Original Article

Study of Hemoglobinopathies in Ajmer region (Rajasthan)

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ABSTRACT

Introduction: *Hemoglobinopathy is an inherited condition with alteration in the structure or quantity of hemoglobin, which reduces the oxygen carrying capacity of blood, manifesting as anemia that is unresponsive to conventional iron therapy.*

Materials and methods: *Data of 190 patients was collected for 6 months, retrospectively, who were screened by using complete blood count (C.B.C.), red cell indices, peripheral blood film (PBF) and High Performance Liquid Chromatography, HPLC (Bio Rad Variant II). Patients with severe anemia were included in the study.*

Result: *A total of 44 (23.15%) cases of various abnormal hemoglobins were detected using combination of red cell indices and HPLC technique. Most common hemoglobinopathy detected was 26 (13.68%) cases of beta-thalassemia minor, 7 (3.68%) cases of delta-beta thalassemia, 5 (2.63%) cases of beta-thalassemia major, 2 (1.05%) cases each of homozygous hemoglobin-D disease and hemoglobin-Q disease, 1 (0.52%) case each of hemoglobin E disease and HbS-beta thalassemia. Out of total cases detected, 23 (52.27%) cases were female patients, and 21 (47.72%) cases were males. The age group of patients ranged from 6 months to 80 years, with most cases of beta-thalassemia minor detected under 5 years of age.*

Discussion and conclusion: *HPLC is an accurate and reliable tool to screen patients for hemoglobinopathies, especially targeting patients with refractory anemia. Screening and identification of these conditions has special importance in our national goal of achieving 12gm% hemoglobin by the age of 12 in all children in the country.*

INTRODUCTION

Hemoglobinopathies are a group of inherited disorders, defined by qualitative and quantitative abnormalities in haemoglobin synthesis.1Thalassemias are the most common monogenic inherited disorder in the world. The haemoglobinopathies are characterised inherited defect 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

It is fortunate that the common haemoglobin variants that have clinical or genetic significance (e.g. Hbs S, C,DPunjab, E and OArab) are readily detectable by electrophoretic and chromatographic techniques.3

Haemoglobinopathies can be classified based on the cause:

1.Anaemias due to structural variation in haemoglobin chain eg: HbS.

2.Anaemias due to deficient chain synthesis eg: Thalassemias.

3.Anaemia due to Hereditary Persistence of Fetal Haemoglobin (HPFH).

MATERIAL & METHODS

The present study was done on patients presenting with chronic anemia, unresponsive to conventional iron therapy and/or requiring regular repeated blood transfusions, suspected hemoglobinopathies. Study

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was carried out at J.L.N. Medical college, Ajmer, Rajasthan. It was a retrospective study from January 2016 to June 2016 and prospectively from July 2016 to December 2016 (total 1 year duration). The study was performed on total 380 patients attending the outdoor or indoor care at our hospital.

Prereqisite for performing HPLC was set that, there should have been no history of blood transfusion in past 3 months. Well mixed EDTA-anticoagulated 5 ml blood sample was collected.Haemoglobin and RBC indices were measured on SYSMEX automated 5-part differential cell counter. Simultaneously peripheral blood smears were also prepared for all the patients. The results of RBC indices were correlated with peripheral smear examination.The HPLC was performed on BIO-RAD 'VARIANT II' (β-thalassemia short program). The software delivers a printed report of chromatogram showing all the haemoglobin fractions eluted, the retention times, the areas of the peaks and the values (%) of different haemoglobin components.

RESULTS

Overall hemoglobinopathies were slightly more common in males (54 cases, 58.06%) than in females (39 cases, 41.94%). The male: female ratio was 1.38:1.

A total 380 cases were studied. Of these, 93 (24.47%) showed abnormal elution patterns of hemoglobins. The most common incidence among the various haemoglobinopathies, was of β-thalassemia minor/trait (BTT) 52 cases (55.92%), followed by δβ-thalassemia 14 cases (15.05%), β-thalassemia major 05 cases (5.38%).Three cases (3.23%) each of HbD trait, Sickle cell trait (HbAS), β-thalassemia intermedia and HbE β-thalassemia. Two cases (2.15%) each of Sickle cell anaemia (HbSS), HbE trait and Hereditary persistence of fetal haemoglobin (HPFH) were noted. One case (1.07%) each of HbD- β- thalassemia, HbQ India, HbS-β-thalassemia and α-thalassemia trait were also diagnosed.

