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Report of Three Bengali Cases with Hemoglobin E Variant in Najran

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Abstract:

INTRODUCTION: Differential diagnosis of hemoglobin (Hb) variants eluting in the A_2 window on high-performance liquid chromatography (HPLC) is of particular importance. Of particular importance is Hb E, which is the most common and the most significant variant. The aim of this study was to study a rare variant infrequently seen in our countries during the routine work.

METHODS: Sixteen Bengali workers at Najran University Hospital, Saudi Arabia, came to the laboratory for routine investigation. CBC, routine blood chemistry, and Hb separation by HPLC were performed.

RESULTS: Three cases out of 16 showed an abnormal Hb peaked on the A_2 window on HPLC consistent with the diagnosis of Hb E. Two of them had Hb E of 29.1% of each. The third case had Hb E around 86%. In addition, one case was consistent with β thalassemia trait had increased Hb A_2 of 5.7%. Five cases were borderline.

CONCLUSION: HbE could be adequately differentiated from other Hb variants eluting in the A_2 window on HPLC by the percentage of the variant, its retention time, the mild clinical presentation, and the ethnic origin of the patient.

Keywords:

Hemoglobin A2, hemoglobin E, high-performance liquid chromatography, Kolkata, variant

Introduction

The δ globin gene is expressed at low levels, approximately 2.0%–3.0% of the total hemoglobin (Hb) in normal individuals. Over 1352 human Hb variants are known up-to-date.[1] The diagnosis of variants elutes in the A_2 window on high-performance liquid chromatography (HPLC) is of particular importance because increased Hb A_2 (around 5%–6%) in the presence of microcytosis and hypochromia of red blood cells (RBCs) is the main characteristic parameter in diagnosing most β-thalassemia carriers.

The increase in Hb A_2 may refer to other structural variants that also migrate in the Hb A_2 window on HPLC,[2] and DNA-based diagnosis is necessary before genetic counseling is undertaken. Of particular importance is Hb E which is the most common and the most significant abnormal Hb eluting in the A_2 window on HPLC. HbE arises from a point mutation in codon 26 in which the normal GAG codon is mutated to an AAG, resulting in a change from glutamic acid to lysine. The β^E mutation activates this cryptic splice site so that it begins to function in a rather efficient way. The result is that about 40% of the splices result in termination at an out-of-frame stop codon. The normally spliced RNA contains the β^E mutation and produces only about 60% of the normal amount of β-globin.[3]

HbE is a mild allele, almost with nearly normal clinical presentation in heterozygote and mild hemolytic anemia in homozygote state.[4] HbE is the most prevalent variant

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in Southeast Asia (Thailand, Myanmar, Cambodia, Laos, and Vietnam). In India, it is clustering in Kolkata and Assam where the carrier frequency is 2%–5% and 6%–51%, respectively.[5,6]

In this study, we have faced with three cases consistent with the diagnosis of HbE trait and HbE disease discovered during a routine investigation of Bengali workers at the Najran University Hospital, KSA. They had abnormal Hb peaks masking Hb A_2 on HPLC. An adequate presumptive diagnosis was done based on the RT, clinical picture, and ethnic origin of the patients. The Hb A2 expression and the diagnosis of β thalassemia carriers in these cases and in the other cases were also discussed.

Materials and Methods

Sixteen male Bengali workers at Najran University Hospital, Saudi Arabia, came to the laboratory for routine investigations. Complete peripheral blood pictures (CBCs) were carried out using Sysmex XS 500i (Sysmex, https://www.sysmex.com/). Serum biochemical analysis was carried out using COBAS C311 (Roche, https://www.roche.com/). Hb separation was carried out by HPLC using the D-10 instrument (Bio-Rad Laboratories Hercules, California, USA). One case, showed a marked increase in expression of abnormal Hb on the A_2 window, was repeated on the Variant II HPLC system ((Bio-Rad) with the use of the Variant II Thalassemia Short Program (Bio-Rad Laboratories Hercules, California, USA).

Results

In this study, 16 Bengali male workers from Kolkata (also written Colcutta) (the capital of the Indian state of West Bengal) were investigated. One case was consistent with β thalassemia carrier. It showed increased Hb A_2 of 5.7% with microcytic hypochromic RBCs and increased RBC distribution width (RDW) [Case 1, Table 1 and Figure 1a]. Five cases were borderline. Although they had Hb A_2 of 4% or slightly more, they had normal total Hb, nearly normal RBC indices, and normal RDW [Cases 2–6, Table 1 and Figure 1b]. Family study, further investigations, and DNA analysis were required to reach to a definite diagnosis. This agrees with Rosnah *et al*.[7] who found that normal HbA_2 level may reach 4%.

