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The Analytical Performances of Four Different Glycated Hemoglobin Methods

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Abstract

Objectives: The analytical performances of the Sebia capillary electrophoresis (CE), Roche turbidimetric inhibition immunoassay (TINIA), Tosoh G8 cation-exchange high-performance liquid chromatography (HPLC) and Premier boronate affinity chromatography methods were evaluated. Capillary electrophoresis (CE) was accepted as a comparative method.

Design and Methods: This study comprised randomly chosen 224 whole blood samples from the diabetic and non-diabetic patients. HbA1c level was quantified using four methods as follows: Roche TINIA, Premier Hb9210 boronate affinity chromatography, Tosoh G8 cation-exchange HPLC and Sebia CE. The analytical performances of the methods were evaluated with imprecion, bias estimation and comparison studies.

Results: The results of all precision studies CV% were under 2.0%. The accepted goals for imprecision are 2.8% (IFCC) and <2.0% (NGSP). Analysis using Spearman test showed good correlation between CE and all three evaluated methods (r=0.99, p=0.001; r=0.98, p=0.001; and r=0.98, p=0.001, respectively). The comparison of the methods with CE was performed using Deming Regression analysis and revealed good agreement between CE and all methods. Although the HPLC method showed a linear relation with CE, it differed significantly from the comparative method because of its confidence interval did not contain 0.

Conclusion: All methods revealed acceptable precision and good accuracy. TINIA and boronate affinity chromatography methods showed good agreement and correlation with the comparative method (CE), whereas a significant difference was obtained between the mean levels of HPLC and CE.

Keywords: Hemoglobin A1c; Electrophoresis; Capillary; Chromatography; High-performance liquid chromatography; Boronate affinity

Introduction

Hemoglobin A1c (HbA1c) assay is accepted as the most useful marker to determine the long-term glycemic control of diabetic patients. This marker has also been recommended for the diagnosis of diabetes mellitus when HbA1c levels are above 48 mmol/mol (6.5%) [1]. The relation between HbA1c levels and diabetic complications has been evaluated by the studies of Diabetes Control and Complications Trial Research Group (DCCT). According to their observations, a 1% decrement in HbA1c level complies with an approximate 30% reduction in developing risk of diabetic complications [2].

Accurate HbA1c results are essential for monitoring and appropriate treatment of diabetic patients. Nowadays, the methods for measuring HbA1c are classified into 3 groups: ion-exchange chromatography electrophoresis and isoelectric focusing which based on charge differences between glycated Hb and non-glycated Hb [2], affinity chromatography and immunoassay which based on structural differences of glycogroups on hemoglobin [3,4] and photometry and electrospray mass spectrometry in which separation is based on chemical reactivity. Many factors may interfere with the HbA1c results causing falsely high or low results depending on the assay methods. While cation exchange methods are not affected from the interference by Schiff base or carbamylated haemoglobin, it may be influenced from hemoglobin variants. Similar interference may also be observed in electrophoretic methods. Boronate affinity measures total glycated hemoglobin consisting of HbA1c and other Hb adducts regardless of charge, and provides very good precision and accuracy [5]. Capillary electrophoresis (CE) and electrospray mass spectrometry were introduced as the candidate reference methods by International Federation of Clinical Chemistry (IFCC) in 2001. This method is based on enzymatic cleavage of N-terminal hexapeptides from the β-chain, then glycated and non-glycated hexapeptides are measured by HPLC/mass spectrometry or HPLC/capillary electrophoresis. Interferences caused by the Hb variants and derivatives in the capillary electrophoresis are less seen than those in the ion-exchange chromatographic methods [6]. The reported stability, reproducibility, and repeatability of this analytical system were very good [5].

