e-ISSN: 0975-1556, p-ISSN:2820-2643

Available online on www.ijpcr.com

International Journal of Pharmaceutical and Clinical Research 2023; 15 (12); 1152-1157

Original Research Article

Clinicohematological Profile of Hemoglobin Lepore

Samira kumar Behera[1], Shushruta Mohanty[2], Swetambari Acharya[3], Swati Das[4], Lipika Behera[5]

[1]Associate Professor, Department of Pathology, MKCG Medical College and Hospital, Berhampur, Odisha

[2,5]Assistant Professor, Department of Pathology, MKCG Medical College and Hospital, Berhampur, Odisha

[3]Senior Resident, Department of Pathology, MKCG Medical College and Hospital, Berhampur, Odisha

[4]Post Graduate, Department of Pathology, MKCG Medical College and Hospital, Berhampur, Odisha

Received: 25-09-2023 / Revised: 28-10-2023 / Accepted: 30-11-2023

Corresponding author: Dr. Lipika Behera

Conflict of interest: Nil

Abstract:

Haemoglobin Lepore is a rare structurally abnormal haemoglobin (Hb) resulting from δβ rearrangements. It is widespread all over the world and in many ethnic groups. Interactions of hemoglobin Lepore with other hemoglobinopathies can lead to various clinical phenotypes and causes diagnostic challenges. These cases can be diagnosed with adequate knowledge of HPLC and CBC interpretation. The incidence of Hemoglobin Lepore in India is very rare due to paucity of data on clinical and hematological literatures. We are here discussing variants of hemoglobin Lepore and its association with other hemoglobinopathies like sickle cell anaemia and thalassemia by analying their clinical, CBC and HPLC findings. Proper diagnosis of this spectrum of rare hemoglobinopathies can assess the disease severity, early recognition of complications and avoidance of overtreatment.

Keywords: Hb Lepore, HPLC.

This is an Open Access article that uses a funding model which does not charge readers or their institutions for access and distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0) and the Budapest Open Access Initiative (http://www.budapestopenaccessinitiative.org/read), which permit unrestricted use, distribution, and reproduction in any medium, provided original work is properly credited.

Introduction

Haemoglobin (Hb) Lepore is a structurally abnormal Hb in which the abnormal globin chain results from an unequal crossover between the δ and β genes, because of a misalignment of homologous chromosomes during meiosis [1]. It was first identified in the Lepore family, an Italian-American family, in 1958. [2]Three variants of Hb Lepore have been identified, each characterized by different gene deletion breakpoints manifesting as various clinical outcomes.[3]

The product of fusion ofδ and β globin genes results in poor synthesis of δβ hybrid chains. This can be manifested as heterozygous β-thalassemia phenotype with mild microcytic hypochromic anemia or homozygosity for Hb Lepore with phenotype of thalassemia major. Also, compound heterozygosity for β-thalassemia and Hb Lepore results in the phenotype of thalassemia major or thalassemia intermedia. Some cases can be co-inherited with HbS resulting in a sickling disorder.[4-6].

HPLC separates different hemoglobin variants based on their molecular properties, such as size and charge. Hemoglobin Lepore will typically appear as an additional peak on the chromatogram, separate from the normal adult hemoglobin (HbA) peak. In the heterozygous condition, Hb Lepore constitutes 6-15% of the total hemoglobin, HbA2 levels are normal or discretely reduced and most subjects have increased HbF levels. [7,8]

The present study was designed to explore the possible identification of the Hb Lepore type by using cation exchange high performance liquid chromatography (CE-HPLC) and to establish its correlation with hematologic variables for identification of various Hb Lepore phenotypes.

Methods

This observational study was done in MKCG medical college and hospital, Berhampur, Odisha during January 2023-June 2023. Study was approved by Institutional Ethics Committee, Berhampur University. Informed consent was obtained from all individual participants included in the study.

Clinically suspected cases of hemoglobinopathy, antenatal, and other cases for family screening were evaluated. Basic hematological data were collected using Sysmex-XN550. CE-HPLC analysis was done on the VARIANT II hemoglobin testing

system using the β Thalassemia Short Program (Bio-Rad Laboratories, USA) in collaboration with multi-disciplinary research unit. For both the instruments, all the possible parameters were analyzed as per the manufacturer guidelines.

