wide geographical area should also be undertaken[4].

HPLC offers the advantages over the routine Hemoglobin electrophoresis as it can more accurately identified and quantitate abnormal hemoglobins. HPLC forms an accurate, rapid and reproducible tool for early detection and management of thalassemia and abnormal hemoglobin variants. This is of utmost importance as there is high incidence of beta thalassemia trait in the Indian subcontinent[5].

*Author for correspondence

Due to interaction of several hemoglobin abnormalities they may present with unusual clinical presentations and their identification may require further investigations. Today, abnormal hemoglobins are generally discovered during a systematic study performed within programmes of prevention of thalassemias or sickle cell disease[6].

2. Aims and Objectives

The present study was conducted to diagnose various hemoglobinopathies among clinically suspected cases.

3. Materials and Methods

Study type/ design: Observational study.

Study settings: The study was done in Department of Pathology in a tertiary care centre.

Duration of study: From August 2017 to December 2019

Sample size: A total of 212 cases were taken into consideration after they met the inclusion and exclusion criteria.

The samples were received in the Central Clinical Laboratory of Dr. Vasantrao Pawar Medical College, Hospital and Research Center, Nashik. Both indoor and outdoor patients were taken into consideration.

Methodology: 2–3 ml of intravenous blood was collected from each individual in an EDTA bulb, after filling the patient information sheet and taking the consent, the sample was then processed on the Beckman Coulter Act-5 Diff Hematology analyzer to check for hematological parameters, then it was collectively run on Bio-Rad D10 Dual Program for hemoglobin fractions, if for some reason the processing was delayed, the sample was stored in refrigerator at 6°C for upto maximum of 1 week.

All the samples were reported on the same day by the Department of Pathology.

4. Results

In our study of 212 cases, we found a prevalence of thalassemia and hemoglobinopathies as 19.8% (Table 1).

42 cases showed positive results on HPLC for either thalassemia or any abnormal hemoglobins.

Out of the 42 cases most cases were Beta thalassemia trait, 17 cases (8%), followed by Sickle cell trait with 10 cases (4.7%), followed by 9 cases (4.24%) of Sickle cell disease, 4 cases (1.88%) of Beta thalassemia major and Double heterozygous Sickle-thalassemia with 2 cases (0.94%) (Table 1).

Gender wise distribution showed that Beta thalassemia trait was more common in females with 4.7% of overall cases - followed by Sickle cell trait with 2.5% cases (Table 2).

On the basis of age groups, most positive cases were seen in the age group 21-30 years with 21 positive cases (9.9%), followed by 0–10 years age group with 15 cases (7%) (Table 3).

Most cases of Beta thalassemia trait (12 cases) were seen in the age group 21–30 years.

All the cases of Beta thalassemia major were seen in pediatrics age group of 0–10 years.

Most cases of Sickle cell trait were seen in age group 0–10 years (5 cases) and that of Sickle cell disease in the age group 21–30 years (4 cases).

Table 1. Hemoglobinopathies diagnosed by HPLC

Diagnosis	No. of cases	%
Normal	170	80.18%
Beta Thalassemia Trait (BTT)	17	8%
Beta Thalassemia Major (BTM)	4	1.88%
Sickle Cell Trait (HbAS)	10	4.7%
Sickle Cell Disease (HbSS)	9	4.24%
Double Heterozygous Sickle-Thalassemia (HbS-BT)	2	0.94%
Total	212	100%

Table 2. Gender wise case distribution

Diagnosis	Male (%)	Female (%)	Total (%)
Normal	74 (35%)	96 (45%)	170 (80%)
Beta thalassemia trait (BTT)	7 (3%)	10 (4.7%)	17 (7.7%)
Beta thalassemia major (BTM)	3 (1.4%)	1 (0.5%)	4 (1.9%)
Sickle cell trait (HbAS)	5 (2.5%)	5 (2.5%)	10 (5%)
Sickle cell disease (HbSS)	5 (2.5%)	4 (2%)	9 (4.5%)
Double heterozygous sickle-thalassemia (HbS – BT)	2 (0.9%)	0 (0%)	2 (0.9%)
Total	96	116	212

Only 2 cases of Sickle-thalassemia were diagnosed one in age group 0–10 and other in group 21–30 years.

