HAEMOGLOBIN E HETEROZYGOUS WITH NEUTROPHILIA- IMPACT ON HbA1c BY HPLC

Ranjith Kumar Chikine[1]

[1]*Associate Professor, Department of Biochemistry, S.V.S Medical College, Yenugonda, Mahabubnagar, Telangana.*

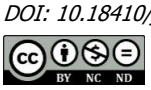
HOW TO CITE THIS ARTICLE: Chikine RK. Haemoglobin E heterozygous with neutrophilia- impact on HbA1c by HPLC. J. Evid. Based Med. Healthc. 2018; 5(15), 1345-1347. DOI: 10.18410/jebmh/2018/279

PRESENTATION OF THE CASE

70 Years female resident of Jadcherla, Mahabubnagar district attended the emergency department of SVS Medical College with complaints of fever since 8 days, 5 episodes of vomiting and epigastric pain since 1 day. She was diagnosed diabetes mellitus type II, 8 years back. She has been using oral hypoglycaemics (Glycomet GP 1) since 3 years. Blood samples were received for routine blood investigations, fasting plasma glucose postprandial plasma glucose were analysed in Beckman coulter AU 480 by hexokinase method, values obtained as 224 mg/dl & 302 mg/dl respectively. For HbA1c, whole blood (K2 EDTA tube) processed in HPLC based Biorad D-10 analyser and found HbA1c value as 14.1% with a variant window value 24.9%. We analysed the same sample for HbA1c in boric acid affinity method and reported as 11.2% and advised for fructosamine. We processed the fresh sample in the Bio-Rad D10 for haemoglobin electrophoresis and found Hb A2 as 20.9%, Foetal Hb F = <0.8%, Hb A0 = 56.3%, P3 = 10.4%. Serum Iron studies as follows Iron = 15 µg/dl, TIBC = 202 µg/dl, UIBC = 182 µg/dl, Ferritin = 290 ng/ml. Complete blood picture (Sysmax XS- 800i) findings as follows Hb = 9gm/dl; MCV = 64.3 fl (N:80-100 fl); MCH = 21.6 pg (N:27 – 32 pg); RDW – SD = 35.6fL; RDW – CV= 15.4%, Neutrophils = 80%, Lymphocytes 15%, Eosinophils 2%,Monocytes = 3%, White blood cells 13,500; platelets 4.3 lacks/ cu.mm and peripheral smear findings as follows microcytic hypochromic anaemia with neutrophilic leucocytosis. Liver function tests as follows total bilirubin = 0.4 mg/dl, direct bilirubin = 0.1 mg/dl, alkaline phosphatase = 80 U/L, alanine transaminase = 20 U/L, total proteins = 6.7 gm/dl, albumin = 3.0 gm/dl, globulin = 3.7 gm/dl.

Serum electrolytes as follows (ISE method) sodium = 129 meq/l, potassium = 5.6 meq/l, chlorides = 101 meq/l. Next day repeated the serum electrolytes, results retained as follows sodium = 133 meq/l, potassium = 5.2 meq/l, chlorides = 102 meq/l. Potassium obtained on higher side of normal range or higher than the normal as of pseudohyperkalaemia.

Financial or Other, Competing Interest: None.
Submission 19-03-2018, Peer Review 22-03-2018,
Acceptance 07-04-2018, Published 09-04-2018.
Corresponding Author:
Dr. Ranjith Kumar Chikine,
Associate Professor, Department of Biochemistry,
S.V.S Medical College, Yenugonda,
Mahabubnagar, Telangana-509002.
E-mail: write2drranjith@gmail.com
DOI: 10.18410/jebmh/2018/279



CLINICAL DIAGNOSIS

Based on laboratory findings concluded as it is a case of Haemoglobin E heterozygous with neutrophilia, because the most of the hemoglobinopathies are asymptomatic.

