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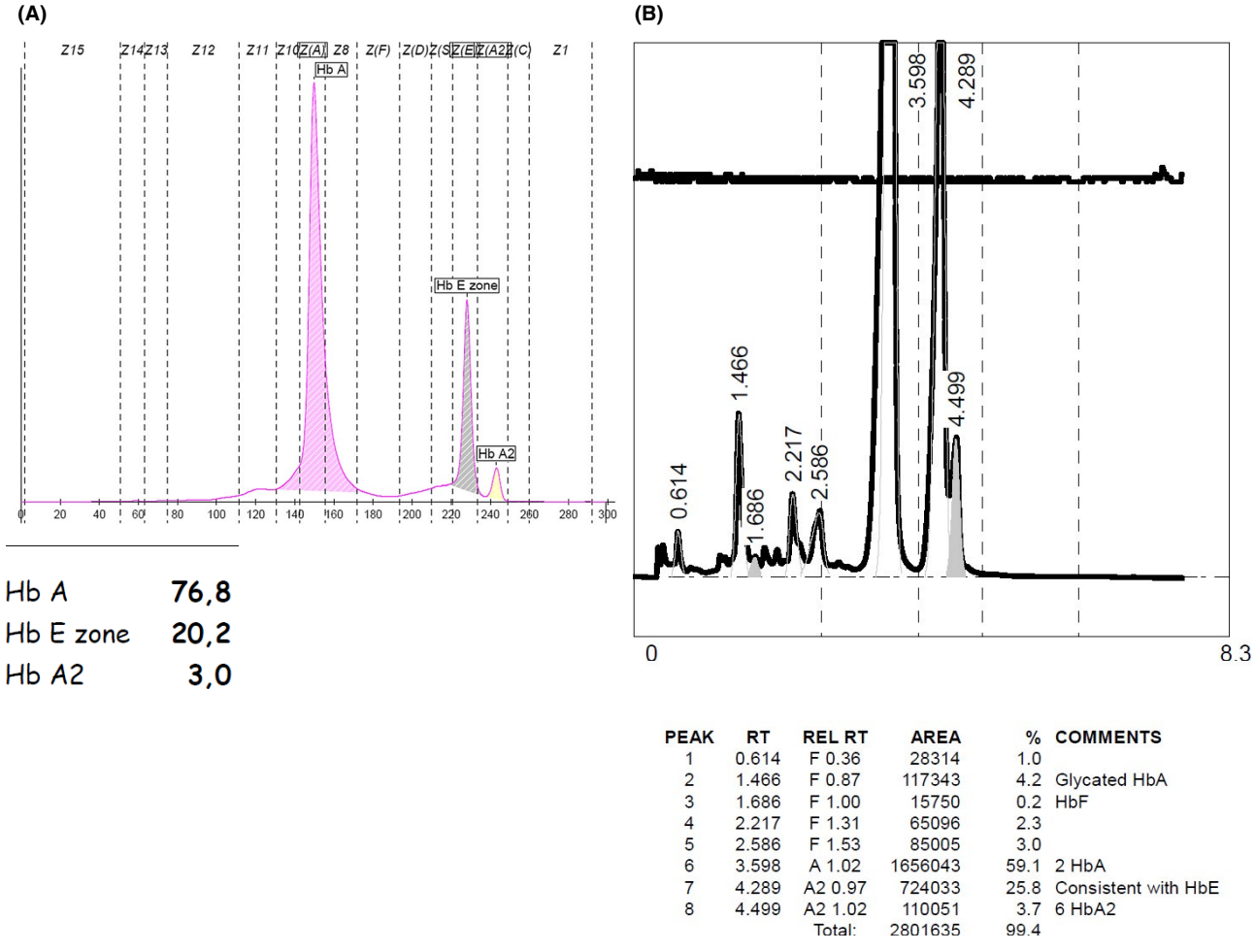
LETTER TO THE EDITOR

The first report of hemoglobin E in combination with the highly unstable alpha-globin variant Hb Adana: The importance of molecular confirmation

