DOI: 10.7860/JCDR/2015/11771.6142

Case Report

Biochemistry Section

Serendipity: A Rare Discovery of Haemoglobin D-Iran in An Indian Female During Routine Antenatal Screening for β-Thalassemia

RITTU SURJIT CHANDEL[1], ABHISHEK ROY[2], LEELA GUL ABICHANDANI[3]

ABSTRACT

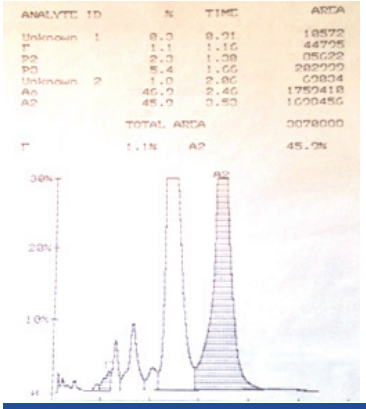
Haemoglobin D is a rare form of haemoglobinopathy in homozygous form. However, the heterozygous form of the disease is clinically silent and relatively easier to find in North-West India, Pakistan and Iran. Haemoglobin D is sometimes found to be coexistent with Haemoglobin S and/or Thalassaemia leading to clinically significant conditions like sickle cell anaemia with mild to moderate splenomegaly. In India the more prevalent form is Haemoglobin D-Punjab (also known as Hb D- Los Angeles) which has a prevalence of 2% in Punjab and around 1% in Gujarat. However, the variant, Haemoglobin D- Iran is very rare in India in heterozygous as well as homozygous forms. This report is of a 36-year-old female, who visited for an antenatal check up. On analysing the blood sample using Agarose Gel Electrophoresis in Alkaline media, the migration of abnormal haemoglobin to haemoglobin S/D/G region was observed. Sickle cell solubility test was negative. On capillary electrophoresis, peak in the Haemoglobin D Zone was seen.

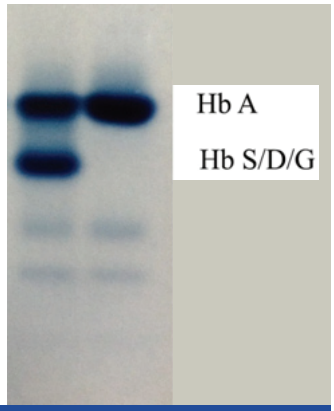
Keywords: Agarose gel electrophoresis, Capillary electrophoresis, Haemoglobinopathy, High performance liquid chromatography

CASE REPORT

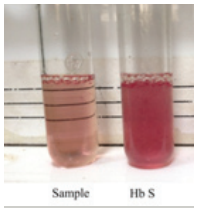
We report a case where we unexpectedly found a patient to have Haemoglobin D-Iran. Our patient was a, 36-year-old Indian Muslim female, who visited Grant Govt. Medical College and Sir J.J. Group of Hospitals for routine antenatal care. She was G4P1L0A3D1 and was in fifth month of pregnancy when she came in for the antenatal checkup. Blood samples of the patient were collected in Department of Biochemistry, under routine screening program for haemoglobinopathies of antenatal mothers in EDTA coated vaccutainers. A complete blood count was done using automated haematology analyser (Abacus 5, Diatron), the findings of which are shown in [Table/Fig-1]. The sample was further subjected to haemoglobin detection using HPLC based Haemoglobin Typing System (BIORAD VARIANT, β-Thalassemia Short Program, BIORAD). The High Performance Liquid Chromatography (HPLC) report displayed Haemoglobin A2 window with variant percentage being 45.9% and retention time of 3.53 min as shown in [Table/Fig-1] (Chromatogram of the patient). In our setup, we routinely get a retention time of 3.60-3.62 min for Haemoglobin A2. The dubious nature of the result and ambiguity with the ethnicity of the patient prompted us to further analyse the sample using Agarose Gel Electrophoresis in Alkaline media (Hydragel 7 Haemoglobin E, SEBIA). Results showed the migration of abnormal haemoglobin to haemoglobin S/D/G region as depicted in [Table/Fig-2].We then went on to do sickle cell solubility test and the test was negative for that sample as shown in [Table/Fig-3].Now we needed to confirm that it was Haemoglobin D-Iran and for that we resorted to automated Capillary Electrophoresis based Haemoglobin Typing System (CAPILLARYS FLEX 2 Piercing, SEBIA). The result showed a peak in the Haemoglobin D zone. However, the peak was not where the Haemoglobin D-Punjab elutes. We also ran a known Haemoglobin D-Punjab sample to show the difference between the peaks. The results are shown in [Table/Fig-4].

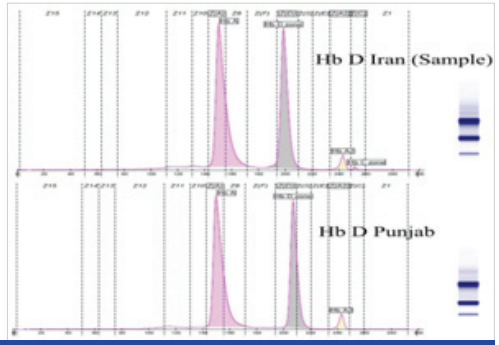
After the diagnosis was made, it was conveyed to the patient. Since she did not have any difficulty or complain related to this finding, it was decided that no intervention was needed, however, we counselled the patient regarding the condition and advised her to have her children screened for the same, to which she disagreed and hence wasn't pursued further.