Table 4 and Table 5 depicts the mean and SD of hemoglobin parameters and hemoglobin fractions of the overall study population. Figures 1 to 5 depict sample HPLCs of Hemoglobinopathies diagnosed in the present study

5. Discussion

Thalassemia and hemoglobinopathy are autosomal recessive inherited disorders, primarily affecting the globin moiety of the Hb molecule. These disorders, which were mainly confined to certain areas, religions, castes and tribes, particularly with consanguineous marriages, are now widely prevalent all over the world. This is because of the migration of various races over the ages and hence, being home to an assortment of sociocultural, linguistic and ethnically diverse people[7].

In India and Mediterranean belt, still thalassemia and hemoglobinopathies are very common causes of morbidity and also exert burden on expenditure. To reduce the burden accurate and reliable screening procedure should be there. The laboratory diagnosis of hemoglobiopathies and thalassemia may be required

(a) To confirm a provisional diagnosis, like sickling disorders or thalassemia major.

(b) To explain a hematologic abnormality such as anemia, microcytosis or polycythemia.

Table 3. Age wise distribution of all positive cases

Age	Beta thalassemia trait (BTT)	Beta thalassemia major (BTM)	Sickle cell trait (HbAS)	Sickle cell disease (HbSS)	Double heterozygous sickle-thalassemia (HbS – BT)	Total
0-10	2	4	5	3	1	15
11-20	1	0	0	2	0	3
21-30	12	0	4	4	1	21
31-40	1	0	0	0	0	1
41-50	1	0	1	0	0	2
>50	0	0	0	0	0	0
Total	**17**	**4**	**10**	**9**	**2**	**42**

Table 4. Complete blood count analysis in overall study population

Diagnosis	Hb (gm/dL) (MeanSD)	RBC (×10^{6}cumm) (MeanSD)	MCV (fL) (MeanSD)	MCH (pg) (MeanSD)	MCHC (g/dL) (MeanSD)	RDW (%) (MeanSD)
Normal	7.5 ± 3.1	3.5 ± 1.3	68.915	22.7 ± 6.8	31.6 ± 4	18.3 ± 4.6
Beta thalassemia trait (BTT)	9.1 ± 2.5	4.8 ± 1.2	60.9 ± 7.3	19.3 ± 2.44	31.7 ± 1.1	17.4 ± 3.1
Beta thalassemia major (BTM)	5.1 ± 0.15	2.7 ± 0.4	61 ± 7.8	19.2 ± 2.9	31.5 ± 1.4	24 ± 4
Sickle cell trait (HbAS)	7.15 ± 2.9	3.3 ± 1.6	72.2 ± 20	23.5 7.6	32 ± 2.4	19 ± 5.5
Sickle cell disease (HbSS)	7 ± 2	2.5 ± 1	82 ± 11	29 ± 5.5	35 ± 5.4	20 ± 5
Double heterozygous sickle-thalassemia (HbS – BT)	8.2 ± 5	3.8 ± 2.5	71.5 ± 3.5	22 1.3	30.8 ± 0.1	20.4 ± 2.8

Table 5. Hemoglobin fractions in overall study population

Diagnosis	HbA % (Mean±SD)	HbA2 % (Mean±SD)	HbF % (Mean±SD)	HbS % (Mean±SD)
Normal	94 ± 9.5	2.9 ± 0.7	3 ± 9.6	-
Beta thalassemia trait (BTT)	92.7 ± 2.3	5.8 ± 0.7	1.6 ± 2.5	-
Beta thalassemia major (BTM)	7 ± 2.4	2.3 ± 0.45	91 ± 2.1	-
Sickle cell trait (HbAS)	66 ± 4.8	3.5 ± 0.75	2.75 ± 2.75	27.6 ± 5
Sickle cell disease (HbSS)	19 ± 14	2.8 ± 1.5	14.5 ± 10	63 ± 11
Double heterozygous sickle-thalassemia (HbS – BT)	6.5 ± 2	5.7 ± 0.3	18.9 ± 0.7	68.9 ± 1.5

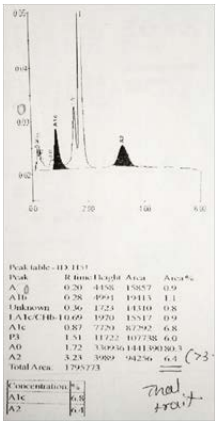

Figure 1. Sample of beta thalassemia trait.

