International Journal of Bioassays
ISSN: 2278-778X
www.ijbio.com

Research Article

COMPARISON OF THREE METHODS FOR MEASUREMENT OF BLOOD HBA1C AS TO RELIABILITY

Boinapalli Sudhakar[1]*, Alluguballi Sreenivas Reddy[2] and JJJ Fallerio[1]
Assistant Professor, Clinical Biochemistry, SBKS Medical College and Dhiraj Hospital, Piparia-391760, Vadodara, India.
Department of Biochemistry, SRM Medical College Hospital and Research Centre, kattankulathur, Chennai, India

Received for publication: January 10, 2014; **Revised:** February 7, 2014; **Accepted:** March 21, 2014

Abstract: To compare three methods for measurement of blood HbA1c as to reliability, ease and time consumption. Measurements of HbA1c were made in blood from 230 patients with pre-diabetes and diabetes using a turbidimetric inhibition immunoassay (TINIA), which also required measurement of total hemoglobin, a particle-enhanced immune turbidimetric assay (PEITT) without measurement of total hemoglobin, and high performance liquid chromatography (HPLC). 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 240 samples was 81 min. for TINIA, 54min. for PEITT and 540 min. for HPLC. 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."

Keywords: HbA1c, Particle Enhanced Immunoturbidimetric Test, Turbidimetric 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

Two hundred and forty patients diagnosed as having pre-diabetes or diabetes were enrolled in the study. They were between 19 and 67 years old (average41 years). One hundred and eight (45%) were women and one hundred and twelve (55%) were men. All patients were being followed up in the all the clinical Departments of Dhiraj General Hospital, Vadodara, India. Patients with Hb-F 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 0C 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,

***Corresponding Author:**
Dr. Boinapalli Sudhakar M.Sc., (Ph.D.).
Assistant Professor,
Clinical Biochemistry,
SBKS Medical College and Dhiraj Hospital,
Piparia-391760, Vadodara, India.

the test requires manual preparation of a sample hemolysate. Samples were mixed with porcine pepsin 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 Randox HbA1c following the kit manufacturer's instructions. For calibration, a randox HbA1c calibrator is used to standardize the reagent and instrument and Bio Rad 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 Randox HbA1C 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 MISPA I Nephlometry instrument by the PEITT method using a mispa kit. For calibration, electronic chip calibrator is used for standardizing the reagent and instrument and Bio-Rad 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 EM-200 automated clinical chemistry analyzer 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 ±1.66 and 7.122 ±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) = 0.7001; $p<0.0001$), 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 = 0.4779; $p<0.0001$).

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 glycated 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 (EM-200) and PEITT (I-2MISPA-NEPHLOMETRY methods and HPLC method on Bio-Rad D-10 HPLC instrument

% HbA1c n=20		Within in run		
	Method	Mean %	SD%	CV%
Control level-1	TINIA	6.24	0.14	2.15
	PEITT	5.26	0.10	1.91
	HPLC	5.59	0.06	1.19
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 EM-200 immuno turbidometry, I-2 MISPA nephlometry (PEITT) methods and HPLC method on Bio-Rad D-10 HPLC instruments respectively.

% HbA1c n=20		Between-day		
	Method	Mean %	SD%	CV%
Control level-1	TINIA	6.30	0.30	4.14
	PEITT	5.28	0.24	3.20
	HPLC	5.62	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

