Study of abnormal haemoglobin variants in patients of anaemia using high performance liquid chromatography (HPLC) in Gujarat, India

Jain R.[1], Saxena S[2]

[1]Dr. Richa Jain, Assistant Professor, [2]Dr. Shubhi Saxena, Assistant Professor, both are affiliated with Department of Pathology, Smt. B.K. Shah Medical Institute and Research Centre, Vadodara, Gujarat, India.

Corresponding Author: Dr. Shubhi Saxena, Assistant Professor, Department of Pathology, Smt. B.K. Shah Medical Institute and Research Centre, Vadodara, Gujarat, India. E-mail: shoobheee@gmail.com

Abstract

Introduction: Haemoglobinopathies are the most common inherited red cell disorders world-wide. Identification of these disorders is immensely important epidemiologically, for early diagnosis, improved management protocols and prevention of disease in upcoming generation. Our aim is to study the abnormal haemoglobin variants in patients of anaemia using High Performance Liquid Chromatography (HPLC) BIO-RAD D-10 Dual Program analyser in Vadodara region of Gujarat. **Methods:** A total of 1890 cases of patient's with anaemia having haemoglobin up to 11 gm% were studied for HPLC for diagnosing any abnormal haemoglobin disorder by BIO-RAD D-10 Dual Program analyser. **Results:** Out of 1890 cases of anaemia studied, 1236 (65.3%) cases showed abnormal haemoglobin, and rest 654 (52.9%) cases showed normal result on HPLC. Of these, 1236 cases, 686 cases of sickle cell trait (55.5%) the predominant abnormality, followed by 247 cases of sickle cell disease (19.9%). There were 208 cases of beta Thalassemia trait (16.8%), followed by 89 cases of double heterozygous state of beta thalassemia and sickle cell trait (7.2%). There were three cases of Thalassemia major with high Hb F (0.24%), two cases of Hb D Punjab heterozygous patients (0.16%) and one case of Hb E heterozygous (0.08%). **Conclusion:** HPLC is an accurate, simple and superior technique in detection of various haemoglobin disorders, which helps in management of patients and prognostic significance. It is also important in premarital and antenatal screening tests, to prevent birth of children with severe Haemoglobin disorders.

Keywords: Abnormal Haemoglobin variants, Anaemia, Gujarat, HPLC.

Introduction

Haemoglobinopathies are the group of genetic disorders of haemoglobin in which there is a quantitative or qualitative abnormal production, or in the structure of haemoglobin molecule [1,2]. Plethora of hemoglobin variants is prevalent in India owing to ethnic diversity of its population with minimal to major clinical significance. These hereditary disorders are major public health problem in many parts of the world including India [2]. The thalassemias are due to a quantitative defect in the globin chain production. Sickle cell anaemia is caused by a point mutation in beta globin chain. Sickle cell disease, beta-thalassaemia major and HbH disease (--/-a thalassaemia) are haemoglobinopathies with severe or intermediate pathology.

The average frequency of HbS and HbD is 4.3% and 0.86%, respectively in Indian population [3]. Detection of asymptomatic carriers by reliable laboratory methods is the cornerstone of prevention of this serious health problem.

Manuscript received: 20th October 2019
Reviewed: 30th October 2019
Author Corrected: 4th November 2019
Accepted for Publication: 8th November 2019

Identification of these disorders is immensely important epidemiologically, for early diagnosis, improved management protocols and prevention of disease in upcoming generation. 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. Awareness about the diagnostic problems as well as their solutions is very important so that one does not miss a single case. As the curative treatment like bone marrow transplantation is costly and so, a prospective prevention through population screening and genetic counselling is the best possible strategy for prevention of these disorders.

Automated cation exchange HPLC is being increasingly used as the initial diagnostic method in diagnosis of Haemoglobinopathies.[4]With increasing global awareness and mass screening programs undertaken at various levels by health care system, the responsibility for laboratory personnel has greatly enhanced in detection and prevention

of this problem. An automated system is the ideal choice for the routine clinical laboratory as by simplifying the labor and technical attention needed to handle internal sample preparation, produces superior resolution, rapid assay throughput, and accurate quantification. The aim of the present study is to determine the common Haemoglobin disorders, analyse its prevalence, and study the abnormal haemoglobin variants in patients of anemia using High Performance Liquid Chromatography (HPLC) BIO-RAD D-10 Dual Program analyser in Vadodara Region of Gujarat and therefore help in prevention, early diagnosis, for improved management protocols.

