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Significance of Elevated LA1c/c Hb1peak in a HbA1c chromatogram: A delimma

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Abstract:

Background and objective: In HbA1c estimation by HPLC method Labile A1c fraction is formed which is unstable and its concentration varies with acute changes in plasma glucose levels. Elevated peak of LA1c/Chb-1 may be due to variation in plasma glucose levels or elution of Hbf in labile A1c fraction indicating hemoglobin disorder.

Methods: HbA1c is estimated in our laboratory by HPLC method on BIORAD D-10 system which is an automated cation exchange HPLC instrument.

Results: Our study focused on a patient's abnormal chromatogram showing abnormal high peak of LA1c/cHb-1 along with missed Hbf in HbA1c estimation and after eliminating preanalytical error patient's report was released with comment on possibility of hemoglobionopathy.

Conclusion: Advantage of HbA1c estimation with HPLC method is to identify hemoglobinopathy can only be achieved by properly analyzing the chromatogram of each and every patient by expert technician or doctor available.

Keywords: Labile A1c, Pre HbA1c, Fetal hemoglobin, HPLC, Glycated hemoglobin, Diabetes mellitus

Introduction:

HbA1c is used as a gold standard method for the assessment of long term glycemic control in patients of Diabetes mellitus. Poor control of diabetes may cause multiple pathological conditions as nephropathy, retinopathy, neuropathy, cardiovascular disease and kidney damage or failure. It is reported that at HbA1c<7%, there is a 76% reduction in the incidence of diabetic retinopathy, 54% of diabetic nephropathy, 60% of peripheral neuropathy and 35% of cardio vascular disease risk.[1]

Glycosylation of Hb is achieved by glucose entering the erythrocyte through the GLUT 1 transporter and its connection to the amino acid valine which is located at the N-terminal of the beta chain of Hb. An initial reversible reaction results information of the aldehyde Schiff base, followed by irreversible Amadorie arrangement to the stable ketoamine.[2] The Schiff base which is formed as an intermediate of non-enzymatic glycation is unstable and known as Labile A1c or pre HbA1c.

HbA1c is not affected by plasma glucose at the time of measurement, where as Labile A1c is affected by plasma glucose at the time of measurement because the reaction from protein to aldimine is reversible which may interfere with accurate detection of HbA1c. The influence of increasing Labile A1c on HbA1c results showed that increased Labile A1c peak lead to a decrease in HbA1c values.[3]Abnormal HbA1c values are observed in various hematologic diseases. Along with this known reason the other causes of increased peak of LabileA1c in chromatogram may be due to some variant also which emphasizes the need to check every chromatogram before releasing the patient report.

Material & methods:

A retrospective analysis of data of HbA1c chromatogram for raised labile A1C peak performed by department of laboratory medicine. 600 samples were received and processed from 1February 2024 to 20 july 2024 and were analysed for glycosylated hemoglobin. 3 ml blood sample was collected in EDTA tube as per CLSI guidelines and analysis performed within 3 hrs. An informed consent was taken from patients. HbA1c was performed on BIORAD D-10(Bio-Rad laboratories,USA) dual HbA2/F/A1c program which is an automated cation-exchange HPLC instrument for screening of HbA1c &inherited hemoglobin disorders which are diagnosed by interpretation of the chromatogram for percentage and retention time.[4] The order of elution of various components on the D-10 analyzer is HbA1a, HbA1b, HbF, LA1c/CHb-1, LA1c/CHb-2, HbA1c, P3,HbA and HbA2.The minor hemoglobin fractions A1a, A1b, A1c, F1, P3 components are post translational modification of the globin chains.[5]

The chromatograms of 600 samples were screened for LA1c/CHb-2 peak.

Results:

Out of 600 samples processed, two samples with abnormal level of Labile A1c/CHb-1 were found during HbA1c analysis by HPLC method. The chromatogram for a first patient with no history of diabetes showed Labile A1c peak with a total area of 28.6 % , no HbF peak and HbA1c with an area of 4.5% on date 18/05/2024. (Figure 1) Finding so high value of labile A1C patient was advised to give another sample to check any possibility of pre -analytical error. On 21/05/2024 repeat sample was given by patient who again showed the same chromatogram of labile A1C of

28.9%, no HbF peakand HbA1c of 4.4% area which eliminated the chances of pre-analytical error. (Figure 2) In the Second sample on 05/07/2024 Labile HbA1c peak was 28.8%, no HbF peak and HbA1c peak was 4.5 %.(Figure 3) Patient history was checked and fasting blood sugar and hemogram reports were also analyzed.

Both the patients were non diabetic and fasting blood glucose were 89.3mg/dL & 93.6mg/dL respectively. Hemogram report of both was within normal limit. When history was taken both patients were related to each other as daughter & mother.

