International Journal of Medical Research Professionals
P-ISSN: 2454-6356; E-ISSN: 2454-6364
DOI: 10.21276/ijmrp

IJMRP

Original Research Article

Study of Haemoglobinopathies and Hb Variants in Ajmer Region, Using HPLC

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ABSTRACT

Introduction: Haemoglobinopathies are the most common hereditary disorders, characterized by abnormalities both quantitative and qualitative in the synthesis of haemoglobin and pose a major health problem in India.

Aims & Objectives: Haemoglobinopathies were studied in relation to age, sex, ethnic group, red blood cell indices and peripheral blood smear examination, using HPLC.

Materials & Methods: A total of 717 patients of suspected haemoglobinopathies were analysed on the Bio-Rad Variant HPLC system by β-thal short program. The retention times, proportion of the haemoglobin (%) and the peak characteristics for all haemoglobin fractions were recorded. The blood samples of patients was analysed for complete haemogram on Auto Haematology Analyser.

Results: A total 717 cases were studied. Of these, 395 (55.09%) had normal and 322 (44.91%) showed abnormal haemoglobin fractions on HPLC. Out of 322, 178 cases (55.28%) were males and 144 cases (44.72%) were females. Of these, most common haemoglobinopathy was β-Thalassemia Minor/BTT 206 cases (28.73%) followed by β-Thalassemia Major 43 cases (5.99%), δβ-Thalassemia 21 cases (2.93%) and HPFH (Hereditary persistence of fetal haemoglobin) 14 cases (1.95%). Other hemoglobinopathies were also identified in smaller proportions. Prevalence of haemoglobinopathies were higher in the Sindhi community of Ajmer region (Raj.) India.

Conclusion: HPLC is very simple, rapid, accurate and superior technique in early detection of various haemoglobin disorders.

Key Words: Haemoglobinopathies, HPLC, Thalassemia.

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Article History:

Received: 02-06-2017, **Revised:** 03-07-2017, **Accepted:** 22-07-2017

Access this article online	
Website: www.ijmrp.com	Quick Response code
DOI: 10.21276/ijmrp.2017.3.4.023	

INTRODUCTION

Haemoglobinopathies are a group of inherited haemoglobin diseases, defined by qualitative (variant Hbs) and quantitative (thalassemia syndrome) abnormalities affecting the globin chains.[1] Classified into two broad groups, the haemoglobinopathies and the thalassemias. The haemoglobinopathies are characterised by the production of structurally defective haemoglobin due to abnormalities in the formation of the globin moiety of the molecule.[2] The thalassemias are characterised by either the reduction or the absence of synthesis of α- or β-globin chains, known as the α-thalassemias and β-thalassemia, respectively.[1] These hereditary disorders are major public health problem in the world including India.[3]

MATERIAL & METHODS

Undertaking and permission was obtained from the ethical committee for conducting the study and collecting information. The present study was done on patients presenting with suspected haemoglobinopathies at tertiary care centre in Rajasthan retrospectively from January 2011 to September 2013 and prospectively from October 2013 to December 2015, (Total 5 years duration). The study was performed on total 717 patients coming to the OPD as well as indoor patients, and from other hospitals also.

Haemoglobin and RBC indices were measured on automated five part differential cell counter using well mixed anticoagulated blood. Peripheral blood smears examination was also done in all the patients. The results of RBC indices were correlated with peripheral smear examination. The tests were performed on BIO-RAD 'VARIANT II' (β-thalassemia short program) which utilizes the principle of high performance liquid chromatography (HPLC). An Hb A2/F calibrator and two levels of controls (BIO-RAD) were analysed at the beginning of each run. The total area acceptable

was between one million to three million. Sample ratio was increased in case of low total area and vice versa. The software delivers a printed report showing the chromatogram, with all the haemoglobin fractions eluted. The integrated peaks are assigned to manufacturer defined "windows" derived from specific retention time (RT). This retention time is the time that elapses from the sample injection to the apex of the elution peak, of normal haemoglobin fraction and common variants. The printed chromatogram 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 predefined, it is labelled as an unknown. Each analytical cycle, from sampling to printing of results takes about 6.5 minutes.[4]

Statistical Analysis

Statistical parameters as mean, standard derivation and chi-square test were used in the study. P value <0.05 was regarded as significant, P value <0.01 was regarded as highly significant. All the statistical methods were carried out through the SPSS for windows (version 12.0).