Materials and Methods

It is a retrospective study. Total 1890 samples of In-Patient Department (IPD) and Outpatient Department (OPD) were examined, out of which 1236 (65.3%) cases showed abnormal haemoglobin, and rest 654 (52.9%) cases showed normal result on HPLC. The study is conducted in patients with one-year period data.

The study is performed in Tertiary health care center,1360 bedded hospital. The machine used for estimating HPLC was Bio-Rad D-10 Dual Program analyser with lot number 70468 (Bio-Rad Laboratories) which is an automated cation exchange HPLC instrument. The Bio-Rad D-10 operates on the principle of HPLC and the column comprises of a small cation exchange cartridge, with a requirement of only 2ml of blood sample, and each sample taking only 6.5 minutes for analysis. The samples are injected into the analysis stream and separated by the cation exchange cartridge using a phosphate ion gradient generated by mixing 2 buffers of different ionic strengths to elute the different haemoglobins. A dual wavelength filter photometer monitors the eluent from the cartridge as it passes through the photometer cell. Changes in optical density at 415nm are measured. A secondary filter at 690nm corrects the effects caused by mixing buffers of different ionic strengths. The data is processed and the report giving the chromatogram where the different peaks are identified in defined windows with relevant information like retention time, relative percentage and area.[5]

Inclusion criteria

1. Patients with haemoglobin less than 11gm% using machine Sysmex KX-21.
2. Age <40 years.
3. Detection of Sickle shaped cells on peripheral smear.
4. Positive sickling solubility test.
5. History of more than 5 blood transfusion in absence of trauma or any clinical morbidity.

Exclusion criteria: There was no exclusion criteria.

Sample type: Whole blood

Sample additives, preservatives: Vacuum collection tube containing EDTA.

Sample storage: Can be stored up to 4 days at 2-8 degree Celsius or 1 day at room temperature (15-30 degree Celsius).

Table-1: Proportion of different haemoglobins in normal individuals and in haemoglobin disorders according to surface area percentage [6].

Condition	HbA	HbF	HbA_2	HbS
Normal Adults	97%	<1%	1-3%	0
Sickle cell trait	56-60%	0	1-3%	40%
Sickle cell anemia	0	5-10%	1-3%	90-95%
β thalassemia trait	90-95%	0-5%	4-7%	0
β thalassemia major	0	95-98%	2-5%	0

[Table 1] show established ranges in which common variants have been observed to elute using extended program.

The printed chromatogram of HPLC shows all the haemoglobin fractions eluted, the retention times, the areas of the peaks and the values (%) of different haemoglobin components. If a peak elutes at a retention time that is not pre-defined, it is labelled as an unknown.

Table-2: Proportion of different haemoglobins in normal individuals according to retention time in minutes [7].

Peak Name	Retention Time (minutes)
HbA	1.55-1.85
HbF	0.38-0.58
HbA2	2.80-3.50
S window	4.02-4.30

[Table 2] show proportion of different haemoglobins in normal individuals according to retention time in minutes [7].

Results

The present study is a retrospective study for a period of 1 year in the department of Pathology of SBKS MI&RC, Sumandeep University. A total of 1890 cases were studied, out of which 1236 (65.3%) cases were detected of abnormal haemoglobin and the results are put forward in tabular form.

Table-3: Age distribution.

Age	No of Patients	%
0-10 years	129	10.4
11-20 years	546	44.2
21-30 years	354	28.6
31-40 years	207	16.8
Total	**1236**	**100**

[Table 3] shows age distribution of 1236 cases screened, in which maximum number of cases were in age group of 11-20 years (44.2%) followed by 21-30 years (28.6%) and 31-40 years (16.8%).

Table-4: HPLC interpretation with age.

