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HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC) STUDY IN PATIENTS SUFFERING FROM HAEMOGLOBINOPATHIES AT A TERTIARY CARE HOSPITAL - AN ORIGINAL ARTICLE.

Pathology

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ABSTRACT

Introduction: Haemoglobinopathies are a group of disorders of hemoglobin. Inherited abnormalities of Hemoglobin synthesis may be divided into two groups: (1) Structurally abnormal hemoglobin variants. (2) One or more of the normal hemoglobin are synthesized at reduced rate.

Aims and Objectives:

1. To assess the pattern of Thalassemia syndrome and Other Haemoglobinopathies by HPLC (High Performance Liquid Chromatography) method in patients of Civil Hospital, Ahmedabad.
2. To study age and gender wise distribution of Haemoglobinopathies in patients of Civil Hospital, Ahmedabad.
3. To study the incidence of Haemoglobinopathies in different castes in patients of Civil Hospital, Ahmedabad according to the caste.
4. To compare the results of present study with the results of other authors similar studies.

Material and Method: The study has been carried out as "Time bound study" to find the pattern of β-Thalassemia and Other Hemoglobinopathies for a period of two years from October 2015 to November 2017.

Observation and Result: Most common HPLC findings in this study was Beta thalassemia Minor (38.47%) followed by Sickle cell trait (21.43%), Sickle cell disease (17.14%), Beta Thalassemia Major (5.71%), Sickle-β thalassaemia (5.71%) and Beta Thal. Intermedia (4.29%).

Conclusion: HPLC is easy, reproducible & accurate in most cases of haemoglobinopathies.

KEYWORDS

High Performance Liquid Chromatography (HPLC), Haemoglobinopathies.

INTRODUCTION:

Haemoglobinopathies are a group of disorders of hemoglobin. Inherited abnormalities of Hemoglobin synthesis may be divided into two groups:

1. Structurally abnormal hemoglobin variants.
2. One or more of the normal hemoglobin are synthesized at reduced rate.[1]

The incidence of β thalassaemia trait and sickle cell haemoglobin opathies varies between 3- 17 %[2] and 0-40%[3] respectively, because of high consanguinity amidst caste and area endogamy, making the disease a major public and genetic health problem in India. Considering schedule tribes, Gujarat is the 5th most populated state of India.

B-thalassaemia is a major monogenic single gene disorder resulting from a reduced or absent synthesis of β-globin chain. This mutant gene is common in communities like Sindhi, Parsee and Lohana and different ethnic groups of Punjabi, Bengali and Gujarati etc. The patients suffering from beta-thalassemia major and Hb E/beta-thalassemia do not survive for more than 5 years without blood transfusion.[4] The present study has been undertaken to find out the burden of haemoglobinopathies and spectrum of these disorders among the hospital-based patients of Civil Hospital, Ahmedabad.

Aims and Objectives:

(1) To assess the pattern of Thalassemia syndrome and Other Haemoglobinopathies by HPLC (High Performance Liquid Chromatography) method in patients of Civil Hospital, Ahmedabad.
(2) To study age and gender wise distribution of Haemoglobin opathies in patients of Civil Hospital, Ahmedabad.
(3) To study the incidence of Haemoglobinopathies in different castes in patients of Civil Hospital, Ahmedabad according to the caste.
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MATERIAL AND METHOD:

The study has been carried out as "Time bound study" to find the pattern of *β*-Thalassemia and Other Hemoglobinopathies for a period of two years from Otober 2015 to November 2017.

Bio-Rad variant II (beta thalassemia short programme) utilizes the principle of high performance liquid chromatography (HPLC). The software delivers a printed report showing the chromatogram with all the Hb fraction eluted, the retention times, the area of peaks and value (%) of different Hb component. If peak elute at a retention time that is not pre-defined, it is labelled as unknown. Each analytical cycle, from sampling to printing of results take about 6.5 minutes. Present study was done in two phase:

1. All indoor patient's samples sent to the central clinical laboratory were subjected to CBC, peripheral smear examination, sickling solubility test and reticulocyte count. Then according to inclusion criteria (mentioned in material and methods) all samples were segregated for further testing.
2. Total 70 samples were selected and were subjected to HPLC for abnormal hemoglobin pattern.

OBSERVATION AND RESULT:

Table No. 1: Approach to diagnosis of haemoglobinopathies with HPLC

Final diagnosis	Elution pattern of HPLC	No.	Percentage (%)
Sickle cell trait	A, S	15	21.43
Sickle cell disease	S	12	17.14
Beta thalassemia Minor	A, A_2	27	38.57
Beta Thalassemia Major	F	04	05.71
Beta Thal. Intermedia	A_2, F	03	04.29
Sickle-β thalassaemia	A,S,A_2	04	05.71
HPFH/ Delta beta thalassemia trait.	A,F	02	02.86
HPFH	A,F	01	01.43
Hb D Heterozygus	A, D	02	02.86
Total	70	70	100

Table No. 2: Percentage of Hb S in cases of Sickle cell trait.

Hb S%	No. of cases
0-10	1
11-20	1
21-30	4
31-40	9
41-50	0
Total	15

Table 2 shows that most of the trait subjects have Hb S% between 31-40%, although there were subject having as low as 9 and as high as 35.4. Average HbS percentage was 28.6 in the screened population.

Table No. 3: Percentage of Hb A2 in cases of Sickle cell trait.

Hb A_2%	No. of cases
0-3.5	13
3.5-5	2
>5	0
Total	15

Table No. 4: Percentage of Hb S in cases of Sickle cell disease.

