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# Hemoglobin EE Disease: A Case Report

By Awad M. Al-Qahtani, Mohamed S. M. Khalil & Essam M. Ahmed

*Najran University*

*Abstract-* *Background:* Hemoglobin E variant results from a G→A substitution resulting consequently in abnormal processing for messenger mRNA. Its interactions with various forms of α and β thalassemia produce a very wide range of clinical syndromes.

*Methods:* A Consent has been taken from a 26-year-old male. CBC, Glucose, Vitamin $B_{12}$, C-peptide, estradiol ($E_2$), follicle stimulating hormone (FSH), free triiodothyronine ($FT_3$), free thyroxine ($FT_4$), luteinizing hormone (LH), prolactin, parathyroid hormone (PTH), testosterone, thyroid stimulating hormone (TSH) and vitamin $D_3$ (25-OH) and HPLC for hemoglobin separation were performed.

*Results:* There was a history of hemolytic anemia due to infection with malaria and just one blood transfusion. There were no significant clinical findings such as organomegaly, icterus, or thalassemic bone changes. C-peptide, $E_2$ and TSH results were slightly above the normal range. Vit D was slightly insufficient. No Helicobacter pylori Antigen is stool and no clinical abnormalties. All the Hb were abnormal. The patient has low HDL-C which could not be explained. Also the slightly increased hormones of E2 and TSH, the slightly increased C-peptide could not be explained and this requires further investigations.

*Conclusion:* The case was reported as abnormal hemoglobin (Hb E) masking the Hb A2 on HPLC.

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# Hemoglobin EE Disease: A Case Report

Awad M. Al-Qahtani [α], Mohamed S. M. Khalil [σ] & Essam M. Ahmed [ρ]

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## I. Background

Around 7% of the world's population comprises hemoglobinopathy gene carriers. Almost a total of 1317 Hb variants have been identified (HbVar database)[1], the four most common worldwide are Hb S, Hb E, Hb C, and Hb D, in the order of decreasing prevalence[2]. Hb E is the most prevalent variant in Southeast Asia (Thailand, Myanmar, Cambodia, Laos, Vietnam), where its prevalence is 30-60%[3,4,5,6]. The prevalence of Hb E in India is about 0-3.5% with an increased clustering in Kolkata (22%) and Assam (50-80%)[7].

Hemoglobin E variant results from a G→A substitution in codon 26 of the β globin gene, this produces an abnormal hemoglobin (glutamate is replaced by lysine) and activates a cryptic splice site at codon 25-27 of the β-globin gene, resulting consequently in abnormal processing for messenger RNA (mRNA). The level of normally spliced mRNA become reduced and because a new stop codon is generated, the abnormally spliced mRNA become nonfunctional [7,8]. Fortunately, only a minor activation of the alternative splicing pathway the mutation is associated with this mutation. Hence there is only a moderate reduction of the normally spliced $\beta^E$ globin mRNA[72].

Hb E trait and Hb EE disease are mild disorders. Although Hb E alone does not cause any significant clinical problems, its interactions with various forms of α and β thalassemia produce a very wide range of clinical syndromes of varying severity[8,9].

Hb E has several compound heterozygotes with common and uncommon β-globin or α-globin gene mutations, the most serious Hb E syndrome is Hb E$\beta^0$-thalassemia. Different phenotypes could be noticed with the compound heterozygote state of Hb Eβ-thalassemia ranging from a complete lack of symptoms to transfusion dependency [7, 8, 3, 10].

Experiments were carried out in vitro at temperatures ranging from 38 to 41°C showed that there was mild instability of Hb E but there is no evidence that this is the case in vivo[15 9]. It is noticeable that, E allele causes mild thalassemia, while $\beta^E\beta^0$ thalassemia shows a severe phenotype, this marked paradox in phenotype could not be explained up till now. It is reported that Hb E is sensitive to oxidative stress {HbVar database}. Does this or other properties of Hb E can contribute to the severity of the disease? This question is still waiting for an answer[11].

The aim of this report is to present a case of Hb EE discovered accidentally during a routine work trying to cast shadow on some parameters.

## II. Case Report

A Consent has been taken from a 26-year-old male, from Kolkata, India, came to Najran University Hospital, Saudi Arabia for routine investigation. He did not complain from anemia or receive treatment. He gave a history of hemolytic anemia because of infection with malaria and only one blood transfusion. On examination, there were no significant clinical findings such as organomegaly, icterus, or thalassemic bone changes.