Age	HPLC Interpretation							
	Sickle cell trait	**Sickle cell disease**	**Thalassemia trait**	**Double heterozygous for thalassemia and sickle cell**	**Thalassemia major**	**Hb D**	**Hb E**	**Grand Total**
0-10 years	50	76	0	0	3	0	0	129
11-20 years	267	162	76	41	0	0	0	546
21-30 years	215	7	96	36	0	0	0	354
31-40 years	154	2	36	12	0	2	1	207
Grand Total	**686**	**247**	**208**	**89**	**3**	**2**	**1**	**1236**

[Table 4] shows HPLC interpretation with age.

Table-5: Gender distribution.

Gender	No of Patients	%
Female	782	63.3
Male	454	36.7
Grand Total	**1236**	**100**

[Table 5] shows gender distribution of 1236 cases screened, in which maximum number of cases were of female (63.3%) followed by male (36.7%).

Table-6: Gender distribution as per specific haemoglobin abnormalities.

Gender	HPLC INTERPRETATION							
	Sickle cell Trait	Sickle cell disease	Thalassemia trait	S-Beta Double heterozygous	Thalassemia major	Hb D	Hb E	Grand Total
Female	439	138	147	56	0	1	1	782
Male	247	109	61	33	3	1	0	454
Grand Total	**686**	**247**	**208**	**89**	**3**	**2**	**1**	**1236**

[Table 6] shows gender distribution of total 1236 cases as per specific haemoglobin abnormalities.

Table-7: HPLC Interpretation with CBC_ Hb(g/dl)

Hb (g/dl)	HPLC interpretation							
	Sickle cell trait	**Sickle cell disease**	**Thalassemia trait**	**S-beta Double heterozygous**	**Thalassemia major**	**Hb D**	**Hb E**	**Grand Total**
<05 (g/dl)	0	9	0	1	3	0	0	13
05-07 (g/dl)	1	117	2	3	0	0	0	123
>07-09 (g/dl)	189	104	14	49	0	0	0	356
>09-<12 (g/dl)	496	17	192	36	0	2	1	744
Grand Total	**686**	**247**	**208**	**89**	**3**	**2**	**1**	**1236**

[Table 7] shows HPLC Interpretation with Haemoglobin (g/dl) out of total 1236 cases, maximum diagnosed cases of sickle cell trait and thalassemia trait were under 9-12 g/dl, followed by sickle cell disease and S-beta double heterozygous state under 7-8 g/dl.

Table-8: HPLC interpretation with CBC TRBC (10^6/ul).

TRBC (10^6/ul)	HPLC interpretation							
	Sickle cell trait	**Sickle cell anemia**	**Thalassemia trait**	**S-beta Double heterozygous**	**Thalassemia major**	**Hb D**	**Hb E**	**Grand Total**
<2 (/ul)	0	2	0	0	3	0	0	5
2-3 (/ul)	89	114	0	2	0	0	0	205
3-4 (/ul)	298	127	5	46	0	1	1	478
>4 (/ul)	299	4	203	41	0	1	0	548
Grand Total	**686**	**247**	**208**	**89**	**3**	**2**	**1**	**1236**

[Table 8] shows HPLC Interpretation with TRBC (10^6/ul) out of total 1236 diagnosed cases, 44.3% cases have TRBC in range of >4 (10^6/ul) , 38.6% cases have TRBC in the range of 3-4 (10^6/ul) and 16.5% cases have TRBC in the range of 2-3 (10^6/ul).

Table-9: HPLC interpretation with CBC_MCV (fl).

MCV (fl)	HPLC interpretation							
	Sickle cell trait	Sickle cell disease	Thalassemia trait	S-beta Double heterozygous	Thalassemia major	Hb D	Hb E	Grand Total
35-45	0	0	0	0	2	0	0	2
45-55	0	35	37	0	1	0	0	73
55-65	16	116	164	13	0	0	0	309
65-75	270	94	5	42	0	1	0	412
75-85	395	2	2	34	0	1	1	435
85-95	3	0	0	0	0	0	0	3
95-100	2	0	0	0	0	0	0	2
>100	0	0	0	0	0	0	0	0
Grand Total	**686**	**247**	**208**	**89**	**3**	**2**	**1**	**1236**

[Table 9] shows HPLC Interpretation with MCV (fl) and out of 1236 total cases, 35.1% have MCV in the range of 75-85 (fl) and 33.3% cases have MCV in the range of 65-75 (fl) and 25% of cases have MCV in the range of 55-65 (fl).