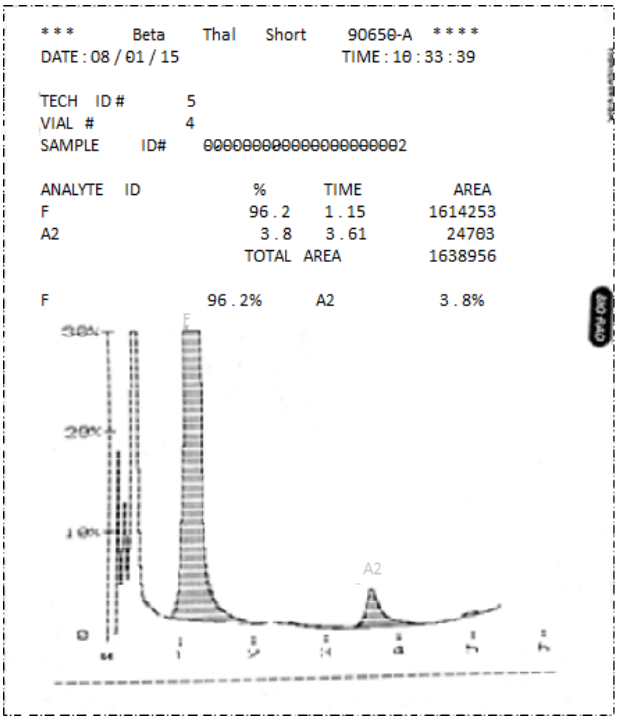

Fig 1: Chromatogram of β-Thalassemia Major. HbA2-3.8%, HbF-96.2%

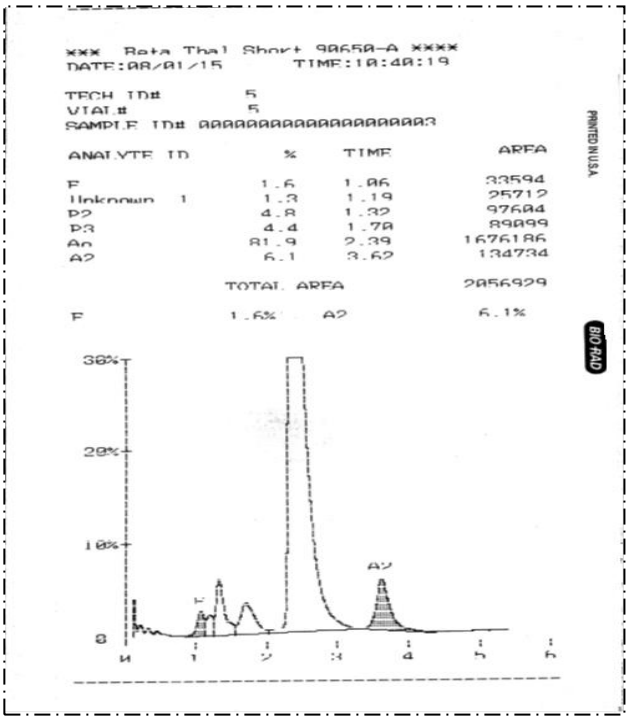

Fig 2: Chromatogram of β-Thalassemia Minor (BTT). HbA2-6.1%, HbF-1.6%, HbA0-81.9%

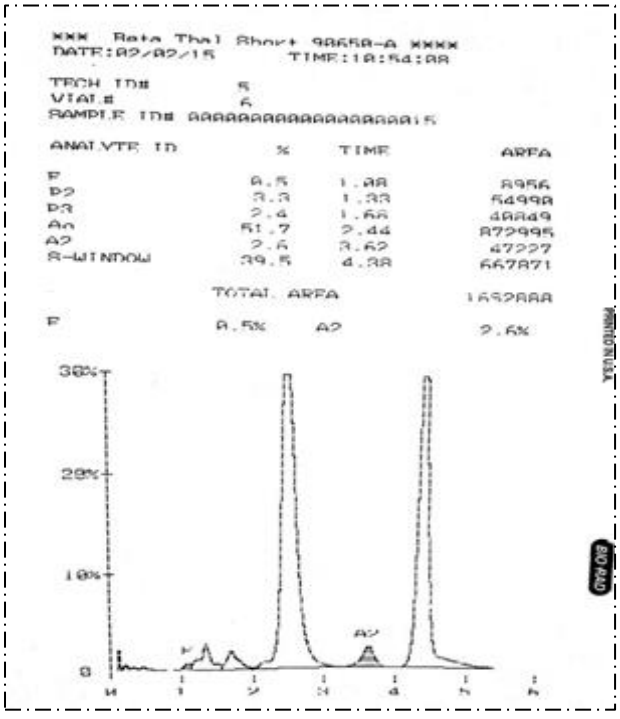

Fig 3: Chromatogram of Sickle cell trait (HbAS). HbA2-2.6%, HbF-0.5%, HbA0-51.7%, HbS-39.5%

