

Hemoglobin A1c Measurement Using Point of Care Testing

Hasta Başı Test Cihazları Kullanarak Hemoglobin A1c Ölçümü

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ABSTRACT

Introduction: Glycated hemoglobin is a sensitive predictor of the long-term complications of diabetes. Hemoglobin A1c (HbA1c) measurements can be performed using point of care testing (POCT) devices, and comparing POCT devices with high-performance liquid chromatography (HPLC) is essential. This study aimed to compare the HbA1c results of POCT devices with HPLC in diabetic patients with and without hemoglobinopathy.

Methods: Twenty-six diabetic patients with hemoglobinopathy and 51 diabetic patients without hemoglobinopathy are included in this study. HbA1c analyzes were performed using Tri-stat POCT analyzer (Trinity Biotech, Ireland) from venous blood and capillary blood, Premier Hb 9210 (Trinity Biotech, Ireland) HPLC method only from venous blood of patients.

Results: HbA1c levels did not differ statistically in patients without hemoglobinopathy between HPLC and capillary blood POCT, HPLC and venous blood POCT, capillary blood POCT, and venous blood POCT, $p=0.392$, $p=0.167$, and $p=0.288$, respectively. HbA1c levels also did not differ statistically in patients with hemoglobinopathy between HPLC and venous blood POCT ($p=0.076$).

Conclusion: Tri-stat POCT analyzer results did not differ statistically in comparison to HPLC results in patients. POCT devices approved by the ministry and accepted by the authorities can be used safely in HbA1c analyzes. Clinicians may prefer both methods in patients with type 2 diabetes who need close follow-up, and whose treatment regimen will be affected by this follow-up.

Keywords: HbA1c, HPLC, POCT, hemoglobinopathy

ÖZ

Amaç: Hemoglobin A1c (HbA1c) diyabetin uzun dönem komplikasyonlarını hassas olarak belirlemektedir. HbA1c ölçümleri hasta başı test cihazları (HBTC) ile yapılabilmekte olup, bu HBTC'lerin yüksek performanslı sıvı kromatografi (HPLC) ile kıyaslanması önem arz etmektedir. Bu çalışmada hemoglobinopatisi olan ve olmayan hastaların HbA1c sonuçlarının HBTC HbA1c cihazı (Tri-stat Analyzer, Trinity Biotech) ve HPLC (Hb Premiere) ile karşılaştırılması amaçlanmıştır.

Yöntemler: Hemoglobinopatili 26 diyabetik, hemoglobinopatisiz 51 diyabetik hasta çalışmaya alındı. Hastaların HbA1c analizleri Tri-stat HBTC kullanılarak (Trinity Biotech, Ireland) venöz ve kapiller kandan, Premier Hb 9210 (Trinity Biotech, Ireland) HPLC kullanılarak sadece venöz kandan çalışılmıştır.

Bulgular: Hemoglobinopatisi olmayan hastaların HbA1c düzeyleri karşılaştırıldığında HPLC - kapiller kan HBTC arasında ($p=0,392$), HPLC - venöz kan HBTC arasında ($p=0,167$) ve venöz kan HBTC - kapiller kan HBTC arasında ($p=0,288$) istatistiksel olarak anlamlı bir fark saptanmamıştır. Hemoglobinopatisi olan hastaların HbA1c düzeyleri karşılaştırıldığında HPLC - venöz kan HBTC arasında da ($p=0,076$) istatistiksel olarak anlamlı bir fark saptanmamıştır.

Sonuç: Hastalarda, Tri-stat HBTC'yi HbA1c sonuçları HPLC ile karşılaştırıldığında istatistiksel olarak farklı değildi. Bakanlıkça onaylı ve otoriteler tarafından kabul gören HBTC'yi HbA1c analizlerinde güvenle kullanılabilir. Klinisyenler, yakın takibe ihtiyaç duyan ve tedavi rejimi bu takipten etkilenen tip 2 diyabetli hastalarda her iki yöntemi de tercih edebilirler.

Anahtar Kelimeler: HbA1c, HPLC, HBTC, hemoglobinopati



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Introduction

Regular monitoring of glycosylated hemoglobin subfraction A1c (HbA1c) in people with diabetes and treatment with glucose-lowering medications to improve glycemic control can reduce the risk of developing complications (1). In the past, the recommended method for the diagnosis of diabetes was through the repeated measurement of fasting plasma glucose or an oral glucose tolerance test (2). More recently, the measurement of the glycosylated fraction (HbA1c) of HbA1c has been recommended to diagnose diabetes (3), in addition to monitoring glycemic control. Glycosylated hemoglobin is a sensitive predictor of the long-term complications of diabetes. High HbA1c levels are strongly linked to increased risk of cardiovascular disease, nephropathy, and retinopathy (4,5) and predict most of the excess mortality risk in men with diabetes (6).