CBC were carried out using Sysmex XS 500i (Sysmex, https://www.sysmex.com/). The results showed Hb of 13 g/dL, red blood cell (RBC) count of 6.8 x $10^{12}$/L, mean corpuscular volume (MCV) of 55.6 fl, mean corpuscular hemoglobin (MCH) of 19.6 pg, MCHC concentration of 36 g/dL, and RBC distribution width (RDW-CV) of 20.7%. Peripheral blood smear

*Author α: Department of Family and Community Medicine, College of Medicine, Najran University, Najran, Saudi Arabia.*
*Author σ: Department of Clinical Pathology, Faculty of Medicine, Assiut University, Assiut, Egypt & College of Medicine, Najran University, Najran, Saudi Arabia.*
*Author ρ: Pharmaceutical Division, National Research Centre, Egypt & Faculty of Applied Medical Sciences, Najran University, Najran, Saudi Arabia. e-mail: mohamedkhalil1967@yahoo.com*



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showed frequent target cells, and spherocytes as shown in Fig.(1). Serum biochemical analysis were carried out using COBAS C311 (Roche, https://www.roche.com/). Results were normal for liver and kidney functions except for mild increase in bilirubin (1.39 mg/dL, mostly indirect of 0.98 mg/dL). Lipid profile was normal except for low high density lipoprotein cholesterol (HDL- C) of 20.6 mg/dL...Iron, UIBC and ferritin were found to be 73.3 ug/dL, 250 ug/dL and 149.6 ug/L respectively. Glucose was within normal ranges. Vitamin $B_{12}$, C-peptide, estradiol ($E_2$), follicle stimulating hormone (FSH), free triiodothyronine ($FT_3$), free thyroxine ($FT_4$), luteinizing hormone (LH), prolactin, parathyroid hormone (PTH), testosterone, thyroid stimulating hormone (TSH) and vitamin $D_3$ (25-OH) were done. C-peptide, $E_2$ and TSH results were slightly above the normal range. Vit D was slightly insufficient. Some parameters were indicated in table (1). Hb A1c did not give results on D-10 HPLC that is why we thought of Hb variant. Hemoglobin (Hb) separation by high performance liquid chromatography (HPLC) using the D-10 instrument (Bio-Rad Laboratories Hercules, California, USA) as in Fig. (2). Nearly all the Hb were abnormal and it was eluted at the Hb A2 window with retention time (rt) of 3.17 minutes. When repeated on Variant II HPLC system ((Bio-Rad ) with use of the Variant II Thalassemia Short Program, it showed 86.1 % abnormal Hb with rt of 3.8 min along with 2.28 % adult Hb (Hb A) and Hb F around 3% of total Hb.

*Table 1:* Some hematological parameters:

| Investigation | Values | Investigation | Values |
|---|---|---|---|
| Hb | 13 g/dl | WBC | $11x10^9$/L |
| RBC | $6.8x10^{12}$/L | Eosinophilia | $1.9 \times 10^9$/L |
| MCV | 55.6 Fl | HDL-C | 20.6 mg/dl |
| MCH | 19.6 pg | C-Peptide | 6.97 ng/ml |
| MCHC | 36.3 g/dl | E2 | 46.88 pg/ml |
| Total bilirubin | 1.39 mg/dl | TSH | 6.8 $\mu$IU/ml |
| Indirect bilirubin | 0.98 mg/dl | Vit-D (25-OH) | 27 ng/ml |
| HDL | 20.6 mg/dl | | |
| UIBC | 250 $\mu$g/dl | | |
| Ferritin | 149 $\mu$g/dl | | |
| Iron | 73.3 $\mu$g/dl | | |