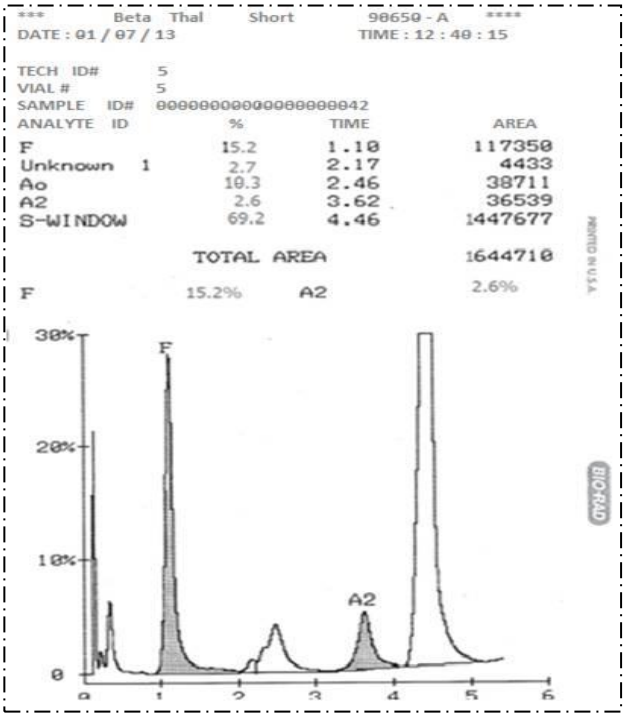

Fig 4: Chromatogram of Sickle cell anaemia (HbSS). HbA2-2.6%, HbF-15.2%, HbA0-10.3%, HbS-69.2%

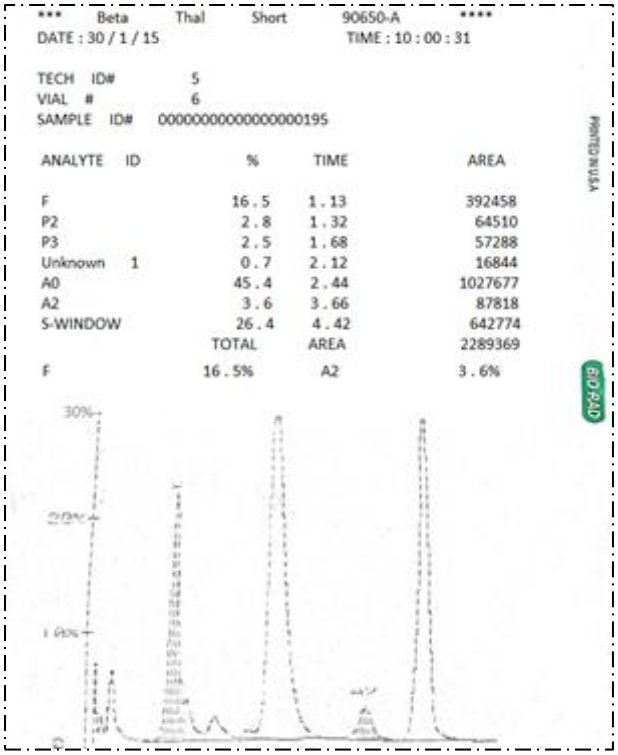

Fig 5: Chromatogram of HbS - β-Thalassemia. HbA2-3.6%, HbF-16.5%, HbA0-45.4%, HbS-26.4%