[Table/Fig-1]: Chromatogram of the sample showing abnormal Hb eluting in the A2 window **[Table/Fig-2]:** Alkaline agarose gel electrophoresis showing migration of abnormal Hb to Hb S/D/G region





[Table/Fig-3]: Sickle cell solubility test was negative for the sample **[Table/Fig-4]:** Capillary Electrophoretogram showing the abnormal Hb peaking in Hb D Zone which is distinct from the peak of a known Hb D Punjab sample

DISCUSSION

It was Itano in 1951 who first described a group of haemoglobinopathies in a white family and classified it as Haemoglobin D (Hb D) [1]. However, after around two decades, Rahbar independently found a substitution of Glutamic Acid → Glutamine (GAA → CAA) at β22 and designated it as Haemoglobin D-Iran (Hb D-Iran) [2]. It should be noted that three other substitutions have been described at β22 position viz. E Saskatoon (Lys), G Coushatta (Ala) and G Taipei (Gly) [3].

In our case,the retention time of the variant haemoglobin on HPLC (3.53 min) correlates closely with the findings of Joutovsky A et al., (3.49 min) and was quite distinct from the retention time of Hb A2 (3.63 min) and Hb E (3.69 min) [4]. In our HPLC instrument, there are separate windows- D Window (for Hb D-Punjab), S Window (for Hb S) and C Window (for Hb C), but HPLC cannot differentiate between Haemoglobin D Iran and Haemoglobin A2 as Hb A2 along with Hb D, Hb Lepore and Hb E elutes in the same A2 window. So we decided to do alkaline electrophoresis where both Hb A2 and Hb D have different mobility.

Hb D-Iran is commonly found to be co-existent with β-Thalassemia and generally doesn't get diagnosed until patient comes for the diagnosis or treatment of the Thalassemia. In homozygous cases, such as one reported by Thornburg CD et al., Hb D-Iran can present with anaemia, poikilocytosis and mild haemolysis [5]. When co-existing β-Thalassemia, the patient's clinical symptoms arise due to the Thalassemia component and not due to the Hb D-Iran counterpart. One such case has been reported by Agrawal MG et al., where Hb D-Iran was co-existing with β-Thalassemia [6]. In heterozygous form with Hb A (Hb D-Iran trait), the patient is usually clinically silent and goes undetected as was evident from this index case. Another case was reported by Gupta et al., where Hb D-Iran was present along with Hb D-Punjab [7]. It should be noted that in all these cases Hb D-Iran was diagnosed by gene sequencing. But, our case is unique because, to the best of our knowledge, the use of capillary electrophoresis for the diagnosis of Hb D-Iran has been done for the first time.

CONCLUSION

With advanced technologies like capillary electrophoresis it becomes easier to detect these rare forms of Haemoglobinopathies as they are less cumbersome to perform. It improves the diagnostic time and reduces the effort drastically. However, gene sequencing still remains the gold standard and can be done for further study in this direction.

REFERENCES

[1] Itano HA. A third abnormal haemoglobin associated with hereditary haemolytic anemia. *Proc Natl Acad Sci U S A*. 1951;37(12):775–84.
[2] Rahbar S. Haemoglobin D Iran: 2 22 glutamic acid leads to glutamine (B4). *Br J Haematol*. 1973;24(1):31–35.
[3] Rohe RA, Sharma V, Ranney HM. Haemoglobin D Iran alpha A2 beta 22 2-Glu leads to Gln in association with thalassemia. *Blood*. 1973;42(3):455–62.
[4] Joutovsky A, Hadzi-Nesic J, Nardi MA. HPLC retention time as a diagnostic tool for haemoglobin variants and haemoglobinopathies: a study of 60000 samples in a clinical diagnostic laboratory. *Clin Chem*. 2004;50(10):1736–47.
[5] Thornburg CD, Zimmerman SA, Schultz WH, Ware RE. An infant with homozygous Haemoglobin D-Iran. *J Pediatr Hematol Oncol*. 2001;23(1):67–68.
[6] Agrawal MG, Bhanushali AA, Dedhia P, Jeswani KD, Dayanand S, Dasgupta A, et al. Compound heterozygosity of Hb D(Iran) (beta(22) Glu-->Gln) and beta(0)-thalassemia (619 bp-deletion) in India. *Eur J Haematol*. 2007;79(3):248–50.
[7] Gupta A, Saraf A, Dass J, Mehta M, Radhakrishnan N, Saxena R, et al. Compound heterozygous haemoglobin d-punjab/haemoglobin d-iran: a novel haemoglobinopathy. *Indian J Hematol Blood Transfus*. 2014;30(Suppl 1):409–12.

PARTICULARS OF CONTRIBUTORS:
1. Junior Resident, Department of Biochemistry, Grant Government Medical College and Sir J.J. Group of Hospitals, Mumbai, India
2. Junior Resident, Department of Biochemistry, Grant Government Medical College and Sir J.J. Group of Hospitals, Mumbai, India
3. Professor, Department of Biochemistry, Grant Government Medical College and Sir J.J. Group of Hospitals, Mumbai, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Abhishek Roy,
Junior Resident, Department of Biochemistry, Grant Government Medical College and Sir J.J. Group of Hospitals,
Byculla, Mumbai-400008, India.
Email: mail@abhishek.ro

FINANCIAL OR OTHER COMPETING INTERESTS: None.

Date of Submission: **Jan 16, 2015**
Date of Peer Review: **Apr 27, 2015**
Date of Acceptance: **May 18, 2015**
Date of Publishing: **Jul 01, 2015**