International Journal of Research in Medical Sciences
Dhanani HJ et al. Int J Res Med Sci. 2025 Mar;13(3):1302-1308
www.msjonline.org
pISSN 2320-6071 | eISSN 2320-6012
DOI: https://dx.doi.org/10.18203/2320-6012.ijrms20250705

Case Report

A rare beta chain variant hemoglobin Deer Lodge; a chance discovery in an Indian family while testing HbA1c by high-performance liquid chromatography

Hiren J. Dhanani[1]*, Gaurav D. Shastri[1], Bharat D. Tandel[1], Hardik Modi[2], Jaysukh D. Kothia[1], Mittal C. Sukhadiya[1], Asmita Patel[1], Srusti C. Dhanani[1], Nandini H. Dhanani[3]

[1]Department of Pathology, Sterling Accuris Laboratory, Surat, Gujarat, India
[2]Department of Hematopathology, National Reference Laboratory, Ahmedabad, Gujarat, India
[3]Sterling Accuris Diagnostics Laboratory, Surat, Gujarat, India

Received: 16 January 2025
Accepted: 12 February 2025

***Correspondence:**
Dr. Hiren J. Dhanani,
E-mail: drhirenjd@gmail.com

ABSTRACT

This case report documents the first reported instance of hemoglobin (Hb) Deer Lodge in an Indian family, discovered during routine HbA1c testing using high-performance liquid chromatography (HPLC). A 34-year-old clinically asymptomatic male (proband) showed an unexpected peak on HPLC, indicative of a Hb variant. Subsequent family screening revealed the same variant in the proband's mother. Multiple analytical platforms (HPLC and capillary electrophoresis) identified a Hb variant with migration patterns inconsistent with common variants. Genetic sequencing confirmed the HBB:c.8A>G mutation in the β-globin gene, corresponding to Hb Deer Lodge, a rare variant previously reported in non-Indian populations. Additional single nucleotide variants (SNVs), including HBB:c.9T>C, were identified in the family, with some showing high prevalence in the eastern Indian population. Functional analysis suggests that Hb Deer Lodge slightly alters oxygen affinity but remains clinically silent. This report emphasizes the importance of comprehensive analysis for Hb variants detected during routine screening, especially in regions with high genetic diversity. Furthermore, it highlights the potential for rare variants to complicate HbA1c measurements, necessitating confirmatory testing and cautious interpretation in clinical practice.

Keywords: Hb Deer Lodge, HPLC, Capillary electrophoresis, Next generation sequencing

INTRODUCTION

Glycosylated Hb or HbA1c, a fraction of Hb, is used to evaluate the average blood glucose level over the preceding 2 to 3 months. The analysis of HbA1c by HPLC or capillary electrophoresis (CE) also gives an indication of the presence of variant Hb; however, the protocol for reporting and management of the incidental detection of Hb variants is not well established. Many clinical laboratories do not report the presence of Hb variants, whereas others report them only if they interfere with HbA1c measurement.[1]

Hemoglobinopathies are important inherited disorders that are very high in prevalence in India, causing significant public health problems. The most predominant variants are Hb S, Hb D, and Hb E, with a cumulative gene frequency of 5.35%.[2,3,4] Severe forms of hemoglobinopathies are typically diagnosed early by clinicians, but carriers often go unnoticed due to lack of symptoms. They are usually identified through family analysis, screening programs, or HbA1c measurement by HPLC and CE. However, it is important to exercise caution when presumptively identifying a Hb variant, as other rare variants may exhibit similar migration patterns on CE or HPLC.[5]

The present report describes two cases (proband and mother) where analysis on three different platforms could not conclusively identify a Hb variant. Molecular genetic analysis subsequently confirmed the variant as Hb Deer Lodge, along with several single nucleotide variants (SNVs). To date, few cases of Hb Deer Lodge have been reported, and this is the first case from India.

CASE REPORT

A 34-year-old clinically asymptomatic male (proband) was referred as part of a comprehensive health check-up. Blood samples from his parents, who were also clinically asymptomatic, were collected for family screening after variant hemoglobin was detected in the proband.

Blood samples were collected in K2 EDTA tubes (Becton, Dickinson, and Company; NJ, US). Hemogram analysis was performed on the BC 6800 Plus analyzer (Mindray; Shenzhen, China) using CDR (Count, Differential, and Reticulocyte) mode.