Table-10: HPLC interpretation with MCH (pg).

MCH (pg)	HPLC interpretation							
	Sickle cell trait	Sickle cell disease	Thalassemia trait	S-beta Double heterozygous	Thalassemia major	Hb D	Hb E	Grand Total
10-20 (pg)	3	71	0	0	2	0	0	76
20-30 (pg)	437	175	189	58	1	1	1	862
30-40 (pg)	246	1	19	31	0	1	0	298
Grand Total	**686**	**247**	**208**	**89**	**3**	**2**	**1**	**1236**

[Table 10] shows HPLC Interpretation with MCH (pg) and out of 1236 cases, 69.7% cases have MCH (pg) in the range of 20-30 (pg) and 24.1% cases have MCH (pg) in the range of 30-40 (pg).

Table-11: HPLC Interpretation with CBC_MCHC(g/dl).

MCHC (g/dl)	HPLC interpretation							
	Sickle cell trait	Sickle cell disease	Thalassemia trait	S-beta Double heterozygous	Thalassemia major	Hb D	Hb E	Grand Total
25-30	428	208	78	38	3	0	0	755
30-35	257	39	128	47	0	2	1	474
35-40	1	0	2	4	0	0	0	7
40-45	0	0	0	0	0	0	0	0
Grand Total	**686**	**247**	**208**	**89**	**3**	**2**	**1**	**1236**

[Table 11] shows HPLC Interpretation with MCHC (g/dl) and out of 1236 total cases, 61.1% of cases have MCHC in the range of 25-30 (g/dl) and 38.3% cases have MCHC in the range of 30-35 (g/dl).

Table-12 - HPLC interpretation with CBC_RDW (CV).

RDW (CV)	HPLC interpretation							
	Sickle cell trait	Sickle cell disease	Thalssemia trait	S-beta Double heterozygous	Thalassemia major	Hb D	Hb E	Grand Total
10-20(CV)	304	24	132	53	0	1	0	514
20-30 (CV)	378	132	76	30	1	1	1	619
30-40 (CV)	4	91	0	6	2	0	0	103
Grand Total	**686**	**247**	**208**	**89**	**3**	**2**	**1**	**1236**

[Table 12] shows HPLC Interpretation with RDW (CV) and out of 1236 total cases, 50.1 of cases have RDW (CV) in the range of 20-30 and 41.5% cases have RDW (CV) in the range of 10-20 (CV).

Table-13: HPLC Interpretation with distribution of total number of cases.

	HPLC interpretation							
	Sickle cell trait	Sickle cell disease	Thalssemia trait	S-beta Double heterozygous	Thalassemia major	Hb D	Hb E	Grand Total
Total Cases	686	247	208	89	3	2	1	1236
Grand Total	**686**	**247**	**208**	**89**	**3**	**2**	**1**	**1236**

[Table 13] shows HPLC Interpretation with distribution of total number of cases. Maximum number of cases are reported to be of Sickle cell trait, followed by Sickle cell anaemia, Thalassemia trait, S-beta Double heterozygous, Thalassemia major, Hb D and Hb E cases.

Table-14: HPLC interpretation with distribution of total number of cases percentage wise.

HPLC interpretation	No of patients	%
Sickle cell trait	686	55.5
Sickle cell disease	247	19.9
Thalassemia trait	208	16.8
S-beta Double heterozygous	89	7.3
Thalassemia major	3	0.24
Hb D	2	0.18
Hb E	1	0.08
Grand Total	**1236**	**100**

[Table 14] shows HPLC Interpretation with distribution of total number of cases percentage wise. Maximum number of cases are reported to be of Sickle cell trait (55.5%), followed by Sickle cell anaemia (19.9%), Thalassemia trait (16.8%), S-beta double heterozygous (7.3%), Thalassemia major (0.24%), Hb D (0.18%) and Hb E (0.08%) cases.