Results

Within a span of 6 months, 7 cases of Hb Lepore were reported from 3 different families of Southern Odisha. Two cases (case 1, 5) presented with fever, arthralgia and hepatosplenomegaly. On CBC, microcytic hypochromic anemia with increased RDW-CV was noted. One of them presented with reticulocytosis while other had reticulocytopenia. HbA2 was found to be 11.2% (RT- 3.47min) and 13.2% (RT-3.48min), respectively in case-1 and 5 with a hump in the downward slope and HbS>60% in each of them. Thus, these two cases were diagnosed as compound heterozygous for HbS and Hb Lepore on HPLC (Fig 1). A 6 years old male child (case 2) presented with clinical picture like that of β thalassemia major which was proved as a case of homozygous Hb Lepore syndrome on HPLC (Fig 2).

CBC finding was severe microcytic hypochromic anemia with marked anisopoikilocytosis and reticulocytosis. On HPLC, fetal hemoglobin was 85.9% and HbA2 was 12% with retention time of 3.43min. Family screening of the case revealed both parents (case 3,4) as Hb Lepore heterozygous. Father has HbA2-10.1% (RT-3.44min) and mother has HbA2-13.8% (RT-3.48min) with a hump in the downward slope of the HPLC graph. (Fig 3)

Another 14 month male child (case 6) with transfusion dependence and clinically diagnosed as β thalassemia major was found to be double heterozygous for Hb lepore and β thalassemia (Fig 4). CBC showed features of severe haemolytic anemia. HPLC showed HbF 36.5%, HbA 43.1% and HbA2 5.2% (RT-3.62min) with a hump in the downward slope. Father of the patient (case 7) was diagnosed as Hb leporeheterozygous (HbA2-12.6%) and mother as β thalassemia heterozygote.

Table 1: Hematological Parameters

	HB	RBC	HCT	MCV	MCH	MCHC	RDW CV	MICRO R	MACRO R	RET%	RET HE
CASE 1	9.7	4.23	29.2	69	22.9	33.2	21.1	32.3	2.7	5.51	18.5
CASE 2	6.3	3.23	20.9	64.7	19.5	30.1	33.8	45.4	2.1	7.81	12.5
CASE 3	12.5	5.69	39.7	69.8	22	31.5	18.4	26.5	3.7	1.47	21.1
CASE 4	10.4	5.39	34.1	63.3	19.3	30.5	14.5	42.8	2	0.96	18.4
CASE 5	6.1	2.81	19.1	68	21.7	31.9	18.6	31.4	1.7	0.49	14.6
CASE 6	3	1.82	12.1	66.5	21.4	32.2	26.5	42.2	1.7	7.36	12
CASE 7	13.5	6.5	43.5	66.9	20.8	31	16.5	30.1	3.8	1.38	19.9

Table 2: HPLC Findings

	HB A %	HB A2%	HB F%	HB S%	RETENTION TIME (SEC)	DIGNOSIS
CASE 1	2.2	11.2	17.7	68.4	3.47	DOUBLE HETEROZYGOUS FOR SCA AND HB LEPORE
CASE 2	0.3	12.0	85.9	--	3.43	HB LEPORE HOMOZYGOUS
CASE 3	72.1	10.1	7.1	--	3.44	HB LEPORE HETEROZYGOUS
CASE 4	75.8	13.8	1.0	--	3.48	HB LEPORE HETEROZYGOUS
CASE 5	2.5	13.2	18.1	65.0	3.48	DOUBLE HETEROZYGOUS FOR SCA AND HB LEPORE
CASE 6	43.1	5.2	36.5	5.8	3.62	DOUBLE HETEROZYGOUS FOR HB LEPORE AND B THALASSEMIA
CASE 7	77.0	12.6	1.6	--	3.50	HB LEPORE HETEROZYGOUS

PEAK NAME	CALIBRATED AREA %	AREA %	RETENTION TIME (MIN)	PEAK AREA
F	18.1*	---	1.12	249167
AO	---	2.5	2.27	34538
UNKNOWN	---	0.9	2.46	12735
A2	13.2*	---	3.48	180308
S-WINDOW	---	65.0	4.37	885142

F CONCENTRATION = 18.1* % Total Area: 1,361,890

A2 CONCENTRATION = 13.2 %

SICKLING POSITIVE

*Values outside of expected ranges
Analysis comments:

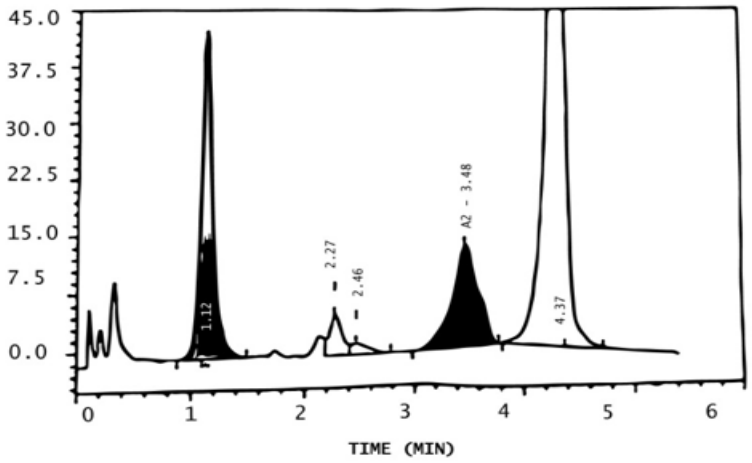

Figure 1: Double Heterozygote for SCA and HB Lepore

PEAK NAME	CALIBRATED AREA %	AREA %	RETENTION TIME (MIN)	PEAK AREA
P1	---	0.5	0.89	6054
F	85.9*	---	1.19	1043926
AO	---	0.3	2.50	4015
A2	12.0*	---	3.43	148008

F CONCENTRATION = 85.9* % Total Area: 1,202,003

A2 CONCENTRATION = 12.0 %

*Values outside of expected ranges
Analysis comments:

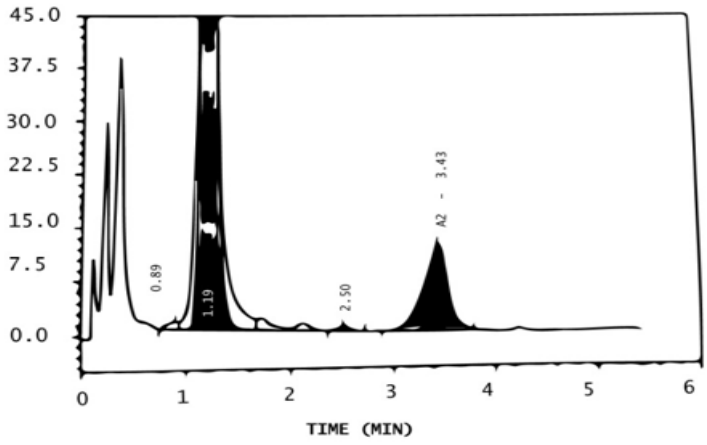

Figure 2: Homozygous Hemoglobin Lepore

PEAK NAME	CALIBRATED AREA %	AREA %	RETENTION TIME (MIN)	PEAK AREA
UNKNOWN	---	0.1	0.99	1341
F	1.0	---	1.11	14270
UNKNOWN	---	0.8	1.26	11614
P2	---	3.6	1.38	53979
P3	---	4.6	1.75	68269
A0	---	75.0	2.55	1122070
A2	13.8*	----	3.48	208823

F CONCENTRATION = 1.0* %

A2 CONCENTRATION = 13.8 %

Total Area: 1,480,456

*Values outside of expected ranges
Analysis comments:

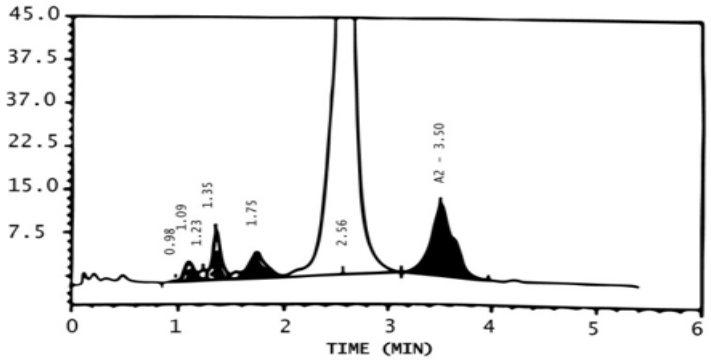

Figure 3: HB Lepore Heterozygote

Peak Name	Calibrated Area %	Area %	Retention Time (min)	Peak Area
F	36.5*	---	1.17	528009
P2	---	5.1	1.34	74111
P3	---	3.9	1.73	56009
A0	---	43.1	2.55	622942
A2	5.2*	---	3.62	79329
S-window	---	5.8	4.28	83997

Total Area: 1,444,397

F Concentration = 36.5* %

A2 Concentration = 5.2* %

*Values outside of expected ranges

Analysis comments:

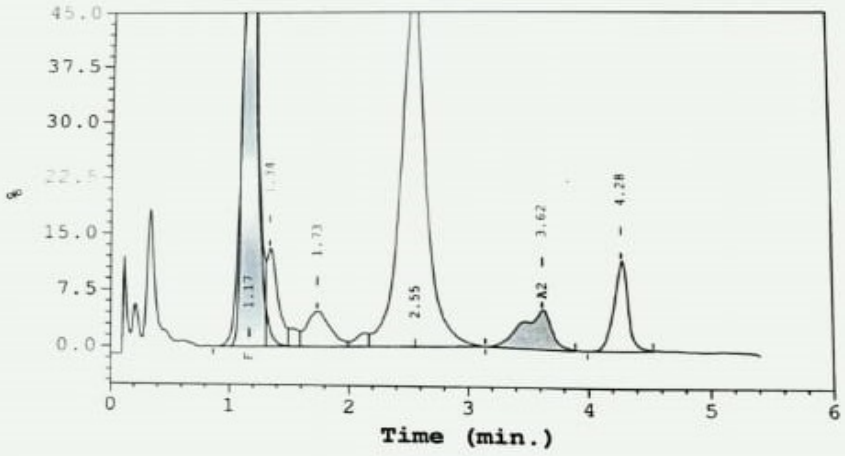

Figure 4: Heterozygote for Beta Thalassemia and HB Lepore

Discussion

Lepore Hemoglobin results from a 7.4kb deletion between the delta and beta globin genes leading to production of a hybrid or fused globin chain comprising an N-terminal amino acid sequence of a delta chain and the C-terminal amino acid sequence of a beta chain. Five Hb Lepore variants were characterized each with a different crossover breakpoint: Hb Lepore Boston Washington, Hb Lepore Hollandia, Hb Lepore Baltimore, Hb Lepore-Leiden and Hb Lepore ARUP [9]. Hb Lepore Boston Washington is the most common Lepore variant. It has been found with a low frequency in a variety of ethnic groups, mainly in Mediterranean countries [10].

In all Hb Lepore variants, the synthesis of the $\delta\beta$ hybrid chain is significantly lower than that of the β-chain, resulting in an overall reduction in non-α-globin chains [6].The Clinicohematological profile of hemoglobin Lepore varies depending on the type of hemoglobin Lepore mutation and the number of abnormal hemoglobin genes present. In general, people with hemoglobin Lepore trait are asymptomatic or have mild symptoms, such as mild anemia and fatigue. People with homozygous hemoglobin Lepore have more severe symptoms, such as moderate to severe anemia, splenomegaly, and jaundice.[3,5]In compound heterozygotes for Hbs S, C, and E with Hb Lepore, the clinical phenotypes are extremely variable but, overall, resemble those of Hbs S, C, or E/β-thalassemia compound state.[6,11]

In the present study the compound heterozygote for Hb Lepore and β thalassemia case was presented with clinical features of β thalassemia major though HPLC shows Hb A level 43.1%. To reach the final diagnosis, HPLC of parents were done. Father of the patient was diagnosed as Hb leporeheterozygous (HbA2- 12.6%) and mother as β thalassemia heterozygote. This concludes in case of diagnostic difficulties for hemoglobinopathies, HPLC of parents give us a better vision for accurate diagnosis.

The Hb Lepore carriers display a β-thalassemic phenotype with microcytosis and hypochromia. The mean cell Hb (MCH) is thought to be the most reliable parameter in the Hb Lepore heterozygotes (range20-25 pg).[12]Our study population mostly presented with mild to moderate anemia (Cases- 1, 3, 4, 7) and severe anemia in 3 cases (Case-2, 5, 6). The red blood cells were often microcytic hypochromic with increased RDW.

In the homozygous state, HbA and HbA2 are absent and hemoglobin is made up of HbF and Lepore only, the level of Hb Lepore ranging from 8% to 30% with a mean value of 15%, the remainder of Hb being HbF. In heterozygous state, the haemoglobin contains HbA, Lepore, HbA2 and a variable amount of HbF, the level of Hb Lepore ranging between 5% and 15%, with a mean level around 10%. [13] The haemoglobin F fraction is usually slightly elevated in Lepore trait as compared to beta thalassemia trait cases. Haemoglobin Lepore homozygous does not have any normal haemoglobin A and usually results in a phenotype ranging from thalassaemia intermedia to major.