HbA1c was initially analyzed on the D10 analyzer (Bio-Rad laboratories; California, United States) using the HbA1c protocol. An unexpected unknown peak on the chromatogram indicated the presence of a hemoglobin variant. Although the HbA1c result was within normal limits and the patient was non-diabetic, reanalysis was performed on the c311 analyzer (Roche; Basel, Switzerland) using the Tina-quant hemoglobin A1c assay.

At our center, a protocol for comprehensive analysis of suspected hemoglobin variants, including family screening, is followed. Variant hemoglobin analysis was conducted on three different platforms; 1) D10 analyzer (Bio-Rad laboratories; California, US) using the HbA2/F protocol. 2) Variant V-II instrument (Bio-Rad laboratories; California, US) using the beta thalassemia short program. 3)Minicap Flex Piercing capillary electrophoresis system (Sebia; Lisses, France).

For final confirmation, HBB gene sequencing was performed on the Genestudio S5 Sequencing instrument using the Ion Torrent™ Ion CarrierSeq™ ECS panel kit. DNA extraction and library preparation were carried out according to the manufacturer's protocol.

As shown in Table 1, the proband and his mother exhibited near-normal hemogram findings, with marginally elevated reticulocyte counts in both cases.

The A1c mode (Bio-Rad D10) chromatogram for proband indicated the presence of variant Hb (43.7%) at a retention time of 1.55 minutes. This was further confirmed in the A2/F mode (Bio-Rad D10) chromatogram, which detected the variant Hb at 39.4% with a retention time of 2.95 minu.

Additionally, the β Thal mode (Bio-Rad VII) chromatogram revealed a similar variant Hb (40.7%) with a retention time of 3.5 minutes (Figure 1).

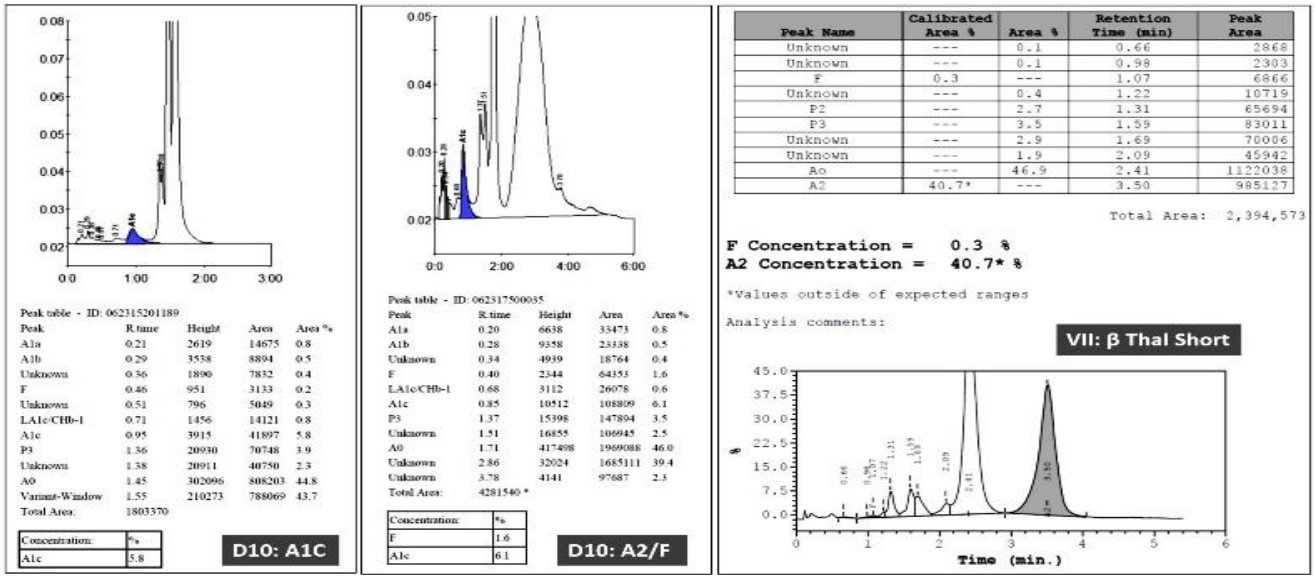

Figure 1: HPLC chromatogram of proband.

