Comparing the HbA1c Assay Results of Architect C 8000 and MQ-2000PT

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ABSTRACT

Objective: Changes in a device and the modification of measurement methods are frequent issues in medical laboratories. The effects of such modifications on assay results should be investigated. HbA1c is a widely used analyte in treatment and in the follow-up of diabetic patients. We aimed to evaluate the effects of two different assay methods on the detection of the percentage of HbA1c.

Methods: We used blood samples with K3-EDTA of 57 diabetic patients who were admitted to our laboratories for the HbA1c assay. HbA1c assays were performed using immunoturbidimetric (Architect C 8000; Abbot Laboratories Inc., Middletown, USA) and ion exchange chromatography (MQ-2000PT; Shanghai Hui Zhong Medical Technology Co. Ltd., Shangai, China) methods. HbA1c assays were repeated two times in both devices. Results were analyzed using MedCalc software.

Results: Mean HbA1c level in immunoturbidimetric and ion exchange chromatography assays were 6.6 (min:4.1 and max:11.4) and 6.9 (min:4.9 and max:11.8), respectively. In the linear regression analysis, we detected an r value of 0.9533 (r<0.975). In Passing–Bablock analysis, we found the following equation, y=0.4+1.0 x (intercept CI:−0.22−0.68; slope CI:0.97−1.09). We did not observe any constant or proportional systematic errors between the assay methods. We found a 0.37 difference between the two methods in the Bland–Altman graphs of mean HbA1c measurements (Bias 5.7%).

Conclusion: Researches on the harmonization of HbA1c are still increasing worldwide. However, at present, there are variations in methods and devices. NGSP suggests that the difference between methods should not exceed HbA1c±0.70. We found that mean HbA1c results were higher by 0.37 times in ion exchange chromatography assay compared with those in immunoturbidimetric assay. This difference is within the range suggested by NGSP. *(JAREM 2015; 5: 52-5)*

Keywords: HbA1c, standardization, method comparison

INTRODUCTION

Hemoglobin is a protein located in red blood cells and is responsible for oxygen transportation. Because of post-translational modifications after the synthesis of hemoglobin, modified hemoglobins are formed, and the most common among them is hemoglobin A1c (HbA1c) (1). It has been known for a long time that the HbA1c level reflects the average blood glucose level 6–8 weeks prior to measurement and that it correlates with the late complications of diabetes (2). In 1988, the American Diabetes Association (ADA) recommended the use of HbA1c in the follow-up of diabetes (3). The estimated treatment goals for HbA1c were determined by the ADA for the first time in 1994 (4). Today, while HbA1c maintains its importance in the follow-up of diabetes, it has also been used as a criterion for the diagnosis of diabetes (5).

HbA1c is a useful parameter because it has low biological variation, does not require any special preparation before the test, is not affected by acute stress, and has high preanalytical stability for diagnosis and follow-up treatment of diabetes (6). For HbA1c analysis, more than 70 methods are reported to be used worldwide (7). As each of these methods measures different fractions of glycated hemoglobin in different ways, the results may differ from each other (8). To ensure standardization for HbA1c measurement methods, the National Glycohemoglobin Standardization Program (NGSP) was established in 1993 by the American Association for Clinical Chemistry (9). In 1995, the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) started standardization studies for HbA1c. In 2001, the reference method that had been established for HbA1c was approved by the IFCC and was started to be used (10). Although the IFCC and NGSP have tried to resolve differences between methods through studies, a standardization that covers all methods that have been used worldwide has not yet been provided (8).

Changes in the device and the modification of measurement methods are frequent issues in medical laboratories. Studies have shown that there is a significant amount of bias between HbA1c levels detected by different methods (11). The effects of such modifications on assay results should be investigated, and clinicians should be informed about these possible changes. We aimed to evaluate the effects of two different HbA1c assays on patient samples.

METHODS

Collecting Blood Samples

For this study, we used the samples of 57 patients who were admitted to the laboratories of Abant İzzet Baysal University Faculty of Medicine Research and Application Hospital for the HbA1c assay. The patients' venous blood samples were taken into tubes that were anticoagulated with K3-EDTA. All samples were incu-

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bated at room temperature, until they had been worked on, and the analysis was completed within 4 h at most.

HbA1c Measurement Methods

HbA1c was measured in accordance with the manufacturer's instructions in both methods. The patients' samples were examined twice in both systems.

