Hemoglobin A1c: A reliable and accurate test for diabetes care? A prospective study in Mexico

José Gerardo González-González, MD, PhD,[1] René Rodríguez-Gutiérrez, MD,[2]
Fernando Javier Lavalle-González, MD,[1] Arnulfo González-Cantú, MD,[1] Héctor Eloy Taméz-Pérez, MD,[1]
Gerardo González-Saldívar, MD,[2] Jesús Zacarías Villarreal-Pérez, MD.[1]

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Abstract

Objective. To compare the concordance correlation coefficient for HbAlc results in an in-field experience. **Materials and methods**. A prospective study in Monterrey, Mexico from April to August 2012 was conducted to evaluate the day-to-day clinical situation when measuring HbAlc. Blood samples from 38 consecutive patients were sent to seven local laboratories and one international reference laboratory. **Results**. Poor concordance was found in 4 out of 7 laboratories, moderate in 2 out of 7, and significant in just one. HbAlc values from three laboratories fluctuated more than 1% above or below the reference laboratory in more than 30% of cases, and more than 2% in 10%-20% of subjects. **Conclusions**. Standardized HbAlc measurement has not occurred worldwide. Physicians should be aware of this issue and be cautious of HbAlc guidelines on diabetes diagnosis or management until proper standardization programs are implemented.

Key words: diabetes mellitus; hemoglobin A glycosylated; laboratory test; Mexico

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Resumen

Objetivo. Comparar el coeficiente de correlación de concordancia de HbAlc. **Material y métodos.** Estudio prospectivo en Monterrey, México, de abril a agosto de 2012 para evaluar la medición de la HbAlc. Participaron 38 individuos y se envió la muestra a 7 laboratorios locales y a uno internacional de referencia. **Resultados.** Se encontró pobre concordancia en 4 de 7 laboratorios, moderada en 2 y una concordancia significativa en uno. Los valores de HbAlc de tres laboratorios fluctuaron más de 1% del laboratorio de referencia en más de 30% de los casos y más de 2% en 10 a 20%. **Conclusiones**. La estandarización de la HbAlc no está concluida. Los médicos deberían tomar con cautela las recomendaciones de las guías para HbAlc en el diagnóstico o manejo de la diabetes hasta que se implementen los programas de estandarización.

Palabras clave: diabetes mellitus; hemoglobina A glucosilada; prueba de laboratorio; México

(1) Servicio de Endocrinología, Hospital Universitario Dr. José E. González, Universidad Autónoma de Nuevo León. Monterrey, Nuevo León, México.
(2) Servicio de Medicina Interna, Hospital Universitario Dr. José E. González, Universidad Autónoma de Nuevo León. Monterrey, Nuevo León, México.

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Corresponding author: Dr. José Gerardo González-González. Servicio de Endocrinología, Hospital Universitario Dr. José E. González, Universidad Autónoma de Nuevo León. Av. Madero y Gonzalitos s/n, col. Mitras Centro. 64460 Monterrey, NL, México.
E-mail: jgonzalezg@investigacion-meduanl.com

β-N-(1-deoxy)-fructosyl hemoglobin, better known as hemoglobin A1c (HbA1c), reveals the mean blood glucose value in the past 8 to 12 weeks.[1,2] It is widely recognized as a keystone in the assessment of the quality of chronic glycemic control and a treatment goal in diabetes care, predicting the risk of severe hypoglycemia and being a constant primary endpoint in diabetes clinical trials.[3-5] The results of the DCCT (Diabetes Control and Complications Trial) and UKPDS (United Kingdom Prospective Diabetes Study) showed that A1c correlated remarkably to predict chronic microvascular complications and demonstrated a trend to predict macrovascular disease.[6,7] Recently, international diabetes organizations have also given support to its use for diabetes screening and diagnosis, although this issue remains debatable.[8-10] Therefore, a reliable and accurate assay for HbA1c measurement, available worldwide, is required for its ideal use.

Recognition of the clinical value of HbA1c in diabetes management led to the creation, around 1990, of different working groups to develop a global reference method as well as a standardization of the HbA1c measurement.[11-13] Some countries achieved a national direct comparison method improving the variation of their HbA1c results but the need for a global strategic program was recognized.[14] Therefore, since 1993, multiple meetings have been carried out by working groups of the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC), the National Glycohemoglobin Standardization Program (NGSP), the American Diabetes Association (ADA), the International Diabetes Federation (IDF) and the European Association for the Study of Diabetes (EASD).[15-19] There have been many advances, such as a global HbA1c reference system, standardization of the procedure and an agreement to inform the results, but the application of these recommendations has not been carried out in some countries that have large populations and a high prevalence of diabetes, such as Mexico.[20] Furthermore, despite the fact that all scientific communications mention the rigorous need of a standardized HbA1c measurement procedure, this has failed to take place in some countries. Many Mexican healthcare providers, on a daily basis, likely rely on a non-standardized HbA1c test for diabetes screening, diagnosis, and treatment.[8,12,13]

As a consequence, we decided to carry out a prospective study to evaluate the day-to-day clinical situation that a type 2 diabetes Mexican patient faces when a blood sample for HbA1c measurement is taken. The primary endpoint was to compare the intraindividual/interlaboratory concordance correlation coefficient (CCC) for HbA1c. Secondary end points were: 1) to estimate the HbA1c CCC and dispersion data at two HbA1c range values and between different laboratories that shared the same HbA1c measurement technique, and 2) to determine the clinical significance of the intraindividual HbA1c results by different laboratories in the physician's interpretation of glycemic control.