The mother's HPLC analysis also demonstrated the presence of a variant Hb with comparable concentrations and retention times: 43% in the A1c (Bio-Rad D10) chromatogram at 1.55 minutes, 50.4% in the A2/F (Bio-Rad D10) chromatogram at 3.01 minutes, and 41.2% in the β-thal (Bio-Rad VII) chromatogram at 3.52 minutes (Figure 2). Based on the available library of Bio-Rad D10 (A2/F mode), potential matches for the retention times include Hb E (RT: 2.87–2.90), Hb Toulon (RT: 2.96), and Hb G Copenhagen (RT: 2.91).

Similarly, the library of Bio-Rad VII (β-thal mode) suggested differential diagnosis like, Hb Toulon (RT:

3.57), Hb D Iran (RT: 3.55), and Hb G Coushatta (RT: 3.48).

Further analysis using capillary electrophoresis (CE) confirmed the presence of a variant Hb in both individuals within Zone-7 or Zone-F (Figure 3).

Manufacturer guidelines suggested possible differentials, including Hb F, Hb Willamette, Hb Alabama, Hb Chapel Hill, Hb Park Ridge, Hb Porto Alegre, Hb Q-Thailand (G-Taichung), Hb Sabine, Hb Bassett, Hb Rampa, Hb G-San José, Hb Barcelona, Hb Geldrop Santa Anna, Hb Richmond, Hb Boumerdes, Hb Swan River, Hb Burke, Hb Tarrant, Hb Presbyterian, Hb Manitoba I, Hb Manitoba II, Hb Port Phillip, and "J-Rovigo" Hb A2 variant, among others.

Since the results from all three instruments did not exhibit concordance, HBB gene sequencing was performed.

The run plan and plugins were applied as per the protocol, and raw data were analyzed using identify digital genetic assistance software. The observed data quality was within acceptable limits (total read aligned 99.99%, uniformity 98.35%, data on target 98.79%).

The proband and his mother were found to be heterozygous for the HBB:c.8A>G mutation, leading to the formation of the variant Hb Deer Lodge (mutation location: exon; hg38: chr11:5,227,014; beta 2(NA2) His>Arg, resulting in the substitution of histidine with arginine).

Additionally, the proband exhibited four single nucleotide variants (SNVs), while the mother displayed five SNVs. Although the father did not carry a variant Hb, he was found to have four SNVs, (Table 2) (Figure 4-6).

Table 1: Hematology and HPLC data of both cases.

Parameters	Proband	Mother
BC 8600 plus		
WBC×10^3/µl	11.21	12.67
HGB gm/dl	15.6	13
RBC×10^6/µl	5.63	5.09
HCT%	47.4	40.3
MCV fl	84.1	79.2
MCH pg	27.7	25.6
MCHC g/dl	32.9	32.3
RDW CV %	13.8	14.6
RDW SD fl	41.3	40.9
PLT×10^3/µl	329	324
MPV fl	9.1	10
PDW	16	15.9
PCT %	0.258	0.314
Retic%	1.76	1.66
Retic# 10^6/µl	0.0992	0.0842
IRF %	4.8	3.9
RHE pg	27	25.7
D10 HbA1c protocol		
A0 @ 1.45 RT	44.80%	44.60%
Unknown Hb @ 1.55 RT	43.70%	43.00%
Hb A1c @ 0.96 RT	5.80%	5.90%
D10 HbA2/F protocol		
A0 @ 1.73 RT	47.30%	47.50%
A2 / Unknown @ 2.95-3.01 RT	39.40%	50.40%
Biorad VII beta Thal short		
A0 @ 2.4 RT	46.90%	48.80%
A2 @ 3.5 RT	40.70%	41.20%
Sebia minicap electrophoresis		
HbA (Zone)	55.00%	54.50%
Unknown Hb (Zone)	42.20%	42.70%
HbA2 (Zone)	2.80%	2.80%

Table 2: HBB Gene mutation data of entire family.