1. Immunoturbidimetric method: In this method, the HbA1c assay was conducted via an autoanalyzer (Architect C 8000, Abbott Laboratories, Inc., Middletown, USA). Before the measurement, samples were subjected to pretreatment with a denaturant (MULTIGENT hemoglobin denaturant). In this way, the decomposition of erythrocytes was achieved by exposing the erythrocytes to osmotic pressure. Afterwards, from the hemolysate that was obtained, two measurements were conducted with the autoanalyzer, including total hemoglobin and HbA1c. The hemoglobin level was measured with the method described by Zander et al. (12). HbA1c microparticle agglutination was immunoturbidimetrically measured with the inhibition method. Calibrator values that were used could be monitored by the IFCC and NGSP. The obtained results were converted to an HbA1c result with the below formula. With the help of this formula, HbA1c results are reported to be correlated to the NGSP-certified method.

$$\frac{[HbA1c\ (g/dL)] \times 100] - 3 + [0.2 \times THb\ (g/dL)]}{THb\ (g/dL)} = \%\ HbA1c$$

2. Ion-exchange chromatography: With this method, HbA1c measurement was conducted with an automatic HbA1c analysis device (MQ-2000PT, Shanghai Hua Zhong Medical Technology Co., Ltd., Shanghai, China) were performed. This device is based on HPLC and uses ion-exchange chromatography. Patient is ionically interacts with HbA1c colon material that was in the sample and is separated from other hemoglobin fractions. Meanwhile, changes in absorbance are measured at 415 nm. HbA1c that was measured was expressed as percentage. This method could be monitored by the reference method used by the IFCC.

Statistical Analysis

Statistical analysis was performed using the demo version of the MedCalc statistical software program. The compliance in the normal distribution of variables was examined with the Kolmogorov–Smirnov test. Values are expressed as average and standard deviation. Differences between averages were assessed by Student's t-test. $P < 0.05$ was considered to be significant. Regression analysis was performed to assess the relationship between results obtained from both methods. The results obtained by ion-exchange chromatography were defined as dependent variables. Because linear regression analysis results in the r-value of <0.975, Passing–Bablock regression analysis was performed. Bland–Altman plots were applied in order for method comparison.

RESULTS

The samples that were examined twice with the immunoturbidimetric method and ion-exchange chromatography were averaged for each method. These results were used for statistical analysis. The results that had been obtained fit in the normal distribution. The average results of HbA1c level in the immunoturbidimetric and ion-exchange chromatography methods were 6.6%

(min 4.1% and max 11.4%) and 6.9% (min 4.9% and max 11.8%), respectively. There was no significant difference between the averages of both methods (p=0.27). In linear regression analysis, the r-value was 0.9533. In the Passing–Bablock analysis, we found the following equation, y=0.4+1.0x (intercept CI: −0.22–0.68; slope CI: 0.97–1.09) (Figure 1). No constant or proportional systematic errors were observed between the assay methods. Deviation from linearity was not observed between the methods (p>0.1). When the two methods were compared in Bland–Altman graphs, HbA1c results obtained by ion-exchange chromatography were found to be higher by an average of 0.37 times than those obtained by the immunoturbidimetric method; this corresponded to 5.7% bias (Figure 2).

DISCUSSION

In this study where the effects of two different measurement methods for HbA1c on patient samples were evaluated, it was reported that between the averages of results obtained by both methods, there was no statistically significant difference. Furthermore, it was determined that there was a linear relationship between the methods and that there was no constant or proportional error. Despite all this, it was suggested that the results obtained by ion-exchange chromatography were found to be higher by an average of 0.37 times and that this corresponded to 5.7% bias.

Today, HbA1c is used in diabetes screening in addition to the diagnosis and treatment of diabetes. Therefore, it must be measured with a high accuracy and reliability (13). Furthermore, for the results that will be obtained in the same laboratory, the provision of its own standardization is particularly important. The method comparison we performed reveals the impact of method change on patient outcomes. In this study, HbA1c levels obtained by ion-exchange chromatography were found to be higher by an average of 0.37 times (5.7%) than those obtained by the immunoturbidimetric method. The NGSP suggests that the difference between the methods should be in the range of HbA1c±0.70 (8). The difference we found is within the limits suggested by the NGSP.