On HPLC analysis with the Bio-Rad Variant Hb testing system, Hb Lepore has a similar retention time (RT) to HbA2 and Hb E. However in a study by Chaibunruanget al, Hb Lepore was identified as having a shorter retention time as compared to Hb A2. The retention time of HbA2 varies between 3.63-3.67 min with a mean of 3.65 whereas Hb Lepore + HbA2 varies from 3.42–3.43 min with a downward hump. The variant haemoglobin is quantified at a higher fraction in A2 window than for beta thalassemia trait.[14]

Treatment of Hb Lepore is mostly symptomatic management. People with Hb Lepore may need blood transfusions to treat anemia. People with hemoglobin Lepore trait typically have a good prognosis. However, people with homozygous hemoglobin Lepore may have a more severe course of disease and may experience complications, such as heart failure.

Conclusion

Diagnosis of spectrum of Lepore hemoglobinopathies may sometimes be challenging and misdiagnosis could occur in a routine setting if only one diagnostic modality is used.

Our observation highlights that the correlation of HPLC results with proper family studies and careful scrutiny of patient's history results in final diagnosis and provide valuable information about the impact of this hemoglobinopathy on their health. Though presumptive diagnosis can be made by HPLC but DNA analysis is essential for accurate diagnosis, hence appropriate clinical management and genetic counseling.

References

1. Ribeiro ML, Cunha E, Gonçalves P, et al. Hb LeporeBaltimore (δ68Leu-β84Thr) and Hb Lepore-WashingtonBoston (δ87Gln-βIVS-II-8) in Central Portugal and Spanish Alta Extremadura. Hum Genet. 1997; 99:669–673.
2. Gerald PS, Diamond LK (September 1958). "A new hereditary hemoglobinopathy (the Lepore trait) and its interaction with thalassemia trait". Blood. 13 (9): 835–44.
3. Bozkurt G, Baysal E, Gu L-H, Huisman THJ (1994) Thalassemia intermedia in two patients with Hb Lepore-b0 -thalassemia (Frameshift codon 8,-AA). Hemoglobin 18(3):247–250

4. Chakova L, Spasova M, Genev E. Thalassemia intermedia in an infant. Folia Med. 1998; 40(1):84–87
5. Sreedharanunni S, Chhabra S, Kaur JH, Bansal D, Sharma P, Das R. b-thalassemia intermedia caused by compound heterozygosity for Hb Lepore-Hollandia and b-thalassemia is rare in the Indian Population. Hemoglobin. 2015; 39(5):362–365
6. T. H. J. Huisman, Compound heterozygosity for Hb S andthe hybrid Hbs Lepore, P-nilotic, and Kenya; comparison of hematological and hemoglobin composition data, Hemoglobin, 1997; 21(3): 249–257.
7. Bunn HF, Forget BG. Hemoglobin: molecular genetics and clinical aspects. Philadelphia: Saunders; 1986.
8. Weatherall D, Higgs DR. The haemoglobino pathies, Bailliere's Clinical Haematology, VI. London: Saunders; 1993.
9. R. H. Nussenzveig, D. L. Vanhille, D. Hussey, and A. M. Agarwal, Development of a rapid multiplex PCR assay for identification of the three common hemoglobin-lepore variants (Boston-Washington, Baltimore, and Hollandia) and identification of a new lepore variant, American Journal of Hematology, 2012; 87(10): E74–E75.
10. M. L. Ribeiro, E. Cunha, P. Gonc¸alves et al., Hb Lepore-Baltimore ((68Leu)-(84Thr)) and Hb Lepore-Washington-Boston ((87Gln)-β(IVS-II-8)) inCentral Portugal and Spanish Alta Extremadura, Human Genetics, 1997; 99(5): 669–673.
11. V. Viprakasit, P. Pung-Amritt, L. Suwanthon, K. Clark, andV. S. Tanphaichitr, Complex interactions of $\delta\beta$ hybrid haemoglobin (Hb Lepore-Hollandia) Hb E (β26 g→a) and α+ thalassaemia in a Thai family, European Journal of Haematology, 2002; 68(2): 107–111.
12. Viprakasit V, Pung-Amritt P, Suwanthon L, Clark K, Tanphaichtr VS. Complex interactions of δβ hybrid Haemoglobin (HbLepore-Hollandia) Hb E (β26G→A) and α+thallasaemia in a Thai family. Eur J Haematol. 2002; 68(2): 107-11.
13. J. Pasangna, E. George, M. Nagaratnam, Haemoglobin Lepore in a Malay family a case report, Malays. J. Pathol. 2005; 27(1): 33–37.
14. Chaibunruang A, Srivorakun H, Fucharoen S, Fucharoen G, Sae-ung N, Sanchaisuriya K. Interactions of hemoglobin Lepore (δβ hybrid hemoglobin) with various hemoglobinopathies: amolecular and hematological characteristics and differential diagnosis. Blood Cells Mol Dis. 2010; 44(3): 140-5.