Short Communication

Standard recommendation for interpretation of HbA1c graph in Ion Exchange HPLC method

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Abstract: Glycated hemoglobin now a day's serve as a prognostic as well as diagnostic marker in diabetic patients and its estimation by high pressure liquid chromatography is commonly used in the laboratories. As the chance of interference increases in this technique few things should be kept in mind before reporting which will decrease the percentage of post analytical errors in the laboratories.
Key words: *Diabetes, HbA1c, hemoglobin variants, ion-exchange chromatography*

Introduction: It is estimated by IDF (International Diabetes Federation) that about 382 million people have diabetes in 2013 all over the world and 80% of them lived in low and middle income countries (Diabetes Atlas 5th Ed). Diabetes mellitus is now a global health problem and is a seventh leading cause of death in U.S. Hence the population at risk should be screened and diagnosed properly. On the other hand known diabetic patients should be monitored promptly to reduce mortality rate due to its complications. Since many years glycated hemoglobin (HbA1c) have been used as a prognostic test and today many clinicians are also using it as a diagnostic test for diabetes mellitus. Number of methods like immunoturbidimetric, ion exchange HPLC, boronate affinity, enzymatic method etc., is available by which glycated hemoglobin can be estimated. Diabetes control and complication trial (DCCT) and National Glycohemoglobin Standardization Programme (NGSP) recommend Ion exchange HPLC method as an acceptable and standard method though it measures all fractions of hemoglobin but this method is affected by hemoglobin variants (HbS, HbE, HbC, HbD and HbF) (Randie, 2009 and Nigam et al., 2006). Hence interpretation of graph by HPLC plays an important role in minimizing the errors which can occur due to abnormal life span of RBC's. There are very few pathologists, biochemist or clinicians who actually interpret the graph of HbA1c in Bio Rad D10 used for Dual HbA1c Program. This article discuss about how to proceed for the data interpretation before reporting the result of HbA1c by ion exchange HPLC (Bio Rad D10) and clinician must also know the interpretation of graph so that patient can be monitored or diagnosed correctly.

Discussion and Recommendation

Firstly check the baseline of the graph which should be straight. Secondly the area count to be within 1.0 million to 5.0 million, if area count is below or above this range it should be diluted with diluent and processed again. Thirdly, check the value of P3 peak if it is more than 8% it means sample is clotted or it was not stored properly and it has to repeat with fresh sample. Next thing which should be checked is retention time of HbA1c and HbA_0 as depicted in Graph1. HbF should be less than 10% while LA1c/CHb-1 and LA1c/CHb-2 peaks should be ≤4.0% and ≤3.5%. Linearity of HbA1c estimation by this method is 3.8% to 18.5%. It means that any value below 3.8% could be due to condition causing RBC lifespan shorter and can interfere with estimation of HbA1c, also value above 18.5% cannot be used for monitoring the diabetic patient. Any unknown peak in variant window due to hemoglobinopathies can also interfere in the analysis of HbA1c which can be ruled out by taking history of the patient and in such cases value of HbA1c result is not reportable. Other conditions like iron deficiency anemia, history of blood transfusion or any condition which alters the life of RBC's should be ruled out before reporting result (Sinha et al., 2012). Lastly but important one is Quality Control which should be within range.

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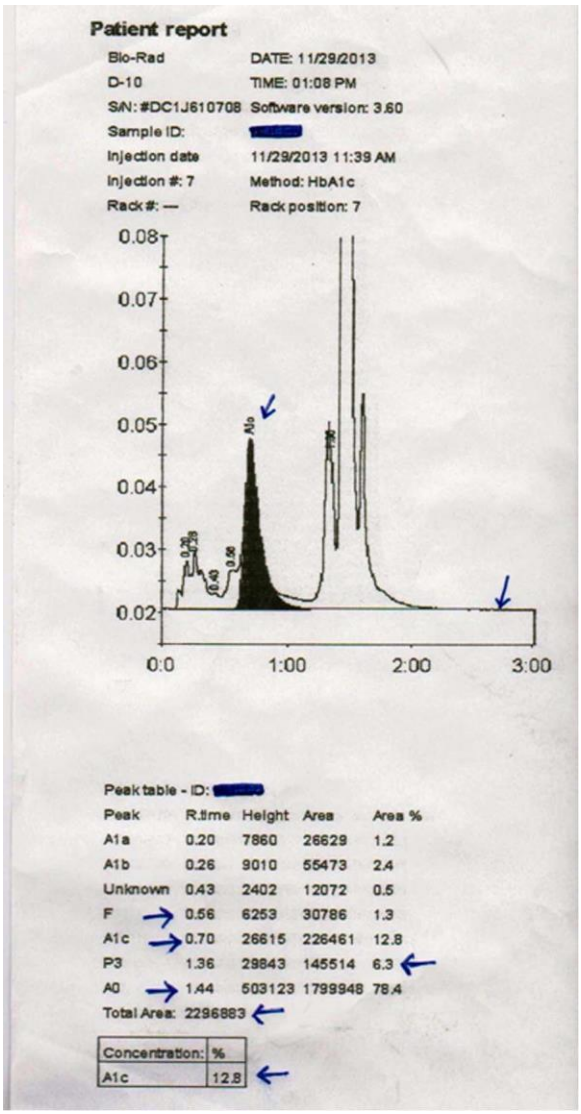

Graph1: Diabetic Sample with elevated HbA1c level

Conclusion: As many laboratories are using ion-exchange HPLC as a technique for monitoring as well as diagnosing diabetic patient by keeping few things mentioned above in mind before reporting will help in reducing the number of errors which can occur in this method. Though most of the information is available in pack insert which seems to be very basic but they are actually important during reporting the HbA1c value by HPLC. Laboratory person should report the cause of error during reporting which will help our clinicians in monitoring the diabetic patients. The number of diabetic population is increasing constantly it became more important to focus on the quality of report.

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