Available online at http://www.ijims.com
ISSN: 2348 – 0343

HbE Trait – A Curious Case Report

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Abstract

This is a curious case of identification of a haemoglobin variant in a patient who was investigated for Glycated haemoglobin (HbA1C). The patient was 8 year old boy from Tanjore,Tamil Nadu, India. This boy was having complaints of fever and myalgia. The boy was treated by a paediatrician who suspecting a infectious pathology requested for Creatine Phosphokinase (CPK) , HbA1C and a peripheral smear. When the sample was analysed for HbA1C by capillary electrophoresis it was found that there was an abnormal variant. Suspecting the credibility of the value the sample was reanalysed in ion exchange High performance liquid Chromatography (HPLC) and the values were in contrast. The sample was then analysed by haemoglobin electrophoresis to identify the variant and it was found that the boy had a variant in the Hemoglobin E (HbE) zone and he was diagnosed as a case of HbE trait (AE).

Keywords: haemoglobin variant, HbE trait,Tamil Nadu, India

Introduction

Hemoglobin E is an abnormal hemoglobin with a single point mutation in the β chain. At position 26 there is a change in the aminoacid from glutamine to lysine. Hemoglobin E is very common in Southeast Asia but has a low frequency in other races around the globe. HbE disease results when the child inherits HbE genes from both parents. At birth, babies homozygous for the hemoglobin E allele do not present symptoms due to HbF (fetal hemoglobin) they still have. In the first months of life, fetal hemoglobin disappears and the amount of hemoglobin E increases, so the subjects start to have a mild β-thalassemia. People who are heterozygote for hemoglobin E (one normal allele and one abnormal allele) do not show any symptoms (there is usually no anemia or hemolysis). Subjects homozygous for the hemoglobin E allele (two abnormal alleles) have a mild hemolytic anemia and mild enlargement of the spleen. Hemoglobin A1c (HbA1c) reflects the glycemic control in diabetic patients and is directly related to the risk of diabetic complications [(1)]. HbE can affect the immunoassays used for HbA1c measurement. Some current HbA1c methods show clinically significant interference with samples containing HbE [(2)]. From existing literature we also understand that there could be significant differences of HbA1c values between normal controls and hemoglobin E containing samples [(3)].

Case Report

Materials and Methods

A 8 year old boy hailing from Tanjore, Tamil Nadu presented to a paediatrician with complaints of fever and myalgia for the past few days. On examination his spleen was faintly palpable just below the left costal margin. There was no significant family history. There was no prior history of blood transfusion. Suspecting an infectious pathology the paediatrician requested for a peripheral smear , Creatine Kinase and HbA1C.

On analysing the sample the haematologist opined the peripheral smear as suggestive of bacterial infection with a neutrophilic preponderance. The Creatine Kinase was analyzed by CK-NAC (N Acetyl Cysteine) activated method in Beckman Coulter Unicel Synchron 660i and was found to be normal 64 IU/L (24-194 IU/L reference range for the kit used). However when analysing the sample for HbA1C by Sebia minicap Flex piercing capillary electrophoresis technique the HbA1C value obtained was 5.0%. Then the sample was analysed by Biorad D10 ion exchange HPLC to cross check the value of HbA1c. However the value obtained by Biorad D10 was in contrast to the one obtained from capillary electrophoresis which was 6.1%. So the sample was subjected to hemoglobin Electrophoresis by another sebia capillarys flex piercing minicap instrument for. The result was there was a variant

