Comparing sensitivity and specificity methods of chemical and baronet affinity measuring glycated hemoglobin with HPLC method

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Abstract

Introduction: Glycated hemoglobin level is a good estimate of the average blood glucose over a period. Therefore, the measurement accuracy is very important. The most commonly available methods for measuring HbA1C are chemistry, immunoassay, enzymatic, electrophoresis, baronet affinity and HPLC.

There is still no standard way that all the laboratories do it. The aim of this study was to compare two methods of chemical and baronet affinity with HPLC method for the verification accuracy.

Methods: This experimental study was done on 50 samples. Glycosylated hemoglobin test performed by the baronet affinity method by Nycocard, HPLC by Bio Rad D-10 and chemical methods by Mahsa Yaran kit.

Results: By HPLC, 21 samples (42%) were normal, 4 cases (8%) were moderate and 25 (50%) were high. The mean and standard deviation and the range of HbA1C of baronet affinity, chemical and HPLC methods were 7.1±1.9 (4.4 to 10.9), 6.9±1.7 (4 to 10,6), and 8.3±2.4 (4.7 to 14.3), respectively. The sensitivity of the baronet affinity method in comparison with HPLC method was 100% and specificity was 58.6%. The sensitivity of chemical method in comparison with HPLC method was 100% and specificity was 62.1%. The correlation coefficient for both was obtained 0.633 which is statistically significant (P<0.0001). Also correlation coefficient for two methods of baronet affinity and chemical was determined 0.852, which is statistically significant.

Conclusions: In this study was observed that between the two methods baronet and chemical there is a coefficient of agreement of 86,9% and even the chemical method has more specifity. Also considering the cost of this method, chemical method can be considered as a suitable method for measuring Glycosylated hemoglobin. But maybe the long-time of doing chemical method is one of the serious limitations of the laboratories to select this method.

Key words: HbA1c, HPLC, Chemical method, Baronet method

{**Citation:** Malekmahmoodi Sh, Ahmadi AR, Behnampoor N, Joshaghani HR. Comparing sensitivity and specificity methods of chemical and baronet affinity measuring glycated hemoglobin with HPLC method. American Journal of Research Communication, 2013, 1(11): 350-356} www.usa-journals.com, ISSN: 2325-4076.

Introduction

Diabetes is one of the most common health problems in the world. At 1995, approximately 4% of the world population were diabetics and estimated in the year 2025 that had increased to 5.4 % (1). In 2008 existed approximately 4 million diabetics in Iran and estimated in the year 2021 to reach 12 million people (2). Glycosylated hemoglobin (GHb) is obtained from the binding of glucose to hemoglobin during a non-enzymatic reaction.

Glycosylated hemoglobin (HbA1C) is created from the binding of glucose to the N-terminal amino acid Valine of beta-globin chain. HbA1C level is a good estimate of the average blood glucose over a period. Therefore, the measurement accuracy is very important (3). Today HbA1C test as a test for long-term monitoring of blood glucose has been accepted. Although it still is not recommended in the testing for the diagnosis or screening for diabetes. A threshold 6 % In 2008 was used for screening–diagnosis of diabetes. A threshold of 6.5% in 2010 was used from the American diabetes association's standards of medical care in diabetes. Determination of HbA1C was also recommended that perform by an approved method by the National glycohemoglobin program (NGPS) (4). On the basis of studies, a single HbA1C test is not sensitive enough to detect type 2 diabetes in older people, but there is a good relationship in patients between the standardized measurement method of HbA1C with retinopathy (5). The most commonly available methods for

measuring HbA1C are chemistry, immunoassay, enzymatic, electrophoresis, baronet affinity and HPLC.

Some hemoglobin variants that may interfere with ion exchange in HPLC method. These methods seem to be abnormal variants of hemoglobin in baronet affinity method have the least interference (6). There is still no standard way that all the laboratories do it. The aim of this study was to compare two methods of chemical and baronet affinity with HPLC method for the verification accuracy.

Materials and Methods

This experimental study was done on 50 samples. In this study of patients that admitted to the medical diagnostic laboratories 5 ml of blood was taken in vials containing anticoagulant EDTA. Glycosylated hemoglobin test performed by the baronet affinity method (Nycocard, Norway), HPLC by Bio Rad D-10 (USA) and chemical methods by Mahsa Yaran kit (Iran). In this study for statistical tests was done from a mean comparison of descriptive and correlation ration tables using of Spss 11.5 software. This study considered 95% confidence interval for all variables.

Results

The mean and standard deviation of glucose samples was 159±66 mg/dl and range of glucose was between 78 to 343 mg/dl. By HPLC, 21 samples (42%) were normal, 4 cases (8%) were moderate and 25 (50%) were high. The mean and standard deviation and the range of HbA1C by baronet affinity, chemical and HPLC methods were 7.1±1.9 (4.4 to 10.9), 6.9±1.7 (4 to 10.6), and 8.3±2.4 (4.7 to 14.3), respectively.