Discussion

Anemia is defined as a reduction of the total circulating red cell mass below normal limits. In general, microcytic hypochromic anaemias are caused by disorders of haemoglobin synthesis. Hereditary haemoglobinopathies are widely distributed worldwide. Sickle cell disease is a common hereditary haemoglobinopathy caused by a point mutation in beta globin that promotes the polymerization of deoxygenated hemoglobin, leading to red cell distortion. Herrick first described a case of sickle cell disease in 1910 [8]. Sickle cell trait is a heterogenous state, where one gene from one parent is for HbS while the other gene is for HbA, the clinical picture is mild and may remain undetected. Sickle cell anaemia is a homozygous state, one gene each from both the parents are inherited. Clinically, these patients manifest early in life since HbS is more than 70% in the red cells [4]. The various factors responsible for development of vascular occlusion include sickle cell deformity, increased blood viscosity, and sickle cell-endothelial cell adherence. However, sickle cell endothelial cell adherence appears to play a significant role in vascular

occlusion which may result in painful crisis. The thalassemia syndromes are a heterogenous group of disorders caused by inherited mutations that decrease the synthesis of either the alpha globin or beta globin chains that compose adult hemoglobin, HbA (alpha2 beta2), leading to anemia, tissue hypoxia, and red cell hemolysis related to the imbalance in globin chain synthesis [9]. The Indian population comprises numerous casts and tribal groups, each revealing different genetic traits [10]. The haemoglobin abnormalities are mainly confined to certain areas, religions, casts and tribes particularly with endogamous norms of marriages, and by knowing the prevalence we can spread awareness regarding the diseases and their outcomes to general population and take measurements to treat them and help in prevention [11]. World Health Organization (WHO) figures estimate that 5% of world population is carrier for haemoglobin disorders [12,13,14]. The prevalence of sickle cell in India and beta Thalassemia trait varies between 1-44% and 3-17% respectively [15,16].

Table-15: Comparative studies of abnormal haemoglobin variants with the present study.

Studies	Total no. of samples analysed	Total no. of normal pattern observed	Total no. of abnormal pattern observed	Most common haemoglobinopathy observed	No. of most common Haemoglobinopathy observed
Campbell et al [17]	25750	24587	1163	Sickle cell trait	568
Sachdev et al [18]	2600	2273	327	Beta thalassemia trait	232
Rao et al [19]	800	553	247	Beta thalassemia trait	145
Chandrashekar et al [20]	543	00	543	Beta thalassemia trait	206
Bhalodia et al [21]	500	457	43	Beta thalassemia trait	26
Pant et al [22]	4800	4510	290	Beta thalassemia trait	216
Mondal et al [23]	119336	104804	14532	Beta thalassemia trait	5488
Banerjee et al [24]	1048	444	604	Beta thalassemia trait	156
Present Study	1890	654	1236	Sickle cell trait	686

This study is in correspondence with study of Campbell et al, where the maximum prevalence of abnormal haemoglobinopathy is Sickle cell trait [Table 15]. Abnormal hemoglobin as a cause of anaemia should be considered, as morbidity and mortality is higher in homozygous conditions of haemoglobinopathies. High Performance Liquid Chromatography (HPLC) is used as a screening test for detection, identification and quantification of haemoglobin variants. In this automated technique, blood sample (haemolysate) is introduced into a column packed with silica gel. Different haemoglobins get adsorbed onto the resin. Haemoglobin fractions are detected as they pass through a detector and recorded by a computer [5]. It is emerging as one of the best methods for screening and detection of various hemoglobinopathies with rapid, reproducible and precise results.

A total of 1890 cases were studied out of which 1236 cases were detected of abnormal haemoglobin. Out of which maximum number of cases are reported to be of Sickle cell trait 686 cases (55.5%), followed by Sickle cell anaemia 247 cases (19.9%), Thalassemia trait 208 cases (16.8%), S-beta double heterozygous 89 cases (7.3%), Thalassemia major three cases (0.24%), Hb D two cases (0.18%), Hb E one case (0.08%).

