



Case Report

Homozygous Lepore Syndrome: A case report

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1. Introduction

Lepore Hemoglobin is a structurally abnormal type of haemoglobin consisting of an abnormal globin chain which is 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 [1]. This abnormal globin chain is a product of a hybrid gene resulting from an unequal crossing over between the delta and beta globin genes because of a mal-alignment of homologous chromosomes during meiosis leading to a 7.4kb deletion between the delta and beta globin genes [1,2]. So far, 3 different Lepore hemoglobins have been identified which are characterized by different delta to beta sequence transitions at the fusion junction [1–3]. These are Hb Lepore Washington Boston (δ87/β116), Hb Lepore Hollandia (δ22/β50) and Hb Lepore Baltimore (δ50/β86). Hb Lepore Washington Boston is the most common and occurs worldwide [1,2]. In all of these variants, the synthesis of these hybrid chains is substantially less than that of the β-chains, resulting in an overall reduction in the non-α globin chains and patients present with a clinical picture of haemolytic anemia. But Hb Lepore can be differentiated from β-Thalassemia by the presence of a distinct Hb Lepore band on cellulose acetate electrophoresis or quantification in High Performance Liquid Chromatography (HPLC) [4].

There are numerous case series and case reports about the heterozygous state of Hb Lepore and compound heterozygous state for Hb Lepore with β Thalassemia and other hemoglobinopathies. But homozygous Hb Lepore is very rare. In fact, this is the only documented case of homozygous Lepore syndrome from Nepal.

The aim of this paper is to raise awareness about this rare disorder which should be included in the differential diagnosis of patients presenting clinically like thalassemia intermedia/major to provide proper prenatal diagnosis, clinical management and genetic counselling.

We describe a case of an 8 year old boy presenting with the clinical picture like that of β-Thalassemia major, which on lab investigations proved to be a case of homozygous Hb Lepore syndrome.

This case report has been reported in line with the SCARE Criteria

2020 [5].

2. Case

A child of age 8 years and 15 days from Morang, Nepal presented to a tertiary care hospital on March 2021 with chief complaints of easy fatigability, decreased appetite and progressive abdominal distension as noticed by parents for 6 months. The patient used to be fatigued by running a short distance, however this had progressed to where he was unable to walk and was breathless even with moving from one room to another. He was breathless but had no orthopnea. He complained of progressive decreased appetite. On examination there was severe pallor, abdomen was non-uniformly distended with major prominence on Left Upper Quadrant region, firm and non-painful. There is no history of bleeding manifestations (epistaxis, bleeding gums, black tarry stool), bone pain, lymphadenopathy, swelling on other body parts, no signs of respiratory distress, noisy breathing, facial puffiness; no headache, vomiting, altered sensorium, focal deficits, abnormal body movements blurring of vision; no history of palpitations, rashes and or yellowish discoloration of body.

After patient presented to the emergency, oxygen saturation monitored and intravenous fluid was administered and investigations were ordered for complete blood count (CBC) with peripheral smear, X-Ray of chest, abdomen and skull, Arterial Blood Gas (ABG), Liver Function Test (LFT) and Ultrasonography (USG). The major clinical findings were pallor, sunken eyes, dry mouth, liver palpated up to 5 cm below right costal margin and spleen palpated up to 15 cm below costal margin, signs of poor growth (short stature), maxillary prominence and abdominal circumference of 34cm. Oxygen saturation 97% on room air, pulse rate was 82 beats per minute, respiratory rate was 24 breaths per minute, blood pressure of 90/50 mm of Hg and temperature was 99° Fahrenheit. Chest X-ray revealed mild to moderate cardiomegaly, abdominal X-ray revealed marked splenic enlargement but skull X-ray (lateral and anterior) was normal. USG abdomen revealed marked splenomegaly. CBC revealed decreased haemoglobin, decreased RBC

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count, hematocrit of 19.3% (decreased), mean corpuscular volume of 68.4 fL (decreased), mean corpuscular haemoglobin concentration of 24.9 gm/dL (decreased), mean corpuscular haemoglobin of 17.4 pg (decreased), total leucocyte count of 24.74×10^3 (increased). Differential count revealed neutrophils 71%, lymphocytes 10%, monocytes 5%, eosinophils 2%, basophils 1%, blast cells 2%, myelocytes 3%, metamyelocytes 6% and proportion of 78nRBC/100 WBC giving a Leukoerythroblastic impression. RBC morphology on peripheral smear revealed moderate to severe anisopoikilocytosis, microcytic, hypochromic picture with tear drop cells and target cells. No hemoparasites were detected on peripheral smear. Platelet count was normal. Serum Lactate Dehydrogenase (LDH) level was 446 IU/L (increased), serum Ferritin level was 434 ng/mL (increased). LFT was normal except for increased Alkaline Phosphatase (ALP) which was found to be 139 IU/L (increased). Serum calcium level was 8.6 mg/dL (decreased). Renal Function Test (RFT) was normal except for creatinine of 0.27 mg/dL (decreased). Echocardiography revealed normal mitral valve, tricuspid valve, pulmonary valve, cardiac chambers and pericardium. The patient was transfused with 3 units of packed red cells. The blood smear of both the parents revealed hypochromia, microcytosis, few target cells and basophilic stippling.