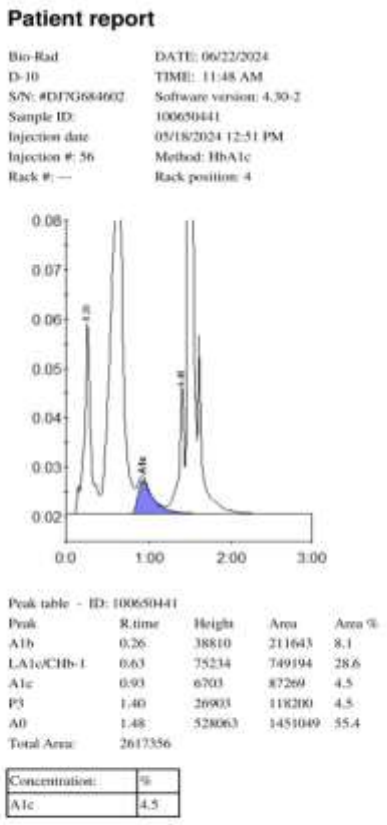
Discussion:

Labile A1c may interfere in HbA1c estimation due to some preanalytical error to detect low value of HbA1c and may cause delay in treatment required if patient is diabetic.[6] In our study we rule out preanalytical error by processing the first sample twice with fresh sample received on different date. The concentration of labile fraction varies with acute changes in the plasma glucose levels but fasting blood sugar levels of both the patients were normal and CBC parameters also showed normal red blood indices with no significant anisopoikilocytosis.

An abnormal HbA1c result is the first indication of an underlying hemoglobinopathy because hemoglobin variants often interfere with the quantification of HbA1c.[7] The chromatogram needs to be screened either for any unknown peak present or the raised concentration of eluted peaks i.e. HbA1a, HbA1b, HbF, LA1c/CHb-1, LA1c/CHb-2, HbA1c, P3, HbA and HbA2.

. Reason of high Labile A1c in both the casesmay be due to the presence

of variant hemoglobin. It has been studied that if value of Labile A1c is higher than 16.5%, HbF may elute in the LA1c/CHb or A1c window and no HbF is reported.[8] In this patient high value of labile A1c (28%) eliminates the chances of pre analytical error and fluctuation of blood sugar levels and shows the possibility of alpha beta thalassemia trait in which HbF has been eluted with LA1c/CHb peak is elevated from 5-20%. Sample also run in HbA2 mode and A2 values were also reduced to 1.9% area (Figure 4), however which can only be confirmed by genetic testing and further investigation which shows the importance of checking the chromatogram of each patient before reporting to not miss such events.



Patient report

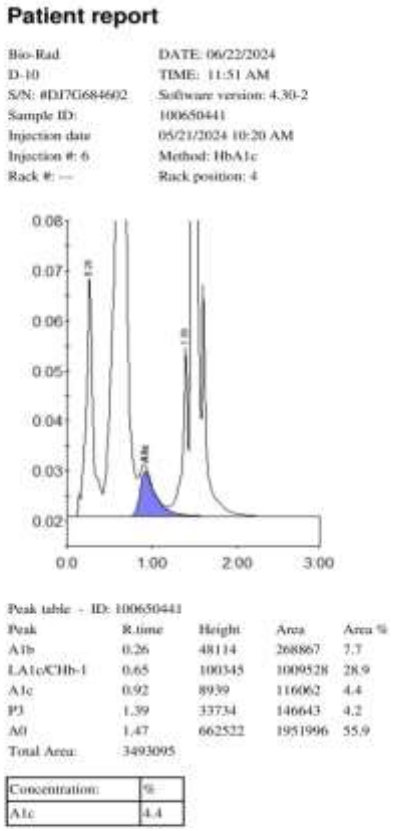
Bio-Rad	DATE: 06/22/2024
D-10	TIME: 11:48 AM
S/N: #DI7G684602	Software version: 4.30-2
Sample ID:	100650441
Injection date	05/18/2024 12:51 PM
Injection #: 56	Method: HbA1c
Rack #: —	Rack position: 4

Peak table - ID: 100650441

Peak	R.time	Height	Area	Area %
A1b	0.26	38810	211643	8.1
LA1c/CHb-1	0.63	75234	749194	28.6
A1c	0.93	6703	87269	4.5
P3	1.40	26903	118200	4.5
A0	1.48	528063	1451049	55.4
Total Area:		2617356		

Concentration:	%
A1c	4.5

Figure 1. Patient's chromatogram on date 18/05/2024



Patient report

Bio-Rad
D-10
S/N: #DI7G684602
Sample ID:
Injection date
Injection #: 6
Rack #: ---

DATE: 06/22/2024
TIME: 11:51 AM
Software version: 4.30-2
100650441
05/21/2024 10:20 AM
Method: HbA1c
Rack position: 4