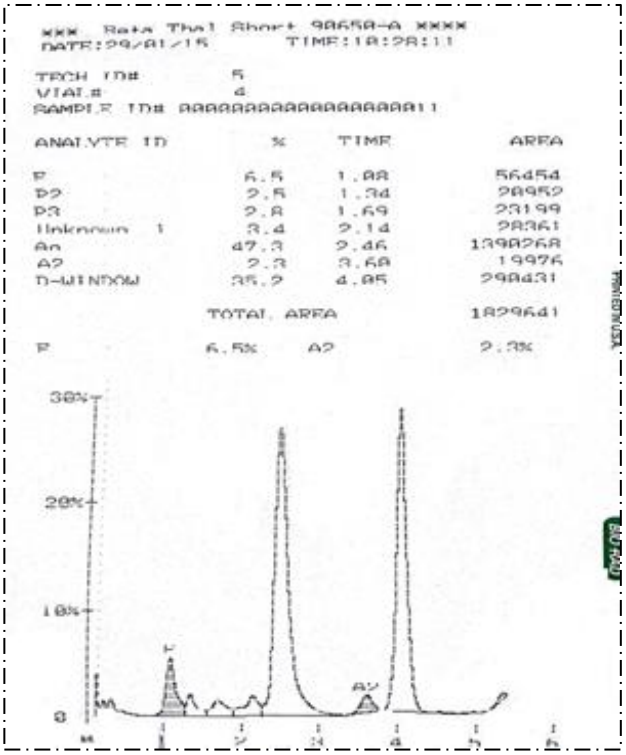

Fig 6: Chromatogram of HbD Punjab trait. HbA2-2.3%, HbF-6.5%, HbA0-47.3%, HbD -35.2%

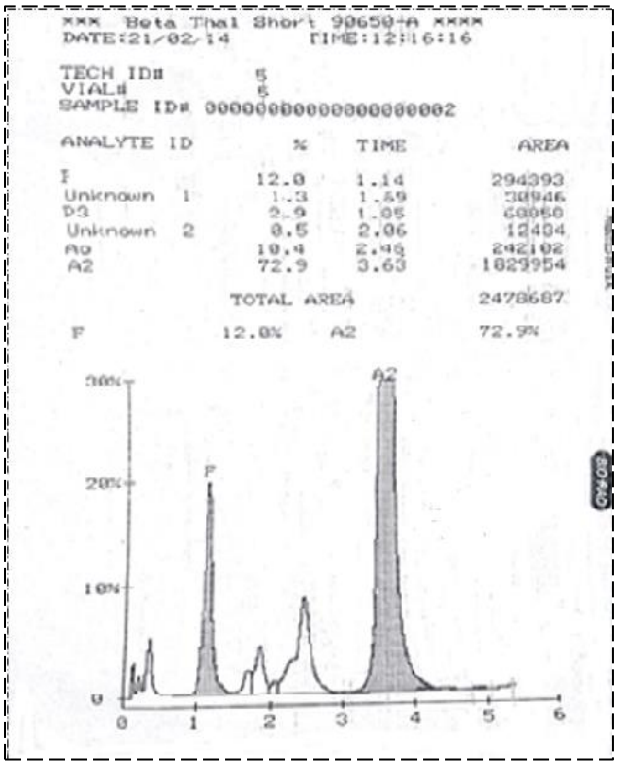

Fig 7: Chromatogram of Homozygous E Disease (HbEE) HbA2+E-72.9%, HbF-12.0%, HbA0-10.4%,

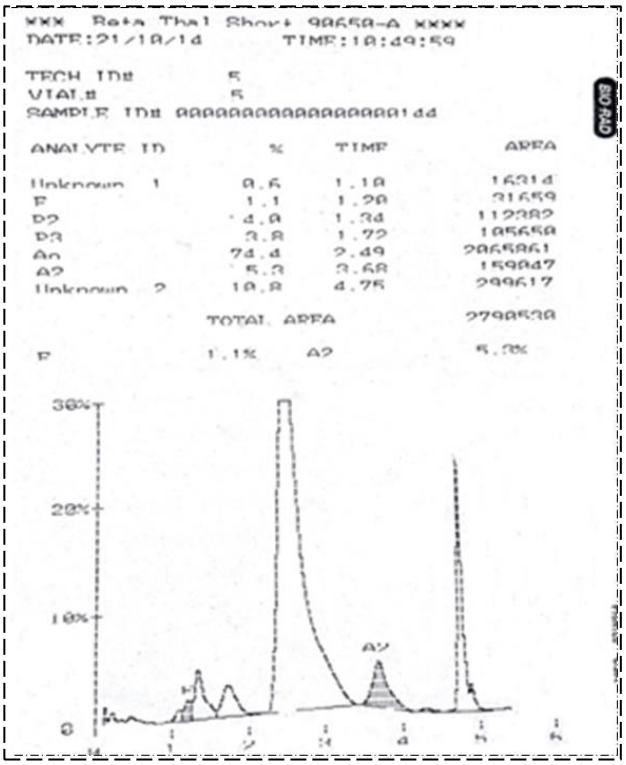

Fig 8: Chromatogram of HbQ India. HbA2-5.3%, HbF-1.1%, HbA0-74.4%, Unknown2-10.8 (RT-4.75min.)