High Performance Liquid Chromatography (HPLC)/Hemoglobin capillary zone electrophoresis test was ordered for patient, father and mother. HPLC of father showed HbA of 77.4% (decreased), HbA2 of 12.8% (increased) and HbF of 1.7% (increased) giving heterozygous Lepore picture (Fig. 1). HPLC of mother revealed HbA of 78.3% (decreased), HbA2 of 11.9% (increased) and HbF of 1.4% (increased) giving heterozygous picture (Fig. 2). HPLC of patient revealed HbA of 0.3 (decreased), HbA2 of 25% (increased) and HbF of 74.4% (increased) verified by two prominent peaks on HbF and HbA2 with retention time of 3.43 minutes suggesting Homozygous variant of Lepore Syndrome (Fig. 3). The patient's parents were advised for splenectomy of the child but they refused and the patient was discharged after symptomatic management. Later after 6 months on September, the patient visited our institute, BP Koirala Institute of Health Sciences (BPKIHS) with similar symptoms but aggravating type to Emergency where ABG, CBC, LFT, RFT, X-Ray, USG tests were ordered and the patient was admitted to paediatrics surgery division immediately. On ER, GCS score was 15/15, pallor was present, respiratory rate was 28 breaths per minute, pulse rate was 120 beats per minute, BP was 100/50 mm of Hg, temperature was 98.2° Fahrenheit and spO2 was 99% on oxygen via face mask. CVS and Respiratory examinations were normal. Abdomen was irregularly enlarged with bosselated appearance on upper abdomen predominantly on left side, extending below umbilicus. The margin of enlargement mass could be well demarcated and was non-painful on palpation. CBC revealed Hb 8.5 g/dL (decreased), PCV 30.4% (decreased), TLC 28000 cells/mm³ (increased), platelets 290,000 cells/mm³. Differential WBC count revealed neutrophils 62%, lymphocytes 16%, monocytes 18%, eosinophils 4%. Blood group was O positive. RFT, LFT and ABG was normal. Serological testing was found to be normal. Peripheral blood smear done 2 days after admission revealed RBC's with anisopoikilocytosis with predominant tear drop cells and schistocytes with microcytes, macrocytes, ovalocytes, elliptocytes, spherocytes and polychromatophils with hypochromia and differential count giving

polymorphonuclear cells 2%, monocytes 3%, neutrophils 60% and lymphocytes 32% (activated lymphocytes 2%), myelocytes 3% giving an overall appearance of leukoerythroblastic blood picture with features of haemolytic anemia. USG revealed massive splenomegaly, hepatomegaly and dilated portal vein. A massively enlarged spleen measuring 33 cm (13 inches) in length and 11 cm in maximum breadth was recovered during splenectomy operation (Fig. 4). 2 weeks prior to operation, the patient's parents were advised to vaccinate their child with Meningococcal and Pneumococcal conjugated vaccine. The child did well after surgery and was discharged 10 days later with possibility of allowance of frequent small dose oral sips of semisolid food.

3. Discussion

Hb Lepore consists of delta-beta hybrid or fused globin chain, it is hybrid gene product (formed due to unequal crossing over between the δ and β globin genes) with a deletion of 7.4 kb between the delta and beta globin genes [1,2]. Three types of Hb Lepore have been identified till date, these are formed due to differences in delta to beta sequence transitions at fusion functions [1–3]. They are Hb Lepore Washington Boston, Hb Lepore Hollandia and Hb Lepore Baltimore, among which Hb Lepore Washington Boston is the most common and occurs worldwide [1,2]. In all these variants, the synthesis of hybrid chain is less than beta chain leading to globin imbalance, excess α -chain production leads to the clinical picture of β -Thalassemia, because of ineffective erythropoiesis and shortened red cell survival [4]. Hb Lepore heterozygotes are usually asymptomatic but according to large Italian series, a few of Greek and Yugoslavian heterozygotes had mild splenomegaly [6,7]. Heterozygotes are usually healthy individuals with only a slight decrease in hemoglobin levels, but with a distinct microcytosis and hypochromia [3]. Homozygotes have clinical findings, ranging from a course similar to transfusion-dependent β -Thalassemia major to Thalassemia intermedia [3]. This variability may be secondary to the degree of imbalance between α and non- α globin chains [3,8–10]. The multifaceted approach for presumptive identification of hemoglobin variants includes series of blood count/red cell indices and hemoglobin analysis. This approach easily identifies Hb Lepore [4]. Hb Lepore has the same retention time (RT) value as HbA2 on HPLC analysis with Bio-Rad variant Hb testing system [2]. Values greater than 10% suggest the presence of variant Hb. The identification of the characteristic type of Hb Lepore requires analysis of globin chains by reversed phase HPLC and DNA studies which confirms the diagnosis of variant Hb [4].