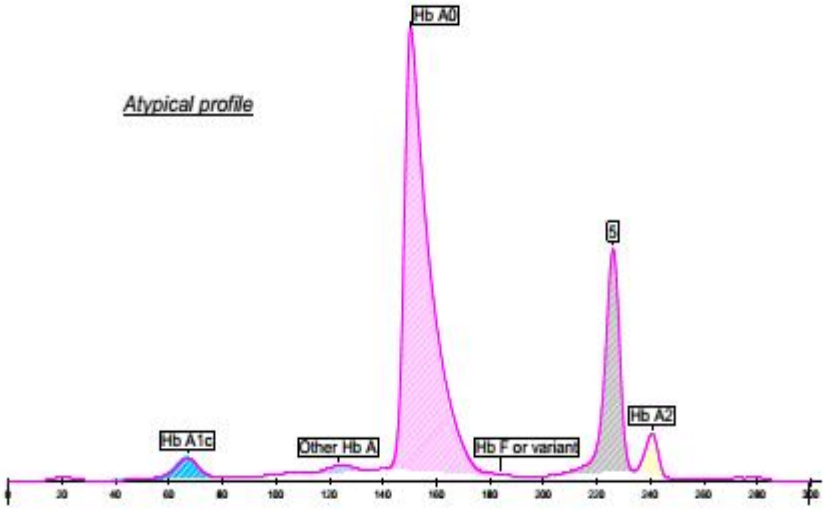
obtained in the HbE zone. Following this the pathologist was consulted again with the peripheral smear for any further findings but there was no evident target cells in the smear. And as the boy didn't exhibit any symptoms suggestive of hemoglobinopathies or has any history of blood transfusion HbE disease was ruled out and he was diagnosed as a case of heterozygote HbE(AE) or HbE trait.

Results

Figure 1

Sample num. **4** Date **3/24/2015** ID : **57822**

Depart : Birth :



A1c Haemoglobin Electrophoresis

Fractions	%	mmol/mol	Cal. %
Hb A1c (*)	-	**31**	5.0
Other Hb A	1.5		
Hb A0	71.6		
Hb F or variant	-		
5	20.4		
Hb A2 (!)	3.2		

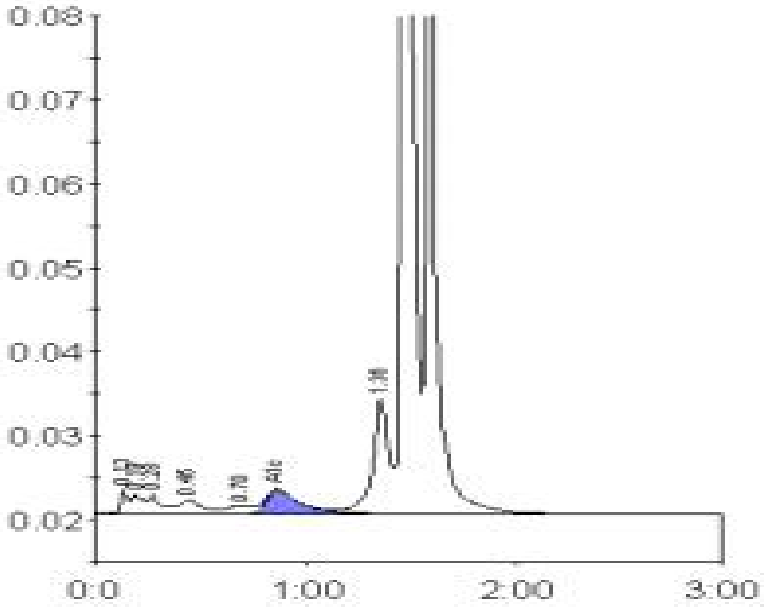
HbA1c (*) = 5.0 %

HbA1c (*) = 31 mmol/mol

(*) Atypical profile

Figure 2

D-10 TIME: 12:13 PM
S/N: #DC1B524417 Software version: 3.60
Sample ID: 57822
Injection date 25/03/2015 07:51 PM
Injection #: 11 Method: HbA1c
Rack #: --- Rack position: 1



Peak table - ID: 57822

Peak	R.time	Height	Area	Area %
Unknown	0.13	2920	6331	0.6
A1a	0.19	2220	9527	0.9
A1b	0.28	2203	8838	0.8
F	0.45	1608	11325	1.1
LA1c/CHb-1	0.70	926	7495	0.7
A1c	0.86	2795	29958	6.1
P3	1.36	13648	72298	6.7
A0	1.46	197983	594834	55.2
Variant-Window	1.58	154029	337519	31.3
Total Area:	1078125			