HbE is the most common variant eluting in the A_2 window on HPLC. It is a mild allele, almost with nearly normal clinical presentation in heterozygote and mild hemolytic anemia in homozygote state.[4] HbE is the most prevalent variant in Southeast Asia (Thailand, Myanmar, Cambodia, Laos, and Vietnam). In India, it is clustering in Kolkata and Assam where the carrier frequency is 2%–5% and 6%–51%, respectively.[5,6] In this study, three cases with abnormal peak in the A_2 window were consistent with the diagnosis of Hb E. Two cases (Cases 7 and 8) [Table 2] showed an abnormal peak of 29.1% for each. They gave nearly the same chromatograph of Hb separation on HPLC [Figure 2a]. They had no complaint and with clinically stable picture, only microcytic hypochromic RBCs.

The third case (case 9) [Table 2, Figures 2b and c and 3], showed abnormal Hb at 86% expression on HPLC, was separated by two instruments (D-10 instrument and Variant II) for confirmation. It showed a mild clinical presentation, a mild microcytic hypochromic anemia, an increase in RDW, a mild hemolysis (mild increase in unconjugated bilirubin), and a mild increase in Hb F (2.7%). Other small Hb peaks were attributed to

Table 1: Descriptive data of 6 cases with increased/ borderline hemoglobin A_2

	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6
RBCs (10^6/ul)	6.89	6.00	5.90	6.09	5.92	5.47
HGB (g/dl)	11.8	15.4	16.6	17.2	15.5	15.4
MCV (fl)	57	82.0	80.7	84.9	80.1	87.2
MCH (pg)	17.1	26.0	28.1	28.2	26.2	28.2
MCHC (g/dl)	30	31.3	34.9	33.3	32.7	32.3
RDW-CV (%)	19.5	12.9	12.7	11.8	11.9	13.1
Serum iron (µg/dl)	83.0	45.1	134.4	67.6	483.7	92.6
Serum ferritin (µg/l)	114.6	100.5	107.9	110.5	95.3	104.4
Hb A (%)	93.4	94.6	95.3	95.2	95.3	95.3
Hb F (%)	0.7	0.7	0.7	0.7	0.7	0.7
Hb A_2 (%)	5.9	4.7	4.0	4.1	4.0	4.0
Total bilirubin (mg/dl)	0.45	0.4	1.2	0.7	0.4	0.8
Direct bilirubin (mg/dl)	0.16	0.1	0.15	0.24	0.13	0.23

RBC=Red blood count; MCV=Mean corpuscular volume; MCH=Mean corpuscular hemoglobin, MCHC=MCH concentration; RDW=Red blood cell distribution width; Hb=Hemoglobin; HGB=Hemoglobin

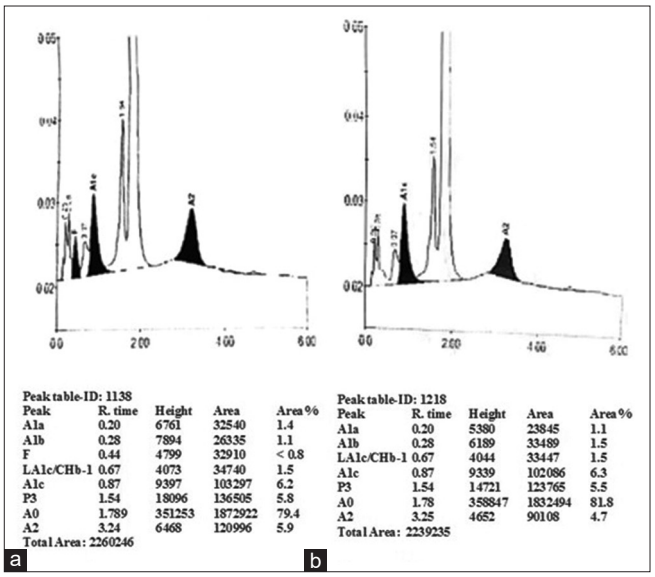

Figure 1: (a) A case consistent with β thalassemia carrier. (b) Borderline case

posttranslational modification of Hb E. This picture was consistent with HbE disease.[8] This patient gave a history of a hemolytic attack once during a high fever period. This was consistent with the reported instability of Hb E in oxidative stress and high fever.[9]

In Eβ^0 thalassemia, Hb E is usually around 50%, yet it can be differentiated with ease by its severe clinical presentation and the marked increase in Hb F.[9] Eβ+ thalassemia is milder and characterized by the presence of Hb A.[10] Retention time of the presumed HbE (3.44 and 3.51 min) [Figure 2] was clearly different from that of Hb A_2 (3.24 and 3.25 min) [Figure 1] on D-10 HPLC. It could be specific for HbE and can be used in differentiation, although it needs more evaluation.

