HbA2 düzeylerinin ölçümünde Lifotronic H9 ve Primus Ultra2 HPLC sistemlerinin karşılaştırılması

Comparison of the Lifotronic H9 and Primus Ultra2 HPLC systems for the detection of HbA2

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ÖZET

Amaç: Beta talasemiler dünya çapında sık görülen otozomal resesif kalıtılan hemoglobinopatilerdir. Beta talasemi taşıyıcılığının belirlenmesinde kullanılan en önemli parametre hemoglobin A2 (HbA2) düzeyleridir. HbA2 ölçümü için en sık kullanılan yöntemlerden biri yüksek performanslı likit kromatografidir (HPLC). Çalışmamızda Lifotronic H9 ve Primus Ultra2 marka HPLC cihazlarının HbA2 ölçüm düzeylerini karşılaştırmayı amaçladık.

Gereç ve Yöntem: Çalışmamıza 19 kadın 32 erkek olmak üzere 51 kişi dahil edildi. Cihazların uyumu Bland-Altman yöntemi ile ortaya koyuldu. Ayrıca iki metodun karşılaştırılması için Passing Bablok regresyon analizi de uygulandı. Regresyon doğrusallığının değerlendirilmesinde, kümülatif toplam (CUSUM) testi kullanıldı.

Bulgular: Bland-Altman analiziyle elde edilen grafik incelendiğinde sonuçların geometrik ortalamasının 0,95 ve %95 güven aralığınında 0,8855 to 1,0090 arasında olduğu gösterildi. Passing-Bablok regresyon analizi sonuçları incelendiğinde oluşturulan model, Y (Lifotronic H9)= 0,52 + 0,8 X (Primus Ultra2) olarak bulundu.

Sonuç: Yeni kullanıma giren Lifotronic H9 cihazının HbA2 ölçümlerindeki performansı Primus Ultra2 ile kıyaslanarak gösterildi.

Anahtar kelimeler: Beta Talasemi; Hemoglobinopati; Yüksek Performanslı Sıvı Kromatografisi; Yöntem Karşılaştırması; HbA2; Lifotronic H9

ABSTRACT

Aim: Beta thalassemias are common autosomal recessive inherited hemoglobinopathy worldwide. Hemoglobin A2 levels are the most important parameter used to determine beta-thalassemia carriers. One of the most commonly used methods for HbA2 measurement is high-performance liquid chromatography (HPLC). We aimed to compare the HbA2 levels of Lifotronic H9 and Primus Ultra2 HPLC systems.

Material and Methods: A total of 51 individuals, 19 females and 32 males were included in our study. The compatibility of the results was demonstrated by the Bland-Altman plot and Passing-Bablok regression analysis.

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Results: The geometric mean ratio of values was 0.95 (95% limits of agreement 0.6 to 1.49) in the Bland-Altman plot. 95% confidence intervals were observed as -1.03 - 1.30 (intercept) and 0.55-1.33 (slope) in Passing-Bablok regression analysis.

Conclusion: We reported first time measuring compatibility of Lifotronic H9 HPLC system in terms of HbA2 levels by using Primus Ultra2.

Keywords: Beta Thalassemia; Hemoglobinopathy; HbA2; HPLC; Lifotronic H9; Method Comparison

INTRODUCTION

The hemoglobin molecule has a tetramer structure formed by the combination of four globin chains. Hemoglobinopathies affects normal hemoglobin structure and cause a family of diseases. Beta thalassemias are inherited autosomal recessive beta chain defects in this family (1). Major and intermedia forms of beta-thalassemia have a heavy-course clinic. The carrier form, which has a mild clinic course and only noticed by special tests by screening, is important for the hereditary transition of the disease (1). In the diagnosis of a hemoglobinopathy, hemoglobin subgroups should be determined. One of the most commonly used methods for this purpose is the high-performance liquid chromatography (HPLC) method. Hemoglobin A2 (HbA2) is the most important parameter that used in beta-thalassemia screenings (2).

In hemoglobinopathy screenings, family history, the examination of complete blood count indices, and HbA2 measurement are generally considered together. Typically, HbA2 levels are elevated in beta thalassemia carriers. Besides this result, thalassemia carriage can be determined by evaluating the erythrocyte indices. However, the slight increase or borderline values of HbA2 in mild beta-thalassemia mutations as well as other accompanying gene defects make it difficult to evaluate. In such cases, the final diagnosis of hemoglobinopathies is possible only by genetic testing (3).