In the Clinical Laboratory of Istanbul Faculty of Medicine which has a very high workflow, an establishment of the accurate, timely and cost effective method with a high precision is essential. Therefore, attempts have been made to evaluate the analytical performances of Roche Tina-quant 3rd generation HbA1c assays based on immunoturbidimetry, Tosoh G8 cation-exchange HPLC and Premier boronate affinity chromatography methods. The Sebia capillary electrophoresis was used as the comparative method in our study.

Materials and Methods

Subjects and samples

This study comprised 224 whole blood samples with a median age of 51 (Range 20-83) randomly chosen from the subjects who were applied to Clinical Biochemistry Laboratory of Istanbul Faculty of Medicine between June to July 2011, for either routine testing or the

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control of the diabetic status. No further selection criteria were used. HbA1c values ranged from 21.3 to122 mmol/mol (4.1% to 13.3%). Of the 224 subjects, 115 (51.3%) were women.

Blood samples were obtained through venipuncture into 2.0 mL BD Vacutainer®, Hemogard tubes with K_2-EDTA (Becton Dickinson, Plymouth, UK) and leftover samples were used to measure the HbA1c levels. All samples were kept +4°C until studied. HbA1c levels were measured with four different methods and assays were completed within four hours following blood sampling. This study was approved by the Ethics Committee of Istanbul University and informed consent forms were obtained from each subject prior to the study (B.08.06. YOK.2.İ.Ü. E.50.0.05.00/23).

HbA1c methods

HbA1c levels from the samples of 224 subjects were determined simultaneously using four commercially available methods. These included the turbidimetric inhibition immunoassay (TINIA) by using Roche E170 autoanalyzer (Roche, Mannheim, Germany), boronate affinity chromatography by Premier Hb9210 (Trinity Biotech, Wicklow, Ireland), cation-exchange high-performance liquid chromatography (HPLC) by Tosoh G8 (Bioscience, San Francisco, USA), and capillary zone electrophoresis with automated Sebia capillary electrophoresis (Sebia, Norcross, USA). All these methods were carried out according to the manufacturer's instructions and certified by the National Glycohemoglobin Standardization Program (NGSP). Sebia capillary zone electrophoresis was used as the comparative method.

Precision studies

Whole blood sample pools which comprised twenty patients' samples at low (33.3 mmol/mol, 5.2 ± 0.05%); and high levels (102.2 mmol/mol, 11.5 ± 0.06%) were used to determine the assay precision. The two-level pools were studied 20 times for within-run and 20 times for between-day precisions on consecutive days, followed by the calculation of variation coefficient (CV%) and standard deviations (SDs).

Statistical analysis

The coefficient of variation (CV) was expressed as the ratio of standard deviation to mean. Statistical analyses were performed using SPSS 15.0 software (SPSS Inc. Chicago, IL, USA). The Spearman correlation analyses were applied for the initial comparison of the methods. Two stage linear regression analysis, Bland Altman and Deming Regression analysis were performed for bias estimation and the method comparisons. These analyses were done by using MedCalc 12.7.2 software (Ostend, Belgium). $P<0.05$ was considered statistically significant.

Results

The findings of precision study carried on the sample pools with low and high levels of HbA1c are shown in Table 1. For high pool (mean value:102.2 mmol/mol, 11.5 ± 0.06%); TINIA assay had within-run CV 0.7% and between–run CV 0.6%, cation-exchange HPLC method had within-run CV 1.8% and between-run CV 2.0%, and affinity chromatography had within-run CV 0.4% and between-run CV 0.1%. Capillary electrophoresis revealed within-run CV 0.8% and between-run CV 0.8%. For the low control (mean value:33. 3mmol/mol,5.2 ± 0.05%); TINIA assay had within-run CV 1.6% and between–run CV 1.6%, HPLC method had within-run CV 1.1% and between-run CV 1.8%, and affinity chromatography had within-run CV 0.5% and between-run CV 0.2%, capillary electrophoresis had within-run CV 1.1% and between-run CV 0.3%. Overall, the results of all precision studies (within-run and between-days) were well below 2.0% CV. The accepted goals for imprecision are 2.8% (IFCC) and <2.0% (NGSP) [7,8].