Concentration:	%
A1c	6.1

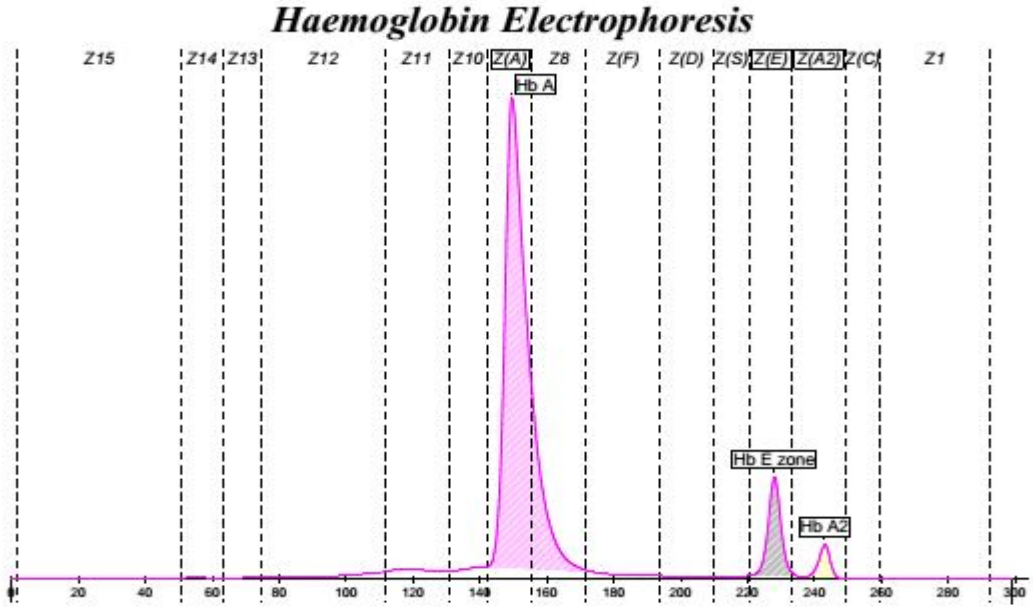
Figure 3

Age / Sex : 8 M

Received On : 23/03/2015

Ref. By : Dr.Lakshmanakumar.,

Reported On : 25/03/2015



Name	%	Normal Values %
Hb A	86.7	
Hb E zone	10.3	
Hb A2	3.0	

Comments

Method : Capillary Electrophoresis

Discussion

In order to standardize GHB/HbA1c results and to ensure that clinical laboratory results are comparable to those reported by the DCCT the NGSP (National Glycohemoglobin Standardization Program) was established. However, the presence of Hb variants affects the accuracy of several HbA1c methods [(4)] and the National Glycohemoglobin Standardization Program does not include

evaluation of interferences as part of the certification program. HbS, HbE, HbC, and HbD are the most common Hb variants worldwide. Most of the commonly used HbA1c methods have already been, and continue to be evaluated for HbS and HbC traits [5] .Limited data is available on the accuracy of HbA1c measurement in the presence of HbE traits.

HbE is the second most prevalent Hb variant worldwide and is found primarily in people from Southeast Asia; prevalence is 30%–40% in some parts of Thailand, Cambodia, and Laos [6]. HbE trait(AE) is a mild disease and homozygous HbE (EE) is a severe disorder . The resistance exhibited by RBCs of patients with HbE trait to invasion by Plasmodium falciparum is most likely the cause for its high prevalence throughout the world [7]. If the laboratory's HbA1c measuring method has interference with samples containing HbE the interpretation of glycemic control using HbA1c in the endemic areas for HbE should be carefully considered. As measuring HbA1c by high performance liquid chromatography in HbEE also has significant interference by HbE [8] a watchful lookout for Hb variants in diabetic patients and identification of the same has a great significance in endemic areas. According to a study by Randie et al [1] none of the immunoassay, enzymatic, or boronate affinity methods investigated showed clinically significant interference, whereas some of the ion-exchange HPLC methods showed interference from HbE or HbD traits.

Conclusion

Laboratories should be aware of the limitations of their methods with respect to these variants and should communicate this information to physicians. Physicians should consider the possibility of interference from an Hb variant if a patient's HbA1c result is significantly different from what is expected. Alternative parameters to evaluate long-term glycemic control in patients with Hb variant should also be created.

Acknowledgement

We greatly acknowledge the help and clinical details shared by paediatrician Dr Lakshmana Kumar from Tanjore, India.

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