Majority of the cases (55 cases, 59.15%) were diagnosed in paediatric age group of 0-18 years, followed by 37 cases (39.78%) in adulthood between ages 19-60, and

Table 1: red blood cell indices in various hemoglobinopathies.

S. No.	Hemoglobinopathy	Hb (gm%)	RBC count (million/cumm)	MCV (fl)	MCH (pg)	MCHC (%)
1.	β-Thalassemia Minor (BTT)	9.26±1.28	5.75±0.77	69.21±7.69	21.44±3.70	27.14±3.37
2.	dβ-thalassemia	9.12±0.82	3.99±0.45	75.49±4.75	23.08±3.45	27.79±2.58
3.	β-Thalassemia major	8.14±1.16	4.22±0.58	62.74±6.75	19.27±3.93	24.44±3.96
4.	Hb-D trait	8.76±0.38	3.6±0.92	69.04±5.64	20.63±3.32	24.61±2.97
5.	Sickle cell trait	8.96±1.96	4.35±0.88	74.81±5.01	22.9±3.63	28.93±4.23
6.	β-Thalassemia intermedia	10.20±0.43	3.83±0.63	69.4±11.39	25.93±4.73	32.16±1.75
7.	Hb-E β-Thalassemia	8.73±1.26	4.96±0.50	71.8±4.8	21.26±3.45	26.2±2.81
8.	Sickle cell anemia	8.85±0.46	4.35±0.88	74.81±5.01	22.9±3.63	28.93±4.23
9.	Hb-E trait	8.20±1.47	3.6±0.75	71.65±8.88	18.85±3.36	24.55±2.65
10.	Hereditary persistence of fetal haemoglobin (HPFH)	9.78±1.37	4.11±0.48	80.38±3.73	27.63±2.44	31.83±2.29
11.	Hb-D β-Thalassemia	9.2±0	4.7±0	53.1±0	18.4±0	34.7±0
12.	Hb-Q India	9.7±0	5.2±0	68±0	23.1±0	26.1±0
13.	HbS- β-Thalassemia	8.9±0	5.14±0	72.44±0	24.54±0	29.31±0
14.	a -Thalassemia	11.10±0	4.6±0	78.5±0	22.6±0	32.3±0

Table 2: HPLC findings in various hemoglobinopathies.

S. No.	Hemoglobinopathy	Hb A2 %	HbF %	Hb S %	Hb D %	Hb Q %
1.	β-Thalassemia Minor (BTT)	5.23±1.13	2.37±2.17	-	-	-
2.	δβ-thalassemia	2.63±0.34	8.9±4.71	-		-
3.	β-Thalassemia major	2.75±1.19	72.52±23.41	-	-	-
4.	Hb-D trait	2.26±0.32	2.16±2.38	-	31.13±5.36	-
5.	Sickle cell trait	3.48±0.33	1.17±0.66	32.66±4.48	-	-
6.	β-Thalassemia intermedia	3.4±1.64	27.87±9.04	-	-	-
7.	Hb-E β-Thalassemia	31.47±14.8	20.1±9.32	-	-	-
8.	Sickle cell anemia	2.3±0.48	1.61±0.02	73.5±5.84	-	-
9.	Hb-E trait	29.15±1.67	2.5±2.73	-	-	-
10.	Hereditary persistence of fetal haemoglobin (HPFH)	2.66±0.47	14.58±9.68	-	-	-
11.	Hb-D β-Thalassemia	4.2±0	1.2±0	84.8±0	-	-
12.	Hb-Q India	5.4±0	1.2±0	-	-	10.9±0
13.	HbS- β-Thalassemia	4.63±0	16.03±0	46.91±0	-	-
14.	α-Thalassemia	0	4.2±0	-	-	-

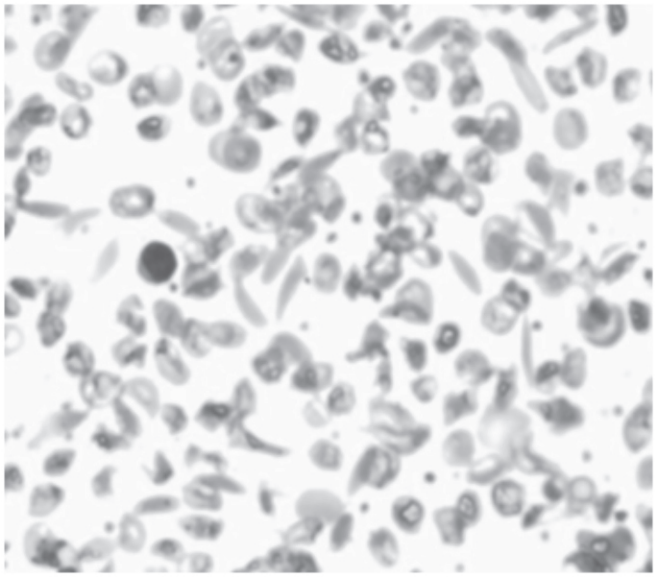

Figure 1: Peripheral blood film of sickle cell anemia. (100X)