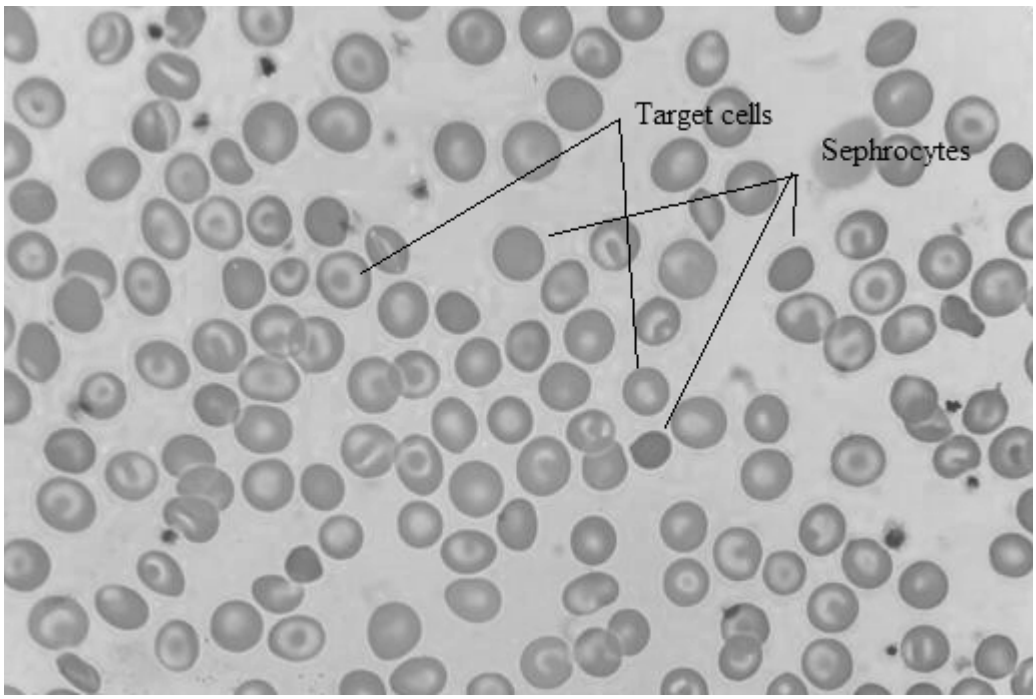



*Fig. 1:* Peripheral blood smear showing spherocytosis and Target cells.



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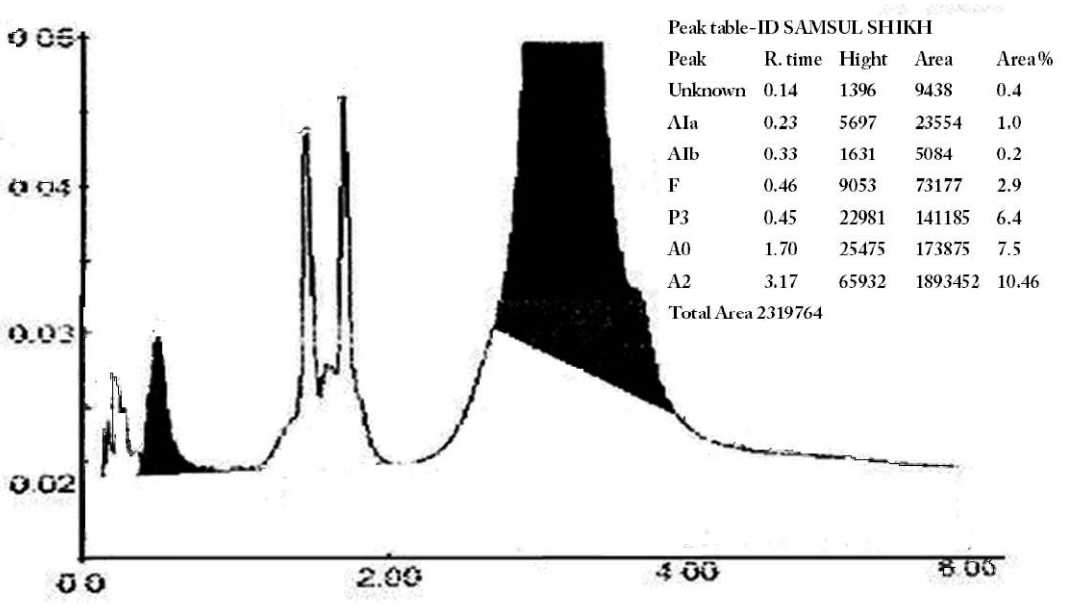



