

Comparison of Three Methods for Measurement of HbA1c

[HbA1c Ölçümünde Farklı Üç Yöntemin Karşılaştırılması]

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ABSTRACT

Aim: To compare three methods for measurement of blood HbA1c as to reliability, ease and time consumption.”

Method: Measurements of HbA1c were made in blood from 120 patients with pre-diabetes and diabetes using a turbidimetric inhibition immunoassay (TINIA), which also required measurement of total hemoglobin, a particle-enhanced immunoturbidimetric assay (PEITT) without measurement of total hemoglobin, and high performance liquid chromatography (HPLC).

Results: There was good concordance between results of PEITT and HPLC methods ($r = 0.9401$, $p < 0.0001$ and by Deming Regression; $y = 0.9978x + 0.24$). The average HbA1c (7.52 ± 1.40 %) measured by HPLC was higher than the other methods (TINIA: 7.12 ± 1.66 % and PEITT: 7.26 ± 1.39 %, $p < 0.0001$). The measured total time spent on 120 samples was 45 min. for TINIA, 39 min. for PEITT and 384 min. for HPLC.

Conclusion: It has been found that, the PEITT method, which is reliable, faster, and easier to perform, can be used as an alternative to TINIA and HPLC measuring system within the known imprecision limits.”

Key words: HbA1c, particle enhanced immunoturbidimetric test, turbidimetric immunoassay, HPLC.

ÖZET

Amaç: HbA1c ölçümünde güvenilir, daha hızlı ve yöntem olarak daha basit olan PGİT yöntemi, TİİA ve HPLC yöntemine alternatif olarak kullanılabilir.”

Yöntem: Pre-diyabet ve diyabet olduğu bilinen 120 hastanın HbA1c düzeyleri üç farklı yöntem kullanılarak ölçüldü. Kan örnekleri, % HbA1c hesaplaması için total hemoglobin ölçümü gerektiren turbidimetrik inhibisyon immunoassay (TİİA), total hemoglobin ölçümünü gerektirmeyen ve yeni bir yöntem olan parçacıkla güçlendirilmiş immunoturbidimetrik test (PGİT) ve yüksek performans sıvı kromatografisi (HPLC) ile ölçüldü.

Bulgular: HPLC yöntemi ile PGİT yöntemi arasında oldukça iyi bir uyum ($r = 0.9401$, $p < 0.0001$ ve Deming Regresyon analizi kullanılarak; $y = 0.9978x + 0.24$) saptandı. HPLC yöntemiyle ölçülen HbA1c ortalaması TİİA ve PGİT yöntemi ile ölçülen HbA1c ortalamasından daha yüksek saptandı (sırasıyla, 7.52 ± 1.40 karşı 7.12 ± 1.66 ve 7.26 ± 1.39 ; $P < 0.001$). TİİA ve PGİT yöntemlerinin toplam çalışma süreleri HPLC yönteminden daha kısaydı (sırasıyla, 45 dakika, 39 dakika ve 380 dakika).

Sonuç: HbA1c ölçümü için daha hızlı sonuç verebilen ve yöntem olarak daha basit olan PGİT yöntemi, HPLC yöntemine alternatif olarak kullanılabilir.

Anahtar Kelimeler: HbA1c, parçacıkla güçlendirilmiş immunoturbidimetrik test, turbidimetrik immunoassay, HPLC.

Introduction

The successful treatment of diabetes depends on keeping blood glucose levels at normal rate over the long term. Many tests have been developed for this purpose. (1-4) While the blood glucose level reflects the current condition of a patient it is inadequate in evaluating the level of glucose regulation. (5, 6) HbA1c level in blood is the most important indicator of the overall glucose level in a patient during a period of two or three months. (2, 7)