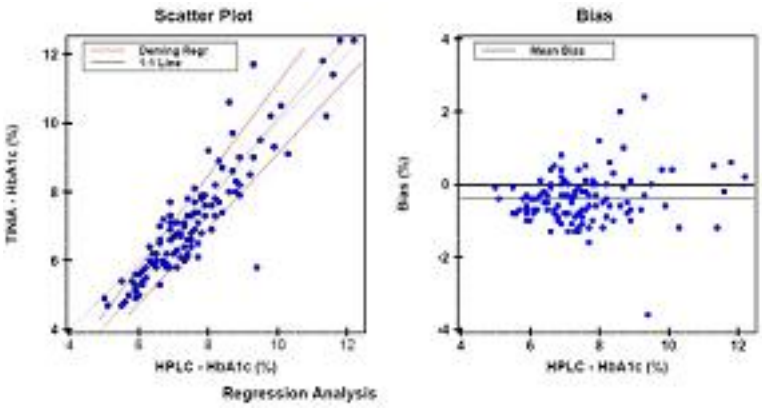
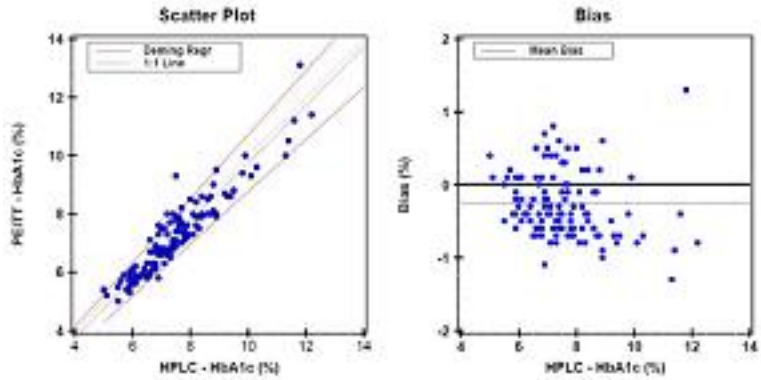


Figure 1. Deming Regression results for the TINIA and HPLC methods. These types of analysis compares one method (y) with another method (x) were obtained the following result. The hypothesis is accepted if the 95% CI for the Intercept contains the value 0. If the hypothesis is rejected, Intercept is 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.

Deming	Regular
Slope : 1.210 (1.116-1304)	1.079 (0.988-1.170)
Intercept : -1.97 (-2.69-1.25)	-0.99 (-1.68to -0.29)
Std err Est: 0.72	0.70

Correlation coefficient (R): 0.9077
Bias : -0.39
X-mean ± SD : 7.52±1.39
Y-mean ± SD : 7.12 ±1.66
STD dev Difference : 0.70



Regression analysis:

Deming	Regular
Slope : 0.966 (0.933 to 1.058)	0.936 (0.874 -0.998)
Intercept :-0.22 (-0.70 -0.26)	-0.22 (-0.25to -0.70)
Std err Est: 0.48	0.48

Figure 2:
Correlation coefficient (R): 0.9401
Bias : -0.26
X-mean ± SD : 7.52±1.39

Y-mean ± SD : 7.26 ±1.39
STD dev Difference : 0.48

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 calibrator's 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.

ACKNOWLEDGEMENT

I thank a lot of thanks to Dr. JJJ. FALLERIO incharge of central laboratory, Department of pathology SBKS Medical College and Research Centre, Pipariya, Vadodara 391760, India.

REFERENCES

1. The Diabetes Control and Complications Trial Research Group. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin dependent diabetes mellitus. N Engl J Med. 1993. 14: 977-986.

2. Krishnamurti U, Steffes MW. Glycohemoglobin: a primary predictor of the development or reversal of complications of diabetes mellitus, clinical chem., 2001, 47, 1157-65.

3. American Diabetes Association Clinical Practice Recommendations. Diabetes Care. 2001. 24 Suppl. 1: S1-133.

4. Nuttall FQ. Comparison of percent total GHb with percent HbA1c in people with and without known diabetes. Diabetes Care, 1998, 21, 1475-1480.

5. Baynes JW, Bunn HF, Goldstein D, Harris M, Martin DB, Peterson C, Winter halter K. National Diabetes Data Group: report of the expert committee on glucosylated hemoglobin. Diabetes Care, 1984, 7, 602-606.

6. Nathan DM, Singer DE, Hurxthal K, Goodson JD. The clinical information value of the glycosylated hemoglobin assay. N Engl J Med 1984. 310: 341-346.

