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Evaluation of Bio-Rad D-10® and arkray adams HA-8160® HPLC analyzers in HbA1c measurement

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Abstract
Glycosylated hemoglobin A1c (HbA1c) is an important parameter used for the assessment of time-dependent glycemic status and the diagnosis and follow-up of diabetes. This study aimed to evaluate the analytical performance of two HPLC analyzers, the Bio-Rad D-10®, and the Arkray Adams HA-8160®. Accuracy and imprecision studies were conducted, and a method comparison study was performed with 105 samples. Samples were collected on five consecutive days and measured on both analyzers within two hours. Bland-Altman and regression analyses were used for statistical evaluation of the data. Low and high-level within-day CV values were calculated as 1.22% and 0.60% for the Arkray analyzer and 1.2% and 0.30% for the Bio-Rad analyzer, respectively. They were calculated as 1.27% and 2.52% for the Arkray analyzer and 2.32% and 3.44% for the Bio-Rad analyzer, respectively, between days. The within-day CV values for both analyzers were below the limit of 2.5% specified by the International Federation of Clinical Chemistry (IFCC). The bias average for three months was 3.2% for the Arkray analyzer and 1.2% for the Bio-Rad analyzer. The Spearman correlation coefficient (r) was 0.973 ($p<0.0001$) between the two measurements, and the slope and intercept values calculated in the regression analyses showed the linear relationship and harmony between the two analyzers [$R^2 = 0.967$, ($y = 0.860x + 0.604$)]. Both HPLC analyzers are shown to be reproducible and accurate for routine biochemistry laboratory use.

Keywords: Diabetes mellitus, HbA1c, HPLC, analytical performance

Introduction

Diabetes mellitus (DM) is a chronic disease that can cause long-term degenerative complications, and it is a significant public health problem of the twenty-first century [1,2]. Diagnosis, treatment, and follow-up of diabetic patients is a global issue and uses considerable resources in laboratories and clinics worldwide [2].

Measurement of blood glycosylated hemoglobin (GHb) and especially its primary component, hemoglobin A1c (HbA1c), has been widely used in the evaluation of retrospective glycemia in patients with DM [2]. HbA1c is formed by the addition of non-enzymatic glucose molecules to the N-terminal valine residue of the beta chain of the HbA molecule and shows the average blood glucose value in the three months before measurement [1,2]. HbA1c is a valuable marker for diagnosis of diabetic patients and for monitoring the glycemic balance in diabetic patients and is therefore widely used [1,2]. HbA1c is an important predictive marker of long-term complications of diabetes. Although HbA1c is used in the diagnosis of DM, it is not the only diagnostic marker used [1,2].

Analytical methods for HbA1c measurements have been available since the 1970s [2]. In July 2009, the International Expert Committee (IEC) recommended 6.5% as the cut-off value for HbA1c in the follow-up of diabetic patients [3]. In January 2010, the American Diabetes Association (ADA) approved this decision. According to the ADA, HbA1c value in adults should be ≥6.5% if used for diagnostic criteria of diabetes, and the target in treatment follow-up should be <7% [4].

Nowadays, HbA1c can be measured with many different methods[2]. Methods of detecting HbA1c are as follows: (1) those based on charge differences between GHb and in-GHb (ion-exchange chromatography, electrophoresis, and iso-electric focusing), (2) those based on structural differences (boronate affinity chromatography and immunoassays), or (3) those found in the chemical difference (electrospray mass spectrometry). In total, more than 30 measurement methods exist [5–7], but the most common of these techniques is the affinity chromatography method. If done according to the instructions for use, the measurements

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made with these methods should be considered correct.

Because the methods measure the GHb fractions in different ways, different HbA1c values can be obtained depending on the technique used [6,8,9]. Therefore, Diabetes Control and Complication Trials (DCCT) have recommended the HPLC method as the reference method, and the National Glycohemoglobin Standardization Program (NGSP) and ADA approved this decision. The WHO and ADA also recommend the HPLC method for HbA1c measurement. At the same time, the ideal CV was accepted as <3% for HbA1c. It was then determined by IFCC that HbA1c should have a CV <2.5% [4,10].

The use of HbA1c in the long-term follow-up and diagnosis of DM requires the measurement method to have adequate diagnostic imprecision or accuracy. The results should also be comparable to other methods [2,8,9].

This study examined the compatibility of HbA1c results with two different analyzers that worked with ion-exchange chromatography in the authors' laboratory. This study aimed to compare the analytical performance of Bio-Rad D-10® Hemoglobin Analyzer and Arkray Adams HA-8160® Hemoglobin Analyzer used in HbA1c measurement.