Initial comparison results of four methods using Spearman test showed good correlation between capillary electrophoresis and cation-exchange HPLC, boronate affinity and TINIA methods (r=0.99, r=0.98, r=0.98, respectively; p=0.001).

The Bland Altman plots of differences between the capillary electrophoresis and other methods are shown in Figure 1 (a,b,c). The mean difference (± 2SD) was -0.25% (-0.67 -0.17) for TINIA, -0.03% (-0.42-0.35) for boronate affinity and, 0.07 (-0.26-0.40) for cation-exchange HPLC method. The mean biases which are measured between the methods were lower than 2.0% as specified by IFCC (<1.1% for NGSP) [7,8].

Data of method comparisons obtained by a set of whole blood samples (n=224) are summarized in Figure 2 (a,b,c), and Table 2. When the Deming regression analysis was evaluated between capillary electrophoresis and other methods, good agreement was observed between capillary electrophoresis and TINIA (y=1.03x+0.016; CI=-0.14 to 0.1821, r=0.9925) and also between capillary electrophoresis and boronate affinity chromatography (y=1.0x+1, 11855, CI=0,0000 to 0,2981, r=0.9974). All values obtained were found to lie in the 95% confidence interval. Although the HPLC method showed a linear relation with CE(y=1,063x -0,52+, CI=-0.6338 – (-0.4625, r=0.9934), a significant difference was obtained between the means of HPLC and comparative methods because of its confidence interval did not contain 0.

Discussion

Overall precision and bias of the methods, the correlation with the reference method and interlaboratory agreement of the results are necessary steps in order to maintain optimum care of the patients and to provide the quality of clinical trials. In our study, the analytical performance of boronate affinity, cation-exchange HPLC and TINIA HbA1c methods were evaluated comparing with CE. The methods indicated a good precision and accuracy. The TINIA and boronate affinity showed good agreement with CE by showing a narrow dispersion around the regression lines. Although the HPLC method showed a linear relation with CE, a significant difference was obtained between the mean levels of HPLC and CE. Because its confidence interval did not contain 0 (CI; 0.63 to -0.4625).

The precision studies revealed within-run CVs lower than 1.7% and between-run CVs lower than 2.0%. This findings are in good agreement with the goals of NGSP (<2.0%) and IFCC (<2.8%) [8].

A previous study evaluating the analytical performances of CE reported intra- and inter-assay CVs were 1.62% and 1.45%, and these findings are in good agreement with our findings, also good agreement

	High pool		Low pool	
Methods	**Within-run CV%**	**Between-day CV%**	**Within-run CV%**	**Between-day CV%**
TINIA	0.7	0.6	1.6	1.6
Cation-exchange HPLC	1.8	2.0	1.1	1.8
	0.4	0.1	0.5	0.2
Capillary electrophoresis	0.8	0.8	1.1	0.3

Table 1: Within-run and between day coefficients of variation (CV%) of HbA1c levels measured by the four methods using whole blood sample pool at low (33. 3 mmol/mol, 5.2 ± 0.05%) and high level (102.2 mmol/mol, 11.5 ± 0.06%).