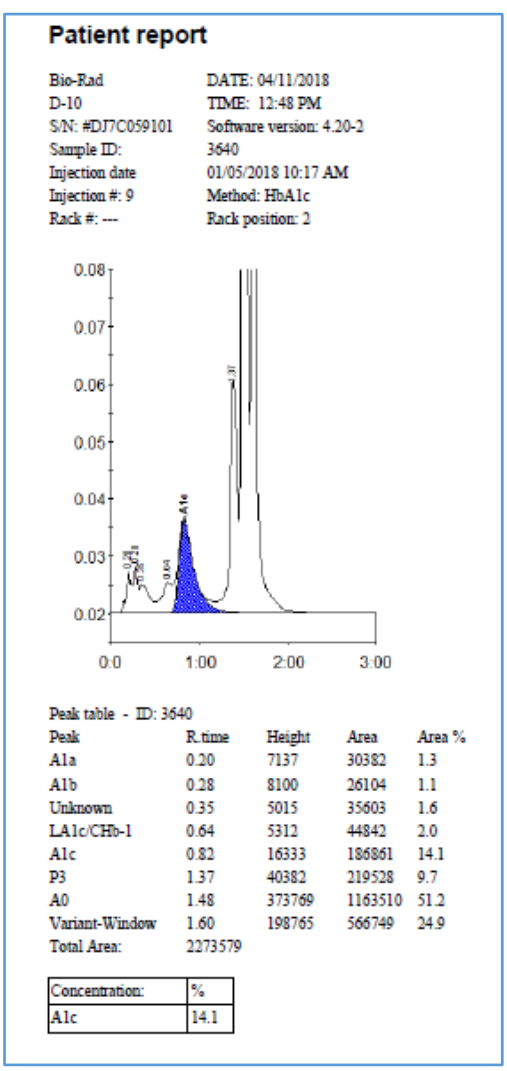
PATHOLOGICAL DISCUSSION

The prevalence of diabetes mellitus (DM) is increasing rapidly worldwide. The World Health
Organization estimated that more than 366 million people will suffer from DM by the year 2030.[1] This increasing trend of DM prevalence emphasizes the need to rely on glycated haemoglobin (HbA1c) for managing glycemic control and long-term monitoring. Large prospective research trials in patients with type I[2] and type II diabetes[3] have established the proportional relationship between A1c levels and risk of diabetes complications, such as retinopathy, neuropathy, and nephropathy. Recently the American Diabetes Association recommended the HbA1c for DM diagnosis.[4] High performance liquid chromatography (HPLC) is a reference method for estimating HbA1c. The principle behind the HPLC is that, the positively charged different Hb fractions were separated based on their ionic interactions with a negatively charged stationary phase in a chromatography column followed by their elution by a mobile phase with phosphate buffers differing in pH and ionic strength. The adsorbed positively charged Hb molecules were eluted from the column into the liquid phase at a rate related to their affinity for the stationary phase. Hbs were identified by their retention time and quantified by computing the area under the corresponding peak in the elution profile.[5] However, there are some clinical situations that make the accurate measurement of HbA1c difficult by HPLC. These conditions include hemoglobinopathies such as thalassaemias and structural Haemoglobin (Hb) variants such as HbE, as well as iron-deficiency anaemia and the use of certain drugs especially ART (antiretroviral) drugs. Even though in India all studies related to this hemoglobinopathies confined to one or more cities or regions, they drawn good output. There is a variation in the prevalence of hemoglobinopathies in different regions and population groups of India. A high frequency of Hb D has been reported in the Punjabi population, Hb E in the eastern region of India and Hb S is mainly reported from populations of tribal origin from different parts of the country.[6] The prevalence of Hb disorders in West Bengal is 12.17%,[7] and the prevalence of thalassemia and hemoglobinopathy of northern India is reported 12.5%.[8] HbE trait prevalence is 3.02%, β thalassemia major/intermedia is 1.66%, and Eβ thalassemia is 1.16 %. Including these found out other variants like HbE disease, sickle-cell disease, sickle β

thalassemia, HbD-Punjab trait, HbQ-India trait, α-thal trait, double heterozygous state of HbS and HbE, double heterozygous state of HbS and HbD, HbJ-Meerut, hereditary persistence of foetal haemoglobin (HPFH), HbH, delta β-thal trait, and HbLepore at smaller numbers.[7]

When come to double heterozygous types, HbE beta thalassemia is the most common type with prevalence 1.16% in the world. In worldwide nearly 50% of severe beta thalassemia major patients are of HbE beta thalassemia type,[9] and among Indians at incidence of 1.44% in the general population.[10]

However, with the migration of the population across the country often for purposes of employment, there is an influx of the tribal population into the surrounding cities or into the larger cities including population movements from East to West, North or South, or vice-versa. With the intermixing of populations, there is likely to be the presence of unexpected haemoglobins in the populations under investigation. It is important that they are looked for when evaluating HbA1c level by a HPLC. But most of the laboratories failed to read the chromatogram while reporting and reporting incorrect values. This type of laboratory behavior misleading the physicians while treating the diabetes and its complications.



