CASE STUDIES

Hide and seek: Efficacy of HPLC and capillary zone electrophoresis as screening tools for hemoglobin disorders

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Abstract

Diabetes and hemoglobinopathy can co-exist, but the extent of diagnosis of such co-existence may depend on choosing the appropriate diagnostic methodology. This case report emphasizes the importance of such methods like HPLC and capillary electrophoresis in diagnosing these disorders.

Keywords: HPLC, Hb D, Hemoglobin disorders, CZE

Introduction

Diabetes is fast gaining the status of a potential epidemic in India with more than 62 million diabetic individuals currently diagnosed with the disease.[1] Presently, hemoglobin A1c (HbA1c) holds the monopoly in monitoring the glycemic control in patients with diabetes mellitus.[2] High performance liquid chromatography (HPLC) has been recognized as one of the reliable methodologies for HbA1c measurement. It has also been extensively used for screening hemoglobinopathies. Hence, efficient use of HPLC may serve as an effective tool for dual diagnosis of diabetes and hemoglobinopathy.

Case report

A 45-years-old female from Andhra Pradesh visited our hospital for master health check-up. Routine laboratory tests, done as a part of health check-up, did not reveal any specific abnormality. HbA1c (using HPLC) done as a part of the master health check-up package yielded results within reference limits (4.8%). HbA1c was performed using HPLC with Bio-Rad D 10 analyzer in HbA1c mode in our laboratory. The laboratory has adopted quality practice of reviewing the HbA1c chromatograms of all patient samples. In the present case, the laboratory made an incidental finding of abnormal hemoglobin in HPLC (HbA1c mode). Processing the sample in HbA2F mode in Bio-Rad D10 also showed an abnormal peak at 2.71 minutes just before HbA2 window. Based on this finding, a reflex testing was performed using capillary zone electrophoresis (CZE, Sebiaminicap flex piercing) after receiving the patient consent. The electrophoretogram showed an abnormal peak in Hb D zone. This was in contrary to the chromatogram picture of the patient. Hemoglobin D, if present, elutes at 3.9 minutes in HPLC, but it was not evident in this patient. These findings raise many questions: What does this abnormal hemoglobin indicate? Considering the presence of clinically silent abnormal hemoglobin, how the diagnosis of hemoglobinopathy benefits the patient?

Discussion

Hemoglobinopathies are a group of disorders characterized by qualitative or quantitative abnormality of hemoglobin. Approximately 7% of the world's population carries an Hb variant, making hemoglobinopathies one of the most common monogenic diseases. Based on the clinical significance, these variants can be classified as clinically silent variants and clinically manifesting variants. The clinically silent variants are relatively underdiagnosed when compared to the manifesting variants, since there are no clinical manifestations to support the laboratory diagnosis. Hence most of these variants are diagnosed through incidental findings in a clinical laboratory.

One of the common approaches towards the incidental discovery of such variants is through testing of HbA1c by HPLC. Moreover, HPLC is one of the commonly used methods across India for HbA1c measurement. In HPLC, HbA1c is measured as a fraction of total hemoglobin. Hemoglobin is separated into several fractions, which appear as peaks in the chromatogram with definite pattern. Apart from HbA1c measurement, HPLC gives value-

added information on presence of abnormal hemoglobins in a patient. In the present case, all biochemical and hematological investigations were within the reference limit except the HPLC chromatogram (A2F mode), which showed the presence of an abnormal peak (40.9%) at 2.71 minutes. This aroused a high index of suspicion of clinically silent hemoglobin (Fig. 1).

As per the British Society of Hematology guidelines (Significant haemoglobinopathies: guidelines for screening and diagnosis), our laboratory has adopted a practice of screening hemoglobinopathies through two methods namely HPLC and CZE.[3] A presumptive diagnosis of hemoglobinopathy is done based on correlation between these two methods.

In the current case, the capillary electrophoretogram showed the presence of an abnormal peak (43.2%) in the Hb D zone, suggestive of the presence of hemoglobin D. In HPLC, hemoglobin D appears at 3.9 minutes, but this did not correlate with the retention time of the abnormal hemoglobin noted in the present patient (2.71 minutes, Fig. 2). There was a diagnostic mismatch between HPLC and capillary electrophoresis. Hence, probing was done to check for the probability of abnormal hemoglobin presenting with this pattern (at Hb D zone in capillary and at 2.71 minutes in HPLC). A review of literature indicated Hb D Iran as the hemoglobin fraction that closely matches with the present case.[4]

Hb D Iran is prevalent in north western parts of India, Pakistan and Iran.[5] There is no convincing evidence on prevalence of Hb D Iran in Southern parts of India. Hence the variant that had been identified in the current patient could be Hb D Iran or any other variants resembling Hb D Iran. The confirmative diagnosis can be arrived only through molecular analysis and this was not performed due to limited resources in the hospital.