RESULTS

A total 717 cases were studied. Of these, 322 (44.91%) showed abnormal Hb fractions. In our study, the most common incidence among the various haemoglobinopathies, was of β-thalassemia minor (BTT) 206 cases (28.73%) (Figure 2), followed by β-thalassemia major 43 cases (5.99%) (Figure 1), δβ-thalassemia minor 21 cases (2.93%), HPFH 14 cases (1.95%), HbS- β-thalassemia 07 cases (0.98%) (Figure 5), HbD trait 07 cases (0.98%) (Figure 6), Sickle cell trait (HbAS) 06 cases (0.83%) (Figure 3), β-thalassemia intermedia 03 cases (0.42%), HbE β-thalassemia 03 cases (0.42%). Two cases (0.28%) each of Sickle cell anaemia (HbSS) (Figure 4), HbS-HbD (double heterozygous), HbE trait and HbE homozygous (Figure 7) were seen. One case (0.14%) each of β-thalassemia minor (BTT)- borderline, HbD- β-thalassemia, HbQ India (Figure 8) and α-thalassemia trait were also diagnosed. (Table 1)

Overall haemoglobinopathies were more common in males (178, 55.28%) than in females (144, 44.72%). The male: female ratio was 1.24: 1. However BTT-borderline and HbD- β-thalassemia cases were diagnosed only in females & HbD trait more common in females than in males.

179 cases (55.59%) of various haemoglobinopathies were most commonly seen in the paediatric age group of 0-15 years followed by 127 cases (39.44%) were in the age group of 16-45 years and minimum numbers of 16 cases (4.97%) were seen in the ≥46 years of age.

Most common age group of presentation of β-thalassemia was found in the age group of 0-15 years with β-thalassemia major most common in 0-15 years and β-thalassemia minor / BTT in 16-45 years whereas thalassemia-intermedia and BTT- borderline cases were seen in the age group of 0-15 years.

In our study the ethnic groups were divided as General (Brahmins, Jains, Maheshwari, Rajput, Baniya), OBC/SC/ST, Sindhi, Punjabi and Muslim community.

Maximum cases of haemoglobinopathies were found commonly in Sindhi community 155 cases (48.13%), followed by General 66 cases (20.50%), OBC/SC/ST 50 cases (15.53%), Muslim 40 cases (12.42%) and 11 cases (3.42%) in Punjabi community.

On studying the mean Hb concentration, RBC count and mean absolute indices, it was found out that their values were on the line of expectation, i.e. in cases of β-Thalassemia Major they were severely reduced whereas in cases of β-Thalassemia-Intermedia and minor they were moderately reduced. (Table 2)

Peripheral blood smear of β-thalassemia minor (BTT) showing microcytic hypochromic RBCs, anisopoikilocytosis, tear drop cells, pencil cells, in cases of β-thalassemia major peripheral blood smear showing microcytic hypochromic RBCs, nucleated RBCs and target cells. Peripheral blood smear of Sickle cell anaemia showing numerous sickled RBCs (Leishman's stain) (Figure 9) and Positive Sickling test in Sickle cell anaemia showing sickled RBCs. (Figure 10)

The various parameters of HPLC are expressed in their mean value. The cut off value of HbA2 for defining β-Thalassemia Minor (BTT) was taken as > 3.5%. (Table 3)

Table 1: Distribution of Various Haemoglobinopathies

Haemoglobinopathies	Male	Female	Total	%
β-Thalassemia Major	28	15	43	5.99
β-Thalassemia Intermedia	01	02	03	0.42
β-Thalassemia Minor (BTT)	108	98	206	28.73
BTT – borderline	-	01	01	0.14
HPFH	09	05	14	1.95
δβ-Thalassemia	12	09	21	2.93
Sickle cell anaemia (HbSS)	02	-	02	0.28
Sickle cell trait (HbAS)	03	03	06	0.83
HbS- βThalassemia	04	03	07	0.98
HbS-HbD (double heterozygous)	02	-	02	0.28
HbD trait	03	04	07	0.98
HbD- βThalassemia	-	01	01	0.14
HbE trait	01	01	02	0.28
HbE Disease Homozygous	01	01	02	0.28
HbE -β Thalassemia	02	01	03	0.42
HbQ India	01	-	01	0.14
α-Thalassemia trait	01	-	01	0.14
Normal HPLC Pattern	209	186	395	55.09
Total	387	330	717	100

P 0.001 (Highly Significant); r = 0.9857; HPFH: Hereditary persistence of fetal haemoglobin; BTT: Beta thalassemia trait.