*Fig. 2:* HPLC separation of Hemoglobin. a) D-10

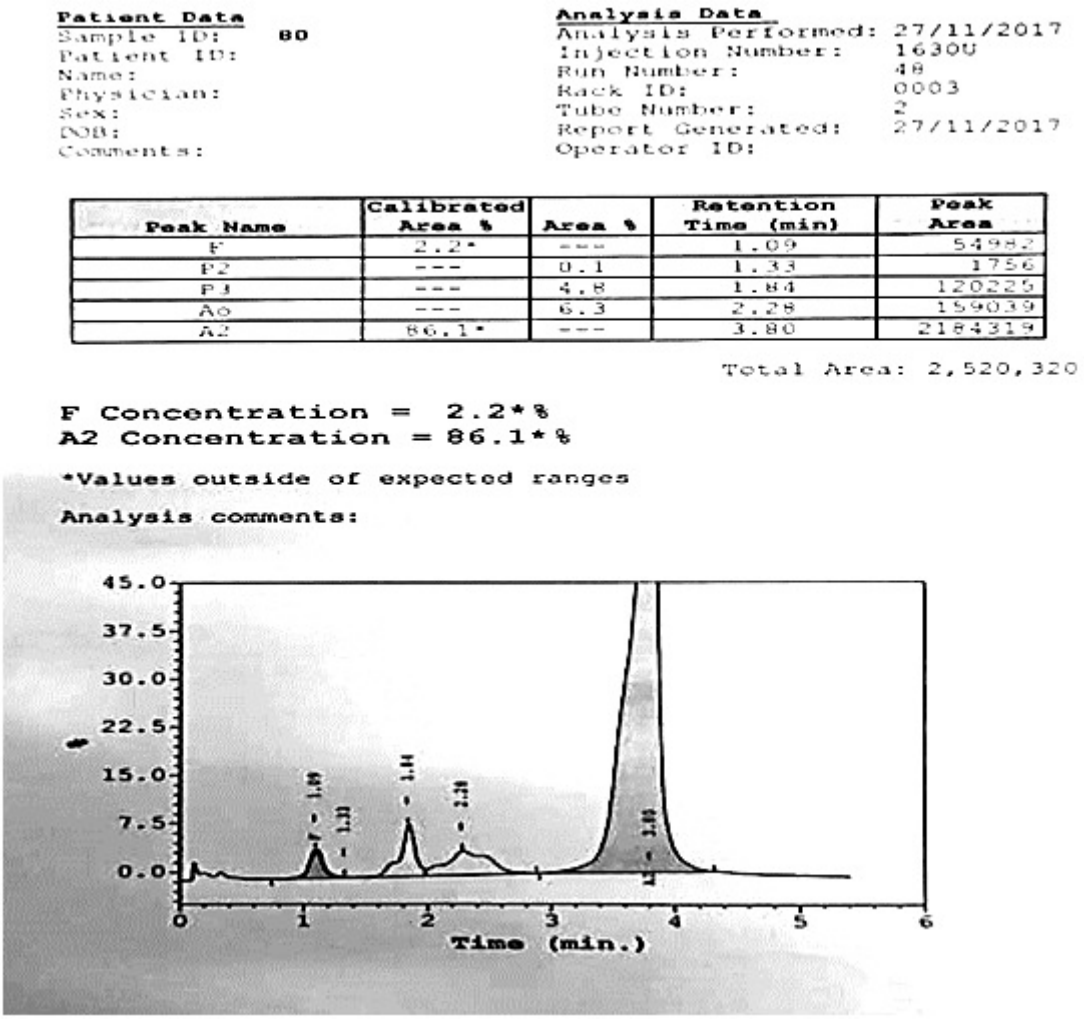



*Fig. 3:* HPLC separation of Hemoglobin. b) Variant II



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Stool examination was negative for parasites. Helicobacter pylori Antigen is stool and Abs in serum were negative by One Step H. pylori test device (ABON Biopharm, China). Malaria Ag in blood was negative by malaria P/F/Pan rapid test device from ABON, China. Clinical examination showed no abnormality.

## III. Discussion

The majority of hemoglobinopathy present in the western and eastern provinces of Saudi Arabia. Abuzenadah et al.[7,3] , 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.

Haemoglobinopathy is not common in Najran city. Prevalence reported by Memish et al., [12] of 14.7 % mostly sickle mutations of 14.1 % [8].

Considering his history, clinical findings and laboratory findings, the diagnosis in this case was homozygous Hb EE disease. The patient showed very mild, clinically asymptomatic, hemolytic microcytic hypochromic anemia with many target cells on peripheral blood smear characteristic of Hb EE disease which consistent with the classical presentation of the disease[9]. The presence of mild increase in Hb F indicates the mildness of the pathophysiology of the disease. The presence of a minute quantity of Hb A on HPLC was explained by post-translational modification of Hb E[13].

The patient gave a history of high fever due to malaria infection, with a hemolytic attack and he received a blood transfusion which could match the published of the instability of Hb E in high fever[14]. We noticed the presence of many spherocytes in the peripheral blood smear with an increase of MCHC. Similar findings occur in Hb C due an increase in the activity of K: $Cl^-$ cotransport that induces the loss of $K^+$ and subsequently of intracellular water[15]. The patient has low HDL-C which could not be explained. Also the slightly increased hormones of E2 and TSH, the slightly increased C-peptide could not be explained and recommended for further investigation.

## IV. Conclusion

Thus we report a case of abnormal hemoglobin (Hb E) masking the Hb A2 on HPLC. Knowledge of such a condition would help in prevention of misdiagnosis. Also we focused on some abnormal findings and recommended them for further research.

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