Mutation	Proband	Father	Mother
HBB:c.8A>G (Hb Deer Logde)	Present (0.51)	Absent	Present (0.52)
HBB:c.9T>C (Exonic SNV)	Present (1)	Present (1)	Present (1)
HBB:c.316-185C>T (Intronic SNV)	Present (1)	Present (1)	Present (1)
HBB:c.315+16G>C (Intronic SNV)	Present (1)	Present (1)	Present (1)
HBB:c.315+74T>G (Intronic SNV)	Absent	Absent	Present (0.5)
HBB:c.*316A>C (Downstream SNV)	Present (1)	Present (1)	Present (1)

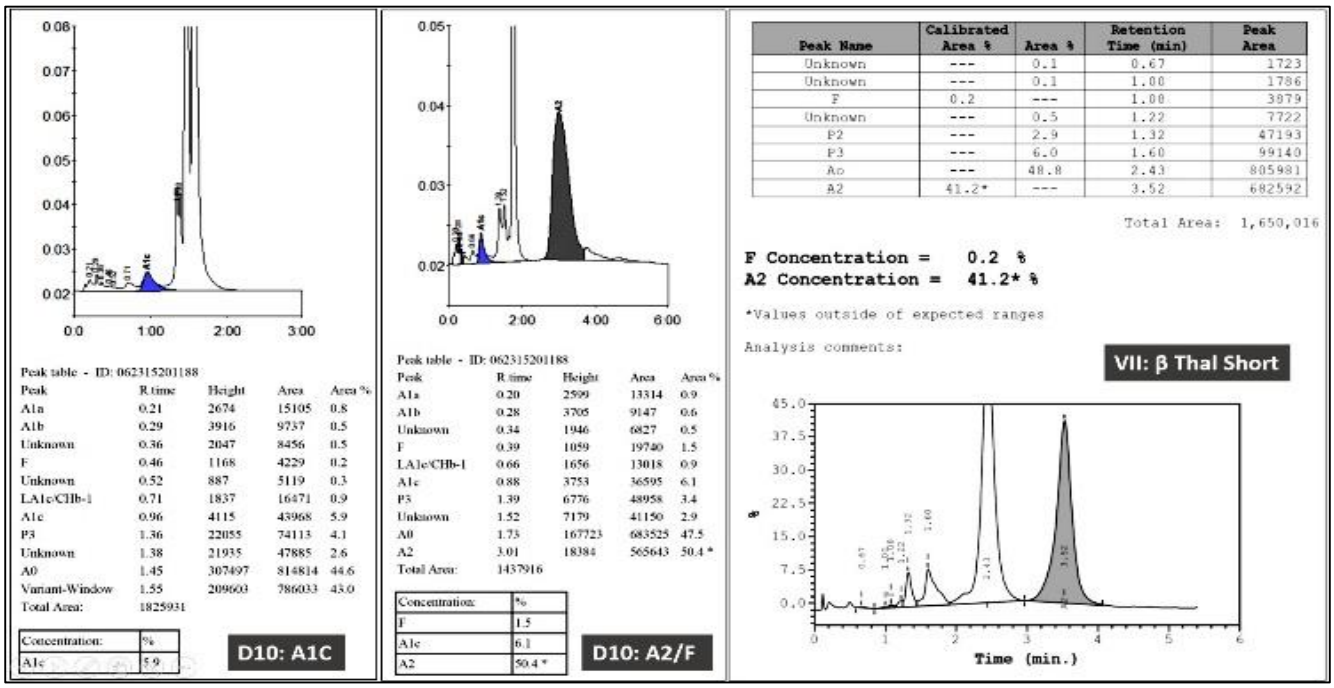

Figure 2: HPLC chromatogram of mother.