The traditional method of testing for glycemic control in primary care involves sending blood samples for laboratory testing and waiting several days for results. In parallel with the developments of nanotechnology in biomedical applications, point of care testing (POCT) of HbA1c is available using a non-invasive, quick and easy analysis. POCT devices are especially useful in emergency departments, in general practitioner's offices or in distant locations to reference laboratories. POCT can also improve the life quality in chronic patients (7,8).

This study aimed to compare HbA1c results of the POCT HbA1c device (Tri-stat Analyzer, Trinity Biotech) with high-performance liquid chromatography (HPLC) (Hb Premiere) in diabetic patients with and without hemoglobinopathy.

Methods

Twenty-six diabetic patients with- and 51 patients without hemoglobinopathy who admit to University of Health Sciences, Bakırköy Dr. Sadi Konuk Training and Research Hospital, outpatient clinic, were enrolled. The American Diabetes Association (ADA) criteria were used to diagnose diabetes (9).

All patients signed an informed consent form before being enrolled, and the study protocol was approved by the University of Health Sciences, Bakırköy Dr. Sadi Konuk Training and Research Hospital Local Ethical Committee (decision no: 2014/231). There was no statistically significant difference between age and sex distributions of the two groups.

HbA1c analyzes were performed using Tri-stat POCT analyzer (Trinity Biotech, Ireland) from venous blood and capillary blood, Premier Hb 9210 (Trinity Biotech, Ireland) HPLC method only from venous blood of patients.

Statistical Analysis

Mean, standard deviation, median, minimum, and maximum values were given for continuous variables. The normal distribution of continuous variables was tested by the Kolmogorov-Smirnov test. For dependent variables showing normal distribution, the Dependent t-test was used in the comparison of two groups. Bland Altman analysis was used for comparisons between methods. The Intraclass Correlation Coefficient (ICC) was calculated for the degree of correlation and agreement between measurements of the methods. P values lower than 0.05, with a 95% confidence interval, were considered as statistically significant.

Results

The median age of the 51 patients without hemoglobinopathy (32 women and 19 men, the ages ranged between 20 and 78 years) was 53.27±11.80 years of age. Only one person in this group had chronic renal failure, who did not receive hemodialysis.

The mean HbA1c level of this group analyzed by the HPLC method, by POCT from venous blood and by POCT capillary blood was 8.45 ± 2.30, 8.54±2.18 and 8.50±2.16, respectively.

No statistically significant difference was observed between HPLC - HbA1C and capillary blood POCT HbA1C measurements (p=0.392).

No statistically significant difference was also observed between the HPLC - HbA1C and venous blood POCT HbA1C (p=0.167). POCT - HbA1C measurements from capillary blood and concurrent venous blood did not differ statistically (p=0.288) (Table 1).

ICC of HPLC - HbA1C and POCT - HbA1C capillary measurements, HPLC HbA1C - POCT HbA1C venous blood and POCT - HbA1C capillary - POCT - HbA1C venous blood is 0.988 (0.980-0.993), 0.989 (0.980-0.994) and

Table 1. Comparison of the capillary and venous blood results of point of care testing analysis with high-performance liquid chromatography method

	Mean ± SD	p
HPLC HbA1C	8.45±2.38	0.392
POCT HbA1C capillary	8.5±2.16	
HPLC HbA1C	8.45±2.38	0.167
POCT HbA1C venous (EDTA)	8.54±2.18	
POCT HbA1C capillary	8.5±2.16	0.288
POCT HbA1C venous (EDTA)	8.54±2.18	

SD: standard deviation, POCT: point of care testing, HPLC: high-performance liquid chromatography, HbA1C: hemoglobin A1c, EDTA: ethylenediaminetetraacetic acid

Table 2. Intraclass correlation of the capillary and venous blood results of the point of care testing analysis and high-performance liquid chromatography

	Intraclass correlation coefficient	95% Confidence interval	
		Lower bound	Upper bound
HbA1C / POCT HbA1C capillary	0.988	0.980	0.993
HbA1C / POCT HbA1C venous (EDTA)	0.989	0.980	0.994
POCT HbA1C capillary / POCT HbA1C venous (EDTA)	0.997	0.995	0.998

POCT: point of care testing, HPLC: high-performance liquid chromatography, HbA1C: hemoglobin A1c, EDTA: ethylenediaminetetraacetic acid

0.997 (0.995-0.998), respectively. All of them indicate strong reliability (Table 2) (Figures 1, 2, 3).