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 Lepore 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%. The mean level of HbA2 is about 2% and the reported values for HbF range from 1 to 14% [4]. HPLC has become the preferred technique for detecting clinically significant structural hemoglobin variants owing to its simplicity of automated system with internal sample preparation, superior resolution, rapid assay time and accurate quantification of hemoglobin fractions making it an ideal methodology for the routine clinical laboratory [11].

HPLC / HB CAPILLARY ZONE ELECTROPHORESIS OF FATHER					
Result of Investigation	Gender	Age Range	Normal Range	Other	Special Remark
HbA ₀ :			(1.500 - 3.500) %	None	
HbA ₂ :					
HbF:	All	1Year(s) - 100Year(s)	(< 1.000) %	None	
Verification Comment:	HPLC findings are suggestive of heterozygous Hb Lepore				

Fig. 1. Heterozygous picture for Lepore in father.

HPLC / HB CAPILLARY ZONE ELECTROPHORESIS OF MOTHER					
Result of Investigation	Gender	Age Range	Normal Range	Other	Special Remark
HbA ₀ : 78.3%	All	0Year(s) - 100Year(s)	(1,500 - 3,500) %	None	
HbA ₂ : 11.9%					
HbF: 1.4%	All	1Year(s) - 100Year(s)	(< 1,000) %	None	
Verification Comment:	HPLC findings are suggestive of heterozygous Hb Lepore				

Fig. 2. Heterozygous picture for Lepore in mother.

Sample Details : DHH-190321011 (Blood)					
HPLC / HB CAPILLARY ZONE ELECTROPHORESIS					
Result of investigation	Gender	Age Range	Normal Range	Other	Special Remark
HbA ₀ : 0.3%					
HbA ₂ : 25.0%	All	0Year(s) - 100Year(s)	(1,500 - 3,500) %	None	Nil
Hb F: 74.4%					
Verification Comment:	Two prominent peaks are present: HbF~74% and HbA ₂ window~25.0% with retention time of 3.43 min				
Comment:	Together with parental HPLC with both parents showing heterozygous Hb Lepore, findings are consistent with homozygous Hb Lepore in the index c.				

Fig. 3. Homozygous picture for Lepore in patient.



Fig. 4. Enlarged spleen removed by splenectomy.

HPLC has the advantage of quantifying HbF and HbA₂ along with detecting other variants in a single screening test [11]. It maintains high sensitivity, specificity and resolution to meet the demands for both screening and conformation purposes highlighting its superiority over electrophoresis for analysis of hemoglobin variants [12]. Hemoglobin capillary zone electrophoresis is another technology and is used along with HPLC for more accurate identification and quantification of haemoglobin variants and A₂ quantification. Both techniques have their advantages and disadvantages and are accurate [12].

The subject of our case is a 8 years old boy who presented with chief complaints of easy fatigability which was progressive, decreased appetite and progressive abdominal distension as noticed by the parents. On further physical examination findings of pallor, splenomegaly and hepatomegaly were evident confirmed by imaging (USG). The patient was initially managed in the line of haematological disorder. CBC, PBS and HPLC of the patient as well as parents confirmed the presence of Homozygous Lepore syndrome, the prime indicator of the diagnosis being the heterozygous nature of the disease pattern in parents as evidenced by findings of PBS, CBC and HPLC.

4. Conclusion

Hb Lepore is structural hemoglobin variant coded by a hybrid gene formed as an unequal crossing over between the delta and beta globin genes because of mal-alignment of homologous chromosomes during meiosis. The homozygous state of this disease is similar to β -Thalassemia intermedia or major clinically.

Usually the presumptive diagnosis can be made in lab by a multi-faceted approach consisting of a series of blood count/red cell indices, Hb electrophoresis and haemoglobin analysis by HPLC. Quantitative analysis for any Hb variant disorder is made by HPLC better than Hb Electrophoresis, the same was done in our case report. However, the confirmation requires DNA estimation which was not done in our setup. But for clinical management of the disease condition, HPLC suffices while DNA estimation can be done for genetic counselling which can also be provided taking into consideration the heterozygous nature of the parents, the same which was done in our case.

Ethical approval

Ethical approval is not required for case report.

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Author contribution

Amrit Bhusal, literature review, follow-up the patient, writing the manuscript, and final approval of the manuscript, Dr. Silan Bhandari, literature review, follow-up the patient, writing the manuscript, and final approval of the manuscript, Dr. Tulika Seth, literature review and final approval of the manuscript, Dr. Rajesh Prasad Sah, Supervisor, literature review, splenectomy of the patient and final approval of the manuscript.

Trial register number

1. Name of the registry:
2. Unique Identifying number or registration ID:
3. Hyperlink to your specific registration (must be publicly accessible and will be checked):

Guarantor

Amrit Bhusal is the Guarantor.

Patient consent

One of the parent(father) gave written consent for the possible publication of this case report.

Provenance and peer review

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Declaration of competing interest

There are no conflicts of interest.

Abbreviations

HPLC High Performance Liquid Chromatography

Hb Hemoglobin
 PBS Peripheral Blood Smear
 CBC Complete Blood Count

Appendix A. Supplementary data

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