

Evaluation of the Analytical Performance of Five Assays in Measurement of Hemoglobin A1c in Iran

Tahereh Keramati¹; Ali Tootee²; Ensieh Nasli Esfahani²; Mostafa Qorbani^{3,4}; Parvin Pasalar⁵; Farideh Razi^{2,*}

¹Endocrinology and Metabolism Research Center, Endocrinology and Metabolism Clinical Sciences Institute, Tehran University of Medical Sciences, Tehran, IR Iran

²Diabetes Research Center, Endocrinology and Metabolism Clinical Sciences Institute, Tehran University of Medical Sciences, Tehran, IR Iran

³Department of Community Medicine, Alborz University of Medical Sciences, Karaj, IR Iran

⁴Non Communicable Diseases Research Center, Endocrinology and Metabolism Population Sciences Institute, Tehran University of Medical Sciences, Tehran, IR Iran

⁵Department of Biochemistry, Faculty of Medicine, Tehran University of Medical Sciences, Tehran, IR Iran

*Corresponding author: Farideh Razi, Diabetes Research Center, Endocrinology and Metabolism Clinical Sciences Institute, Tehran University of Medical Sciences, Tehran, IR Iran. Tel: +98-2188334192, Fax: +98-2188334195, E-mail: farideh.razi@gmail.com

Received: December 30, 2014; **Revised:** February 26, 2015; **Accepted:** February 28, 2015

Background: Hemoglobin A1c (HbA1c) testing devices are widely used to evaluate glycemic control in diabetic patients. Despite international effort on standardization of HbA1c tests, different levels of variation in different assay results are still present.

Objectives: The purpose of this study was to investigate the comparability of the results of various HbA1c instruments used in Iran.

Materials and Methods: This cross-sectional study was conducted in the endocrine and metabolic institute of Tehran University of Medical Sciences from October 2013 to December 2013. Fresh blood samples taken from 45 diabetic type 2 patients with different HbA1c levels (4.8-12.7%) were tested with five assays; NycoCard Reader II (Axis-Shield), CERA STAT 2000 (Ceragem Medisys Inc), DS5 (Drew Scientific Inc), Biosystems (BioSystems S.A.) and Pars Azmoon (Pars azmoon), and the results were compared to those obtained from TOSOH G8 Ion exchange (Tosoh Bioscience) as a reference method. Clinical Laboratory Standardization Institute (CLSI) protocols (EP-15A2 and EP-9A2) were used for designing the study and evaluation of the results. Microsoft Excel 2007 and SPSS version 15 packages were used for computation and analysis of the data.

Results: Interassay imprecision of all assays was less than 3.4%. There was a significant linear correlation between test methods and TOSOH G8 results ($r: 0.86$ to 0.96). The mean values of all different methods were significantly different from the reference method (P value < 0.01).

Conclusions: Although the results obtained with the use of different methods show an excellent correlation, when one particular sample is tested by different assays, the results are significantly different. It can be concluded that the national standardization program of HbA1c measurement is necessary.

Keywords: Hemoglobin A1c; Chromatography; High Pressure Liquid; Diabetes Mellitus

1. Background

Diabetes mellitus, being one of the most prevalent endocrine disorders, is a major public health concern both in developed and developing countries. The prevalence of diabetes was estimated 12.9% among the population older than 20 in the United States based on a survey carried from 2005 to 2006 (National Health and Nutrition Examination Survey) (1). The prevalence of diabetes is also high in Iran and it is estimated that 7.7% of the adults aged 25 to 64 years old suffer from diabetes, half of them arguably being undiagnosed (2). In another study the prevalence rates of diabetes and prediabetes were 7.04% and 8.58%, respectively (3). Measurement of Hemoglobin A1c (HbA1c) blood levels is considered as a reliable indicator of glycemic control in diabetic patients since 1977 (4). Hemoglobin A1c measurement is currently considered as the main method for monitoring long-term glycemic control in both type 1 and type 2 diabetic patients. Moreover, it is demonstrated that HbA1c blood levels also can

predict the risk of developing diabetes micro- and macrovascular complications (5). Approximately, 100 different HbA1c assays have been invented and are currently in use in different countries. The instruments utilize different technologies, which range from low-throughput research laboratory systems to high-throughput automated systems invented exclusively for the measurement of blood HbA1c levels. Therefore, the HbA1c results reported for a same blood sample can considerably differ unless they are standardized according to a common reference system (6). In response to this need, National Glycohemoglobin Standardization Program (NGSP), which is partly sponsored by the American Diabetes Association, was established in 1996 with the primary goal of standardization of HbA1c assays. Based on the NGSP requirements, manufacturers of A1c testing devices are awarded a certificate of traceability to the Diabetes Control and Complications Trial (DCCT) reference method provided that

their assay methods are precise and accurate enough to pass the stringent test administered which needs to be renewed on a yearly basis (7, 8). Notwithstanding the relative success of NGSP, it is reported that the presence of inter method variability among NGSP-certified methods could still decrease the clinical usefulness of HbA1c testing. This can cause even more dramatic results in mismanagement of diabetic patients when different methods are used interchangeably, which may be quite inevitable when patients change health providers and diagnostic laboratories (9).