Detail investigation of anaemia keeping in mind the possibilities of detecting abnormal haemoglobin is very much helpful in finding out more carriers of different haemoglobinopathies. Combined approach of primary and secondary prevention needs to be followed. It will prove to be cost effective by preventing the birth of child with genetic homozygous inheritance disease. It is a common practice among clinicians that to give iron therapy in all anaemic patients. It can lead to unnecessary iron overload in patients of haemoglobin variants. In India premarital screening is still considered taboo. So, the best approach would be to target those patients attending the anaemic OPD, the antenatal population and extended family members.

Person having positive report for carrier state should be counselled regarding the nature of the disease and implications of being carrier which can help in preventing birth of child with homozygous inheritance of haemoglobinopathies. The limitations of the present study is that, only patients of Vadodara and patients coming for treatment in Vadodara were evaluated, with haemoglobin less than 11gm%, with age less than 40 years, whose abnormal haemoglobin was detected using cation exchange HPLC BIO-RAD D-10 Dual Program analyser.

Conclusion

In our country major cause of anaemia is nutritional deficiencies which can be treated by medications. Abnormal hemoglobin as a cause of anaemia should also be considered, as morbidity and mortality is higher in homozygous conditions of haemoglobinopathies.

The haemoglobin abnormalities are mainly confined to certain areas, religions, casts and tribes and by knowing the prevalence we can spread awareness regarding the diseases and their outcomes to general population and take measurements to treat them and help in prevention. Automated cation exchange HPLC is being increasingly used as the initial diagnostic method in diagnosis of Haemoglobinopathies. It is an accurate, simple and superior technique in detection of various haemoglobin disorders, which helps in management of patients and prognostic significance. It is also important in premarital and antenatal screening tests, to prevent birth of children with severe haemoglobin disorders.

What the study adds to the existing knowledge?

This study adds to the existing knowledge that the prevalence of abnormal haemoglobin variants in patients of anaemia in Vadodara region of Gujarat, India, using high performance liquid chromatography (HPLC) BIO-RAD D-10 Dual Program analyser and is therefore important for the formulation of specific diagnostic, preventive and therapeutic strategies and meet the future challenges.

Author's contribution

Dr. Richa Jain: Experimental studies, Data acquisition, Data analysis, Statistical analysis, Manuscript preparation
Dr. Shubhi Saxena: Concept, Design, Definition of intellectual content, Literature search, Manuscript preparation

Funding: No funding sources
Conflict of interest: None declared
Ethical Approval: This study was approved by the Institutional Ethics Committee

Abbreviations

HPLC: High Performance Liquid Chromatography, **HbA:** Adult Haemoglobin, **HbF:** Foetal Haemoglobin, **HbS:** Sickle Haemoglobin, **CBC:** Complete Blood Count, **No.:** Number, **Hb:** Haemoglobin, **TRBC:** Total Red Blood Cell, **MCV:** Mean Corpuscular Volume, **MCH:** Mean Corpuscular Haemoglobin, **MCHC:** Mean Corpuscular Haemoglobin Concentration, **RDW(CV):** Red cell Distribution Width (Coefficient of Variation).

References

1. WHO. Management of haemoglobin disorders. Report of joint WHO-TIF meeting on the management of haemoglobin disorders. Nicosia, Cyprus, 16-18 November 2007. World Health Organization 2008; 1-2. Available from: http:// www. who. int/ genomics/ WHO TIF genetics.

2. Vaz FE, Thakur CB, Banerjee MK, Gangal SG. Distribution of beta-thalassemia mutations in the Indian population referred to a diagnostic center. Hemoglob. 2000; 24 (3):181-194. doi: 10.3109/03630260008997526.

3. Balgir RS. Genetic epidemiology of the three predominant abnormal hemoglobins in India. J Assoc Physicians India. 1996;44(1):25-28.

4. Singh Tejindar. Atlas and Text of Hematology. 4th ed. New Delhi: Avichal Publishing Company; 2018.

5. Sood SK, Bhargava M, Shirish, Colah Roshan, Chandra S, Saxena Renu et al. Underlying principle of D-10TM dual mode. www.bio-rad.com/diagnostics.