HbA2 analysis in HPLC is based on the principle of separating hemoglobin subtypes from each other according to their molecular properties by injecting samples treated with special solutions onto the column (4). So HPLC method has also abilities to diagnose other hemoglobinopathies.

Nowadays, both developments of technology and the widespread use of health services have caused to raise interests in Hb testing systems. The existence of a wide range of systems needs comparison studies. Therefore, we aimed to compare HbA2 levels of HPLC devices from Lifotronic H9 (Lifotronic Technology, Shenzhen, China), which is a relatively new use, and Primus Ultra2 (Primus Corporation, Kansas, USA).

MATERIAL AND METHODS

A total of 51 individuals, 19 females and 32 males were included in our study. Venous blood taken from these individuals was taken into EDTA (Ethylene Diamine Tetra Acetic Acid) blood tubes. Samples were stored at 2-8 ° C until analysis. Samples were run once in both systems and results were recorded. Since both methods have automated dilution and hemolysis steps, any pretreatment procedure wasn't applied. For accuracy and precision requirements, internal control and calibration studies were performed at certain intervals or when necessary.

In these systems, Hb subgroups are separated from each other in accordance with ion-exchange chromatography method and reported via chromatograms. The peaks formed in the Lifotronic H9 system can be named in the output as HbA1a, HbA1b, HbF, LA1c, HbA1c, P3, P4, HbA0, HbA2, HbE, D, S, and C (5). In the Primus Ultra2 system, the resulting peaks are classified as F, A, S, and C, and the variants matching within the database are indicated. The Primus Ultra2 system also has two modes, quick scan, and high-resolution mode. Only quick scan mode was used in our study.

Before the study, approval was received from the local ethic committee (October 2020 and 153 session number).

Statistical Analysis

The results were statistically analyzed using SPSS v22 (IBM, NY, USA) and MedCalc v18 (Ostend, Belgium). The normality for distribution of continuous variables was tested with the Kolmogorov-Smirnov test. The HbA2 cut-off value was considered as 3.5% to determine beta-thalassemia carriers. The determination rates were evaluated by McNamer and Cohen's Kappa tests. The Wilcoxon test was used to determine the difference in HbA2 measurements. Intraclass, and concordance correlation coefficients and Spearman test were used to determine the correlations.

The limit of agreement (LoA) was determined with Bland Altman plot. Since there wasn't any reference method for HbA2 analysis yet, the means of HbA2 results were used for comparison. Also, Passing Bablok regression analysis was applied for the comparison. Cumulative sum test (CUSUM) was used to evaluate regression linearity. The statistical significance level of alpha was generally accepted as $p < 0.05$.

RESULTS

5 (9.8%) and 7 (13.7%) individuals in Lifotronic H9 and Primus Ultra2, respectively, were detected as beta-thalassemia carriers. According McNemar test, there was no significant difference between the rates of finding thalassemia carriers ($p=0.5$). In Cohen's Kappa test, the compatibility between results was found statistically significant ($p < 0.001$; $\kappa = 0.812$).

Additionally, there was also no significant difference in Wilcoxon test (z: -1.616, $p > 0.05$) (as shown in Table 1). The intraclass correlation (two-way random) coefficient of the measurements was 0.740, and the concordance' was 0.567. So on, it was found 0.303 in Spearman analysis ($p < 0.05$).

If there is no statistical relationship between the differences and averages, and if the differences distribute approximately normally, LoA can be examined by using the Bland-Altman method (6). As a result, it was seen that the differences distribute normally, but the differences and averages were related to each other. Therefore, it was considered appropriate to place the ratios of measurements on y-axis while creating the Bland-Altman plot (6). The geometric mean ratio of values was 0.95 (LoA: 0.6-1.49) (Figure 1.). The 95% confidence interval (0.8855-1.0090) contains 1 value for a plot using ratios. So it indicates compatibility.

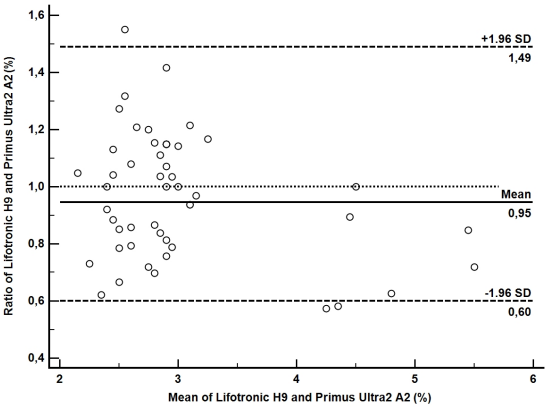

Figure 1. Ratio of HbA2 measurements plotted against their average (Bland-Altman difference plot). The geometric mean ratio of values by Lifotronic H9 and Primus Ultra2 was 0.95 with 95% limits of agreement (0.6 to 1.49) relating to the ratios of measurements by the two methods.