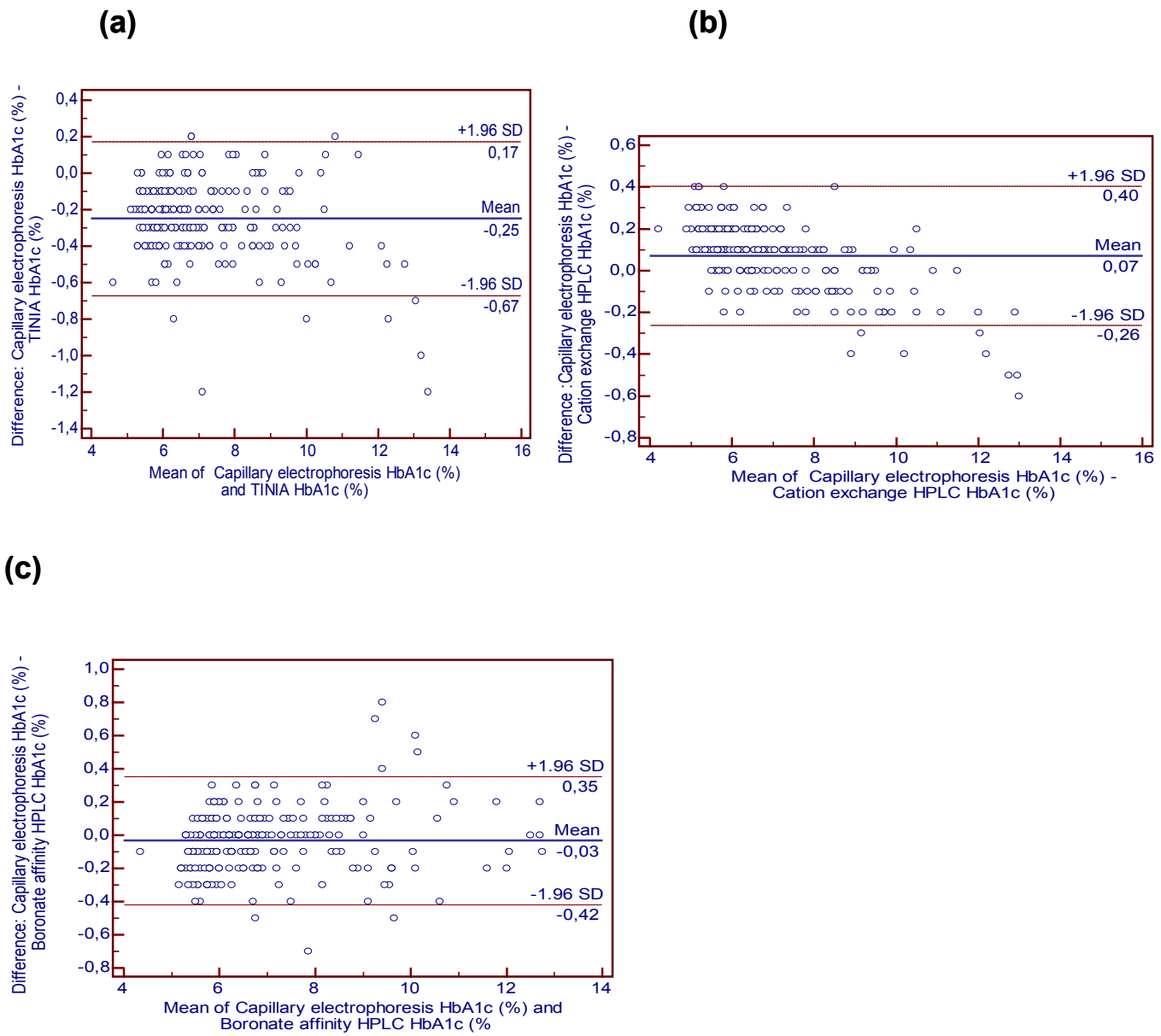
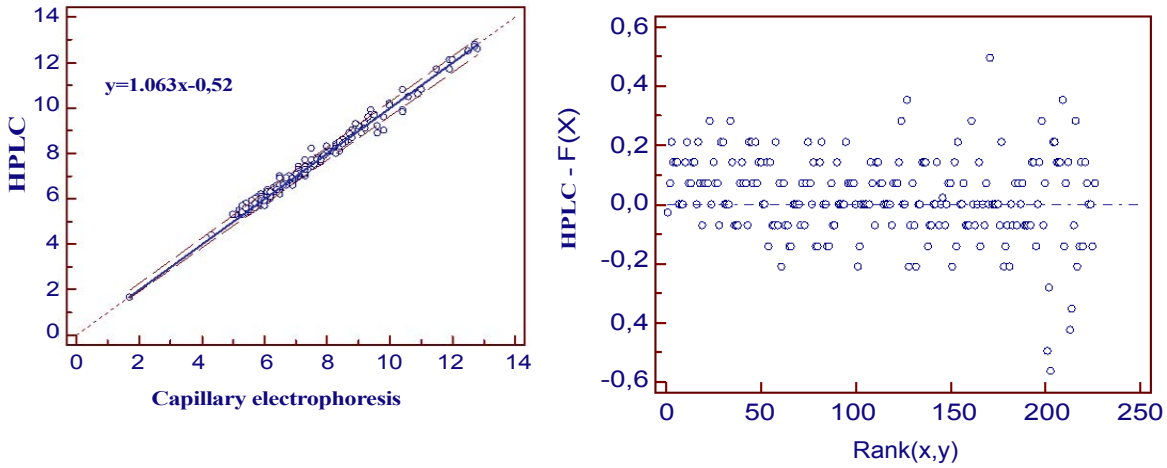