Hb S%	No. of cases
< 50	0
51-60	2
61-70	4
71-80	4
81-90	2
91-100	00
Total	12

Table No. 4 shows that maximum Sickle Cell Disease has HbS% in range of 60% - 80%. Average HbS% was 69.10%.

Average A_2 in screened population was 3.35.

Table No. 5: Percentage of HbA2 in cases of Sickle cell disease.

Hb A_2%	No. of cases
0-3.5	8
3.5-5	4
>5	0
Total	12

Table No. 6: Percentage of Hb F in cases of Sickle cell disease.

Hb F%	No. of cases
0-10	5
11-20	3
21-30	4
31-40	00
>40	00
Total	12

Average value of fetal Hb in Sickle Cell Disease in present study was 12.9% and most of had fetal hemoglobin level between 10-30%.

Table No. 7: Age and Gender wise Distribution of Haemoglobinopathies.

Type of haemoglobinopathies	Age and Gender wise distribution							Percentage (%)
	0-12		13-36		37-60		Total	
	M	F	M	F	M	F		
Sickle cell trait	0	0	4	7	3	1	15	21.43
Sickle cell disease	3	5	1	3	0	0	12	17.14
B Thalassemia Minor	0	6	7	10	3	1	27	38.57
B Thalassemia Major	1	3	0	0	0	0	4	5.71
Sickle-β Thalassaemia	1	1	1	1	0	0	4	5.71
HbD Heterozygous	0	0	1	1	0	0	1	2.86
HPFH/ Delta Beta Thalassaemia	0	0	0	1	0	0	1	1.43
HFPH	0	0	2	0	0	0	2	2.86
Beta Thal . Intermedia	0	0	2	0	1	0	3	4.29
Total	5	15	18	24	6	2	70	100
Total (Including both gender)	20 (28.57%)		42 (60%)		8 (11.43%)		70 (M=29,F=41)	100

Table No. 8: Caste Wise Distribution of Haemoglobinopathies.

Type of haemoglobinopathies	Hindu General Category	Schedule Caste	Schedule Tribe	Muslims
Sickle cell trait	3	1	11	0
Sickle cell disease	4	1	5	2
B Thalassemia Minor	18	3	3	3
B Thalassemia Major	1	1	0	2
Sickle-β Thalassaemia	1	0	2	1
Hb D Heterozygous	1	0	0	1
HPFH/ Delta Beta Thalassaemia	1	0	0	0
HFPH	2	0	0	0
Beta Thal. Intermedia	2	0	0	1
Total	33	6	21	10

DISCUSSION:

Table No. 9: Comparison of distribution of various haemoglobinopathies of present study with other published studies*

Type of haemoglobinopathies	M.Vasaikar et al[5]	Seema Rao et al[6]	R.S. Balgir et al[7]	Jain BB et al[8]	M.M. Udin et al[9]	Present study
Sickle cell trait	82.7	4.4	45.3	2.1	1.1	21.4
Sickle cell disease	9.9	1.6	11.6	0.5	0.0	17.1
Beta thalassaemia trait	6.7	58.7	27.8	55.9	36.9	38.6
Thalassaemia major	0.1	9.3	8.1	9.5	0.9	5.7
Double heterozygous for HbS and Beta thalassaemia	0.4	2.4	2.5	1.3	0.0	5.7
HbD trait	0.1	2.8	0.3	0.3	0.0	2.86
Double heterozygous for HbS & HbD	0.0	0.4	0.3	0.1	0.0	0.0
HbE homozygous	0.0	0.4	0.4	0.6	15.8	0.0
Suspected α thalassemia	0.0	4.4	0.0	0.0	0.0	0.0
HPFH/ Delta beta thalassemia trait	0.0	2.4	1.3	0.7	0.9	1.4
Hereditary Persistant Fetal Hb. Heterozygous	0.0	0.0	0.0	0.0	0.0	2.9
Beta Thal. Intermedia	0.0	0.0	0.0	0.0	0.0	4.3
Other hemoglobinopathies	0.1	13.0	2.4	28.9	44.4	0.0

* - All above mentioned studies includes normal cases also. For the purpose of comparision with present study normal cases are excluded and percentage of positive cases are adjusted accordingly in all other studies.

In present study most common hemoglobinopathies were having sickle cell abnormality (44.2% of total cases) out of which Sickle cell trait were 21.4%, Sickle cell Disease were 17.1% and double heterozygous for sickle β thalassaemia were 5.7%. The patient drainage of the hospital is from central Gujarat and Madhya Pradesh. Central Gujarat is also known as a Sickle belt of Gujarat. So, there is a high frequency of sickle cell anemia cases in Present study. The results of present study correlate with results of M Vasaikar et al[5] and R S Bargil et al[7]. Studies of M Vasaikar et al[5] and R S Balgir et al[7] were carried out on Patients of Rural area of Maharashtra (Nandubar, Dhule, Nasik and Jalgao District) and Central east coast of India (Orrisa) respectively, where there is high prevalence of sickle cell anemia. On other hand other three studies, Seema Rao et al[6], Jain B B et al[8] and M M Udin et al[9] were done at Delhi, West Bengal and Dhaka, Bangladesh respectively. All these area have very high prevalence of thalassemia and HbE Disease. So, all these studies had very low prevalence of HbS.

CONCLUSION:

From present study, it can be concluded that HPLC is easy, reproducible & accurate in most cases. But one has to be cautious when dealing with compound heterozygous states of sickle & β thalassaemia, β thalassaemia & Hb D. These states require family study to have exact presumptive diagnosis. So, we can conclude that all abnormal hemoglobin finding should follow family study and HPLC for diagnosis.

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