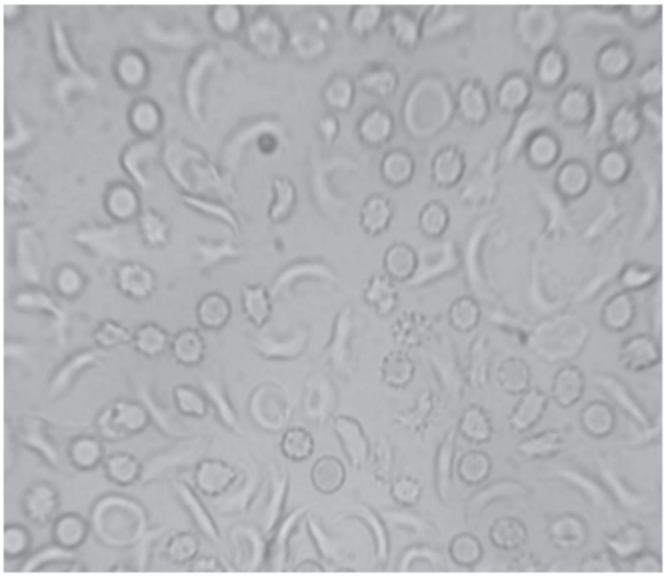

Figure 2: Positive sickeling test showing many sickled RBCs (40X)