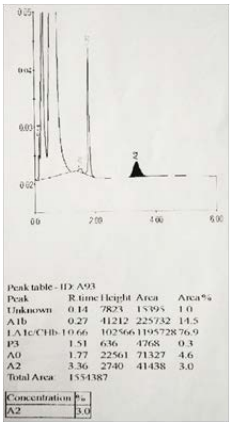

Figure 2. Sample of beta thalassemia major.

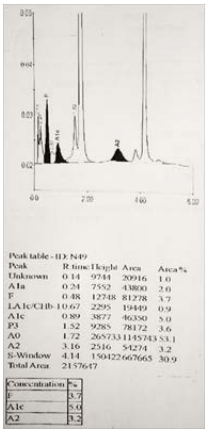

Figure 3. Sample of heterozygous sickle cell disease.

(c) To identify an abnormality through neonatal screening.
(d) To predict serious disorders of globin-chain synthesis in the fetus.
(e) To permit genetic counselling of prospective parents.

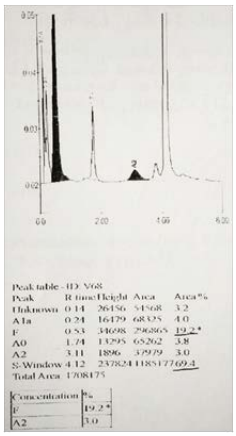

Figure 4. Sample of homozygous sickle cell disease.

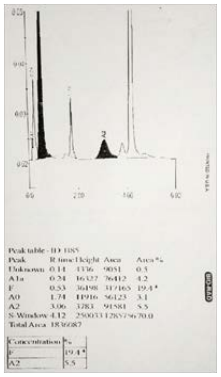

Figure 5. Sample of double heterozygous sickle-thalassemia.

(f) To allow preoperative screening for the presence of sickle cell Hb[8].

Today abnormal hemoglobins are generally discovered during a systematic study performed within programs for prevention of thalassemias or sickle cell disease[6].

Accurately and timely detection of different Hb variants which includes beta thalassemia trait can prevent occurrence of many serious disorders like thalassemia major in newborns, interactions between other rare Hb variants in heterozygous state may lead to serious homozygous Hb variant in the offspring. Double heterozygous states between different variants can lead to adverse hematological defects[5].

Cation exchange HPLC has emerged as one of the best methods for screening and detection of various hemoglobinopathies with rapid, precise and reproducible results[9].

Other analytical procedures include, alkaline gel electrophoresis and acid gel electrophoresis, ion exchange

Table 6. Comparison of prevalence with other similar studies

Hemoglobinopathies	Sarvaiya et al	Narang et al	Buch et al	Mondal et al	Sachdev et al	Iqbal et al	Baruah et al	Shrivastav et al	Patel et al	Present study
Beta Thalassemia Trait (BTT)	10.61%	6.5%	3.7%	4.6%	8.9%	7.72%	3.48%	11.55%	1.95%	8%
Beta Thalassemia Major (BTM)	0.83%	4%	0.02%	1.66%	0.6%	0.38%	0.36%	4.02%	-	2%
Sickle Cell Trait	4.71%	6%	0.2%	0.38%	-	10.8%	2.1%	2.95%	6.54%	5%
Sickle Cell Disease	0.93%	0.5%	0.2%	0.32%	0.04%	2.3%	2.26%	1.17%	-	4%
Double Heterozygous Sickle-Thalassemia	0.68%	22%	0.28%	0.26%	0.08%	-	0.59%	0.72%	-	1%
Hemoglobin E trait	0.04%	-	0.02%	3.02%	0.19%	-	25.48%	0.15%	-	-
HPHF	0.34%	-	-	0.12%	-	0.2%	0.06%	0.11%	-	-

column chromatography and alkali denaturation method[10].