2. Objectives

This study aimed to investigate the compatibility of various HbA1c assays used in Iran, both NGSP-certified and non-NGSP certified.

3. Materials and Methods

This cross-sectional study was conducted in diabetes and metabolic clinic of endocrine and metabolic institute of Tehran University of Medical Sciences from October 2013 to December 2013. A total of 45 fresh blood samples from diabetic type 2 patients with different HbA1c levels (4.8 - 12.7%) were collected in sterile tubes containing K₂ Ethylene Diamine Tetraacetic Acid (EDTA). Patients with any kind of hemoglobinopathy were excluded. No further inclusion/exclusion criteria were used. The study was approved by the ethics committee of endocrinology and metabolism research institute and a written informed consent was obtained from all participants.

The samples were tested by five devices within the same day otherwise refrigerated up to two days. The HbA1c assays, which were evaluated in this study included:

1. TOSOH G8 Ion exchange (Tosoh Bioscience, Japan),

High performance liquid chromatography (HPLC)

2. NycoCard Reader II (Axis-Shield, Norway), Boronate affinity

3. CERA STAT 2000 (Ceragem Medisys Inc, Korea), Boronate affinity

4. DS5 (Drew Scientific inc, France), Ion exchange chromatography

5. Biosystems (BioSystems S.A, Spain), Ion exchange chromatographic (manual)

6. Pars Azmoon (Pars azmoon, Iran), Immunoturbidometry

TOSOH G8, NycoCard Reader II and CERA STAT 2000 are NGSP-certified assays. TOSOH G8 was considered as the Standard Reference Method (SRM) and comparability of HbA1c results was evaluated by the analysis of TOSOH G8 results.

The Clinical Laboratory Standardization Institute (CLSI) EP 15-A2 and EP-9 A2 protocols were used to investigate assay imprecision and compare the results of the methods (10, 11). Microsoft Excel 2007 and SPSS version 15 packages (SPSS, Chicago, IL, USA) were used to analyze the data. Total Error (TE) was calculated as: bias \pm 1.96 SD of differences.

4. Results

Total imprecision of TOSOH G8, NycoCard Reader II, CERA STAT 2000, DS5, Biosystems and Pars Azmoon in different levels of HbA1c were less than 0.5%, 3.1%, 3%, 3.3%, 3.4% and 2.3%, respectively. A significant difference between SRM and the mean of each test method was evident (Table 1). The results were classified into three groups (less than 6.5%, 6.5% - 8% and more than 8% of HbA1c) and in each group, the means of the assays, partitioned bias, SD of difference and TE (percent) were calculated (Table 2).

Table 1. Correlation of Different Kit/System Results With TOSOH G8

Assays	Mean \pm SD	Linear Regression	r	r ²	P Value
TOSOH	7.27 \pm 1.69	-	-	-	-
Pars Azmoon	6.36 \pm 1.40	Y = 0.81x + 0.45	0.98	0.96	< 0.001
Biosystems	7.55 \pm 1.42	Y = 0.78x + 1.87	0.93	0.87	0.004
NycoCard Reader II (Venous blood)	6.73 \pm 1.35	Y = 0.77x + 1.12	0.97	0.94	< 0.001
CERA-STAT 2000 (Venous blood)	6.3 \pm 1.68	Y = 0.94x - 0.56	0.95	0.89	< 0.001
DS5	6.82 \pm 1.91	Y = 1.1x - 1.16	0.97	0.94	< 0.001

Table 2. Total Error in Percent in Different Levels of HemogolobinA1c

Assays	HbA1c Level		
	< 6.5%	6.5 - 8%	> 8%
Pars azmoon	-20.91	-17.85	-22.73
Biosystems	28.57	22.07	-9.95
NycoCard Reader II (Venous blood)	-10.65	-18.04	-19.64
CERA-STAT 2000 (Venous blood)	-27.04	-27.51	-27.45
DS5	-26.06	-19.70	-14.88