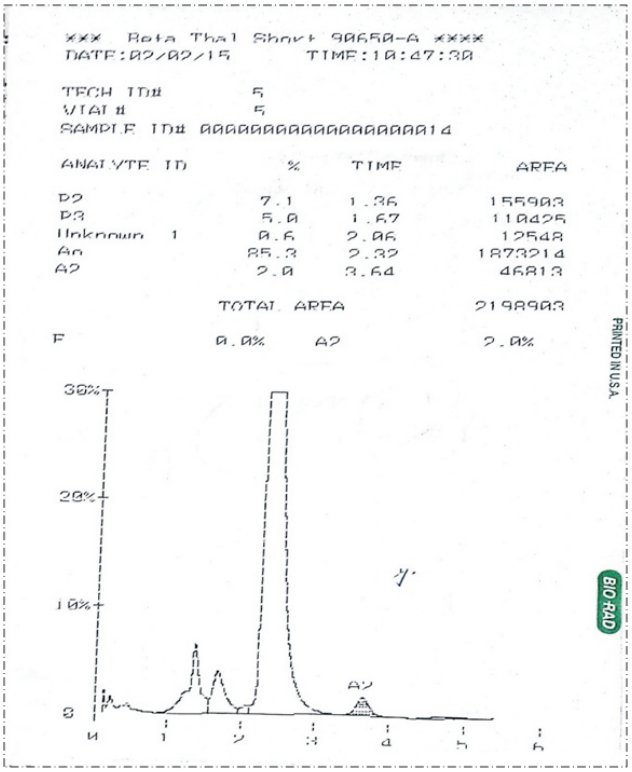

Fig.3 Chromatogram of Normal HPLC Pattern
HbA2-2.0%, HbF-0.0%, HbA0-85.3%`1

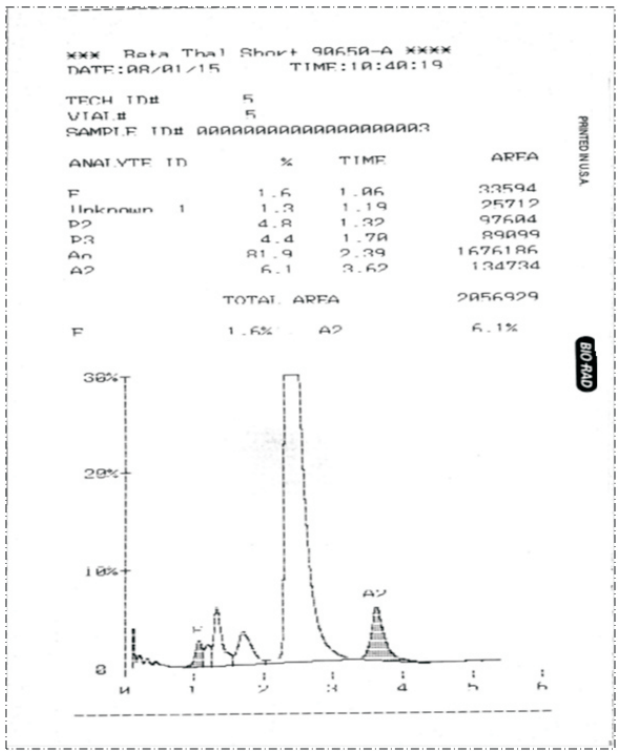

Fig.4 Chromatogram of β-Thalassemia Minor (BTT)
HbA2-6.1%, HbF-1.6%, HbA0-81.9%

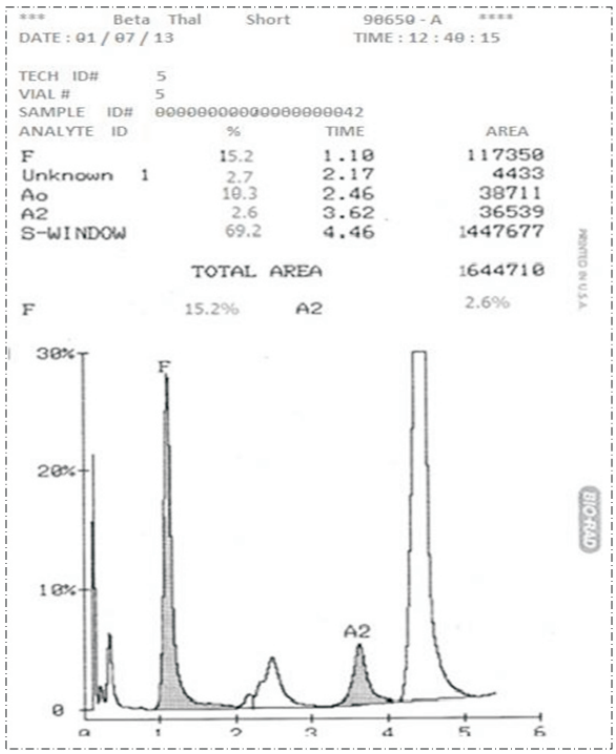

Fig. 5 Chromatogram of Sickle cell anaemia (HbSS)
HbA2-2.6%, HbF-15.2%, HbA0-10.3%, HbS-69.2%

just 1 (1.07%) case was seen in elderly age group above 60 years 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 0-18 years and β-thalassemia minor / BTT in 19- 60 years. Thalassemia-intermedia cases were seen in the age group of 0-15 years.

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.

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 and Positive Sickling test in Sickle cell anaemia showing sickled RBCs.

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%.

DISCUSSION

Thalassemia being a heritable genetic disorder, its distribution of specific disorders varies by geographics and by ethnicity. World Health Organization (WHO)states that 7% of the world population is a carrier for Hemoglobindisorders.5 Every year 10,000 children are bornwith thalassemia major in India, which accounts for 10% of the total incidence in the world.7

A large number of haemoglobin variants prevalent in the populations of Ajmer region indicate the population of the Ajmer region is genetically heterogeneous. Resulting in multi-ethnic backgrounds resulting in introduction of newer genetic alterations in future genomes. Thalassemia group of disorders have been found to be common in muslim population of Ajmer region, and are continuing to propagate to future generations in families due to still continuing practice of consanguineous marriages.

Male:female ratio of 1.38:1 was concluded by our study, which was similar to Dangi CBS et al (2013)13, 1.22:1.

In our study, the most common age group of presentation (59.15%) was 0-18 years which was similar to that published in 2012 byUddine MM et al12, (55.7%). More than 50% of the patients presenting for medical attention was in the age group of 0-15 years due to early onset anemiathat is refractory to therapy. Hence, there is a higher chance of detection ofhaemoglobinopathiesof various etiologiesin this age group.

Chopra GS et al (2008)8 in their study found that overall incidence of abnormal hemoglobins out of all cases received was 25% (258 out of 1032). Our study showed concordant results with overall incidence of 24.47%. They also showed β-thalassemia trait to be the commonest hemoglobinopathy (68.22%) encountered which was similar to our outcomes.

In our study, Sindhi community 155(48.13%) was having higher incidence of haemoglobinopathies than those reported by Sinha S et al (2004).14 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 It may be due to the reason that sindhi community forms a significant part of population in Ajmer and surrounding areas. 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 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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