Peak table - ID: 100650441

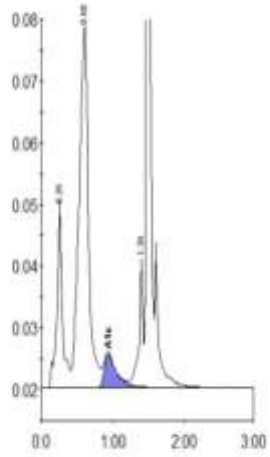
Peak	R.time	Height	Area	Area %
A1b	0.26	48114	268867	7.7
LA1c/CHb-1	0.65	100345	1009528	28.9
A1c	0.92	8939	116062	4.4
P3	1.39	33734	146643	4.2
A0	1.47	662522	1951996	55.9
Total Area:		3493095		

Concentration:	%
A1c	4.4

Figure 2. Patient's chromatogram on date 21/05/2024

Patient report

Bio-Rad	DATE: 07/15/2024
D-10	TIME: 01:36 PM
S/N: #D07G684602	Software version: 4.30-2
Sample ID:	100675367
Injection date	07/05/2024 01:48 PM
Injection #: 54	Method: HbA1c
Rack #: —	Rack position: 1



Peak table - ID: 100675367

Peak	R.time	Height	Area	Area %
A1b	0.26	30606	156131	8.4
LA1c/CHb-1	0.60	58521	537649	28.8
A1c	0.94	5300	63169	4.5
P3	1.39	21202	88934	4.8
A0	1.47	391748	1021762	54.7
Total Area:		1867645		

Concentration:	%
A1c	4.5

Figure 3. Patient's mother chromatogram on date: 05/07/2024

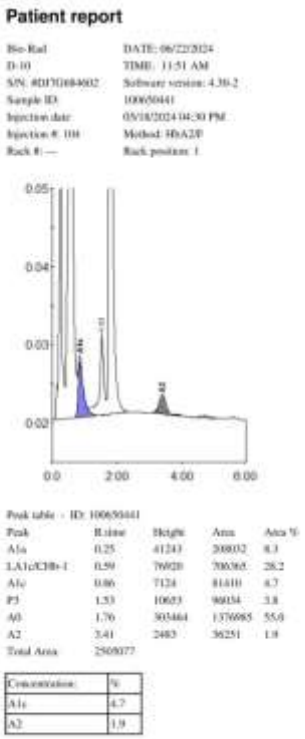


Figure 4. Patient's chromatogram on HbA2/F mode

Conclusion:

Over the years the quality of method to detect HbA1c has been improved and most of the analytical methods are interference free, however advantage of HPLC to detect HBA1c to find hemoglobinopathies make it the best method among all only if chromatogram is analyzed properly which can be done carefully looking after missed or elevated peaks and variant windows. More training of the users (Technicians and pathologists) regarding the peaks generated and possibility of co-elution of different hemoglobin is required to avoid misinterpretation. Further investigations also should be performed to rule out before making a therapeutic decision.

Declarations:

Ethics approval and consent to participate: Not applicable

Consent for publication: Not applicable

Availability of data and materials: Available in laboratories LIS system.

Competing interests: The authors declare that they have no competing interests

Funding: Not required

Authors contributions: Dr Amita k gadhok analysed the HbA1c chromatogram and history and major contribution in writing the manuscript and Dr Poonam sahni analysed the chromatogram in HbA2 mode to analyze the possible thalaseemia. All the authors read and approved the final manuscript.Dr Parul Singla and Dr Naveen Singh contributed in writing the manuscript, review of literature.

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References:

1) Nathan DM. The diabetes control and complications trial/epidemiology of diabetes interventions and complications study at 30 years: overview. Diabetes Care 2014;37:9–16.

2) Bunn HF, Haney DN, Kamin S, Gabbay KH, Gallop PM. The biosynthesis of human hemoglobin A1c: Slow glycosylation of hemoglobin invivo. J Clin Invest. 1976;57:16529.

3) Labile glycated haemoglobin and carbamylated haemoglobin are still

critical points for HbA1c measurement. Biochemia Medica 2017;27(2):378–86.

4) Joutovsky A, Hadzi-Nesic J, Nardi MA. HPLC retention time as a diagnostic tool for hemoglobin variants and hemoglobinopathies: a study of 60000 samples in a clinical diagnostic laboratory. Clin Chem. 2004;50:1736–1747.doi:10.1373/clinchem.2004.034991.

5) Chandrashekar V, Soni M. Hemoglobin Disorders in South India. ISRN Hematology 2011;1-6.

6) Goyal R, Sardana V. Labile Hemoglobin - A Biochemical Entity. J Clin Exp Invest. 2020;11(1):em00732.

7) Shrestha A, Pant V, Gautam K, Pyakurel D, Pradhan S. Detection of Hemoglobinopathies by HPLC in a referral Clinical Laboratory in Nepal. Nepalese Medical Journal 2020;3:306-8.

8) Hemoglobin A1C/A2/F. Bio-Rad D10 dual program. UCSF Clin Labs Chemistry. Cited 6 Apr 2018.