Original Research Paper

VOLUME-6 | ISSUE-4 | APRIL - 2017 • ISSN No 2277 - 8179 | IF : 4.176 | IC Value : 78.46

Relevance of HPLC for HbA1c estimation

Pathology

KEYWORDS: Haemoglobin A, Glycosylated, HbA1c, Standards, High performance liquid chromatographic method, Immunological method.

Anshu Jamaiyar Associate Professor of Pathology, Rajendra Institute of Medical Sciences,Ranchi

ABSTRACT

Diabetes Mellitus (DM) has become a global epidemic. Diabetes Mellitus (DM) has become a global epidemic whose incidence is increasing and which already affects one in twelve people (1)

HbA1c can be measured by different methods. Enzymatic, boronate affinity chromatographic and cation-exchange high performance liquid chromatographic (HPLC) methods are considered as gold standard methods.Other methods include Immune assay and Capillary electophorosis(10)

The accuracy of HbA1c methods can be affected adversely by the presence of hemoglobin (Hb) variants or elevated levels of fetal hemoglobin (HbF). The effect of each variant or elevated HbF must be examined with each specific method.

Glycohemoglobin (GHb), measured as HbA1c, is an invaluable tool for monitoring long-term glycemic control, and as such it is a key issue in diabetes care

Glycosylated haemoglobin (HbA1c) has been in use since 1980s as the 'gold standard' for monitoring glycaemic control and as a predictor of diabetic complications. Even though several conditions, such as, haemolytic anaemia (lowers HbA1c) and aplastic anaemia (raises HbA1c) tend to confound and interfere with HbA1c measurement, in most circumstances HbA1c is a valid and reliable index of glycaemic status

Introduction

HbA_{1c} is an ideal parameter for diagnosis and control of DM because there is a direct relationship between average blood glucose levels and HbA_{1c} concentration (4)

ADA 2010 recommends the use of HbA1c as a diagnostic marker for diabetes and categories for increased risk of diabetes (formerly known as prediabetes). Persons with HbA1c of 6.5% and above are to be diagnosed as diabetes and HbA1c between 5.7-6.4 are considered to have categories for increased risk of diabetes.

HbA_{1c} is a derivative of hemoglobin, a minor fraction that is generated as a result of non-enzymatic reaction between the aldehyde group of the open structure of the glucose molecule and the free amino group in the terminal valine of the hemoglobin β chain

The most common Hb variants worldwide are HbS, HbE, HbC, and HbD. All of these Hb variants have single amino acid substitutions in the Hb β chain. HbF is the major hemoglobin during intrauterine life; by the end of the first year, HbF falls to values close to adult levels of approximately 1%.(13)

We used the Bio-Rad D-10TM Hemoglobin A1c program which is intended for the percent determination of HbA1c in human whole blood.

Material & Methods

The study was done at Rajendra Institute ofMedical Sciences. A 49yr.old man refd. From opd medicine came with normal CBC with no family H/O of anemia &no known hemoglobinopathy. Patient had a normal haemoglobin value of 12.6g/dl,with anisopoikilocytosis and microcytic normochromic rbc. The sample was run on Bio-rad variant and HPLC revealed a unknown peak of 21.7% at 4.34 minutes &A2 of 4.5%.

Discussion

HbA1c results (DCCT or IFCC) will be misleading in certain situations e.g. a variety of haematological conditions where there is abnormal red cell turnover also diffs. The results can also vary depending on the HbA1c method used by a particular laboratory. Some methods for HbA1c can give more reliable results in some haemoglobinopathies, but if this or any other condition leads to a change in red cell survival, then HbA1c measurement by any means can, at best, be used to track changing trends in glycaemia. Other measures of glycaemia may then be required, such as more reliance on self monitored blood glucose values or the use of a serum fructosamine assay, if available.erent other disease conditions.

Before advising for HbA1c, clinicians should also remember that unstable haemoglobin variants may interfere with the HbA1c measurement.

Abnormal hemoglobinemia should be considered when a major discrepancy between the levels of HbA1C and fasting plasma glucose is observed.

A major limitation of these HbA1c immunoassays is that they do not definitely detect the presence of abnormal haemoglobin variants. As because the red blood cells with abnormal haemoglobin variants have shortened life spans, the reported HbA1c value may not reflect the preceding 2 – 3 months blood glucose control. When there is a discrepancy between HBA1c and blood glucose values,conditions that affect the red cell lifespan and possibility of hemoglobinopathy must be investigated.

The glycated hemoglobin variants exist due to congenital disorders 114 of globin chain synthesis, called "hemoglobinopathies". Although 115 some variants can directly interfere with the HbA1c test, some variants 116 interfere by causing premature turnover of red blood cells. In cases 117 where an individual is heterogeneous, the individual will be asymptomatic and have normal red cell survival. For example, HbS homozygosity 119 leads to sickle cell anemia, which involves premature turnover of red 120 blood cells, whereas those who are heterozygous for the sickle cell allele 121 are asymptomatic [14]

Correlation between HbA1c level and mean plasma glucose levels : HbA1c (%) 6(135mg/dl) 7(170mg/dl) 8(205mg/dl) 9(240mg/dl) 10(275mg/dl) 11(310mg/dl) 12 (345MPG (mg/dL).corresponding MPG is correlated with HbA1c.