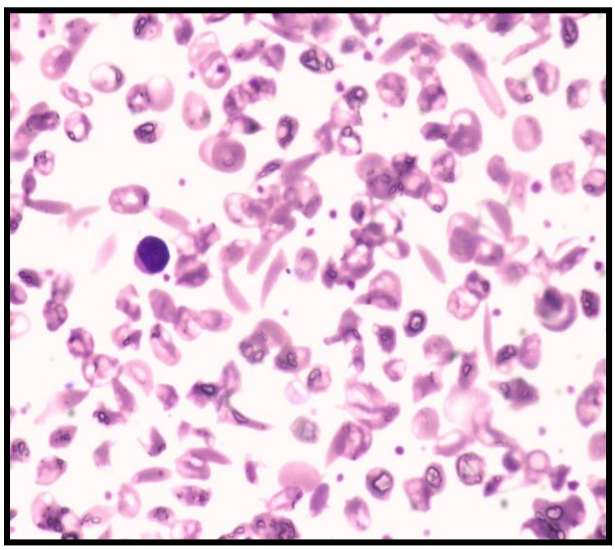

Figure 9: PBF of Sickle cell anaemia showing numerous sickled RBCs (Leishman's stain, 100X)

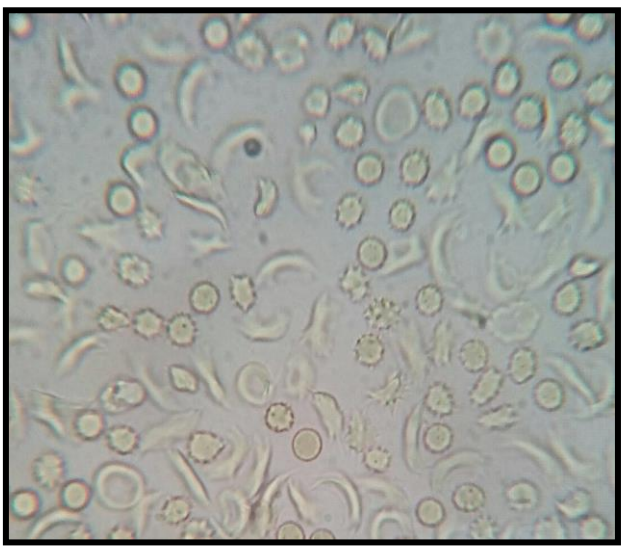

Figure 10: Positive Sickling test showing sickled RBCs (40X)

Table 2: Red Blood Cells Indices in Various Haemoglobinopathies

Haemoglobinopathies	Hb(g/dl) Mean±SD	RBC count (mill/cmm) Mean±SD	MCV(fl) Mean±SD	MCH (pg) Mean±SD	MCHC (%) Mean±SD
β-Thala Major	8.04±1.26	4.12±0.68	62.64±6.85	19.17±4.03	24.34±4.06
β-Thal Intermedia	10.1±0.53	3.73±0.72	69.3±11.49	25.83±4.83	32.26±1.85
β-Thal Minor(BTT)	9.16±1.38	5.65±0.87	69.11±7.79	21.34±3.80	27.04±3.47
BTT-borderline	9.2±0	3.66±0	83.1±0	25.1±0	30.3±0
HPFH	9.88±1.57	4.01±0.58	80.28±3.83	27.53±2.54	31.73±2.39
δβ-Thalassemia	9.02±0.92	3.89±0.55	75.39±4.85	22.98±3.55	27.69±2.68
Sickle cell anaemia (HbSS)	8.95±0.35	3.8±0	67.5±2.33	25.5±1.27	28.5±0.28
Sickle cell trait (HbAS)	8.86±2.06	4.25±0.98	74.71±5.11	22.8±3.73	28.83±4.33
HbS- βThal	8.98±1.32	5.04±1.82	72.34±10.42	24.44±1.77	29.21±2.62
HbS-HbD	8.6±0	6.5±0	59.8±0	19.2±0	23.4±0
HbD trait	8.66±0.48	3.5±1.02	68.94±5.74	20.53±3.42	24.51±3.07
HbD- βThal	9.4±0	4.6±0	53±0	18.3±0	34.6±0
HbE trait	8.3±0	3.5±0.85	71.55±8.98	18.75±3.46	24.45±2.75
HbE Disease Homozygous	8.5±1.99	3.1±0.28	68.95±4.88	21.05±5.16	28±2.26
HbE -β Thal	8.63±1.36	4.86±0.60	71.7±4.9	21.16±3.55	26.1±2.91
HbQ India	9.9±0	5.1±0	67.9±0	23±0	26±0
α-Thal trait	11.2±0	4.5±0	78.4±0	22.5±0	32.2±0