Table 2: Descriptive data of the three cases with abnormal peaks in the A_2 window on high-performance liquid chromatography

	Case 7	Case 8	Case 9
RBCs (10^6/ul)	6.36	6.25	6.16
HGB (g/dl)	14.7	13.9	12.3
MCV (fl)	71.7	69.0	55.0
MCH (pg)	23.1	22.2	20.0
MCHC (g/dl)	32.2	32.3	36.3
RDW-CV (%)	14.3	14.3	20.1
Serum iron (µg/dl)	123.2	80.6	80.1
Serum ferritin (µg/l)	256.2	290.2	141.5
Hb A (%)	70.3	70.5	11.4
Hb F (%)	0.6	0.4	2.7
Abnormal Hb peak (%)	29.1	29.1	86
Total bilirubin (mg/dl)	0.94	0.5	1.39
Direct bilirubin (mg/dl)	0.28	0.17	0.41

RBC=Red blood count; MCV=Mean corpuscular volume; MCH=Mean corpuscular hemoglobin, MCHC=MCH concentration; RDW=Red blood cell distribution width; Hb=Hemoglobin; HGB=Hemoglobin

Discussion

The majority of hemoglobinopathy present in the western and eastern provinces of Saudi Arabia, particularly in the southwestern province. Abuzenadah *et al*.[11] reported a great heterogeneity at the molecular level in the western province and attributed this to the large population of immigrants there. Hb E was one of the seven common β-thalassemia alleles reported. The prevalence of Hb E trait was the least frequent (0.85%) compared to the other investigated Hb disorders in Jedda (57 Hb E carrier from 6750 cases studded).[12] Only three cases (2.4% of cases studied) at Taif city were reported by Dahlawi *et al*.[13] Najran city, located in the south of KSA, had the least prevalence of β Thalassemia trait (2.4%) and zero β thalassemia disease in a study done by Alsaeed ES *et al*.[14] 2011–2015. As far as we know, there is no report about Hb E in Najran.