Physicians want the HbA1c analysis of diabetic patients on a regular basis, and they assess results under the guidance of current guidelines on fixed threshold values (8). Possible differences between successive results are interpreted as the effects of diabetic regulation. According to the common idea, 0.5 of change in HbA1c in the patient's results is interpreted as a clinically significant change (14). However, there are several factors resulting from biological and analytical variations that could lead to differences in results between the two measurements. If two consecutive measurements that are compared with each other are measured with two different methods, there is no doubt that one of the reasons for the difference observed between the results is the difference between methods (15). The 0.37 HbA1c difference we obtained between the two measurement methods in our patient group remains essentially below 0.5 HbA1c change level, which is considered to be clinically significant. However, it should be noted that when effects that may arise from biological and analytical variations are added, the changes between successive results may exceed the 0.5 HbA1c limit. The effect of the biological variation for HbA1c is given as <2% (14).
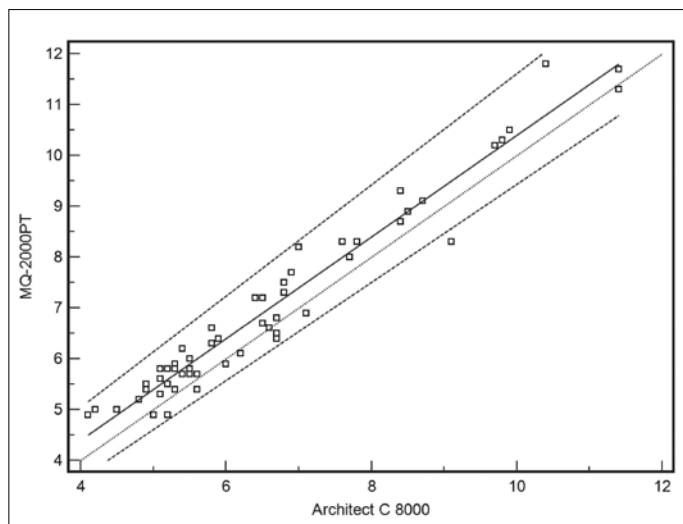
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Figure 1. Passing–Bablock regression graph. In Passing–Bablock analysis, the following equation was obtained: y=0.4+1.0 x (intercept CI: −0.22–0.68; slope CI: 0.97–1.09).

Figure 2. Bland–Altman graph. HbA1c results obtained by ion-exchange chromatography were found to be higher by an average of 0.37 times than those obtained by the immunoturbidimetric method.

Therefore, it has little effect on patient outcomes. Although the impact of analytical variation varies from method to method, the coefficients of variation of the two measurement methods that we compared were also reported to be lesser than 2%. Under these circumstances, in consecutive results, biological and analytical variations appear to be a factor that will adversely affect the evaluation of the results alone. However, it should be remembered that when the impact of differences in methods is also included, clinical decision making might be adversely affected. Therefore, when assessing the results after the change of method, the total impact of all these factors on patient outcomes must be taken into account, and the results should be accordingly interpreted.

The methods used for HbA1c measurement have two basic principles (16). The first one includes methods such as chromatography or electrophoresis that separate HbA1c from other hemoglobin fractions. The other approach is immunochemical methods, in which antigens targeting HbA1c are used. Results obtained with these methods are not correlated to each other, and there are some differences between methods and devices. To eliminate the probable negative impacts of these differences on patient outcomes, studies are underway worldwide on HbA1c harmonization. However, there is no standardization that covers all methods and devices (17). The calibrators that were used in both methods we compared can be monitored by the IFCC and NGSP. Although the results were compatible with each other, there is still a difference to be considered between the two methods. This difference highlights that it is necessary to assess the impact of this difference on the results when there is a change of device in laboratories, although the methods can be monitored with a standardized method.

The strength of this study is using the Bland–Altman analysis that is considered to be the golden standard in the statistical analysis of method comparison studies. According to this statistical analysis method, the measurement differences of both methods are presented, and interpreting the admissibility level of these differences is left to the clinician's judgement.

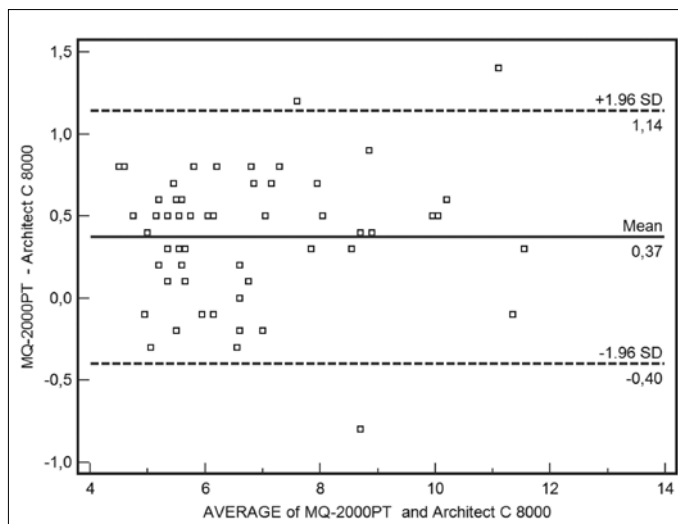The weakness of this study is its single-center design and the limited patient population. Furthermore, no recurrence study was performed for methods that were used.

CONCLUSION

The results of this study show that HbA1c results obtained by the MQ-2000PT device were 0.37-times higher in the ion-exchange chromatography assay than those in the immunoturbidimetric assay that used ARCHITECT C 8000. This difference is within the range suggested by the NGSP. Nevertheless, we believe that informing clinicians about these differences would be useful for the follow-up and treatment of diabetic patients.

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