Material and Method

HbA_{1c} measurements were determined using the Bio-Rad D-10® HPLC analyzer (Bio-Rad Laboratories, CA, USA) working with ionic exchange high-pressure liquid chromatography and the Arkray Adams HA-8160® HPLC analyzer (Arkray, Inc., Kyoto, Japan) working with reverse-phase cation exchange chromatography in our hospital biochemistry laboratory. Both HPLC analyzers are equipped with cap-piercing and onboard hemolysis systems allowing the use of a closed primary tube with whole blood. All the two HPLC analyzers are equipped with cap-piercing and on-board hemolysis systems enabling the use of a closed primary tube with whole blood. All the two systems using IFCC calibration and providing derived NGSP value were certified by the NGSP [8]. The study was declared under the number DC-2018– 223 and approved by the local ethics committee on 07.02.2019.

Analytical Performances
Imprecision and Accuracy

In the first phase of our study, low and high-level control studies were performed for Arkray analyzer 5.3%, 11.4%, and for Bio-Rad analyzer 5.4%, 9.6%, respectively. Within-run and between-day imprecision were calculated using low- and high-quality controls supplied by each manufacturer and measured on each analyzer once a day during 20 days. Mean, standard deviation and coefficient of variation (CV %) values were calculated using a total of 80 data. CV% was calculated as CV% between two different levels of quality control results for each test. CV% is the percentage ratio of the standard deviation obtained from the internal quality control data to the average. Three samples provided from Clinical Biochemistry Experts Association External Quality Control (KBUDEK) HbA1c Program (8. period) were used for evaluating accuracy. Percentage difference from the published target mean (bias%) was calculated with formula: ((measured result - mean) / mean x 100) [11,12].

Method Comparison

In the second stage of our study, 105 patients who were sent to our hospital's biochemistry laboratory for HbA1c measurement, were included in this study. Method comparison studies were performed according to EP9-A2 protocol of the National Committee for Clinical Laboratory Standards (NCCLS) published by the Clinical Laboratory Standards Institute. In this protocol, it is recommended that at least 40 data are collected for the method comparison, some of them are outside the reference range, and the study is performed at least in five days [9,13]. Under these recommendations, our study was performed in 10 days with low-level controls, high-level controls, and 105 subjects. Method comparison was determined using the venous blood samples of randomly selected from the routine laboratory with 35 subjects (≤6%), 35 subjects (6-9%) and 35 subjects (≥9%) levels of HbA1c. The venous blood samples were taken to the K2-EDTA tubes (BD Vacutainer, Plymouth, UK) after 10-12 hours of fasting. HbA1c concentrations were measured in Bio-Rad D-10® and Arkray Adams HA-8160® HPLC autoanalyzer in clinically healthy and diabetic subjects. The HbA_{1c} levels were measured in duplicate with both analyzers without waiting.

Data were analyzed using SPSS (Statistical Package for Social Science) program. The Kolmogorov-Smirnov test was used to test normality. Descriptive statistics for numerical variables are expressed as the mean ± standard deviation for normally distributed parameters and as median ± IQR (25th, 75th). The linear correlation between the two variables was analyzed by Pearson and Spearman correlation analysis in the data with a normal distribution. Variability coefficients (CV) were calculated for the reproducibility study. Linear regression and Bland-Altman graph were used to analyze the method comparison. The results were evaluated with a 95% confidence interval, and $p < 0.05$ was considered significant statistically.

Results

We took one hundred five participants with HbA1c levels between 4.8%–15.0% that had been submitted to biochemistry laboratory for analysis. The HbA1c results (median of 25th-75th) of Bio-Rad D-10® [6.70% (5.85%-9.75%)] were slightly higher than the results of the Arkray Adams HA-8160® [6.5% (5.70%-8.90%)]. When the results of the patients were compared with each other, a statistically significant difference was observed ($p < 0.05$).

Analytical Performances
Imprecision and Accuracy

In the present study for the Arkray Adams HA-8160® analyzer, within days CV% values for low (5.3%) and high (11.4%) controls were calculated as 1.22% and 0.60%, respectively, and between days CV% values for the same controls were calculated as 1.27% and 2.52%, respectively. In the Bio-Rad D-10® analyzer for low (5.4%) and high (9.6%) levels of control, within days CV% values were found as 1.23% and 0.30% and between days CV% values were 2.32% and 3.44%, respectively. Results Table 1-2 and Figure 1-2 also summarized. The within-day CV% values in all the two analyzers were below the limit of 2.5% specified by IFCC. The bias average for three months was 3.2% for the Arkray analyzer and 1.2% for the Bio-Rad analyzer.