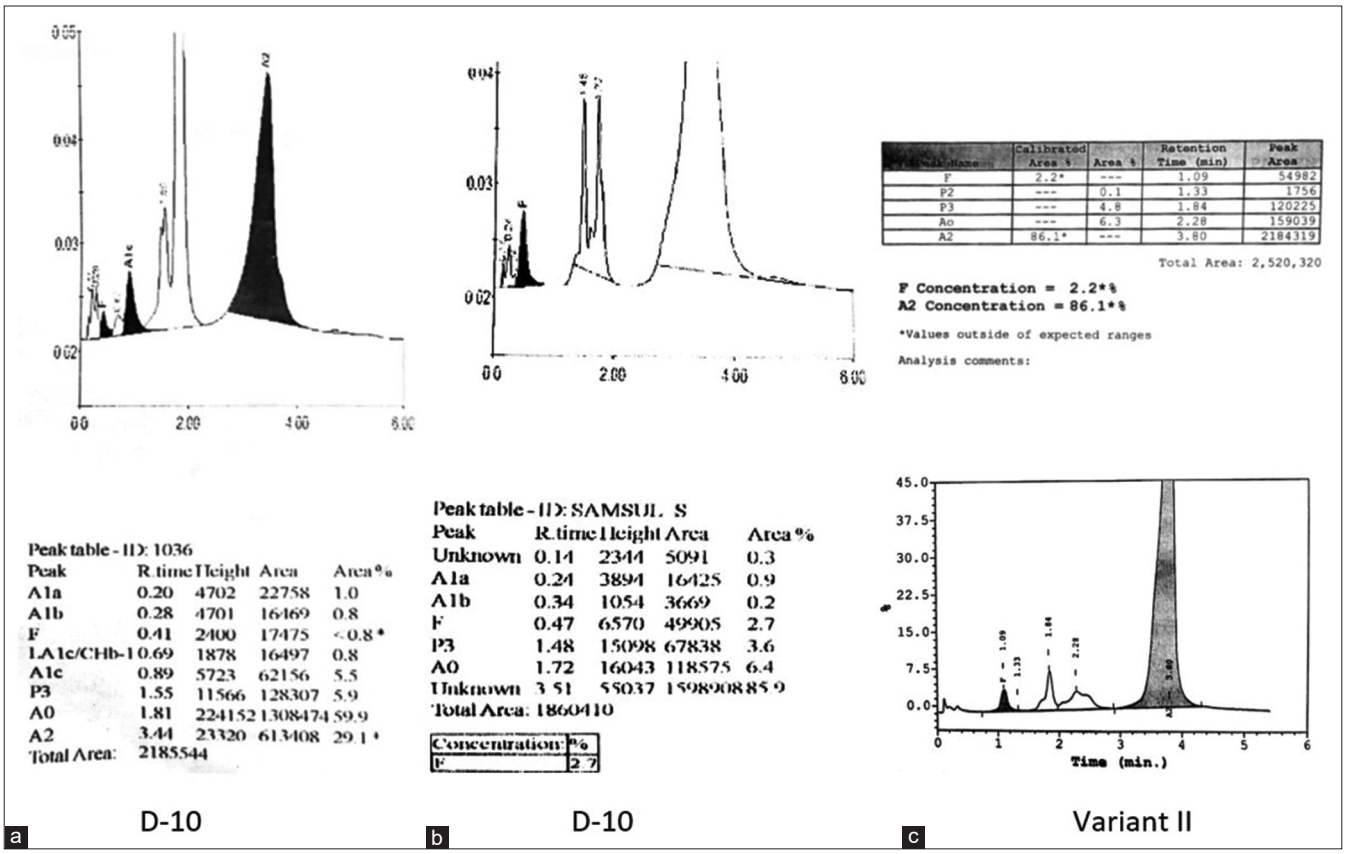

Figure 2: High-performance liquid chromatography of the three cases with abnormal peaks on the A2 window. (a) Case 7 or 8 (b and c) case 9

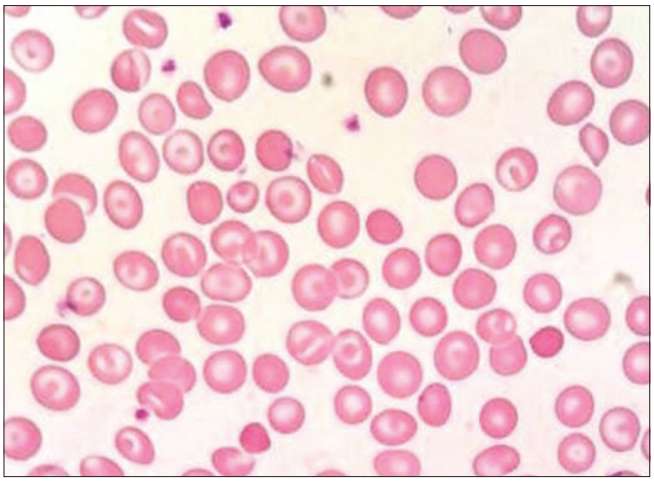

Figure 3: Peripheral blood smear of case 9

Although HbE is not prevalent in KSA, physician awareness by diagnosis and the clinical course is important, especially because of the pilgrimage and pilgrims, and the presence of sustainable employment, especially from East Asian countries. Better understanding of Hb phenotypes is crucial in the diagnosis and would help in simple and cheap DNA diagnosis by ARMS PCR or restriction digest rather than the expensive and laborious sequencing.

In this study, we focused on the phenotype of three cases consistent with the diagnosis of HbE and rare variants, two heterozygotes, and one homozygote. The abnormal Hb was masking the Hb A_2 on HPLC. The ethnic origin of the patients was crucial in confirming the diagnosis of Hb E. It was impossible to determine the Hb A_2 expression on HPLC in these cases. The diagnosis of a mild β thalassemia carrier is compromised and only can be excluded by DNA diagnosis in such cases. The awareness of such dilemma is important, especially as regards the premarriage programs in KSA and the Middle East countries where Hb E is not prevalent. Furthermore, this study highlighted the importance of the clinical picture, ethnic origin, family history, and the DNA techniques in the diagnosis of hemoglobinopathies.

Conclusion

HbE could be adequately differentiated from other Hb variants eluting in the A_2 window on HPLC by the percentage of the variant, its retention time, the mild clinical presentation, and the ethnic origin of the patient. Adequate presumptive diagnosis in hemoglobinopathy is a preliminary step to a definite diagnosis by DNA analysis.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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