Our study was carried out in a tertiary care center in Nashik, Maharashtra, and consisted of 212 cases selected according to the inclusion and exclusion criteria.

5.1 Beta Thalassemia Trait

In our study 8% cases were positive for Beta thalassemia trait. Narang *et al.*[4] had done a study on 200 patients in Indore and found out 6.5% prevalence, Sachdev *et al.*[5] had done a study on 2600 patients in Haryana and found a prevalence of 8.9% and Iqbal *et al.*[11] had done a study on 518 patients in Davangere and reported a prevalence of 7.72% for Beta thalassemia trait.

All these study shows a comparable prevalence of Beta thalassemia trait with the present study.

5.2 Beta Thalassemia Major

In our study only 4 cases showed Beta thalassemia major which comprises to 1.88%.

Mondal *et al.*[12] had done a study on 119336 patients in Kolkata and quoted a prevalence of 1.66% for Beta thalassemia major which is comparable to our study. Iqbal et al and Sarvaiya *et al.*[13] quoated a prevalence of 0.02% and 0.83% for Beta thalassemia major.

5.3 Sickle Cell Trait

In our study the prevalence for sickle cell trait was found out to be 4.7%, which is very much comparable with the study done by Sarvaiya *et al.*[13] which showed a prevalence of 4.71%. But other studies showed a relatively less prevalence like Buch *et al.*[3] who had done a study on 3465 patients in Pune and quoted a prevalence of 0.2%.

Other studies show relatively high prevalence of Sickle cell trait like Narang *et al.*[4] and Iqbal et al who have quoted a prevalence of 6% and 10.8% respectively.

5.4 Sickle Cell Disease

In the present study prevalence of Sickle cell disease was 4.24% which is highly significant as compared to other studies.

Buch *et al.*[3] quoted a prevalence of 0.2%, Sarvaiya *et al.*[13] 0.93, Narang *et al.*[4] 0.5%, Mondal *et al.*[12] 0.32%, Sachdev *et al.*[5] 0.04%, Baruah *et al.*[14] 2.26%, Iqbal *et al.* 2.3% and Shrivastav *et al.*[15] quoted a 1.17% prevalence for sickle cell disease.

5.5 Double Heterozygous for Sickle-Thalassemia

In the present study prevalence for Sickle-thalassemia was found out to be 0.94% which is comparable to the study done by Shrivastav *et al.*[15], Baruah *et al.*[14] and Sarvaiya *et al.*[13] quoting a prevalence of 0.72%, 0.59% and 0.68% respectively.

We did not find any case of other abnormal hemoglobins such as HbE, HbD, HbC, HbQ and other double heterozygous in our study.

6. Conclusion

Hemolytic anemias due to defects in haemoglobin synthesis (Thalassemia) and abnormal hemoglobins (Hemoglobinopathies) have a particular pattern of

inheritance and thus transmitted to the offsprings. At present, since there is no definitive treatment for these disorders, prevention plays an important role in bringing down the incidence of these disorders.

In a country where nutritional deficiencies are considered most common cause of anemia, we should also consider the presence of abnormal Hb types, which should be diagnosed promptly to prevent morbidity and mortality.

In the present study we used HPLC which reflected the magnitude of thalassemia and hemoglobinopathies in a small hospital based population which may be in fact the tip of an iceberg, but this type of study can definitely help to increase awareness among both healthcare givers and general population.

Mass screening and continuous awareness programs of the population especially childbearing age and school going children will help in early detection of heterozygous states.

Larger studies spread over a wide geographical area should also be undertaken.