In this study sample were divided into normal and abnormal and HPLC method was considered as the gold standard, the sensitivity of the baronet affinity method in comparison with HPLC method was 100% and specificity was 58.6%. Positive predictive value (PPV) and negative predictive value (NPV) of the baronet affinity method in comparison with HPLC method was 63.6% and 100%, respectively. The sensitivity of chemical method in comparison with HPLC method was 100% and specificity was 62.1% . Also, PPV and NPV of chemical method in comparison with HPLC method was 65,6% and 100%, respectively. In this study to determine the extent of correlation between two methods of

baronet affinity and HPLC and chemical method with HPLC was used by a correlation coefficient. The correlation coefficient for both was obtained 0.633 which is statistically highly significant (P<0.0001). Also correlation coefficient for two methods of baronet affinity and chemical was determined 0.852, which is statistically highly significant. Phi coefficient of agreement was between two methods of baronet affinity and chemical

And between the two methods of 86.9, between the two methods of baronet affinity and HPLC 61.1 Chemical and HPLC was 63.8.

Discussion

In this study, the sensitivity of the two methods of chemical and baronet affinity was high in contrast to HPLC method, but the specificity of chemical method was obtained higher than the baronet affinity method. In the study of Alaodolehi and coworkers performed in Babol showed that an average of GHb with electrophoresis method in fasting state 10.1% and in non-fasting state was 11.4% and average of this index with the calorimetric method in fasting state 362.4 nmol/grHb and in non-fasting state was 361.5 nmol/grHb. The significant difference between the average of GHb in two conditions in the calorimetric method was not found, while this difference was significantly in electrophoresis method (p< 0.001) (7). In another study that performed by Garcia and colleagues in Mexico, comparison of one measuring method of the sensitivity and specifity and positive and negative predictive value was with the HPLC method that they concluded that although there is a correlation between the two methods (p≤ 0.0001) but the results were used in comparison with HPLC method is in the status far from desirable level (8). Bannon and coworkers reported that glycosylated hemoglobin with ion exchange chromatography method in uremic patients is more accurate (9). In another study John and colleagues various devices are available to measure Glycosylated hemoglobin (HbA1c), including: DCA, GDX, Nycocard that compared to the laboratory reference method ranged from − 0.31% to + 0.39%. Only the DCA device had a between batch imprecision of less than 5% and the analytical performance obtained by laboratory staff was similarly better for the Nycocard device. In this study the two methods of chemical and baronet affinity in comparison with HPLC method were highly sensitive, but the chemical method for measuring glycosylated hemoglobin had more specifity (10).

Many factors can affect the accuracy of chemical method that following them will increase the accuracy of the results. Including issues that must be followed are washing the blood samples before were kept and is using the curved tubes in the stage that samples are placed in a boiling water bath.

The comparison of baronet affinity with HPLC It follows that the 33 cases in which the method of baronet affinity normal is measured, 8 cases (24.2%) by HPLC in the high range, and 4 samples in the range of intermediate were obtained, but the values of intermediate and high from baronet affinity method all were matched with HPLC method that shows intermediate and high of baronet affinity method is matched 100% with HPLC method but more than 30% of normal values in this method with HPLC method more than the normal level is achieved.

From the interpretation of these results it comes to that the normal values require more study. Also from the comparison of chemical method with with HPLC it comes on that of 32 samples were obtained with chemical method in the normal range 7 samples by HPLC method in the high range and 4 samples in the range of intermediate were obtained but the values of intermediate and high from chemical method all were matched with gold standard method that shows intermediate and high of chemical method is matched 100% with HPLC method but more than 30% of normal values in this method with gold standard method more than the normal level is achieved. From the interpretation of these results it comes to that the normal values of chemical method require more study.

From the results of baronet and chemical methods for HPLC method, it comes with that high values of a natural level of baronets and chemical methods all were matched with HPLC method and are reliable but in more than 30% of values, natural level of baronets and chemical methods are not reliable and it is not matched with HPLC method and this inconsistency can be due either to weakness in both the above methods and lack of accurate cutoff point that is selected.

It seems that cutoff point on the leveling of consequences for patients requiring serious review and given the importance of this issue is needed not only this method but also the existing methods in the country laboratory compared with reference method and a cutoff point of each level can be derived from the current methods and it seems using of stated values in the brochure of imported kits or resource is not possible generalize to the results of the patients within the country.

Conclusions

Despite the initial impression that consider chemical method as a traditional method, in this study was observed that between the two methods baronet and chemical there is a coefficient of agreement of

86,9% and even the chemical method has more specifity. Also considering the cost of this method, chemical method can be considered as a suitable method for measuring Glycosylated hemoglobin. But maybe the long-time of doing chemical method is one of the serious limitations of the laboratories to select this method.

Acknowledgement

This research supported by Deputy of Research and Technology of Golestan University of medical sciences.

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