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Original Research Article

Study of High Performance Liquid Chromatography in Thalassemia and Hemoglobinopathies at Tertiary Care Centre

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Abstract:

Background: A hemoglobinopathy is an inherited blood disorder in which there is an abnormal form of hemoglobin or decreased production of hemoglobin. Proper timely identification of these disorders is immensely important epidemiologically and to prevent clinically severe hemoglobinopathies.

Aims: (1) For identification and quantification of haemoglobin fractions. (2) Accurate diagnosis of various hemoglobinopathies and thalassemia by HPLC to know the spectrum of hemoglobin abnormalities.

Material and Method: The study was conducted at Department of Pathology of P.D.U. Medical College and Hospital, Rajkot from August 2017 to July 2020. EDTA samples were received at Central Clinical Laboratory from patients in whom clinicians suspect possibility of any hemoglobinopathy. HPLC was performed on all these samples on BIORAD VARIANT-II HPLC machine.

Result: 386 patients (positive sickle solubility or positive NESTROFT or highly suspected hemoglobinopathy) were analyzed with HPLC. 316 (81.9%) patients found to have hemoglobinopathy. Most common detected hemoglobinopathy was Beta-Thalassemia Trait (BTT) (57.0%). Second most common was Sickle Cell Heterozygous (23.4%).

Conclusion: HPLC forms a rapid, accurate and reproducible tool for early detection of hemoglobinopathies.

Keywords: HPLC, Hemoglobinopathies, Thalassemia, Sickle cell anemia.

Introduction

A hemoglobinopathy is an inherited blood disorder in which there is an abnormal form of hemoglobin (variant) including HbS, HbE, and HbC or decreased production of hemoglobin (thalassemia) [1].

Most of the early studies on epidemiology of hemoglobinopathies in different parts of the India used the sickling or the solubility test or NESTROFT and, in many reports, this was followed by Hb electrophoresis to determine the phenotypes. However, in recent years, high performance liquid chromatography (HPLC) analysis has been used in many large programs to identify carriers of both β-thalassemia as well as sickle hemoglobinopathies [2].

Aims and Objectives:

- For identification and quantification of haemo-globin fractions
- Accurate diagnosis of various hemoglobinopa-thies and thalassemia by HPLC to know the spectrum of hemoglobin abnormalities

Material and Methods

Study Design: The study was conducted at Department of Pathology of P.D.U. Medical College and Hospital, Rajkot from August 2017 to July 2020.

Inclusion Criteria: Samples of all patients in whom hemoglobinopathies are suspected by treating clinician and in which family history was positive

Exclusion Criteria:

- Infants less than 28 days of age
- Patients who had history of blood transfusion within 1 month at the time of blood collection

Laboratory Analysis:

EDTA samples were received at Central Clinical Laboratory from patients in whom clinicians suspect possibility of any hemoglobinopathy. Complete blood count on a hematology 3-part analyzer (NIHON KOHDEN Celltac α) was performed. Then, NESTROFT and Sickle Solubility Test performed on samples.

Samples for performing HPLC were selected include NESTROFT or solubility test positive or patients in whom clinicians had asked for HPLC even if NESTROFT OR Sickle Solubility test was negative. HbA2/F calibrator and controls were analyzed at the beginning of each run on HPLC. HPLC was performed on all these samples on BIORAD VARIANT-II HPLC machine. For each sample, chromatogram was obtained. The software delivers Hb fractions eluted as a printed report in the form of a chromatogram.

Result:

386 patients (positive sickle solubility or positive NESTROFT or highly suspected hemoglobinopathy) were analyzed with HPLC. Sickle solubility was performed in 386 patients. Out of that, solubility was positive in 112(29%) samples and negative in 274(71%) samples.

NESTROFT was performed in 386 patients. Out of that, NESTROFT was positive in 218(56%) samples and negative in 168(44%) samples. Out of 386 patients analyzed with HPLC, 316 (81.9%) patients found to have hemoglobinopathy.

Table 1: Age wise distribution of all cases of hemoglobinopathies:

Age (years)	No. of Patients	Percentage of patients
<10	86	27.2%
11 – 20	66	20.9%
21 – 30	130	41.1%
31 – 40	17	5.4%
41 – 50	9	2.8%
51 -60	6	1.9%
>60	2	0.6%
Total	316	100.0%

In our study, most common age group affected by hemoglobinopathies was 21-30 years. Females (52%) were affected more than males (48%).

Table 2: Sex wise distribution of all cases of hemoglobinopathies:

Sex	No. of patients
Female	164
Male	152
Total	316

In our study, Females (52%) were affected more than males (48%).