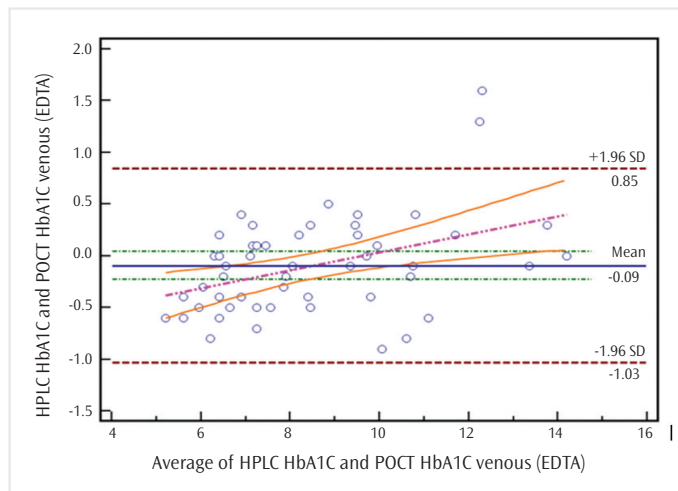


Figure 1. Bland Altman analysis of HPLC HbA1c - POCT HbA1c venous blood measurement

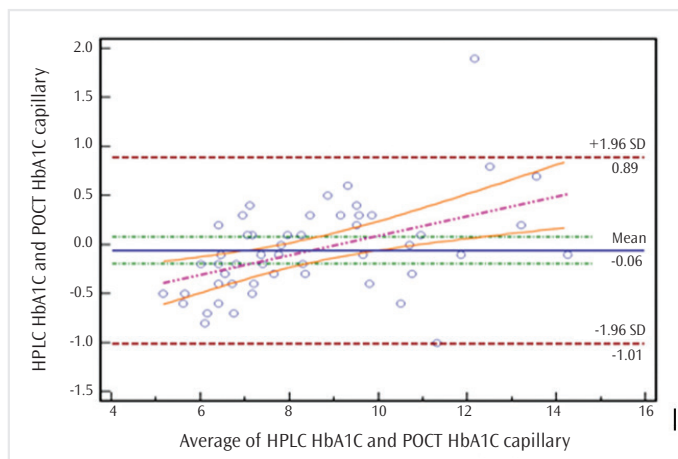


Figure 2. Bland Altman plot of HPLC- HbA1c and POCT- HbA1c capillary blood measurement

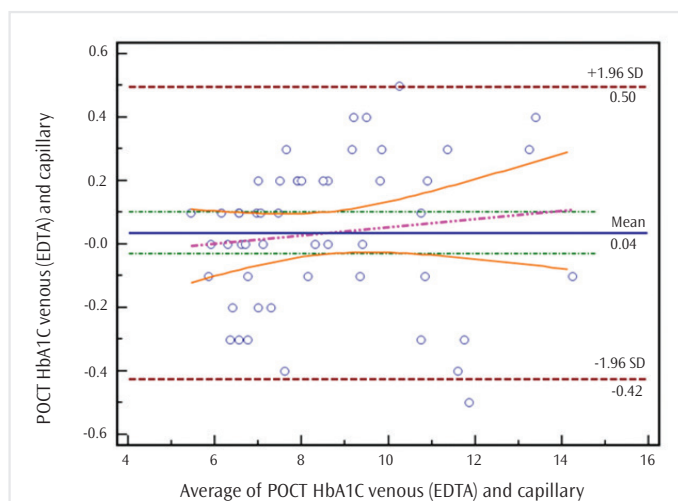


Figure 3. Bland Altman plot of POCT HbA1c capillary - POCT HbA1c venous blood measurement

Mean HbA1c level of 26 patients with hemoglobinopathy and type 2 diabetes analyzed by the HPLC method and by POCT was 6.78 ± 1.45 and 6.45 ± 1.38 , respectively. No statistically significant difference was observed ($p=0.076$) (Table 3).

ICC of HPLC - HbA1c and POCT - HbA1c is 0.994 (0.990-0.997), which indicates strong reliability (Table 4).

Discussion

The ADA guidelines (2010) recommend the use of glycosylated hemoglobin (HbA1c) levels for screening and diagnosis of diabetes (10). Thus, accurate measurement of HbA1c levels is essential for the optimal management of diabetes (11-13).

In recent years, international standardization organizations such as the National Glycohemoglobin Standardization Program and the International Federation of Clinical Chemistry have helped to improve the quality of HbA1c determination (14).

HbA1c measurement is an essential tool in the management of patients with diabetes mellitus. In this context, the possibility of having rapid and accurate methods for HbA1c evaluation is significant.