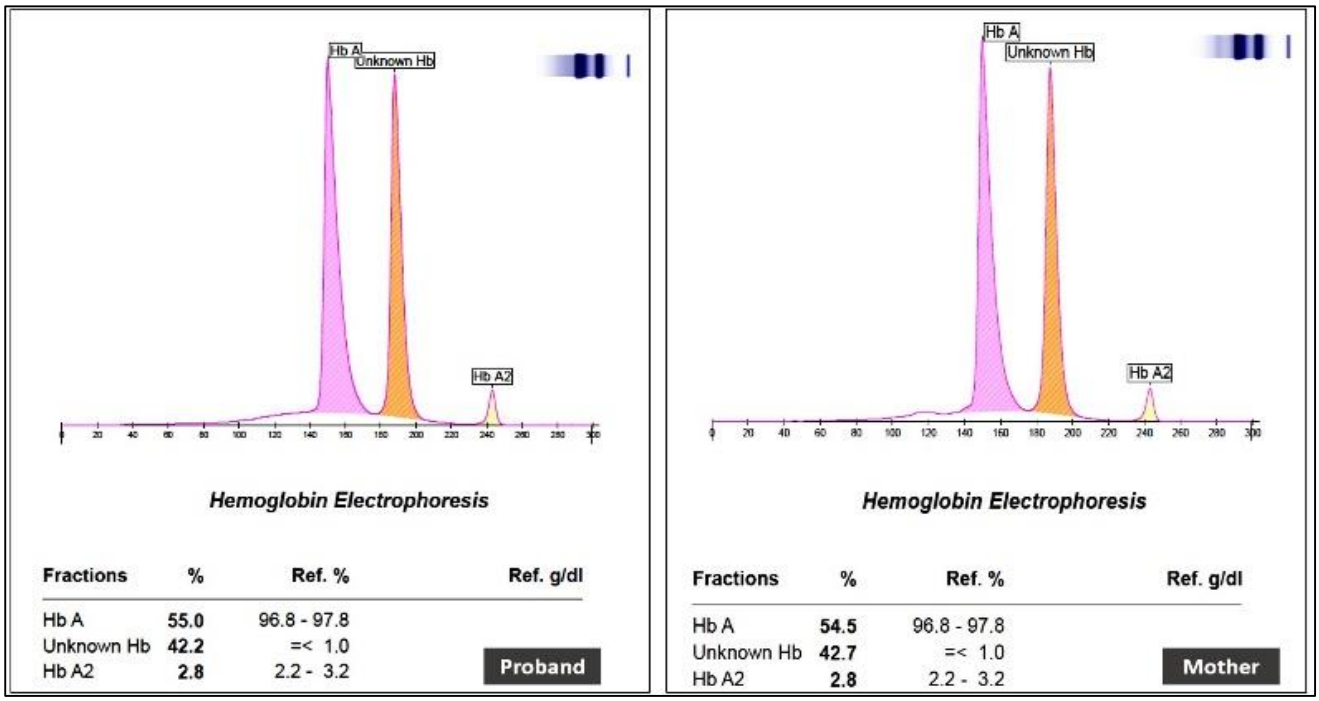

Figure 3: Capillary electrophoresis patterns of proband and mother.

Figure 4: HBB gene sequencing of proband.