The Passing-Bablok regression analysis was also performed to evaluate the relationship between the methods (Figure 2a). According to the results of the CUSUM test conducted to investigate the linearity assumption, it was found that there was no deviation from linearity ($p > 0.05$) (Table 2). In Passing-Bablok regression analysis, the regression equation was found as Y (Lifotronic H9) = 0.52 + 0.8 X (Primus Ultra2) (Figure 2a). In addition, 95% confidence intervals were observed as -1.03 - 1.30 (intercept) and 0.55-1.33 (slope), respectively (Table 2). As can be seen, the confidence intervals include the numbers 0 (for intercept) and 1 (for slope). Since there is no accepted reference measurement yet, the means (Hb mean) were used for X-axis (target value) for bias calculation (Figure 2b-2c) (7). 3.6% was deemed appropriate as the decision limit in the bias calculation (3). The bias values for Lifotronic H9 and Primus Ultra2 were 1.96% and 7.2%, respectively (Table 2).

Table 1. Statistical data for HbA2 measurements

Method	N	Minimum	Maximum	Mean	SD	Median	95% CI (median)	Interquartile range	p value
Lifotronic H9	51	1,8	5	2,906	0,6491	2,9	2,600 to 3,000	2,425 to 3,100	0.106*
Primus Ultra2	51	2	6,4	3,129	1,022	2,8	2,700 to 3,000	2,600 to 3,200	

* Wilcoxon test (paired samples) p<0.05, N: Number of samples, SD: Standard Deviation, CI: Confidence Interval

Table 2. Passing-Bablok regression analysis results

Comparison Group	Passing-Bablok regression analysis					
	Bias (%)**	Slope (95% CI*)	Intercept (95% CI*)	R (correlation coefficient)	p value (CUSUM test)	Figure
Lifotronic H9 vs Primus Ultra2		0,8 (0,50 to 1,34)	0,52 (-1,18 to 1,33)	0,303	0,68	a.
Lifotronic H9 vs Hb mean	1,96	1,14 (0,88 to 1,54)	-0,44 (-1,57 to 0,29)	0,838	0,14	b.
Primus Ultra2 vs Hb mean	7,2	1,37 (1,17 to 1,66)	-1,06 (-1,88 to -0,47)	0,729	0,89	c.

* CI: Confidence Interval, ** Bias at specific decision level (for HbA2: 3,6%), Hb mean: average of HbA2 measurement results