Since these hemoglobins do not manifest clinically (Fig.

Fig. 1: Hemoglobin D Iran in HPLC

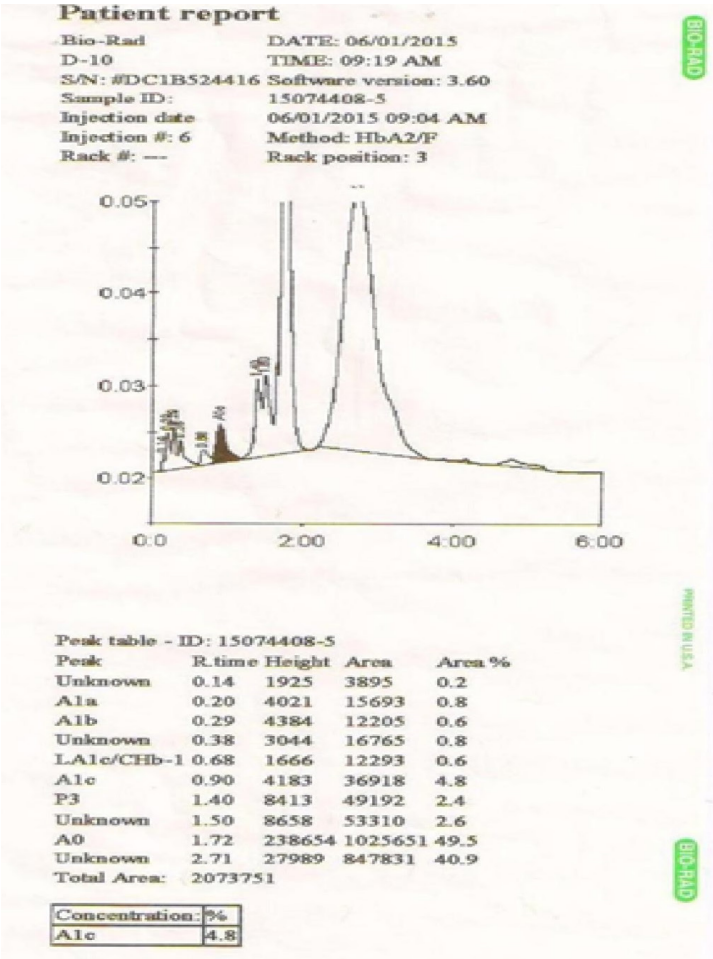


Fig. 2: Hemoglobin D Iran in capillary electrophoresis

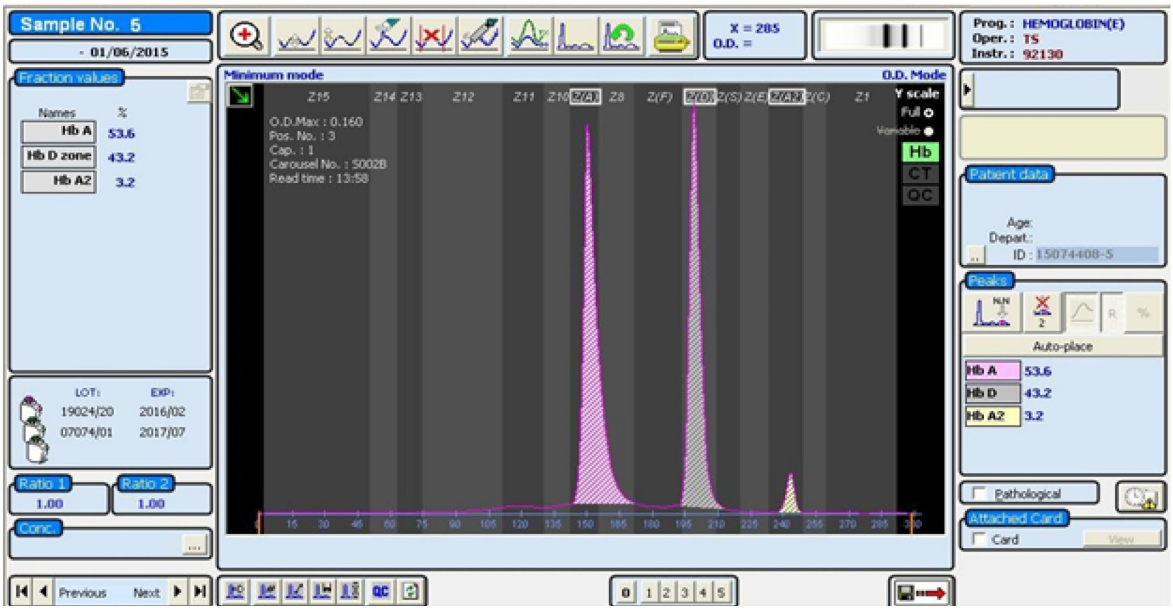
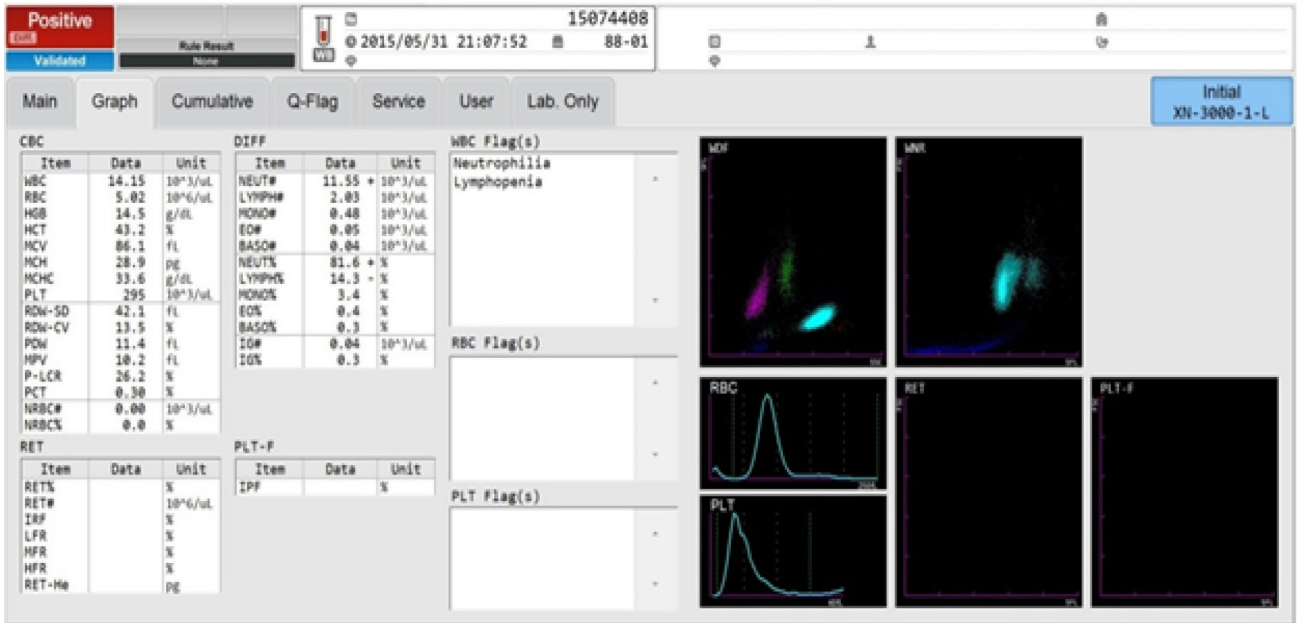


Fig. 3: Hemoglobin D Iran- Complete blood count



3), how such incidental findings benefit the patient? Hemoglobinopathies are inherited disorders that can be transmitted across generations. Co-occurrence of clinically virulent hemoglobins like Hb S and Hb C with clinically silent variants can worsen the course of the disorder.[6] This can occur through consanguineous marriages.

Conclusion

This case report may assist in creating awareness in laboratories regarding the presence of abnormal hemoglobins (some of them are clinically silent) in local population. Hence, it is necessary for the laboratorian to have a basic knowledge on understanding and interpreting the chromatograms and electrophoretograms, and this guideline should be implemented in the laboratory practice. To conclude, hemoglobinopathies are preventable, though not treatable, for which implementation of an appropriate diagnostic method with in-depth understanding is required. In addition, to prevent the transmission of these disorders, proper genetic counselling of family members is warranted.

Competing interests

The authors declare that they have no competing interests.

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