Hb: Haemoglobin; RBC: Red blood cell; PCV: Packed cell volume; MCV: Mean corpuscular volume; MCH: Mean corpuscular haemoglobin; MCHC: Mean corpuscular haemoglobin concentration; RDW: Red cell diameter width; HPLC: High performance liquid chromatography; SD: Standard deviation; HPFH: Hereditary persistence of fetal haemoglobin; BTT: Beta thalassemia trait; thal: Thalassemia.

Table 3: HPLC Findings in Various Haemoglobinopathies

Haemoglobinopathies	HbA2% Mean±SD	HbF% Mean±SD	HbS% Mean±SD	HbD% Mean±SD	HbQ% Mean±SD
β-Thal Major	2.65±1.29	72.42±23.51	-	-	-
β-Thal Intermedia	3.3±1.74	27.77±9.14	-	-	-
β-Thal Minor(BTT)	5.13±1.23	2.27±2.27	-	-	-
BTT-borderline	3.5	0.68	-	-	-
HPFH	2.56±0.57	14.48±9.78	-	-	-
δβ-Thal minor	2.53±0.44	8.8±4.81	-	-	-
Sickle cell anaemia (HbSS)	2.2±0.56	1.51±0.01	73.4±5.94	-	-
Sickle cell trait (HbAS)	3.38±0.43	1.07±0.76	32.56±4.58	-	-
HbS- βThalassemia	4.53±0.76	15.93±8.82	46.81±18.4	-	-
HbS-HbD	4.15±0.21	4.15±0.07	41.85±2.33	42±4.66	-
HbD trait	2.16±0.42	2.06±2.48	-	31.03±5.46	-
HbD- βThalassemia	4.1	1.1	84.7	-	-
HbE trait	29.05±1.77	2.4±2.83	-	-	-
HbE Disease Homozygous	79.75±6.69	6±8.48	-	-	-
HbE -β Thalassemia	31.37±14.9	20±9.42	-	-	-
HbQ India	5.3	1.1	-	-	10.8
α-Thalassemia trait	0	4.1	-	-	-

HPFH: Hereditary persistence of fetal haemoglobin; BTT: Beta thalassemia trait; thal: Thalassemia; SD: Standard deviation.

DISCUSSION

The β-thalassemia and their interaction with structural haemoglobin (Hb) variants, beta HbS, HbE and HbD are a major public health problem in India. The distribution of specific disorders varies geographically and by community. World Health Organization figures estimates that 7% of the world population is a carrier for Hb disorders.[5] The prevalence of β-thalassemia trait and sickle cell in India varies between 3-17% and 1-44% respectively in general population.[3,6] Every year 10,000 children with thalassemia major are born in India, which constitutes 10% of the total number in the world.[7]

A large number of haemoglobin variants prevalent in the populations of Ajmer region indicate that haemoglobinopathies are not uncommon at birth and also their related complications. This scenario of haemoglobinopathies reflects that the population of the Ajmer region is genetically heterogeneous and so many ethnic elements have absorbed into the main stream of people along with the original inhabitants with varied genetic heritages, resulting in population diversity with the passage of time.

Chopra GS et al (2008)[8] in their study found that, the incidence of HbS- β-thalassemia was 06 case (0.6%), HbD trait 10 cases (01%), HbE trait 08 cases (0.8%) and HbE homozygous 06 cases