Method Comparison

A good fit and correlation were observed in the comparison of the two analyzers. Spearman's correlation coefficient r = 0.973 between two analyzers was statistically significant (p <0.0001). In the linear regression analysis, the value of R2 was calculated 0.97 (y = 0.860x + 0.604) for all cases. In the regression equation, the calculated cutoff values of 0.60 and slope 0.86 showed the linear relationship and coherence between the two analyzers (Figure 1).

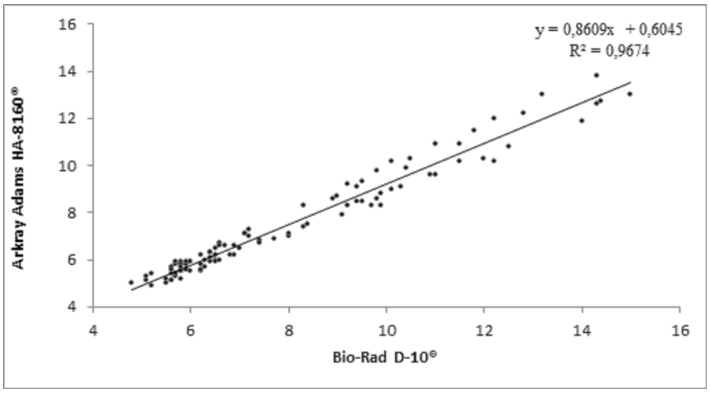

Figure 1. Regression graph, and R 2 =0.967, n=105, Arkray Adams HA-8160® = 0.860 x(Bio- Rad D-10®) + 0.604

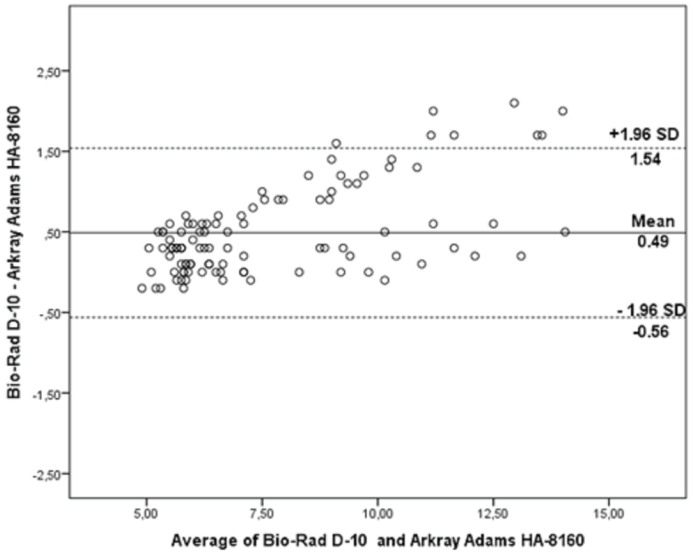

Figure 2. Bland –Altman plot of Bio-Rad D-10® against Arkray Adams HA-8160®

According to the Bland-Altman method, it is concluded that there is a harmony between these two analyzers because the average of the differences is spread around zero (Figure 2).

Table 1. Within-run imprecision of Bio-Rad D-10® and Arkray Adams HA-8160® Hemoglobin analyzers

Controls (n=20)	Bio-Rad D-10®		Arkray Adams HA-8160®	
	Level 1 (5.4%)	Level 2(9.6%)	Level 1(5.3%)	Level 2(11.4%)
Within-run Mean HbA_{1c} (%) ± SD	5.54 ± 0.068	10.19±0.03	5.3±0.06	11.34±0.06
Within-run CV(%)	1.23	0.30	1.22	0.60

Table 2. Between-day imprecision of Bio-Rad D-10® and Arkray Adams HA-8160® Hemoglobin analyzers

Controls (n=20)	Bio-Rad D-10®		Arkray Adams HA-8160®	
	Level 1 (5.4%)	Level 2 (9.6%)	Level 1 (5.3%)	Level 2(11.4%)
Between-day Mean HbA_{1c} (%) ± SD	5.66±0.13	10.67±0.37	5.36±0.068	11.23±0.28
Between-day CV(%)	2.32	3.44	1.27	2.52

Discussion

HbA_{1c} measurement, which gives information about the total glucose value in plasma retrospectively, is required for the follow-up of long-term glucose control in DM patients[14]. HbA1c is considered the gold standard for monitoring diabetes, but the current guidelines of the American Diabetes Association (ADA) recommend using it as a diagnostic tool for diabetes [15]. A small systematic error in the measurement of HbA1c is significant in the interpretation of HbA_{1c} because the clinical decision limit HbA1c for diabetes diagnosis (6.5% in NGSP units) is close to the upper limit of the non-diabetic reference range (6.0%) [16]. Because the HbA1c measurement is recommended for use in the long-term follow-up of diabetes and the diagnosis of diabetes, the HbA1c measurement method must have sufficient diagnostic imprecision and accuracy, and it is important that the results should be comparable with other methods[17].