7. Hoelzel W, Weykamp C, Jeppsson JO, Miedema K, Barr JR, Goodall I, Hoshino T, John WG, Kobold U, Little R, Mosca A, Mauri P, Paroni R, Susanto F, Takei I, Thienpont L, UmemotoM, Wiedmeyer HM. IFCC reference system for measurement of hemoglobin A1c in human blood and the national standardization schemes in the United States, Japan, and Sweden: a method-comparison study. Clin Chem. 2004, 50, 166-174.

8. Santiago JV. Lessons from the Diabetes Control and Complications Trial. Diabetes. 1993; 42: 1549-1554.

9. Weykamp CW, Penders TJ, Muskiet FA, van der Slik W. Influence of hemoglobin variants and derivatives on glycohemoglobin determinations, as investigated by 102 laboratories using16 methods. Clin Chem, 1993, 39, 1717-1723.

10. Weykamp CW, Penders TJ, Siebelder CW, Muskiet FA, van derSlik W, Interference of carbamylated and acetylated hemoglobinsin assays of glycohemoglobin by HPLC, electrophoresis, affinity chromatography, and enzyme immunoassay. ClinChem, 1993, 39, 138-142.

11. Little RR, Tennill AL, Rohlfing C, Wiedmeyer HM, KhannaR, Goel S, Agrawal A, Madsen R, Goldstein DE. Can glycohemoglobin be used to assess glycemic control in patients with chronic renal failure? Clin Chem, 2002, 48,784-786.

12. Metus P, Ruzzante N, Bonvicini P, Meneghetti M, ZaninottoM, Plebani M. Immuno turbidimetric assay ofglycated hemoglobin. J Clin Lab Anal, 1999, 13, 5-8.

13. Chang J, Hoke C, Ettinger B, Penerian G. Evaluationand interference study of hemoglobin A1c measured by turbidimetric inhibition immunoassay. Am J Clin Pathol, 1998,109, 274-278.

14. Goodall I. HbA1c standardisation destination globall FCC Standardisation. How, why, where and when--a tortuous pathway from kit manufacturers, via inter-laboratory lyophilizedand whole blood comparisons to designated national comparison schemes. Clin Biochem Rev, 2005. 26, 5-19.

15. Davie SJ, Gould BJ, Yudkin JS. Effect of vitamin Con glycosylation of proteins. Diabetes 41 (2): 167-173.

16. Rohlfing CL, Connolly SM, England JD, Hanson SE, Moellering CM, Bachelder JR, Little RR. (2008) The effect ofelevated fetal hemoglobin on hemoglobin A1c results: five commonhemoglobin A1c methods compared with the IFCC reference method. Am J Clin Pathol, 1992, 129, 811-814.

17. Little RR, England JD, Wiedmeyer HM, Goldstein DE, Effects of whole blood storage on results for glycosylated hemoglobin as measured by ion-exchange chromatography, affinity chromatography, and colorimetry. Clin Chem, 1983, 29, 1113-1115.

18. Peterson CM, Jovanovic L, Raskin P, Goldstein DE. Acomparative evaluation of glycosylated haemoglobin assays: feasibility of references and standards. Diabetologi, 1984, 26, 214-217.

19. Geistanger A, Arends S, Berding C, Hoshino T, Jeppsson JO, Little R, Siebelder C, Weykamp C. Statistical methods for monitoring the relationship between the IFCC reference measurement procedure for hemoglobin A1c and the designated comparison methods in the United States, Japan, and Sweden, Clin Chem, 2008, 54, 1379-1385.

20. Sacks DB, Bruns DE, Goldstein DE, Maclaren NK, Mc Donald JM, Parrott M. Guidelines and recommendations for laboratory analysis in the diagnosis and management of diabetes mellitus, Clin Chem, 2002, 48, 436-472.

21. Weykamp CW, Penders TJ, Muskiet FA, van der Slik W. Effect of calibration on dispersion of glycohemoglobin values determined by 111 laboratories using 21 methods. Clin Chem, 1994, 40, 138-144.

22. Weykamp CW, Penders TJ, Miedema K, Muskiet FA, van der Slik W. Standardization of glycohemoglobin results and reference values in whole blood studied in 103 laboratories using20 methods. Clin Chem, 1995, 41, 82-86.

Source of support: Nil
Conflict of interest: None Declared