Figure 1: Bland-Altman plot of difference in HbA1c levels analyzed by **1a)** TINIA and Capillary electrophoresis. **1b**) Cation Exchange HPLC and Capillary electrophoresis. **1c)** Boronate affinity HPLC and Capillary electrophoresis

was obtained between Variant II HPLC method and CE method with no interfering effect of fetal hemoglobin, labile HbA1c, and high urea concentration in this study [9]. However, another study, the CV% of Variant II HPLC and Roche Integra 800 with TINIA were reported higher than 2.0% for low control and the bias % for Variant II and Tosoh G8 were found -4.9% and 3.9, respectively [10]. In our study, estimated mean difference of the methods from CE were found very small; -0.25 % (-0.67 - 0.17) for TINIA, -0.03 % (-0.42- 0.35) for boronate affinity and, 0.07 % (-0.26-0.40) for cation- exchange HPLC method. Allowable bias for HbA1c was suggested by Rohlfing in 2008 as a ≤ ± 1.5% desirable and a ≤ ± 0.8 % optimal [11]. The values obtained in our study were at desirable level. In our previous study included 2917 subjects, second generation TINIA assay was well associated with the HPLC by Arkray Adams HA-8160, mean bias was 0.19% [12]. In the study of Abadie et al. which included 80 patients with HbS or HbC, the second generation TINIA assay exhibited a better agreement with HPLC method than first generation TINIA assay [13]. In another study, no interferences were seen due to the presence of HbD and HbE traits in the immunoassay, enzymatic, boronate affinity or capillary electrophoresis whereas a significant interference occurred in some ion-exchange methods [14].

In this study, four current methods for the HbA1c measurement were evaluated, and all methods revealed acceptable precision and good accuracy. TINIA and boronate affinity chromatography methods showed good agreement and correlation with the comparative method (CE), while a significant constant difference was obtained between the mean level of the HPLC and CE methods. The size and characteristics of our study population were not suitable for an investigation of possible interference of hemoglobin variants and various metabolites such as urea and triglycerides on HbA1c levels. Further studies by the interference analysis are needed to examine the effect of such factors on the glycated hemoglobin measurements.

2a.



