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CORRESPONDENCE

First Observation of Compound Heterozygosity for Hb S/Hb Lepore-Hollandia in India

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Abbreviations

PICU	Paediatric Intensive care unit
COVID-19	Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)
CRP	C-Reactive Protein
SGOT	Serum glutamic-oxaloacetic transaminase
SGPT	Serum glutamic-pyruvic transaminase
HbF	Fetal Hemglobin
HbS	Sickle haemoglobin
HbA_0	Normal Adult Hemoglobin/non-glycated hemoglobin
HbA_2	Normal variant of hemoglobin A
GAP-PCR	Gap-polymerase chain reaction

Dear Editor,

Compound heterozygotes of HbS and Hb Lepore Boston or Hollandia have been reported from various parts of the globe in low incidence [1]. The clinical severity in these double heterozygotes is variable from mild to severe depending on the Lepore variant. So far no compound heterozygote for HbS/Hb Lepore has been reported from India. Here we report first identification of HbS/Hb Lepore-Hollandia from Central India.

Case

A two-year-old propositus with acute febrile illness due to suspected malaria/dengue with rashes all over the body, cough, and high respiratory rate was admitted to paediatric intensive care unit (PICU) of NSCB Govt Medical College, Jabalpur. The child exhibited pallor, icterus, and poor general condition with clear chest and positive S1S2 with oxygen saturation of 97% but with marginally lower blood pressure. Patient was negative for COVID-19. Haematological indices of proband at the time of admission showed microcytic anemia with Hb of 5.8 g/dL. The patient was CRP positive with high SGOT (792 mg/dL), SGPT (98 mg/dL) blood urea (57 mg/dL). Cultures of blood and urine showed no growth. Patient was treated with artesunate and doxycycline for suspected malaria and other infection. One unit of packed red blood cell was also transfused. Since patient had a history of blood transfusion, he was referred to us for differential diagnosis of hemolytic anemia. Alkaline cellulose gel hemoglobin electrophoresis revealed presence of HbF, HbS and a slow moving Hb variant marginally trailing the HbS controls (Fig. 1A). Cation exchange-high performance liquid chromatography showed HbS (32.2%); HbA_0 (37.5%); HbF (15.4%) with split HbA_2 peak of 6.3% with retention time of 3.47 min. Earlier studies have shown that retention time of Hb Lepore + HbA_2 varies from 3.42–3.43 min with a mean of 3.4 min; in contrast the retention time of HbA_2 varies between 3.63–3.67 min with a mean of 3.65 min suggestive of Hb Lepore. Family screening of propositus revealed 5-year-old brother as heterozygous for elevated A_2 (9.6%),