7. References

1. Borgna-Pignatti C, Galanello R. Thalassemias and related disorders: Quantitative disorders of haemoglobinopathies synthesis. Wintrobe's Clinical Haematology. 13th ed. Vol 1. Philadelphia: Lippincott Williams & Wilkins; 2014. p.1990–2119.
2. Bhalodia JN, Oza HV, Modi PJ, Shah AM, Patel KA, Patel HB. Study of hemoglobinopathies in patients of anemia using High Performance Liquid Chromatography (HPLC) in Western India. Natl J Comm Med. 2015; 6(1):35-40. https://doi.org/10.1155/2015/792485. PMid:25692053. PMCid:PMC4321080
3. Buch A, Iqbal B, Bordawekar R, Jain A, Jariwala P, Rathod H. Patterns of haemoglobinopathies diagnosed by high-performance liquid chromatography in and around Pune (Western Maharashtra, India): A pilot study. J Med Soc. 2016; 30:111–5. https://doi.org/10.4103/0972-4958.182921
4. Narang S, Singh A, Nema S. Spectrum of haemoglobinopathies in a suburb of Indore (India): A two-year study. Indian J Pathol: Research and Practice. 2017; 6(2):378–82. https://doi.org/10.21088/ijprp.2278.148X.62(pt-II)17.6
5. Sachdev R, Dam AR, Tyagi G. Detection of Hb variants and hemoglobinopathies in Indian population using HPLC: Report of 2600 cases. Indian J Pathol Microbiol. 2010; 53(1):57–62. https://doi.org/10.4103/0377-4929.59185. PMid:20090224
6. Wajcman H, Moradkhani K. Abnormal haemoglobins: Detection and characterization. Indian J Med Res. 2011 Oct; 134(4):538–46.
7. Patne SC, Shukla J. Hemoglobin E disorder in Eastern Uttar Pradesh. Indian J Pathol Microbiol. 2009; 52:110–2. https://doi.org/10.4103/0377-4929.44991. PMid:19136801
8. Working party of the general haematology task force of the British committee for standards in haematology. Guideline: The laboratory diagnosis of haemoglobinopathies. Br J Haematol 1998; 101:783–92. https://doi.org/10.1046/j.1365-2141.1998.00809.x. PMid:9674756
9. Bravo-Urquiola M, Arends A, Montilla S, Velasquez D, Garcia G, Alvarez M, et al. Advantages in the use of high performance chromatography technique for screening hemoglobinopathies in Venezuela. Invest Clin. 2004; 45:309–15.
10. Gupta PK, Kumar H, Kumar S, Jaiprakash M. Cation exchange high performance liquid chromatography for diagnosis of hemoglobinopathies. Med J Armed Forces India. 2009; 65:1. https://doi.org/10.1016/S0377-1237(09)80051-8
11. Iqbal MS, Tabassum A, Chatura KR. Spectrum of thalassemia and hemoglobinopathies in a tertiary care diagnostic center. J Adv Clin Res Insights. 2016; 3:161–5. https://doi.org/10.15713/ins.jcri.129
12. Mondal SK, Mandal S. Prevalence of thalassemia and haemoglobinopathy in Eastern India: A 10-year high-performance liquid chromatography study of 119,336 cases. Asian J Transfus Sci. 2016 Jan-Jun; 10(1):105–110. https://doi.org/10.4103/0973-6247.175424. PMid:27011683. PMCid:PMC4782486
13. Sarvaiya AN, Chauhan SC. Variant hemoglobin spectrum by cation exchange high performance liquid chromatography: A study of 2035 subjects. Ann Pathol Lab Med. 2017 May; 4(3):297–302. https://doi.org/10.21276/APALM.1393
14. Baruah MK, Saikia M, Baruah A. Pettern of hemoglobinopathies and thalassemias in upper assam region of North East India: High Performance Liquid Chromatography studies in 9000 patients. Indian J Pathol Microbiol. 2014; 57(2):236–43. https://doi.org/10.4103/0377-4929.134680. PMid:24943756
15. Shrivastav A, Patel U, Joshi JR, Kaur A, Agnihotri AS. Study of hemoglobinopathies and Hb variants in population of Western India using HPLC: A report of 7,000 cases. J Appl Hematol. 2013; 4:104–9. https://doi.org/10.4103/1658-5127.123308

How to cite this article: Bajaj P. and Gupta A. Spectrum of Hemoglobinopathies Diagnosed by High Performance Liquid Chromatography at a Tertiary Care Centre: An Observational Study. MVP J. Med. Sci. 2020; 7(2):240-245.