(0.6%). Results were comparable to our study. Chaudhury SR et al (2013)[9] in their study reported the incidence of β-thalassemia Intermedia as 36 cases (0.25%), HbS- β-thalassemia 135 cases (0.95%), HPFH 269 cases (1.9%), HbAS 39 cases (0.27%) and HbE homozygous 18 cases (0.13%). Results were similar to our study. Mannan A et al (2013)[10] in their study the incidence of β-thalassemia major was 11 cases (5.30%) and β-thalassemia minor (BTT) was 63 cases (30.43%). Results were comparable to our study. Mandal PK et al (2014)[11] in their study the incidence of HbAS was 284 cases (0.65%) and HbE- β-thalassemia 213 cases (0.42%). Results were similar to our study. Bhalodia JN et al (2015)[4] in their study the incidence of HbAS was 06 cases (1.2%), HbD trait 02 cases (0.40%), HbD- βThalassemia 01 case (0.20%), HbE- β-thalassemia 01 case (0.20%) and HbE homozygous 01 case (0.20%). Results were comparable to our study. In our study, the most common age group of presentation (55.59%) was 0-15 years which was similar to that of Uddine MM et al (2012)[12], (55.7%). More than 50% of the patients presenting for medical attention was in the age group of 0-15 yrs. Hence there was higher chance of detection of various haemoglobinopathies in this age group.

In our study, male to female ratio was 1.24:1 which was similar to Dangi CBS et al (2013)[13], 1.22:1. This may be due to the prevalent socio-cultural factors in our society, that more male patients seek medical attention.

In our study, Sindhi community 155(48.13%) was having higher incidence of haemoglobinopathies than those reported by Sinha S et al (2004)[14] and lower in the General 66(20.50%) and OBC/SC/ST 50(15.53%) community. The result of our study among the Punjabi 11 (3.42%) and Muslims 40 (12.42%) were comparable to that of Sinha S et al (2004).[14] In our study the various haemoglobinopathies were most prevalent among the Sindhi community. These findings were similar to those reported in various other studies conducted in the past in different regions. It may be due to the reason that the bulk of the population is being formed by the Sindhi community. The incidence of β-thalassemia has been mainly attributed to its high prevalence in the migrant populations of Sindhi and Punjabi origin.

Uddine MM et al (2012)[12] in their study, they found that, in β-thalassemia minor (BTT) the Mean ± SD of Hb was 9.23±2.96 and MCH was 19.5±3.31. Results were comparable to our study. Philip J et al (2013)[15] in their study, they found that, in β-thalassemia minor (BTT) the Mean ± SD of Hb was 9.8±2.4, RBC 5.6±0.9, MCV 68.5±6.2, MCH 21.3±2.6 and MCHC 28.3±1.8. Results were similar to our study. Shrivastav A et al (2013)[7] in their study, they found that, in β-thalassemia minor (BTT) the Mean ± SD of RBC was 5.38±0.91. Result was similar to our study. Brush MK et al (2014)[16] in their study, they found that, in β-thalassemia minor (BTT) the Mean ± SD of Hb was 7.9±3.4. Result was similar to our study. Bhalodia JN et al (2015)[4] in their study, they found that, in β-thalassemia minor (BTT) the Mean ± SD of Hb was 8.3±2.5. Result was comparable to our study. β-thalassemia trait is commonly first suspected by a specific pattern in the red blood cell counts and red blood cell indices generated by an automated blood counter. In this condition, the haemoglobin (Hb) level is normal or minimally reduced, the red cell count raised, the mean corpuscular volume (MCV) and the mean corpuscular haemoglobin (MCH) being less than 75 fl and 27 pg respectively. In our study also similar findings were seen.

CONCLUSION

It can be concluded from our study that haemoglobinopathies impose high clinical, psychological and economical burden on the patients and their families in Ajmer region. The Indian subcontinent is a rich reservoir of thalassemia and various abnormal haemoglobinopathies. Reliable detection and identification methods are required for various haemoglobinopathies. HPLC is single, highly reproducible system, making it an excellent technology to screen various haemoglobinopathies. HPLC has a high degree of reproducibility and precision. All cases of anaemia should undergo HPLC screening. There should be an initiative towards population screening, genetic counselling and pre-natal diagnosis to counter the magnitude of problem. More efforts are needed to increase awareness in high risk communities like Sindhis to control haemoglobinopathies. Also, an important public strategy would be to promote an early detection and diagnosis of anaemia in the first year of life, aiming at early treatment and preventive measures.

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Source of Support: Nil.

Conflict of Interest: None Declared.

Cite this article as: Geeta Pachori, Radha Kishan Meena, Ravi Kant Sunaria, Pramendra Pachori, Tushar Bayla. Study of Haemoglobinopathies and Hb Variants in Ajmer Region, Using HPLC. Int J Med Res Prof. 2017; 3(4):111-17.
DOI:10.21276/ijmrp.2017.3.4.023