Many methods have been used for the determination of HbA1c, but in recent years, great efforts have been made to standardize the detection of HbA1c by the National Glycohemoglobin Standardization Program (NGSP) and International Clinical Chemistry and Laboratory Medicine (IFCC), as different methods show different characteristics and performances. Standardization studies of HbA1c measurement methods at an international level continue. One benefit of standardization is the possibility that HbA1c will have interchangeable methods without affecting the results[18]. It has been shown that other methods, as well as HPLC systems, are acceptable but HPLC analyzers have superior analytical performance. The study of HbA1c in the HPLC system is important because of its high specificity and sensitivity as well as the usefulness of detecting abnormal hemoglobin in the HPLC system. Bio-Rad D-10® and Arkray Adams HA-8160® HbA1c analyzers are based on ion-exchange HPLC method which is certified by IFCC There are three accepted methods for the HbA1c in the Joint Committee for Traceability in Laboratory Medicine (JCTLM) database. These are NGSP-HPLC, IFCC mass spectrometry / capillary electrophoresis (MS/CE) and liquid chromatography-isotope dilution-mass spectrometry (LC-ID-MS) methods [19,20].

We evaluated the analytical performance of Arkray Adams HA-8160® HbA1c analyzer currently used in our laboratory which is based on HPLC aiming for a CV of < 2.5% as specified by the IFCC working group for HbA1c standardization. We have also compared this analyzer with before used Bio-Rad D-10® in our laboratory. Within-run coefficient of variation (CV) for low and high HbA1c levels were ≤1.23 % for all the two systems tested. The between days CV% values for little control in all two analyzers were below the limit of 2.5% specified by IFCC. Data from the imprecision study were summarised in Table 1 and Table 2, respectively. İn this study, the bias average for three months was 3.2% for the Arkray

analyzer and 1.2% for the Bio-Rad analyzer. In our study, the median (25th-75th) values were found 6.70% (5.85% -9.75%) and 6.5% (5.70% -8.9%) as a result of the comparison of the effects of Bio-Rad D-10® and Arkray Adams HA-8160® analyzers.

According to the NCCLS EP9-A2 protocol, it is recommended to perform a regression analysis for the correlation between the analyzers. Thus, the relationship between analyzers is expressed by equation [9,13]. According to the results of this study; The regression standard error (Sy/x, the ideal is near 0) 0.41 and the correlation analysis r = 0.973 value obtained by the analyzers are compatible with each other. The fact that the change in any of the two analyzers examined can be explained by the other analyzer is also evaluated by the coefficient of determination (R^2). As this coefficient approaches 1, the suitability increases. In our regression analysis, Arkray Adams HA-8160® showed good correlation with Bio-Rad D-10® with Arkray Adams HA-8160® = 0.860 Bio-Rad D-10+ 0.604 (R^2=0.967). Spearman correlation coefficient was r = 0.973 ($p < 0.0001$) between two measurements. In this case, there is linearity of 86% between the two analyzers examined. Based on this result, it can be shown that there are two alternative analyzers.

Roth et al. Despite their study with IFCC-approved HbA_{1c} methods, they found up to 0.5 % difference between 4 laboratories [21]. This difference was clinically significant and reported that it could lead to misdiagnosis. In our study, two methods were correlated, and these studies were conducted in the same laboratory.

For the method comparison studies, according to NCCLS protocols, it is not necessary for interference studies, but the lack of hemoglobin variants and interference studies is a missing aspect of our research. Hemoglobin (Hb) variants can interfere with HbA_{1c} testing and may lead to misdiagnosis or inappropriate treatment [22]. In this study, we found the correlation between the analyzers appropriate; this result suggested that the interference is low.

Conclusions

As a result, both HPLC analyzers are reproducible and accurate for routine biochemistry laboratory use. Although the HbA1c measurements obtained from two different analyzers using two same methods are compatible, accurate and reliable, it is appropriate for laboratories to select HPLC method for routine use, as well as for interferences such as variant Hbs, in addition to factors such as speed and cost concluded.

Conflict of interest
The authors declare that there are no conflicts of interest.

Financial Disclosure
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Ethical approval
The study protocol has approved from local ethic committee

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