Ion exchange HPLC method is based on the charge of the globin component of hemoglobin (Hb). Although it measures all types of Hb and is affected by abnormal and minor Hb fractions, the Diabetes Control and Complications Trial (DCCT) and the National Glycohemoglobin Standardization Program (NGSP) have recommended it as an acceptable standard. (4, 7-10) Recently, new HPLC systems have been developed using more modern chromatographic materials in order to reduce the effects of abnormal and minor Hb fractions. (11) HPLC is more expensive than turbidimetric immunoassays because it needs more technical staff, expensive equipment and time. On the other hand the HbA1c antibody used in turbidimetric immunoassays reacts only with HbA1c and the result can be measured easily with a turbidimeter. (12, 13) Furthermore, these methods are easier to adapt to biochemical devices, cheaper in cost and faster in producing results than HPLC. Despite all these advantages, turbidimetric immunoassays have lower precision than HPLC. (14)

The purpose of this study was to compare PEITT, which is a new method, with TINIA and HPLC for measurement of blood HbA1c to find out which method is easier to adapt to biochemical instruments, faster, practical and reliable.”

Material and Methods

One hundred and twenty patients from Turkey diagnosed as having pre-diabetes or diabetes were enrolled in the study. They were between 19 and 67 years old (average 41 years). Fifty four of them (45%) were women and 66 (55%) were men. All patients were being followed up in the Internal Medicine and Cardiology outpatient clinics of Gümüşsuyu Military Hospital. Patients with HbF levels higher than 10%, serum triglyceride levels higher than 800 mg/dl and patients using high doses of aspirin, vitamin C or alcohol, were excluded. (10, 13, 15, 16) Blood was collected into EDTA from all patients over four days and kept at 4 °C in order to preserve the stability of the blood samples (17, 18) until fifth day when measurements were conducted in one day.

Analytical procedures were conducted according to the following three methods:

1. HbA1c levels of the patients were measured using a Bio-Rad D-10 HPLC instrument, whose compliance with the latest Diabetes Control and Complications Trial (DCCT) reference method has been documented by the

National Glycohemoglobin Standardization Program (NGSP). (7) Two levels of Bio-Rad calibrators and controls were used for the calibration of the instrument.

2. Before performing automated analysis on samples, the test requires manual preparation of a sample hemolysate. Samples were mixed with tetradecyltrimethylammonium bromide containing hemolyzing reagent (10µl whole blood + 1000µl hemolyzing reagent) for 5 minutes in accordance with the testing method. The HbA1c values of hemolyzed samples were measured by TINIA using a Diasis Diagnostic Systems (DDS) kit following the kit manufacturer's instructions. For calibration, a DDS calibrator and control set were used. Total hemoglobin (THb), which is needed for estimation of % HbA1c, was measured in another channel of the same instrument with the DDS kit.

3. For the PEITT method sample hemolysates were first prepared as made described above. Samples were mixed with containing the hemolyzing reagent (10µl whole blood + 500µl hemolyzing reagent) for approximately 5 minute or until lysis was completed in accordance with the testing method. The HbA1c values of the blood samples were measured directly without measurement of THb on an Olympus AU 400 instrument by the PEITT method using a DDS kit. For calibration, a DDS calibrator and control set were used. HbA1c, THb, and HbA1c in hemolyzed sample were bound with equal affinity to solid-phase particles in reagent. Subsequently mouse anti-human HbA1c monoclonal antibody was added to attach to particle bound HbA1c. Goat anti-mouse IgG polyclonal antibody then reacted with the monoclonal mouse anti-human HbA1c antibody to produce the agglutination reaction. Finally absorbance, which is proportional to the HbA1c bound to particles, was measured by comparison to the calibrator set.

All results from each of the immunoturbidimetric methods (TINIA and PEITT) were calculated according to DCCT/NGSP (as follows) (7, 19) and compared with those of the HPLC method.

Calculation according to International Federation of Clinical Chemistry (IFCC):

$$HbA1c\% = HbA1c (g/dl) \times 100 \div Hb (g/dl)$$

Calculation according to DCCT/NGSP:

$$HbA1c\% = 0.915 \times IFCC + 2.15$$

The imprecision of TINIA and PEITT methods were expressed as the coefficient of variation (CV) for within-run and between-days. Two levels of HbA1c control materials (Diasis Diagnostic Systems, DDS) were assayed 20 times consecutively within a single day to determine within-run CV and 20 times on consecutive days to determine between-day CV for these methods.