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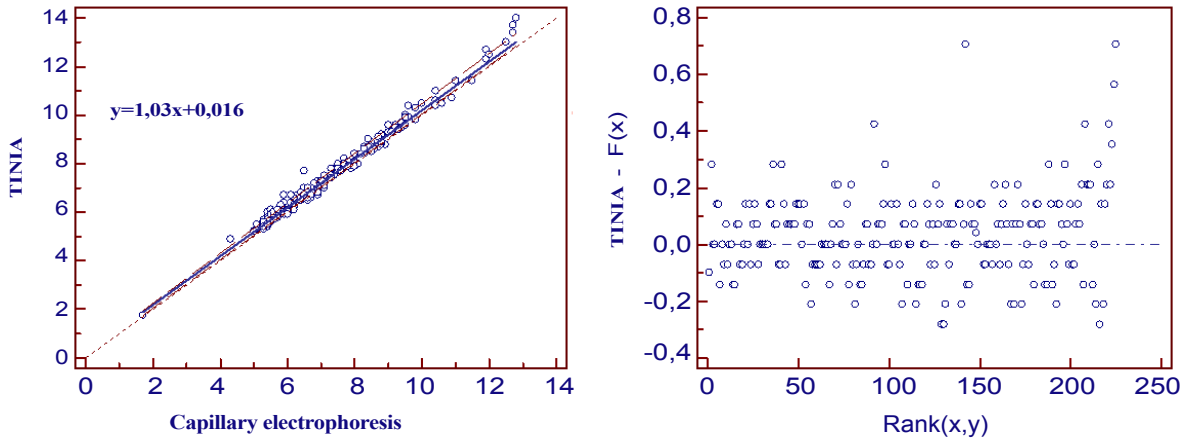

2c.

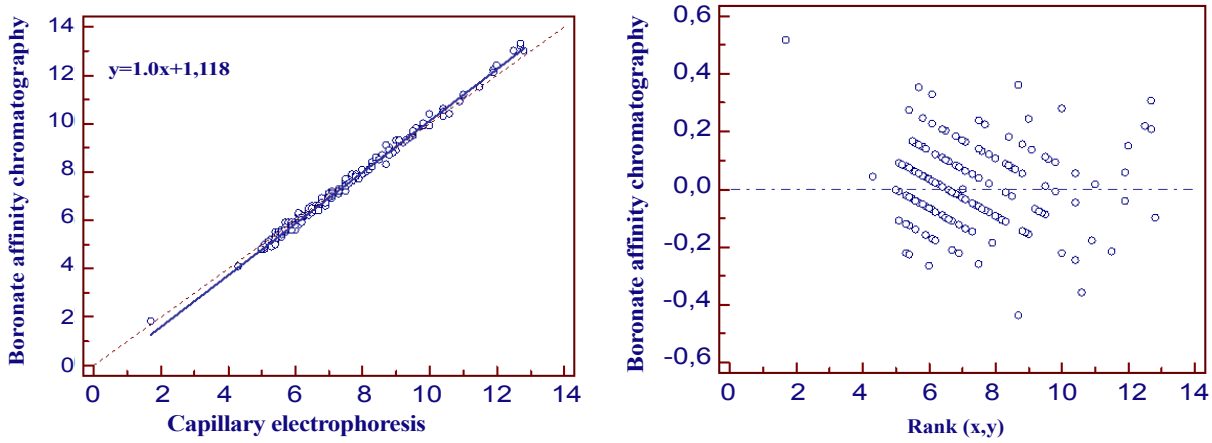

Figure 2: Comparison of the results of HbA1c methods **2a)** TINIA vs Capillary electrophoresis. **2b**) Boronate affinity HPLC vs Capillary electrophoresis **2c)** Cation Exchange HPLC vs Capillary electrophoresis.

Methods	Bias %	Slope	Intercept	SE	95 CI %	r
CE- TINIA	-0.25 -0.67 -0.17	1.03	0.016	0.089	-0.14 - 0.1821	0.9925
CE–HPLC	0.07 -0.26-0.40	1.063	-0.52	0.048	-0.63-(-0.46)	0.9934
CE- Affinity chromatography	-0.03 - 0.42-0.35	1.00	1.1185	0.065	0.0 -0.29	0.9974

Table 2: Comparison of capillary electrophoresis with TINIA, boronate affinity chromatogram and cation-exchange HPLC method: Individual results for the method-comparison studies (n=224).

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