Patient report

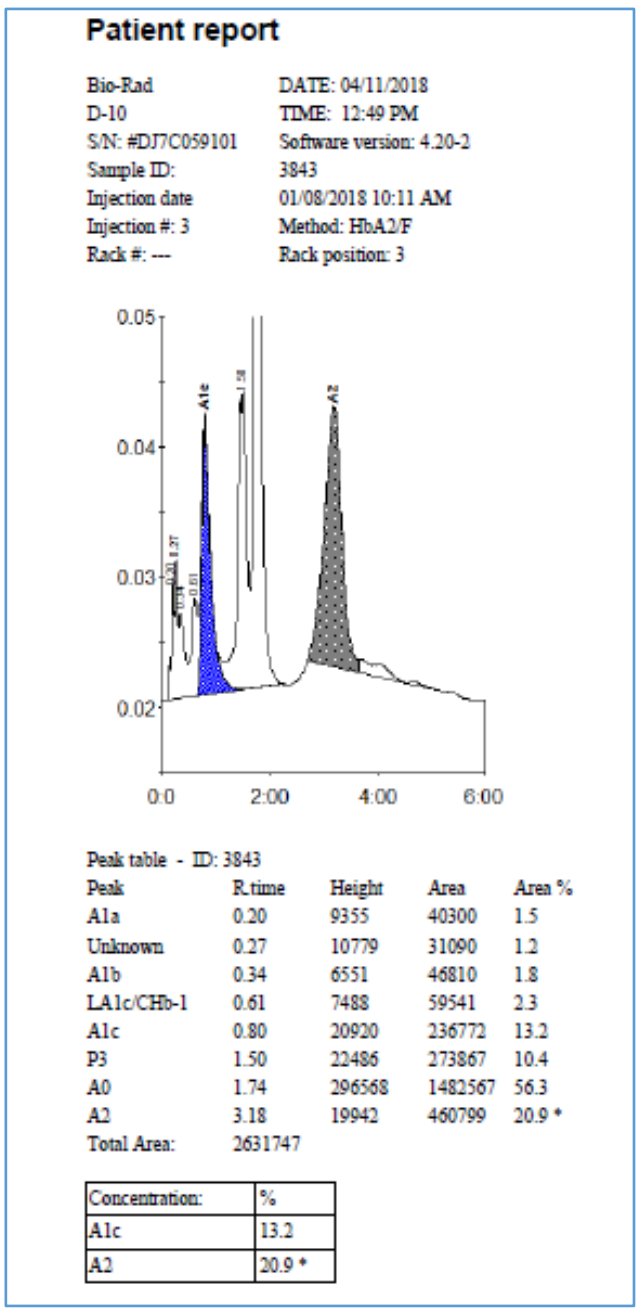
Bio-Rad	DATE: 04/11/2018
D-10	TIME: 12:48 PM
S/N: #DJ7C059101	Software version: 4.20-2
Sample ID:	3640
Injection date	01/05/2018 10:17 AM
Injection #: 9	Method: HbA1c
Rack #: ---	Rack position: 2

Peak table - ID: 3640

Peak	R.time	Height	Area	Area %
A1a	0.20	7137	30382	1.3
A1b	0.28	8100	26104	1.1
Unknown	0.35	5015	35603	1.6
LA1c/CHb-1	0.64	5312	44842	2.0
A1c	0.82	16333	186861	14.1
P3	1.37	40382	219528	9.7
A0	1.48	373769	1163510	51.2
Variant-Window	1.60	198765	566749	24.9
Total Area:		2273579		

Concentration:	%
A1c	14.1

Figure 1. HbA1c Value of Patient in HPLC Method



Patient report

Bio-Rad	DATE: 04/11/2018
D-10	TIME: 12:49 PM
S/N: #DJ7C059101	Software version: 4.20-2
Sample ID:	3843
Injection date	01/08/2018 10:11 AM
Injection #: 3	Method: HbA2/F
Rack #: ---	Rack position: 3

Peak table - ID: 3843

Peak	R.time	Height	Area	Area %
A1a	0.20	9355	40300	1.5
Unknown	0.27	10779	31090	1.2
A1b	0.34	6551	46810	1.8
LA1c/CHb-1	0.61	7488	59541	2.3
A1c	0.80	20920	236772	13.2
P3	1.50	22486	273867	10.4
A0	1.74	296568	1482567	56.3
A2	3.18	19942	460799	20.9 *
Total Area:		2631747		