All the data were evaluated with statistical software of InStat3. The paired t-test was used to compare and linear regression analysis was used to determine the coefficient of correlation. We also used the Deming regression

analysis for the comparison of these three methods.

Results

The manual preparation time spent on 120 samples was approximately 40 min., for each of TINIA and PEITT methods. The total measuring time spent on 120 samples for TINIA was 45 min., for PEITT 39 min. (TINIA and PEITT methods were conducted using Olympus AU 400 device) and for HPLC 384 min. using Bio-Rad D-10 device. Therefore, measurement time was approximately 1/5 of the time required for the HPLC method. When the data obtained from the control level 1 and 2 were examined, we found that CVs for within-run and between-day for PEITT method were lower than TINIA method (Table 1a and 1b).

HbA1c values measured with the HPLC method were higher than those HbA1c values measured with TINIA and PEITT methods (7.52±1.40 vs. 7.26±1.39 and 7.12±1.66, respectively). Although the difference was very small, it was statistically significant ($p < 0.0001$). No difference was found between the results of TINIA and PEITT methods ($p = 0.0777$).

When the Deming Regression analysis between TINIA and HPLC methods was examined, the confidence interval for slope did not contain the value 1. Compared to the HPLC method, in the TINIA method the margin of error was greater especially for the values under 8 %. This explains why the TINIA method was not concordant with the HPLC method (Figure 1). In addition, linear regression analysis showed that the relationship TINIA

and HPLC methods was strong (Correlation Coefficient (r) = 0.9077, Bias = -0.39, Standard deviation of residual from line ($Sy.x$) = 0.6991; $p < 0.0001$), although TINIA method was not suitable.

Deming Regression analysis showed that PEITT method was found to be compatible with HPLC method (Figure 2). By linear regression analysis, it also has a stronger correlation with HPLC method than TINIA method ($r = 0.9401$, Bias = -0.26, $Sy.x = 0.4774$; $p < 0.0001$).

Discussion and Conclusion

Several studies have reported a perfect relationship between HbA1c methods based on different principles when the methods were standardized using a widespread reference model and calibrated with the same calibrator. Otherwise important differences were seen between the glycosylated hemoglobin measured with different methods. (7, 20-22) Furthermore, by using the same calibrator for the TINIA and PEITT methods, we have found that both method results were very similar to each other.

CV values for TINIA, PEITT and HPLC methods were higher than those given by the manufacturers. This difference was attributed to the different user and instrument. However, they were in agreement with referenced CVs for within-run and between-day (<3% and <5%, respectively) of other reports. (20, 23-25) Moreover, the lower the CV values obtained from PEITT method were found more suitable than TINIA method.

The finding that HbA1c values measured with HPLC

Table 1a. Within run coefficients of variation of HbA1c measurements obtained by the TINIA and PEITT methods on Olympus AU 400 and HPLC method on Bio-Rad D-10 HPLC instrument

%HbA1c, n=20		Within run		
	Method	Mean (%)	SD (%)	CV (%)
Control Level 1	TINIA	6.24	0.14	2.18
	PEITT	5.26	0.10	1.91
	HPLC	5.63	0.06	1.14
Control level 2	TINIA	9.25	0.18	1.94
	PEITT	9.75	0.13	1.36
	HPLC	9.87	0.08	0.83

Table 1b. Between-day coefficients of variation of HbA1c measurements obtained by the TINIA and PEITT methods on Olympus AU 400 and HPLC method on Bio-Rad D-10 HPLC instrument