6. Kawthalkar SM. Essentials of clinical pathology. Jaypee Brothers, Medical Publishers Pvt. Limited; 2018.

7. BIO-RAD: D-10 Dual Program. Customer Notification: Release of cartridge Resin Lot no. 70468. United States; 2018.

8. Kawthalkar SM. Essent Haematol. 2nd ed. New Delhi: Jaypee Brothers Medical Publishers; 2013.

9. Kumar V, Abbas AK, Fausto N, Aster JC. Robbins and Cotran pathologic basis of disease, professional edition e-book. Elsevier Health Sci; 2014.

10. Patel AG, Shah AP, Sorathiya SM, Gupte SC. Hemoglobinopathies in South Gujarat population and incidence of anemia in them. Indian J Hum Genet. 2012; 18 (3): 294-298. doi: 10.4103/0971-6866.107979.

11. Patne SC, Shukla J. Hemoglobin E disorders in Eastern Uttar Pradesh. Indian J Pathol Microbiol. 2009; 52 (1): 110-112.

12. Baruah MK, Saikia M, Baruah A. Pattern of hemoglobinopathies and thalassemias in upper Assam region of North Eastern India: high performance liquid chromatography studies in 9000 patients. Indian J Pathol Microbiol. 2014; 57(2): 236-43. doi: 10. 4103/0377-4929. 134680.

13. Patel U, Shrivastav A, Joshi JR, Agnihotri AS, Kaur A, Thakkar B. Detection of hemoglobinopathies and thalasemias in population of Gujarat State using HPLC: Analysis of 2022 cases. Pathol Lab Med. 2012;4(2):80-84.

14. Philip J, Sarkar RS, Kushwaha N. Microcytic hypochromic anemia: Should high performance liquid chromatography be used routinely for screening anemic and antenatal patients? Ind J Pathol Microbiol. 2013;56(2):109-113. doi: 10.4103/0377-4929.118699.

15. Patel AP, Naik MR, Shah NM, Sharma N, Parmar P. Prevalence of common hemoglobinopathies in Gujarat: An analysis of a large population screening programme. Natl J Community Med. 2012;3(1):112-116.

16. Parikh UR, Goswami HM, Mehta RC, Patel PS, Gonsai RN. Incidence of hemoglobinopathies in women attending antenatal clinics in their first trimester. NHL J Med Sci. 2014;3(1).

17. Campbell M, Henthorn JS, Davies SC. Evaluation of cation-exchange HPLC compared with isoelectric focusing for neonatal hemoglobinopathy screening. Clin Chem. 1999; 45(7):969-975.

18. 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. doi: 10.4103/0377-4929.59185.

19. Rao S, Kar R, Gupta SK, Chopra A, Saxena R. Spectrum of haemoglobinopathies diagnosed by cation exchange-HPLC & modulating effects of nutritional deficiency anaemias from north India. Indian J Med Res. 2010; 132:513-519.

20. Chandrashekar V, Soni M. Hemoglobin disorders in South India. ISRN Hematol. 2011;2011:748939. doi: 10. 5402 /2011/ 748939. Epub 2011 Jun 28.

21. 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 Community Med. 2015; 6 (1): 35-40.

22. Pant L, Kalita D, Singh S, Kudesia M, Mendiratta S, Mittal M, Mathur A. Detection of abnormal hemoglobin variants by HPLC method: common problems with suggested solutions. Int Scholar Res Not. 2014;2014. doi: http://dx.doi.org/ 10.1155/2014/257805.

23. 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(1):105-110. doi: 10. 4103/0973-6247.175424.

24. Banerjee S, Singh RK, Shrivastava RK, Mahto SK. Study of haemoglobinopathies in patients of anaemia using High Performance Liquid Chromatography (HPLC) in rims (a premier institute of Jharkhand). J Evol Med Dent Sci-JEMDS. 2016; 5(46): 3029-3033. doi: 10.14260/jemds / 2016 / 681.

How to cite this article?

Jain R, Saxena S. Study of abnormal haemoglobin variants in patients of anaemia using high performance liquid chromatography (HPLC) in Gujarat, India. Trop J Path Micro 2019;5(11):925-933doi:10.17511/jopm.2019.i11.15