Concentration:	%
A1c	13.2
A2	20.9 *

Figure 2. Haemoglobin Electrophoresis by HPLC Method

DISCUSSION OF MANAGEMENT

Here we report a rare case of heterozygous hemoglobinopathy of Hb E (haemoglobin Iran) with neutrophilia. Haemoglobin E (HbE) is an abnormal haemoglobin with a single point mutation in the β chain of globulin. Hb E is caused by a substitution of glutamic acid by lysine at codon 26 of the β-globin gene. Mutation in β-globin gene that creates an alternate splice site which leads to decreased production of an abnormal globin chain. Haemoglobin E is very common among people of Southeast Asian, Northeast Indian, Sri Lankan and Bangladeshi descent (internet source). Heterozygotes (HbE trait) and homozygotes (HbE disease) are asymptomatic. The MCV is reduced and target cells are seen on peripheral blood smear. Mild anaemia is seen with HbE disease and less commonly

with HbE trait. Important to distinguish HbE disease from HbE/β-thalassemia as the latter is clinically significant.

Hemoglobinopathies are known to affect HPLC measurement of HbA1c[11,12] by at least 2 possible methods. One is the presence of an abnormal peak on chromatography, making the estimation of the fraction of HbA1c unreliable. Second, some abnormal forms of Hb (e.g., β-thalassemia and sickle cell trait) make red blood cells more susceptible to haemolysis. Increased haemolysis corresponds with decreased red cell lifespan. This decreases the time available for glycosylation of Hb. In subjects with a hemoglobinopathy, choose of alternative methods for HbA1c like enzymatic, boric acid affinity method, turbidimetric method or use of fructosamine to monitor diabetes may be more reliable. If discrepant results are found on an HbA1c assay (either higher or lower than expected), hemoglobinopathy should be considered as a possible cause.

Elevated immature platelets and leucocytes cell walls have labile cell membrane, they are easy to fragile on centrifuge when compare to the normal cells, and releases the intracellular K^+ in to serum. Haemoglobin E heterozygous presented with neutrophilia is rare.

CONCLUSION

Long term glycemic control indicator, HbA1c estimation by HPLC method is not a suitable investigation to diabetes mellitus with asymptomatic hemoglobinopathies patients but it acts as screening test. There is no chance to make differential diagnosis even after haemoglobin electrophoresis, but genetic testing has the importance. Once diagnosis was made, patients should be advised to get haemoglobin electrophoresis of patient's parents and siblings. Pre-marriage haemoglobin electrophoresis of couples may decrease the prevalence of the abnormal hemoglobinopathies.

REFERENCES

[1] Wild S, Roglic G, Green A, et al. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. Diabetes Care 2004;27(5):1047-1053.

[2] The DCCT Research Group: The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. N Engl J Med 1993;329:977-986.

[3] Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). UK Prospective Diabetes Study (UKPDS) Group. Lancet 1998;352(9131):837-853.

[4] American Diabetes Association. Standards of medical care in diabetes--2011. Diabetes Care 2011;34 Suppl 1:S11-S61.

[5] Bain BJ. Hemoglobinopathy diagnosis. 2nd edn. Massachusetts USA: Blackwell Publishing 2006:26-62.

[6] Sukumaran PK, Master HR. The distribution of abnormal haemoglobins in the Indian population. In: Proceedings of the First Conference of the Indian Society of Human Genetics. Human population genetics in India. Mumbai: Brient Longman 1973:91-111.

[7] Mondal SK, Mandal S. Prevalence of thalassemia and hemoglobinopathy in eastern India: A 10-year high-performance liquid chromatography study of 119,336 cases. Asian J Transfus Sci 2016;10(1):105-110.

[8] Sachdev R, Dam AR, Tyagi G. Detection of Hb variants and hemoglobinopathies in Indian population using HPLC: report of 2600 cases. Indian J Pathol Microbiol 2010;53(1):57-62.

[9] Olivieri NF, Pakbaz Z, Vichinsky E. Hb E/beta-thalassaemia: a common and clinically diverse disorder. Indian J Med Res 2011;134(4):522-531.

[10] Mohanty D, Colah RB, Gorakshakar AC, et al. Prevalence of β-thalassemia and other haemoglobinopathies in six cities in India: a multicentre study. J Community Genet 2013;4(1):33-42.

[11] Gunton JE, McElduff A. Heterozygous hemoglobin Hamadan affects HbA1c assay. Diabetes Care 1999;22(1):177.

[12] Wolfsdorf JI, Anderson BJ, Pasquarello C. Treatment of the Child with Diabetes. In: Kahn CR, Weir GC, eds. Joslin's diabetes mellitus. 13th edn. Philadelphia: Lea and Febiger 1994:530-551.