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BRIEF REPORT

Labile hemoglobin A_{1c}: A factor affecting the estimation of glycated hemoglobin

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ABSTRACT

Labile hemoglobin A1c (also known as pre-HbA1c or LA1c or pre-glycohemoglobin) an unstable form, is a Schiff base formed during non-enzymatic glycation of hemoglobin. The concentration of labile fraction varies with acute change in plasma glucose level. Labile hemoglobin A1c fraction cannot be separated from the stable ketoamine fraction by most of the methods available for estimation of glycated hemoglobin (HbA1c). Though ion-exchange HPLC (high pressure liquid chromatography) method can separate the labile HbA1c fraction, but in some situation abnormally high level of this fraction may serve as a pre-analytical error interfering in estimation of glycated hemoglobin. By focusing on the hemoglobin chromatogram, reporting of this pre-analytical error can be minimized in HPLC method.

Key words: Diabetes, Glycated hemoglobin, HPLC, Labile hemoglobin

INTRODUCTION

Glycated hemoglobin (GHb or HbA_{1c}) is formed by non-enzymatic attachment of glucose to the N-terminal valine residue of the β-chain of adult hemoglobin (HbA). An initial reversible reaction result in formation of the aldehyde Schiff base, followed by the irreversible Amadori rearrangement to the stable ketoamine [1]. The Schiff base formed as an intermediate of non-enzymatic glycation, is unstable known as labile hemoglobin A_{1c} (also termed as pre-HbA_{1c} or LA_{1c} or pre-glycohemoglobin) [2]. The concentration of labile fraction varies with acute change in plasma glucose level [3]. The labile hemoglobin A_{1c} cannot be separated from the stable ketoamine fraction by most of the available methods which may result in pre-analytical error during for estimation of glycated hemoglobin, though it is uncommon.

Glycated hemoglobin a prognostic marker for diabetes mellitus, used as a gold standard for the assessment of long term glycemic control (2-3 months) and also predicts the risk of microvascular and macrovascular complications in diabetic patients [4]. Three basic methods are used for estimation of HbA_{1c} based on difference in ionic charges, structural characteristics and chemical reactivity[5]. The methods based on differences in ionic charge are commonly used in clinical practice. Although the ion-exchange method does not achieve the standards for accuracy, still it plays an important prognostic role in monitoring average plasma glucose level of diabetic patient [5]. Recently, electronspray ionization mass spectrometry (ESI-MS) have been used as the reference method for HbA_{1c} estimation [6]. As the method is not cost effective and needs sophisticated technologies ESI-MS does not play role in routine laboratories.

Different laboratories use different methods for measuring HbA_{1c} which are either not affected by labile hemoglobin A_{1c} fraction (immunoassay, affinity chromatography) or separate it during the analysis (high pressure liquid chromatography) [7]. Though the HPLC method can separate the labile hemoglobin A_{1c} fraction, in some conditions (blood transfusion) it may result in misinterpretation of HbA_{1c} values [7-8]. The

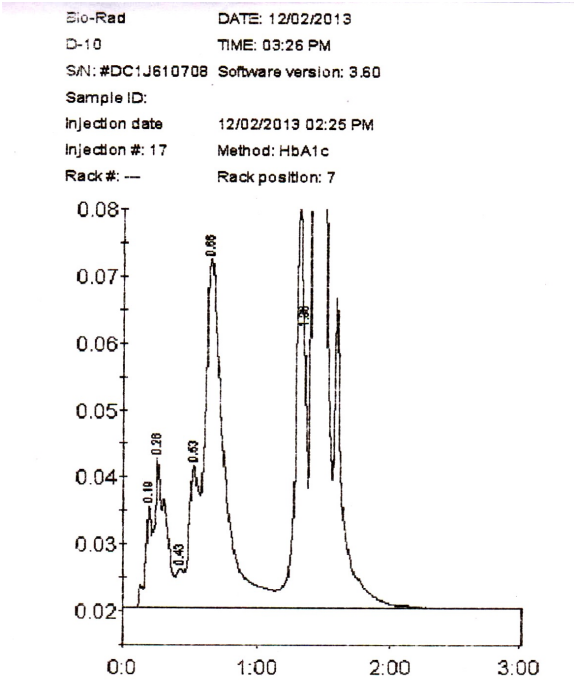
percentage of labile hemoglobin A_{1c} should be taken into account while using HPLC technique in the laboratories for HbA_{1c} estimation.

MATERIALS AND METHODS

Over a period of one year, we processed approximately 3000 samples for HbA_{1c} in the Department of Biochemistry, King George's Medical University, Lucknow Uttar Pradesh India. 3ml blood sample was collected in vacutainer containing ethylenediaminetetraacetic acid (EDTA) anticoagulant and HbA_{1c} was analyzed on the same day. HbA_{1c} levels were estimated by using HPLC technique (Bio-Rad D10) after processing internal quality control.