HbE is a common hemoglobin variant causing only minimal red cell abnormalities in carriers. Alpha-globin gene defects may cause decreased MCV and reduction of the percentage of HbE.[1] Molecular analysis involves gap-PCR for the most common alpha-thalassemia rearrangements and covers over 80% of all alpha-thalassemia defects. Less frequently point mutations or unknown deletions are involved, which can be detected by Sanger sequencing and MLPA. Sometimes, the combination of HbE and alpha-thalassemia may go undetected if molecular analysis is not standardly performed, especially if the HPLC does not show a reduced percentage of HbE. Depending on the type of Hb variant or alpha-thalassemia defect, this could have serious consequences for counseling. Hb Adana



PEAK	RT	REL RT	AREA	%	COMMENTS
1	0.614	F 0.36	28314	1.0	
2	1.466	F 0.87	117343	4.2	Glycated HbA
3	1.686	F 1.00	15750	0.2	HbF
4	2.217	F 1.31	65096	2.3	
5	2.586	F 1.53	85005	3.0	
6	3.598	A 1.02	1656043	59.1	2 HbA
7	4.289	A2 0.97	724033	25.8	Consistent with HbE
8	4.499	A2 1.02	110051	3.7	6 HbA2
		Total:	2801635	99.4	

FIGURE 1 A, Capillary electrophoresis results showed 76% HbA, 3% HbA2, 0 and 20.2% HbE. B, High-performance liquid chromatography results showed 59.1% HbA, 3.7% HbA2, 0.2% HbF, and 25.8% HbE. The HbE and HbA2 peaks were not completely separated in HPLC which could explain the slight overestimation of the HbE level in our patient. With CE, the HbE level was slightly decreased compared to HPLC and to the literature for a plain HbE carrier. No HbH or Hb Bart's have been shown in the two graphs [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 1 Hematological, biochemical, and DNA results of our patient

Parameters	Patient results	Normal reference range	Reference range for HbE carriers in our department
Hb (g/dL)	14.1	M: 13.0-18.0	11.3-14.3
MCV (fL)	83.0	80.0-96.0	73.0-86.0
MCH (pg)	25.1	27.4-33.8	23.0-32.0
ZPP (μmol/mol heme)	47.0	<100.0	<100.0
CE			
HbA (%)	76.0	96.8-97.8	-
HbA2 (%)	3.0	2.2-3.2	-
HbF (%)	0.0	0.0	-
HbE (%)	20.2	0.0	23.3–26.2
HPLC			
HbA(%)	59.1	>80.0	-
HbA2(%)	3.7	2.3-3.3	-
HbF(%)	0.2	0.0	-
HbE(%)	25.8	0.0	23.4–28.4
β-sequencing	het. HBB:c.79G>A (p.Glu27Lys)(HbE)		
α2-sequencing	het. HBA2:c.179G>A (p.Gly60Asp)		

CE, capillary electrophoresis; Hb, hemoglobin; HPLC, high-performance liquid chromatography; MCH, mean corpuscular Hb; MCV, mean corpuscular volume; ZPP, zinc protoporphyrin; α2-sequencing, alpha 2-globin gene sequencing; β-sequencing, beta globin gene sequencing.

(c.179G>A), a highly unstable alpha-globin gene variant, interacts with deletional and nondeletional mutations to produce HbH disorders with varying clinical manifestations from asymptomatic to severe anemia with significant hepatosplenomegaly. The most severe form of HbH disorders is the HbH hydrops fetalis, where the fetus usually dies *in utero* or shortly after birth due to a severe intrauterine hemolytic anemia.[2] Here, we report, to our knowledge, the first case of a carrier of HbE (c.79G>A p.Glu27Lys) and the highly unstable Hb Adana variant (HbA2: c.179G>A p.Gly60Asp). We present a case of a 53-year-old man with normal MCV, a slightly decreased MCH without anemia. Biochemical assays showed a borderline decreased percentage of HbE in capillary electrophoresis (CE) but normal HbE levels in high-performance liquid chromatography (HPLC) which could easily have been mistaken for a regular HbE carrier. Hb Adana was detected by alpha-globin gene sequencing following a negative gap-PCR result for the seven most common alpha-thalassemia deletions.