5. Discussion

The UK Prospective Diabetes Study results have demonstrated that the risk of retinopathy, nephropathy, and potentially neuropathy is significantly reduced when blood sugar levels in type 2 diabetes are kept strictly low by means of tight glycemic control, which is defined by a median HbA1c of 7.0% in contrast with the standard glycemic control with a median HbA1c of 7.9%. It was found that with the tight glycemic control monitored by continuous measurement of blood HbA1c levels; the overall microvascular complication rate was decreased by 25% (12). Therefore, considering the severity of diabetes complications, in diabetes control, low imprecision (CV of 1.9%) of analytical methods is considered critical (13). In our study, imprecision of all evaluated assays (except TOSOH G8) was higher than 1.9%. Although a strong correlation was observed in comparison study (r : 0.86 to 0.96), the mean of different methods were significantly different from that of the reference method (P value < 0.01). Moreover, in a great majority of them, a negative bias was observed, which was not dependent to concentration. Besides, calculated TEs for all assays ranged from 9.65% to 28.68%, which were remarkably higher in comparison with optimal ranges (NGSP and CAP criteria are less than 7% and 6%, respectively) (14, 15). Furthermore, our findings demonstrated that the results reported by NGSP-certified and non-NGSP certified devices can differ significantly when the same sample is assayed. The findings of our study demonstrated that the differences between results reported by different HbA1c assays in Iran are too large to be acceptable when used interchangeably and this can lead to confusing clinical interpretations. Moreover, our results indicated that the routine assays used in laboratories in Iran show different degrees of negative bias. According to the NGSP criteria as described before, the calculated bias is quite significant. The extent of bias among the evaluated methods was large and highly suggestive that unacceptable intermethod differences can most commonly be observed when various HbA1c assays are utilized for the analysis of the same sample. In conclusion, it seems that national programs are needed to standardize HbA1c assays. In the current situation, for better monitoring of diabetic patients using the same laboratory for HbA1c measurement is recommended. To shed more light on this issue and its potential to distort clinical decisions in diabetes care, further studies with larger sample sizes are warranted.

Authors' Contributions

Parvin Pasalar and Farideh Razi proposed the concept of the study. Farideh Razi and Tahereh Keramati did the

analytical aspects of the study. Mostafa Qorbani and Farideh Razi analyzed the results. An initial draft of the manuscript was written by Ensieh Nasli Esfahani and Ali Tootee, which was reviewed and edited by Parvin Pasalar and Farideh Razi. All authors read and approved the final manuscript.

Funding/Support

This project has been funded by the diabetes research center, endocrinology and metabolism clinical sciences institute of Tehran University of Medical Sciences as a part of an endocrinology subspecialty fellowship thesis.

References

- Cowie CC, Rust KF, Ford ES, Eberhardt MS, Byrd-Holt DD, Li C, et al. Full accounting of diabetes and pre-diabetes in the U.S. population in 1988-1994 and 2005-2006. *Diabetes Care*. 2009;**32**(2):287-94.
- Esteghamati A, Gouya MM, Abbasi M, Delavari A, Alikhani S, Alaedini F, et al. Prevalence of diabetes and impaired fasting glucose in the adult population of Iran: National Survey of Risk Factors for Non-Communicable Diseases of Iran. *Diabetes Care*. 2008;**31**(1):96-8.
- Vahdat Shariatpanahi Z, Shahbazi S. Prevalence of Diabetes Mellitus and Pre-Diabetes in Sarableh City, Ilam. *Thrita*. 2014;**3**(2).
- John WG, Mosca A, Weykamp C, Goodall I. HbA1c standardisation: history, science and politics. *Clin Biochem Rev*. 2007;**28**(4):163-8.
- Sacks DB, Bruns DE, Goldstein DE, Maclaren NK, McDonald JM, Parrott M. Guidelines and recommendations for laboratory analysis in the diagnosis and management of diabetes mellitus. *Clin Chem*. 2002;**48**(3):436-72.
- Berg AH, Sacks DB. Haemoglobin A1c analysis in the management of patients with diabetes: from chaos to harmony. *J Clin Pathol*. 2008;**61**(9):983-7.
- Goldstein DE, Little RR, Lorenz RA, Malone JJ, Nathan D, Peterson CM, et al. Tests of glycemia in diabetes. *Diabetes Care*. 2004;**27**(7):1761-73.
- Weykamp C. HbA1c: a review of analytical and clinical aspects. *Ann Lab Med*. 2013;**33**(6):393-400.
- Holmes EW, Ersahin C, Augustine GJ, Charnogursky GA, Gryzbac M, Murrell JV, et al. Analytic bias among certified methods for the measurement of hemoglobin A1c: a cause for concern? *Am J Clin Pathol*. 2008;**129**(4):540-7.
- Krouwer J, Tholen D, Garber C, Goldschmidt H, Harris Kroll M, Linnet K. *Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline, ed 2, document EP9-A2*. Wayne: Clinical and Laboratory Standards Institute; 2002.
- Carey RN. *User Verification of Performance for Precision and Trueness: Approved Guideline*. Clinical and Laboratory Standards Institute; 2006.
- American Diabetes A. Implications of the United Kingdom Prospective Diabetes Study. *Diabetes Care*. 2000;**23** Suppl 1:S27-31.
- Lenters-Westra E, Schindhelm RK, Bilo HJ, Slingerland RJ. Haemoglobin A1c: Historical overview and current concepts. *Diabetes Res Clin Pract*. 2013;**99**(2):75-84.
- Little RR, Rohlfing CL, Sacks DB, National Glycohemoglobin Standardization Program Steering C. Status of hemoglobin A1c measurement and goals for improvement: from chaos to order for improving diabetes care. *Clin Chem*. 2011;**57**(2):205-14.
- Woodworth A, Korpi-Steiner N, Miller JJ, Rao LV, Yundt-Pacheco J, Kuchipudi L, et al. Utilization of assay performance characteristics to estimate hemoglobin A1c result reliability. *Clin Chem*. 2014;**60**(8):1073-9.