%HbA1c, n=20		Between-day		
	Method	Mean (%)	SD (%)	CV (%)
Control Level 1	TINIA	6.22	0.26	4.14
	PEITT	5.27	0.17	3.20
	HPLC	5.66	0.08	1.46
Control level 2	TINIA	9.19	0.28	3.00
	PEITT	9.76	0.27	2.79
	HPLC	9.87	0.13	1.34

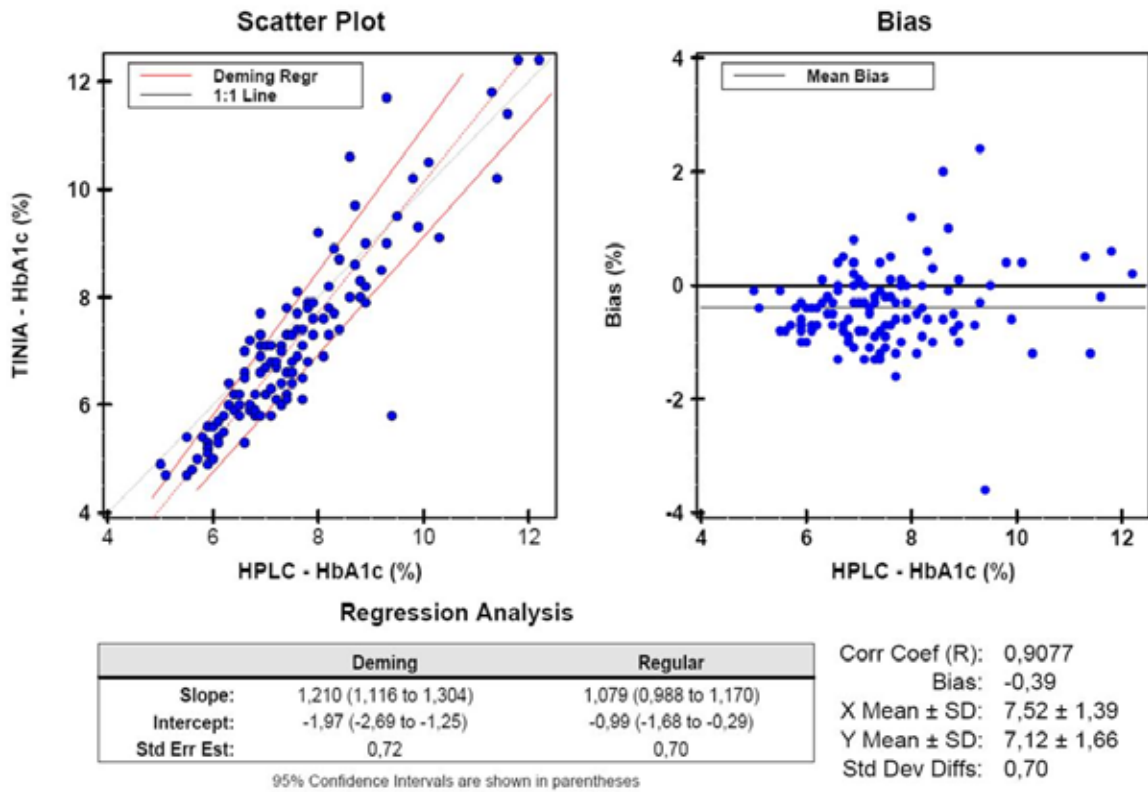


Figure 1. Deming Regression results for the TINIA and HPLC methods. These types of analysis compares one method (y) with another method (x) were obtained the following result. The hypothesis is accepted if the 95% CI for the Intercept contains the value 0. If the hypothesis is rejected, Intercept is significantly different from 0 and both methods differ at least by a constant amount. The hypothesis is accepted if the CI for Slope contains the value 1. If the hypothesis is rejected, Slope is significantly different from 1 and there is difference between the two methods.