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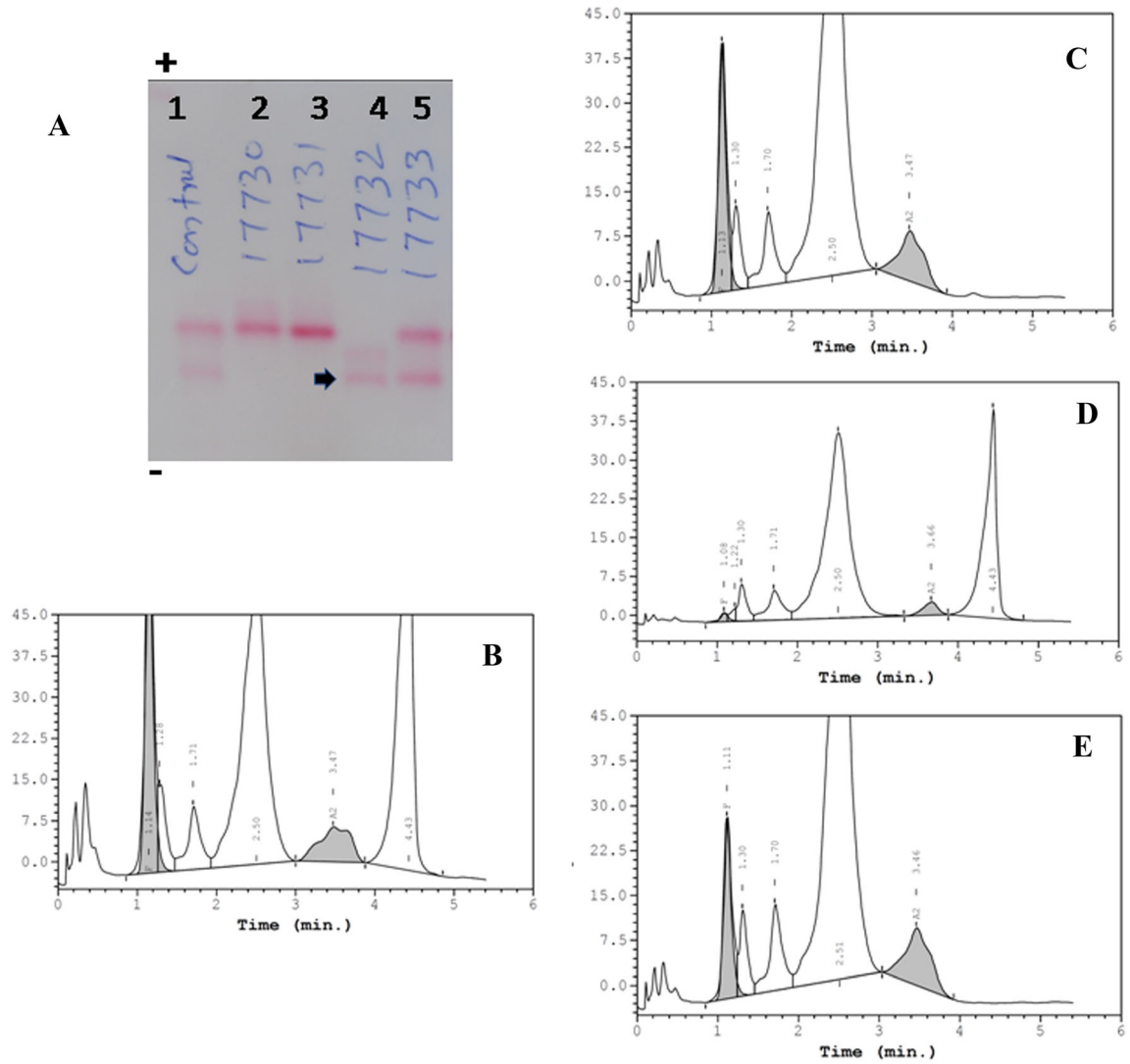

Fig. 1 Cellulose gel electrophoresis of Propositus (**A**) Lane 1-Control, Lane-2–3-Normal Hb, Lane 4-Propositus & L5 HbAS. HPLC chromatograms of the propositus (**B**) and his mother (**C**) father (**D**) and Brother (**E**)

father as sickle carrier and mother with elevated A_2 (8.8%) (Fig. 1B–E). Hematological indices of mother and brother were lower than normal warranting molecular diagnosis. PCR was performed using primers flanking the break point of deletion producing Hb Lepore [2] (Supplemental Figure 1). PCR confirmed the presence of the Hb Lepore mutation in heterozygous state in propositus, mother and brother (Fig. 2). DNA sequencing of the mutant PCR fragment confirmed the presence of Hb Lepore Hollandia (NM_000518.4(HBB):c.-7305_92 + 16del), delδ22Ala/β50Thr) variant.

Hb Lepore($\alpha_2(\delta\beta)_2$) is a hemoglobin variant in which non-α globin peptides are coded by the hybrid δβ globin gene. This hybrid gene is formed by large deletion between δ and β globin genes. Depending upon the breakpoint regions at 5′ of the δ globin gene and 3′ of the β globin gene, three major type of Hb Lepore namely Hb Lepore–Washington–Boston (δ87Gln/βIVSII-8 or δ87Gln/β116His (NG_000007.3:g.63632_71046del)), Hb Lepore–Baltimore (δ50Ser/β86Ala (NG_000007.3:g.63564_70978del) or δ68Leu/β84Thr orδ59Lys/β86Ala) and Hb Lepore–Hollandia (δ22Ala/β50Thr, NG_000007.3:g.63290_70702) have been reported from different parts of the world [3–5].