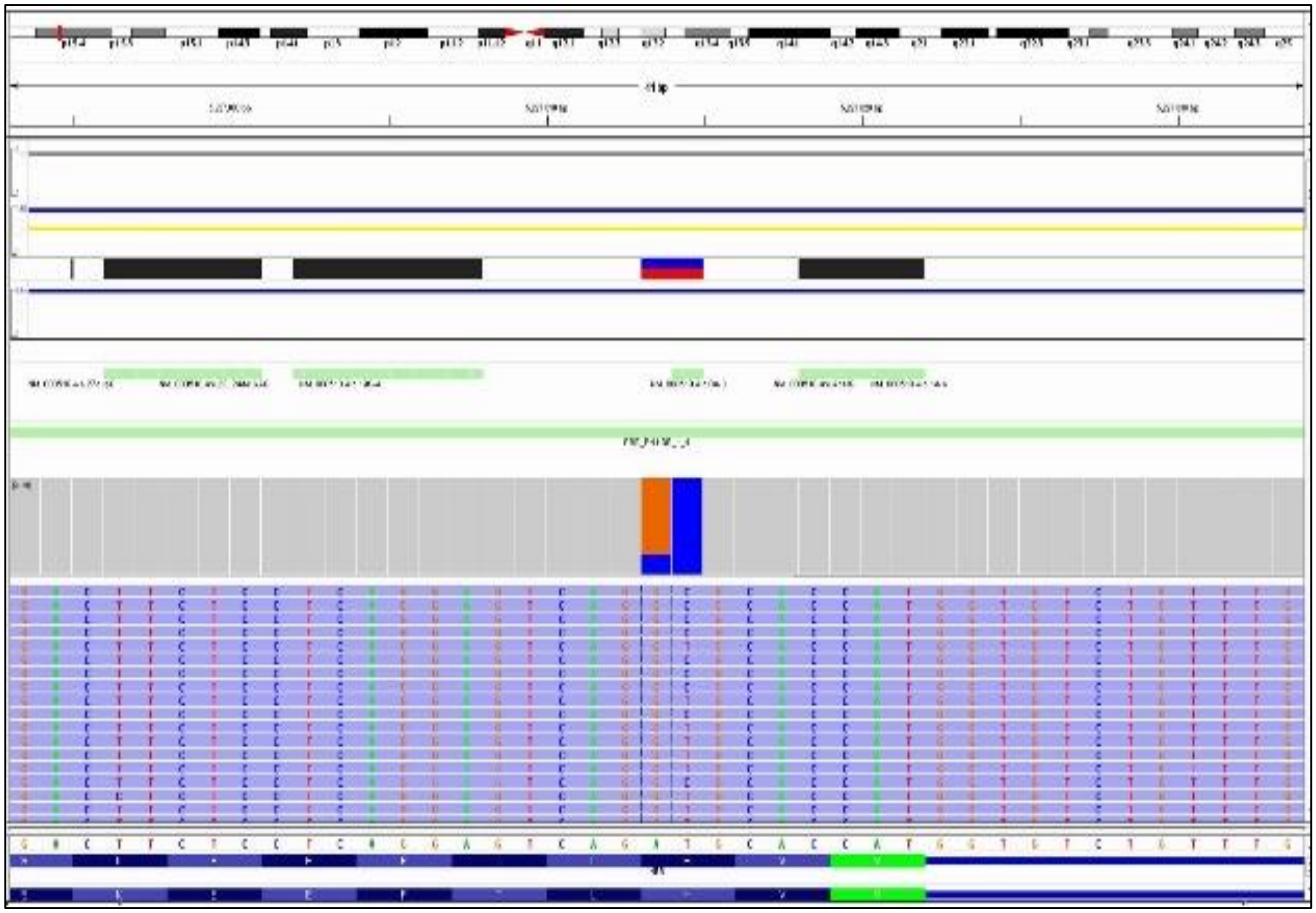

Figure 5: HBB gene sequencing of mother.

Figure 6: HBB gene sequencing of father.

DISCUSSION

The name Hb Deer Lodge was given when it was first time detected in Deer Lodge hospital, Winnipeg in a 50-year-old Canadian male of Welsh-Dutch-English ancestry, where it showed different electrophoretic mobility on alkaline pH. Purification and characterization of the variant fraction showed that it contained an arginine in place of a histidine residue in the position next to the N-terminal residue of the beta polypeptide chain. The novel variant was named Hb Deer Lodge.[6] Since the first reported case, few cases of Hb Deer Lodge have been sporadically reported in Caucasian American, Black, and Venezuelan populations, all of them were clinically silent.[7-9] Upon extensive reviewing the literature, we could not find a documented case in an Indian family.

The mutations at the β2 (NA2) position of the Hb beta chain demonstrate a variety of amino acid substitutions that impact Hb's structural and functional properties. Notably, the substitution of histidine with arginine in Hb Deer Lodge (HBB:c.8A>G) alters the 2,3-diphosphoglycerate (2, 3-DPG) binding site, resulting in slightly increased oxygen affinity, reduced cooperativity, and an amplified Bohr effect.[10] Similarly, other mutations at this position include Hb Fukuoka (HBB:c.7C>T; His→Tyr), Hb Franklin Park (HBB:c.7C>A; His→Asn), Hb Agrigente (HBB:c.8A>C; His→Pro), Hb Graz (HBB:c.8A>T; His→Leu), Hb Marseille (also known as Hb Long Island-Marseille; HBB:c.8A>C; His→Pro), and Hb Okayama (HBB:c.[9T>A or 9T>G]; His→Gln). Among these, Hb Agrigente and Hb Okayama elute in the Hb A1c zone during HPLC analysis, leading to falsely elevated HbA1c levels, which could complicate glycemic control assessment.[11-14]

Interestingly, a silent mutation, HBB:c.9T>C, at the β2 (NA2) position does not result in an amino acid change (His→His). This polymorphism, first identified in Odisha, India, is present in approximately 20% of the state's population, suggesting a significant single nucleotide polymorphism prevalence in this region. The present study also detected this mutation in its homozygous form in all three family members.[15]

The present study also found the HBB:c.316-185C>T and HBB:c.315+16G>C are intronic SNVs, also with a prevalence of 12% in the eastern part of India. The HBB:c.315+74T>G (intronic SNV) and HBB:c.*316A>C

(downstream SNV) were not found in published data from India.[15]