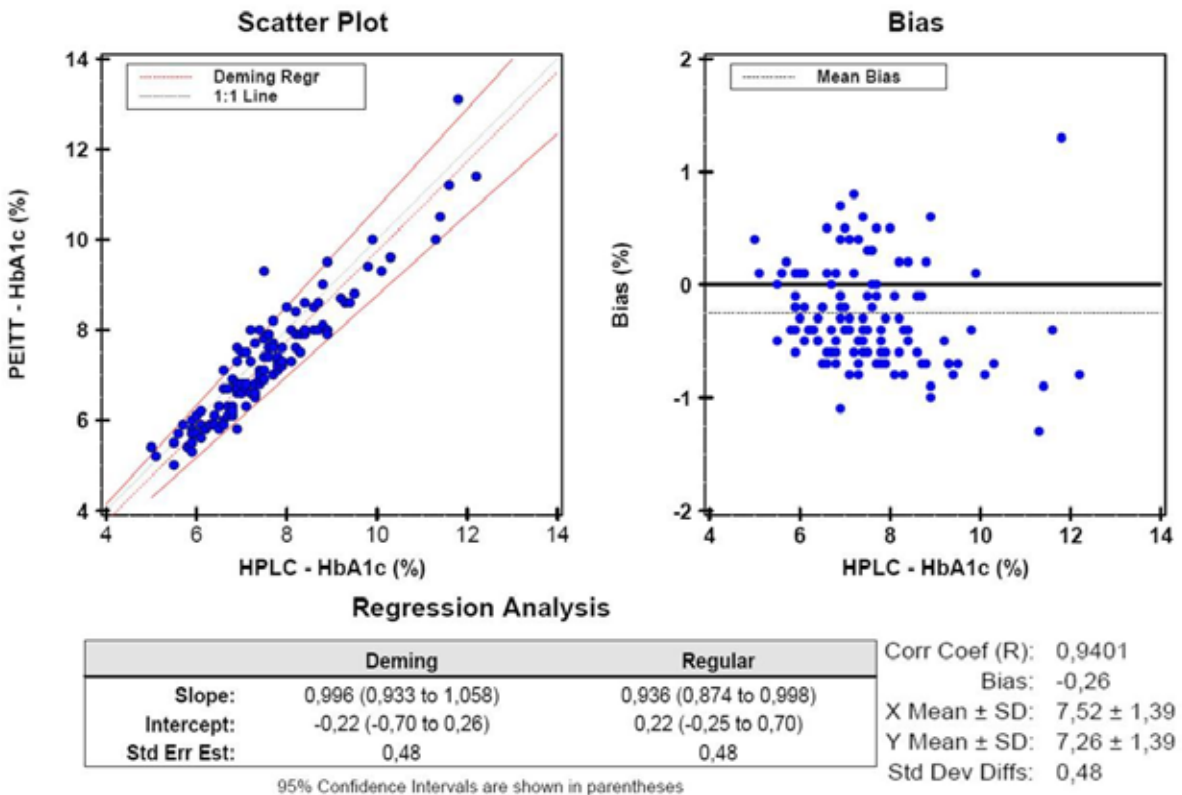


Figure 2. Deming Regression results for the PEITT and HPLC methods.

were statistically higher than those measured with TINIA and PEITT methods may reflect that the HbA1c peak was affected by other substances and by abnormal Hb variants, because the HPLC method is less specific than two the other methods. These results are similar to those obtained in other studies. (4, 7, 9, 16, 26-28) Perhaps this difference between HPLC and two other methods may be due to the use of different calibrators or differences in sample preparations.

The good relationship and concordance between the PEITT and the HPLC methods, as indicated in other studies, (12, 26-28) support the reliability of properly standardized immunoturbidimetric methods same as PEITT. Moreover, HbA1c values measured with this method do not require further correction equations, though each laboratory should determine its own reference values according to its results. (29, 30)

Although up to date, most of studies concluded no superiority between lots of methods,^{20, 23-25} we found that PEITT method was faster, because it does not require measurement of THb, than the other two methods.

Consequently and in conclusion, the PEITT method, which is reliable, faster, and easier to perform, can be used as an alternative to TINIA and HPLC measuring system within the known imprecision limits.

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