The complete blood count (CBC) (Horiba medical, ABX Micros ES60) showed normocytic hypochromic parameters without anemia (Hb = 14.1 g/dL, MCV = 83 fl, MCH = 25.1 pg), Zinc protoporphyrin (ZPP) (AVIV Hematofluorometer Model 206) was normal (ZPP = 47), CE (Sebia) and HPLC (Trinity Biotech Premier Hb9210™) showed a low level of HbE (20.2% and 25.8%, respectively) (Figure 1; Table 1). Direct sequencing of the HBB gene (ABI PRISM™ 3730 DNA sequencer) confirmed the presence of the β^E mutation (HBB:c.79G>A p.Glu27Lys). Normally Hb E carriers without iron deficiency show HbE levels between 25% and 30%.[1] The HPLC result showed 25.8% HbE, which is concordant with a HbE carrier. However, the HbE percentage on CE was slightly decreased (20.2%) which was suggestive of an underlying alpha-thalassemia. As the seven most common alpha deletions were excluded by multiplex gap-PCR, we decided to perform alpha-globin gene sequencing and we discovered a heterozygous Hb Adana variant in the alpha 2-globin gene (HbA2: c.179G>A p.Gly60Asp).To our knowledge, this is the first report of a double heterozygote HbE/Hb Adana. The point mutation causative of Hb Adana has been reported in the literature occurring on the alpha 1- and alpha 2-globin genes (codon 59 GGC → GAC). The mutation results in a Gly → Asp substitution in the alpha-globin chain of Hb Adana.[3] This amino acid substitution replaces a small noncharged glycine with a large charged aspartic acid molecule, which compromises alpha-globin chain stability and leads to abnormal precipitation and red cell membrane damage, hemolysis, and ineffective erythropoiesis.[4] Especially when on the dominantly expressed alpha 2-globin gene, this mutation may even cause HbH hydrops fetalis in homozygosity or in combination with alpha 0-thalassemia.[2,5]

Due to its unstable nature, heterozygosity for Hb Adana is not visible by routine biochemical assay such as HPLC and CE,[6] but the carrier does present with microcytic hypochromic parameters. As carriers of HbE may also present with mild microcytic hypochromic parameters, the co-inheritance of alpha-thalassemia defects could be easily overlooked unless the HbE percentage is taken into account, which is usually slightly decreased in those cases. In our case, the decrease in percentage of HbE was only visible on CE, while the hematology (normal MCV) and the percentage of HbE on HPLC

(25.8%) were not suggestive of an additional alpha-globin defect (Table 1).

A complicating feature in the quantification of the HbE fraction may be the overestimation of HbE due to the inability to separate it from HbA_2 by many HPLC systems presently used for hemoglobinopathy diagnostics.[7] The Premier High Resolution (Trinity Biotech) HPLC system used is capable of separating HbE from HbA_2; however, as shown in Figure 1, the separation between HbA_2 and HbE is better in CE, which explains why the HPLC measurement of the HbE is within the normal range of the HbE carrier without alpha-thalassemia. Therefore, borderline low HbE levels and low MCV and MCH should not be ignored and should be investigated for co-inherited alpha-thalassemia defects.

The identification of a highly unstable alpha-globin variants such as Hb Adana has genetic implications for counseling to prevent a severe form of alpha-thalassemia such as a HbH—hydrops fetalis. A recent literature study revealed that cases of Hb Adana associated with hydrops fetalis did not follow the classical alpha-thalassemia paradigm where four alpha-globin gene deletions are typically causal of Hb Bart's hydrops fetalis syndrome. If the partner is a carrier of Hb Adana on the alpha 2-globin gene as well or is carrier of SEA, in which alleles are particularly frequent in the Indonesian population (16% and 4%-14%, respectively), there is a 25% risk of having a HbH hydrops fetalis in the offspring.[2,5,8] In addition, the alpha 2-globin variant (c.179G>A) found in our patient has been more associated, in the literature, with severe phenotypes in homozygous or in compound heterozygous states than alpha 1-globin gene Hb Adana mutation [2,9-11] which underlines the importance to determine the exact genotype of these variants in carriers.

In conclusion, our case confirms that hematologists evaluating patients with anemia should perform a thorough evaluation for alpha-thalassemia, which, besides the commonly used gap-PCR for the seven most common deletions, should also include alpha-globin gene sequencing. Careful evaluation of both hematological, biochemical separation, and DNA analysis is essential to diagnosis all combinations of beta and alpha-globin gene defects in order to determine precise genetic risk in the carriers to provide adequate genetic counseling.[12]

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