*Mean whole blood glucose results are 10%-15% lower. Most blood glucose meters are calibrated to read as plasma glucose HbA1c= glycosylated haemoglobin; MPG=mean plasma glucose

Iron deficiency anaemia has been known to cause a rise in HbA1c of up to 2% and this has been shown to be reversed with iron supplementation. Given that iron deficiency anaemia is a common finding, especially in pre-menopausal women, caution should be exercised while interpreting HbA1c results in these patients. Haemolytic anaemia has the opposite effect to iron deficiency and a reduction in HbA1c is observed in affected individuals. This occurs due to reduced red cell survival, meaning a reduction in the availability of haemoglobin for glycation (12)

As HbA1c is based on Hb, both quantitative and qualitative variations in Hb can affect the HbA1c value. [5] If the Hb substitution causes a net change in charge of the Hb (as with Hb variants S, C, D,

and G), or if Hb variants cannot be separated from HbA/HbA1c will produce spuriously increased or decreased results by HPLC. [5]

A single case report of a diabetic case with Hemoglobin J-Meerut and low HbA1c levels has been reported in the literature. [4] However, the presence of Hb variants may falsely produce low values for HbA1c or spuriously increased HbA1c values.

The identification of Hb variants is, therefore, important to avoid inaccurate GHb results. In addition, several other factors besides the presence of genetic variants or presence of chemically modified derivatives of Hb 7, such as drugs, anemia, uremia, and alcoholism, may falsely lower GHb results. Decreased red blood cell survival and mean erythrocyte age falsely lower GHb values (6).

Blood loss, hemolytic anemia, sickle cell anemia, and chronic renal disease affect the life span of red blood cells and are known to be associated with underestimated GHb values (6) . In contrast, iron and B12 vitamin deficiency have been reported to overestimate GHb results (9 .)

It is essential that clinical laboratories be aware of the limitations of their HbA1c assay methods as well as the importance of visual inspection of ion exchange chromatograms to detect abnormalities (extraordinary peaks or non-separation of each peak) caused by Hb variants.

Conclusions

All HbA1c methods are inappropriate for the assessment of glycemic control in patients homozygous for HbS or HbC, with HbSC disease, or with any other condition that alters erythrocyte survival. Laboratorians should be aware of the limitations of their method with respect to interference from the most prevalent Hb variants. They can also select new methods that are less likely to have interference. If an ionexchange HPLC method is used, then careful inspection of chromatograms may identify the presence of aberrant peaks produced by variants. If possible, all patients should have at least once HPLC assay for HbA1c to rule out the presence of interfering haemoglobin varients. The high prevalence of iron deficiency, especially in women in India, also renders HbA1c a poor choice as a diagnostic test. (3)HbA1c continues to be the best available option to monitor glycaemic control in patients receiving treatment for DM.

Referances:-

1. F. Aguiree, A. Brown, N.H. Cho, G. Dahlquist, S. Dodd, T. Dunning, et al. IDF Diabetes Atlas (sixth edition)International Diabetes Federation, Basel (2013)
2. K.H. Gabbay, K. Hasty, J.L. Breslow, R.C. Ellison, H.F. Bunn, P.M. Gallop Glycosylated hemoglobins and long-term blood glucose control in diabetes mellitus J. Clin. Endocrinol. Metab., 44 (5) (1977), pp. 859–864
3. Reddy A,etal,clinical applications of glycosylated hemoglobin
4. Yagame M, Jinde K, Suzuki D, Saotome N, Takano H, Tanabe R, et al. A diabetic case with Hemoglobin J- Meerut and low HbA1c levels. Intern Med 1997;36:1
5. Little RR, Roberts WL. A review of variant hemoglobins interfering with hemoglobin A1c measurement Diabetes Sci Technol 2009;3:446-51. HYPERLINK " http://www.ijpmonline.org/article.asp?issn=0377-4929;year=2012;volume=55; issue=2; spage=270; epage=271;aulast=Sharma" \l "ft5"
6. Bry L, Chen PC, Sacks DB. Effects of hemoglobin variants and chemically modified derivatives on assays for glycohemoglobin. Clin Chem 2001; 47: 153-63.
7. National Glycohemoglobin Standardization Program (NGSP). University of Missouri, http://www.missouri.edu/,diabetes/ ngsp/factors.htm (accessed in June 2006).
8. Smaldone A.Glycemic control and hemoglobinopathy: when A1c may not be reliable.Diabetic Spectrum2008;21:46-9
9. Gram-Hansen P, Eriksen J, Mourits-Andersen T, et al. Glycosylated haemoglobin (HbA1c) in iron and vitamin B12 deficiency. J Intern Med 1990; 227: 133-6.
10. Gabriele H, Katzensteiner S, Schnedl W, Purstner P,Pieber T. Comparative evaluation of three assay systems for automated determination of haemoglobin A1c. Clin Chem 1997;43:511-517.
11. Khuu H, Robinson A, Goolsby K et al. Evaluation of a Fully Automated High – Performance Liquid Chromatography Assay for Hemoglobin A1c. Arch Pathol Lab Med 1999;123:763-767.
12. Jiao Y, Okumiya T, Saibara T, Park K, Sasaki M. Abnormally decreased HbA1c can be assessed with erythrocyte creatine in patients with a shortened erythrocyte age. Diabetes Care 1998;21:1732-5.
13. RandieR.Little ,William L.roberts ,A review of variant hemoglobins interfering with HA1c measurements.
14. Bain BJ,Haemoglobinopathy diagnosis: algorithms, lessons &pitfalls.
15. Dam A,Ahuja A,SinghM etal,liitationin HPLC methodology forHBA1c estimation.