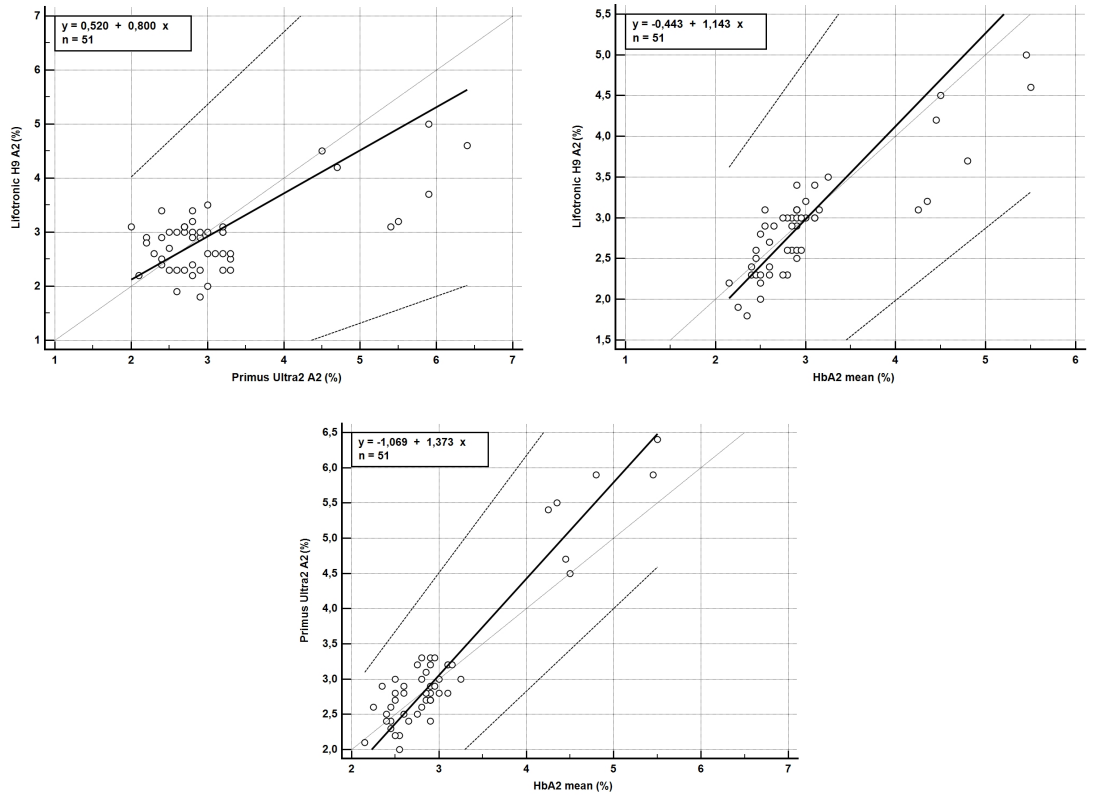

Figure 2. a. Passing-Bablok regression plot for Lifotronic H9 vs Primus Ultra2 HbA2 (%) levels. **b.** Passing-Bablok regression plot for Lifotronic H9 vs HbA2 mean (%) levels **c.** Passing-Bablok regression plot for Primus Ultra2 vs HbA2 mean (%) levels

DISCUSSION

In method comparison studies, it is aimed to investigate the results that can be used interchangeably. Even if all conditions are kept constant, it is difficult to obtain the same results from samples that are repeated or studied with different methods. However, the compatibility between these results can be tested. Although classical correlation studies came to mind first for this purpose, they can give misleading information. Because, samples with a broad distribution show higher correlations than those with a narrower distribution (6,8). For example, HbA2 levels on which our study is based have a distribution between 1.5 to 3.5%, while glucose levels have a broader distribution such as 70 - 110 mg/dl. This is a disadvantage for correlation calculations in HbA2 comparison studies. However, finding a correlation in samples with a large distribution width may overlook the mismatch caused by measurement differences. While there is no random error between the measurements, there may be a proportional error that the correlation coefficient cannot find. Moreover, using least-squares regression analysis in method comparisons can cause false results due to measurement errors (9). So it may be nonuseful. The method developed by Bland-Altman, which uses LoA and includes visual graphics, has been used frequently in method comparison studies (8,10). Besides, Passing-Bablok plots, one of the nonparametric regression analysis, is used in method comparison studies because it is less affected by the distribution width and outliers (11,12). For the narrow distribution width in our study, the correlation values between the methods may have been found to be low. Moreover, when LoA was evaluated in the Bland-Altman plot, it was observed that there was an compatibility between the two methods. Also, the relationship and systematic bias were evaluated using the Passing-Bablok analysis, and the two methods were found to be compatible (Figure 2a).

Bias calculations are one of the important factors in the research of new methods. In a study conducted in 2013, the acceptable bias value for HbA2 measurements was reported as 2.9% by Mosca et al. (13). Accordingly, one system (1.96%) meets this criterion, while the other one (7.2%) cannot, in this situation. However, there is not any internationally accepted reference method that can be used in comparisons. So it is hard to calculate an accurate bias. Another limitation of our study is HbF, as it is known, the other parameter used in beta thalassemia screening. It could have been better if it was included in the comparison.

Furthermore, there are a lot of factors affecting HbA2 analysis such as incorrect calibration, presence of abnormal Hb variants, and sample-to-sample contamination (14). However, only the optimization of the analysis phase is not enough. Preanalytical factors and post-analytical factors such as temperature and waiting of samples may affect the accuracy.

HPLC systems are often used for thalassemia screenings worldwide. The measurement of HbA2 levels is the most important issue for these screenings. At the state-of-art, many systems were developed for this purpose. As a result, we reported first time measuring compatibility of Lifotronic H9 HPLC system in terms of HbA2 levels by using Primus Ultra2.

Conflict of interest

None of the authors received any type of financial support that could be considered potential conflict of interest regarding the manuscript or its submission.

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