RESULTS

Out of total samples processed, two samples with abnormal level of labile hemoglobin A_{1c} fraction were encountered during the analysis of HbA_{1c} by using HPLC method. The values for HbA_{1c} given by the analyzer were zero in both the samples. Both the patients were known cases of diabetes mellitus type 2 and were requested for HbA_{1c} analysis from outpatient department (OPD). Next day fresh samples were collected and processed with same method, again the values of HbA_{1c} were zero with labile hemoglobin A_{1c} fraction 12.6% and 10.0%. The chromatogram with abnormal peak of labile hemoglobin A_{1c} fraction and a normal chromatogram are shown in Figure 1 and 2.

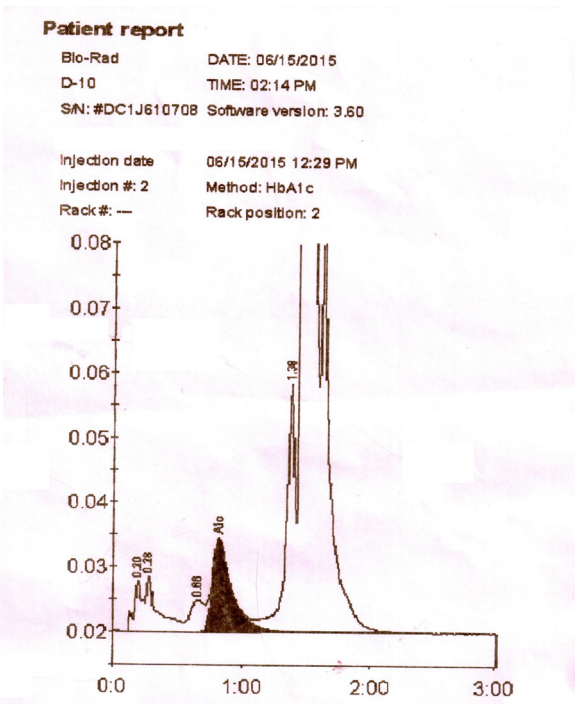


Peak table - ID: BHAGWATI

Peak	R.time	Height	Area	Area %
A1a	0.19	15400	46024	1.1
A1b	0.26	22420	129580	3.2
Unknown	0.43	5735	21550	0.5
F	0.53	21198	107461	2.6
LA1c/CHb-1	0.66	52330	515898	12.6
P3	1.36	60058	298609	7.3
A0	1.43	809572	2982010	72.7
Total Area:		4101133		

Concentration:	%
---	0.0

Figure 1. A normal chromatogram



Peak table

Peak	R.time	Height	Area	Area %
A1a	0.20	7857	34115	0.9
A1b	0.28	8569	53299	1.4
LA1c/CHb-1	0.66	4831	38443	1.0
A1c	0.82	14081	145184	5.5
P3	1.36	38468	181296	4.9
A0	1.47	756674	3284037	87.9
Total Area:		3736374		

Concentration:	%
A1c	5.5

Figure 2. The chromatogram with abnormal peak of labile hemoglobin A_{1c} fraction

DISCUSSION

We studied the chromatogram in detail and compared with normal chromatogram as shown in figure 1 and 2. Baseline in the graph was well constructed. Area count (1 million to 5 million) and P3 peak (<8%) were within acceptable range. Retention time of HbA_{1c} and HbA_0 were checked and we found that peak of HbA_{1c} was missing in abnormal chromatogram. It is well documented in the literature of manufacturer that the peak of HbF<10% (fetal hemoglobin) and LA_{1c}/CHb-1 ≤4% (labile hemoglobin A_{1c}) does not interfere in the analysis of HbA_{1c} while using HPLC. We noted that the peak of labile hemoglobin A_{1c} in abnormal chromatogram was very high (12.6% and 10.0%) as compared to normal chromatogram (1%). Unknown peaks in variant window were also inspected. Corbe-Guillard E etal, in their study reported that labile hemoglobin A_{1c} interfere in HbA_{1c} assay [9]. Though in their study labile hemoglobin A_{1c} was 3.1% and 3.6% while in our study it was abnormally high i.e 12.6% and 10.0%. Hence, after focusing on all the peaks of chromatogram we found that the abnormally high level of labile hemoglobin A_{1c} fraction resulted in misinterpretation of HbA_{1c} results which may be due to some acute change in the blood glucose level.

In conclusion, labile hemoglobin A_{1c} does not play role in diagnostic purpose. But to reduce the potential source of pre-analytical error due to labile hemoglobin A_{1c}, its percentage should be taken into account while estimating HbA_{1c} by HPLC technique. Each hemoglobin chromatogram needs attention by clinician as well as pathologist/ biochemist which will reduce the pre-analytical error, misinterpretation of report and inappropriate clinical decision while managing the patient.

Declaration of Conflicting Interests: The authors declare that they have no conflict of interest.

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