In the heterozygous condition, Hb Lepore shows phenotype similar to heterozygous β thalassemia with marginally elevated HbF. Co-inheritance of Hb Lepore with other β-globin variants such as HbS, HbE etc. may influence clinical presentation. [6–8] Coinheritance of HbS and Hb Lepore have been reported from Mediterranean region, Caribbean islands and USA and Europe [9–11]. So far, no evidence of coinheritance of Sickle and Lepore has been reported from India. Shaji et al. [7] first ever reported Hb Lepore Hollandia in three unrelated individuals with

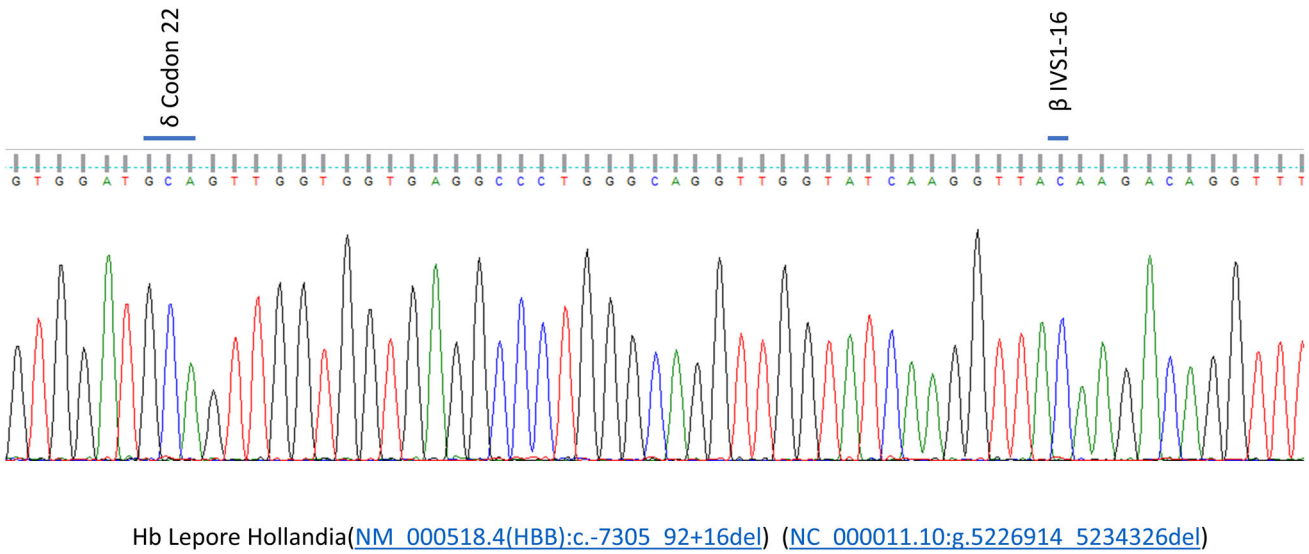

Fig. 2 **A** Hb Lepore mutation by PCR analysis. Lane 1–Propositus, L2-Mother, L3-Marker 100 bp. L4-Father, L-5 Sibling, L6—Control for Hb lepore & L-7 Marker. **B** DNA sequencing of the mutant PCR fragment showing a δβ hybrid gene producing the Hb Lepore Hollandia genotype

variable phenotype from North India. Later, Edison et al. [12] reported a compound heterozygous case of HbE and Hb Lepore Hollandia in a 15-year-old boy from West Bengal. Nadkarni et al. [6] reported Hb Lepore Hollandia in 3 unrelated families.. Sreedharanunni et al. [13] reported a case of compound heterozygous Hb Lepore-Hollandia and β-Thalassemia in a 4-year-old boy presented as thalassemia intermedia phenotype. Previously we reported Hb Lepore Hollandia in a 1.6-year-old male child belonging to primitive Baiga tribe from central India.

Here we report the presence of Hb Lepore Hollandia along with HbS in a compound heterozygous state for the first time from India..The clinical severity of HbS-Lepore has been reported to vary with Lepore deletions. This variability is thought to occur due to imbalance in the alpha and non-alpha chains. In the current study, propositus required hospitalization due to suspected malarial infection rather than HbS-Lepore disease symptoms. Patient was discharged with advice of hydroxyurea, folic acid and multivitamin for treatment of sickle cell disease. The regular monitoring and follow up of the subject is essential to ascertain clinical manifestation due to the HbS-Lepore double heterozygosity.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s12288-021-01475-0.

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Declarations

Conflict of interest The authors declare no conflict of interest.

Ethical Approval Study was approved by Institutional Ethics Committee. All procedures performed in the study involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed Consent Informed consent was obtained from all individual participants included in the study.

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