Several techniques are used for laboratory determination of serum HbA1c levels. The most frequently used form is affinity chromatography, both manual chromatography, and affinity HPLC. As affinity chromatography in principle measures not only the specific glycation on the β -N-terminal, but also on the α -N-terminal and ϵ -residues of the total hemoglobin molecule, the end-result is total glycated hemoglobin or glycohemoglobin (15).

There are some disadvantages in clinical practice, although the reliability, widespread use, and standardization of laboratory methods are possible.

Physicians in ambulatory settings routinely send blood samples to laboratories for HbA1c testing and wait several days for the HbA1c test results. Thus, patient counseling and treatment adjustments based on HbA1c levels are delayed, and at times follow-up can be lost entirely.

Table 3. Hemoglobin A1c levels of patients with hemoglobinopathy (high-performance liquid chromatography and point of care testing)

	Mean	SD	p
HbA1c (HPLC)	6.78	1.45	0.076
HbA1c (POCT)	6.45	1.38	

SD: standard deviation, HbA1c: hemoglobin A1c, POCT: point of care testing, HPLC: high-performance liquid chromatography

Table 4. Intraclass Correlation Coefficient of high-performance liquid chromatography and point of care testing of patients with hemoglobinopathy

HPLC / POCT	Intraclass correlation coefficient	95% Confidence interval	
		Lower bound	Upper bound
HbA1c	0.994	0.990	0.997

POCT: point of care testing, HPLC: high-performance liquid chromatography

POCT devices can provide excellent convenience for patients receiving home care services, or in places where healthcare facilities are not easy to access. These devices are small, portable, and not expensive. They are easy to use and do not require extensive training.

Several HbA1c POCT devices are currently available for use in clinical practice, including Bayer's A1CNow+® Multi-test A1C System (A1CNow+), the in2it (II) Analyzer (in2it; Bio-Rad Laboratories), the DCA Vantage Analyzer (DCA Vantage; Siemens Healthcare Diagnostics, Inc.), Tri-stat POCT analyzer (Trinity Biotech, Ireland) and the Afinion AS100 Analyzer System (Afinion; Axis-Shield Point-of-Care).

Although new devices are developed every day, the standardization problem and the lack of adequate studies negatively affect the usage of these devices widely.

Our study evaluates a fast and easy way to perform Tri-stat POCT analyzer measurements of glycated hemoglobin HbA1c in comparison with an immunoassay on an automated biochemistry analyzer, Premier Hb 9210 (Trinity Biotech, Ireland), the methods routinely used in our clinical laboratories for the measurement of HbA1c.

In previous studies, POCT analyses were consistent with the HPLC method, but the POCT -HbA1c level was lower than HPLC. In their study, Schwartz et al. (16) compared the performance of the BIO-RAD Micromat II POCT device with a laboratory-based HPLC method (Primus Model 386) for measurement of HbA1c. For each of the laboratory methods, the correlation coefficient was lower than the 0.96 reported by the manufacturer. HbA1c results were also consistently lower than those obtained from laboratory analysis.

Grant et al. (17) compared HPLC and Quo-Test analyzer and obtained similar results. In another study, Arsie et al. (18) reported similar results after comparing HPLC and DCA 2000 POCT analyzer.

In our study, although the results of HPLC and Tri-stat were strongly correlated, however, POCT HbA1c levels were not lower than the results of HPLC, as in the studies mentioned above, even slightly higher. These results did not change in capillary and venous blood samples. These almost identical results may encourage clinicians to rely on this method, which is less invasive for the patient and allows bedside analysis.

Although Tri-stat POCT analyzer results did not differ statistically in comparison to HPLC results in patients with hemoglobinopathy in our study, they were lower than HPLC. This condition may be associated with the shortened lifespan of erythrocytes. Haliassos et al. (19) obtained similar results like ours. In this study, HbA1c levels of patients with hemoglobinopathy were compared using DCA 2000 of Bayer Diagnostics (Tarrytown, NY, USA) and HPLC.

As a result, Tri-stat POCT analyzer results did not differ statistically in comparison to HPLC results in patients. POCT devices approved by the ministry and accepted by the authorities can be used safely in HbA1c analyzes.

Conclusion

Clinicians may prefer both methods in patients with type 2 diabetes who need close follow-up, and whose treatment regimen will be affected by

this follow-up. The POCT analyzers should be evaluated for diagnostic purposes by professional organizations developing clinical guidelines.

Ethics Committee Approval: The study protocol was approved by the University of Health Sciences, Bakırköy Dr. Sadi Konuk Training and Research Hospital Local Ethical Committee (decision no: 2014/231).

Informed Consent: All patients signed an informed consent form before being enrolled.

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