CONCLUSION

This case report presents the first documented instance of Hb Deer Lodge in an Indian family, identified incidentally during HbA1c testing. The reluctance of many clinical laboratories to report Hb variants detected during HbA1c analysis, possibly due to their clinically silent nature, highlights a significant gap in practice. The authors advocate for the routine reporting of such variants and recommend that clinical laboratories actively engage with patients and clinicians to facilitate further investigations, including comprehensive family screening when appropriate. The diagnostic workflow employed in this case, encompassing HPLC, CE, and molecular genetic analysis, underscores the critical role of multidisciplinary approaches in accurately identifying rare Hb variants. While Hb Deer Lodge is clinically silent, other Hb variants can significantly impact HbA1c interpretation, underscoring the necessity of confirmatory testing when anomalous chromatographic peaks are observed. This report contributes to the expanding knowledge of hemoglobinopathies in the Indian population and highlights the need for further research to assess the prevalence and clinical relevance of rare Hb variants across diverse ethnic groups.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: Not required

REFERENCES

1. Dhanani HJ, Sukhadiya MC, Dhanani NH, Kothia JD, Tandel BD. Accidental detection of clinically silent compound heterozygous Hb D Punjab/Hb Q India while analyzing HbA1c by high-performance liquid chromatography. Int J Res Med Sci. 2021;9(11):3468-71.
2. Desai D, Dhanani HJ, Shah M, Dayal N, Kapoor A, Yeluri S. Hemoglobin D disease: a case report. Internet J Pathol. 2003;3(1):1-4.
3. Desai D, Dhanani H. Sickle cell disease: history and origin. Internet J Hematol. 2003;1(2):1-4.
4. Balgir RS. Genetic epidemiology of the three predominant abnormal hemoglobins in India. J Assoc Physicians India. 1996;44(1):25-8.
5. Harteveld CL, Achour A, Arkesteijn SGJ, Ter Huurne J, Verschuren M, Bhagwandien-Bisoen S, et al. The hemoglobinopathies, molecular disease mechanisms, and diagnostics. Int J Lab Hematol. 2022;44(1):28-36.
6. Labossiere A, Vella F, Hiebert JM, Galbraith PA. Hemoglobin Deer Lodge: α2β2 2 His → Arg. Clin Biochem. 1972;5(1-4):46-50.
7. Powars D, Schroeder W, Shelton J, Evans L, Vinetz R. An individual with hemoglobins S and Deer Lodge. Hemoglobin. 1976;1(1):97-100.
8. Shulman L, Bunn H. Hb Deer Lodge in a Caucasian American: effect of iron deficiency on level of variant. Hemoglobin. 1988;12(2):197-9.
9. Arends T, Garlín G, Pañez de Perera A, Castillo O. Hemoglobin Deer Lodge [alpha 2 beta 22 (NA2) His → Arg] in a Venezuelan family. Hemoglobin. 1984;8(6):621-6.
10. Bonaventura J, Bonaventura C, Sullivan B, Godette G. Hemoglobin Deer Lodge (β2 His replaced by Arg): consequences of altering the 2,3-diphosphoglycerate binding site. J Biol Chem. 1975;250(24):9250-55.
11. Harano T, Harano K, Ueda S, Imai K, Ohkuma A, Koya Y, et al. Hb Fukuoka [β2(NA2)HISTYR]: A New Nutation at the 2,3-Diphosphoglycerate Binding Site. Hemoglobin. 1990;14(2):199-205.
12. Liu JS, Molchanova TP, Gul LH, Wilson JB, Hopmeier P, Schnedl W, et al. Hb Graz or α2β22(Na2)His→Leu; a new β Chain Variant Observed in four Families from Southern Austria. Hemoglobin. 1992;16(6):493-501.
13. Boi S, Hendy J, Goodall I, Gilbert A, Fleming P, Hughes WG. First report of HB Long Island-Marseille in Australia - a chance discovery. Hemoglobin. 1989;13(5):515-20.
14. Frers CR, Dorn S, Schmidt W, Kochhan L, Simon-Schultz J, Arndt R. Falsely increased HbA1c values by HPLC and falsely decreased values by immunoassay lead to identification of Hb Okayama and help in the management of a diabetic patient. Clin Lab. 2000;46(11-12):569-73.
15. Sahoo SS, Biswal S, Dixit M. Distinctive mutation spectrum of theHBBGene in an urban Eastern Indian population. Hemoglobin. 2013;38(1):33-8.

Cite this article as: Dhanani HJ, Shastri GD, Tandel BD, Modi H, Kothia JD, Sukhadiya MC, et al. A rare beta chain variant hemoglobin Deer Lodge; a chance discovery in an Indian family while testing HbA1c by high-performance liquid chromatography. Int J Res Med Sci 2025;13:1302-8.