Table 3: Percentage of various hemoglobinopathies among patients underwent HPLC:

Hemoglobinopathy	No. of patients	Percentage of patients
Beta Thalassemia Trait	180	57.0%
Sickle Cell Heterozygous	74	23.4%
HbS Homozygous	16	5.1%
HbD-Punjab Heterozygous	13	4.1%
HbE Heterozygous	10	3.2%
Double Heterozygous for HbS and Beta Thalassemia	10	3.2%
HPFH heterozygous	4	1.3%
Double Heterozygous for HbE and Beta Thalassemia	3	0.9%
Double Heterozygous for HbS and HbD	3	0.9%
Beta Thalassemia Major	3	0.9%
Total	316	100%

Most common detected hemoglobinopathy was Beta-Thalassemia Trait (BTT) (57.0%). Second most common was Sickle Cell Heterozygous (23.4%).

Discussion:

Hemoglobinopathies are common disorders which are genetically inherited and exert significant burden on various developed and developing countries of world including India. Hence, adequate measures and screening procedures are required for confirmation [3]. Hemoglobin disorders are responsible for complex clinical phenotypes. Thalassemia and Sickle cell Anemia can cause chronic ill health and life threatening situations, so it is very important to have reliable detection and identification methods for hemoglobin variants and because this can lead to the prevention of more severe disorders [4]. Diagnosis of hemoglobinopathies in most centers in India relies upon conventional methods like, clinical and family history, complete blood counts (CBC), red cell indices, HbA2, NESTROFT, sickling test, and Hb electrophoresis.

Many limitations of these methods have been seen. One of difficulty in the identification of Hb variants with same electrophoretic mobility. Another issue comes up while diagnosing certain compound heterozygous states such as, HbD + HbE, HbS + β thalassemia, HbS + HbD, HbE + β thalassemia, HbD + βthalassemia [5]. There are many types of HPLC like partition chromatography, normal-phase chromatography, displacement chromatography, reversed phase chromatography, size-exclusion chromatography, ion-exchange chromatography, bio affinity chromatography, etc. Among them we used ion exchange chromatography. Cation exchange HPLC is one of the best methods for screening and detection of various hemoglobinopathies. It has the advantages of quantifying HbF and HbA2 and screening of hemoglobin variants in single and highly reproducible system.

The simplicity of the automated system with internal sample preparation, superior resolution, rapid assay time, and accurate quantification of hemoglobin fractions make this an ideal methodology for routine clinical laboratory [6].

Table 4: Comparison of most commonly affected age group in hemoglobinopathies in various studies.

Study	Sample size	Commonly affected age group
Jawarkar et al [7]	200	11-20 years
Mistry et al [8]	1186	21-30 years
Present study	386	21-30 years

In our study, most common age group affected in various hemoglobinopathies was 21-30 years. Results are comparable with study done by Mistry et al.

Table 5: Comparison of sex wise distribution of hemoglobinopathies with different studies

Study	No of cases	Male	Female
Tambse et al [4]	7093	45.66%	54.34%
MinalThakre and Kiran Bharti [9]	5295	40.3%	59.7%
Ajjak et al [10]	70	49.4%	51.6%
Present study	386	48%	52%

Females were affected more than males in our study which is comparable with study done by Minal Thakre and kiranbharti, Ajjak et al and Tambse et al.

Table 6: Comparison of percentage of various hemoglobinopathies with other studies

Hemoglobinopathies	Present study	Jain et al study [11]	Shrestha et al study [12]	Khera et al study [13]
Beta Thalassemia Trait	57.0	55.84	69.1	56.3
Sickle Cell Heterozygous	23.4	2.14	2.4	0.9
HbS Homozygous	5.1	0.54	0.8	
HbD-Punjab Heterozygous	4.1	0.36	0.8	7.2
HbE Heterozygous	3.2	15.7	4.9	6.3
Double Heterozygous for HbS and Beta Thalassemia	3.2	1.07	0.8	7.2
HPFH heterozygous	1.3	0.71	0.8	-
Double Heterozygous for HbE and Beta Thalassemia	0.9	11.95	0.8	-
Double Heterozygous for HbS and HbD	0.9	-	-	-
Beta Thalassemia Major	0.9	9.46	2.4	5.4

Comparison with other studies, Beta Thalassemia Trait (BTT) is Most Common hemoglobinopathy.

The Indian population comprises numerous castes and communities, each revealing different genetic traits. The distribution of beta thalassemia is variable in Indian subcontinent.

The highest frequency of BTT is reported in Gujarat (10-15%), Sindh (10%), Punjab (6.5%) and Tamilnadu (8.4%) [14]. In our study, there was highest percentage of thalassemia patients.

Conclusion

Hematological parameters along with HPLC findings aid in detecting and quantifying hemoglobin variants. HPLC forms a rapid, accurate and reproducible tool for early detection of hemoglobinopathies.

HPLC detect life threatening cases like double heterozygous for HbS and HbD and double heterozygous for HbE and BTT. So, it is beneficial to the patient, society and next generation.

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