Materials and methods

Subjects

We studied 38 consecutive participants from April to August of 2012 of the Diabetes Clinical Research Unit of the Dr. José E. González University Hospital in Monterrey, Mexico. Approval was obtained from the Institutional Review Board and informed consent was obtained from all participants. Male or female patients between 18 and 70 years of age with diabetes mellitus were included. Pregnant women and all clinically well-recognized situations that may lead to erroneous HbA1c values were excluded.[1] After an overnight fasting, a blood sample was taken for HbA1c measurement. The sample was sent to eight laboratories.

Measurements

After an overnight fast, a blood sample for HbA1c was taken between 0800 and 0900 hours in all participants and then sent to each laboratory within the next three hours at a temperature between 4 and 8ºC. The seven laboratories were the largest routine clinical laboratories in the metropolitan area of Monterrey, Mexico. Table I shows the methods for HbA1c determination used in each selected clinical laboratory. Three used ionic exchange high-pressure liquid chromatography (IE-HPLC) with different commercial products; two laboratories used cationic exchange resin by spectrophotometry (S-CER); one laboratory used an immunoassay of cationic exchange resin (I-CER), and the last one utilized turbid metric immunoassay (TI). The manufacturer for IE-HPLC (D10 short and extended) was Bio-Rad Laboratories, clinical diagnostics group and for G8 Tosoh was Bioscience Inc. The manufacturer for S-CER was Stanbio laboratory. The manufacturer for I-CER (DCA 2000) was Siemens healthcare diagnostics Inc., and for TI, Biolabo. As a reference for comparison, the eighth laboratory was a central, certified laboratory for HbA1c measurement in the United States (Quintiles, Durham, NC). This laboratory used the IE-HPLC technique (Bio-Rad variant II turbo A1c, Bio-Rad laboratories clinical diagnostics group). The intraassay and interassay coefficient of variation (CV) of the laboratories participating in this study are shown in Table I.

Table I
Hemoglobin A1c measurement method and variability parameters in all selected laboratories. Monterrey, Mexico, April-August 2012

				Intra-laboratory CV	
Lab	*Manufacturer*	*Method*	*Technique*	*Intraassay (%)*	*Interassay (%)*
1en	Bio-Rad	D-10 extended	IE-HPLC	0.46-0.78	0.52-0.53
2en	Siemens	DCA 2000	I-CER	2.20-3.70	0.90-4.30
3mo	Bio-Rad	D-10 short	IE-HPLC	0.46-0.78	0.52-0.53
4an	Stanbio	Spectrophotometry	S-CER	1.70-2.70	4.10-4.60
5al	Biolabo	Turbidimetry	TI	1.43-1.72	2.67-2.77
6le	Tosoh	G8	IE-HPLC	0.78-1.89	1.67-2.58
7ro	Stanbio	Spectrophotometry	S-CER	1.70-2.70	4.10-4.60
8*	Bio-Rad	Variant II Turbo A1c	IE-HPLC	0.54-0.82	1.68-2.58

*Reference laboratory

CV= coefficient of variation
IE-HPLC= ion-exchange high-pressure liquid chromatography
I-CER= immunoassay of cationic exchange resin
S-CER = spectrophotometry of cationic exchange resin
TI= turbid metric immunoassay

Statistical analysis

All results are reported as means ± standard deviations unless otherwise indicated. A $p \leq 0.05$ was considered statistically significant. Descriptive statistical analysis was used for quantitative variables, measures of central tendency and dispersion. In the case of qualitative variables, frequencies were obtained. For concordance correlation analysis, Lin CCC was calculated.[21] In the case of continuous variables, such as in our study, a value greater than 0.99 means almost perfect concordance, between 0.95 and 0.99 significant concordance, between 0.90 and less than 0.95, moderate concordance and when less than 0.90, poor concordance.[21] The Bland & Altman method was used to show agreement between each local clinical laboratory and the reference laboratory. Using a formula for means equivalence studies with a K value $(z\,\alpha + z\,\beta)^2$ of 13, an alpha value of 0.05 two-tailed, a power of 95%, accepting an error of 1, a sample size of 31 participants was calculated. The statistical analysis was performed with IBM SPSS Statistics 20.0 and MedCalc Software bvba.[22]

Results

Study population

The mean age of the participants was 45.7 ± 12.2 years (range, 19-66). Twenty-eight cases (73.7%) were women. All participants had type 2 diabetes. Selecting the result of the reference laboratory, there were nine participants with an HbA1c value between 5.0 and 7.0%. A value greater than 7.0% and below 9.0% was found in 19 cases, and a value greater than 9.0 % but less than 11.0% was found in 10 subjects. There were no cases with a value greater than 11.0%.

Variation, dispersion and concordance of the HbA1c results

Table II shows the CV and CCC in the HbA1c results of the local clinical laboratories when compared to the reference laboratory. Laboratory 7 had the highest CV (30.35%). On the other hand, the lowest CV was found in laboratory 4 (15.28%). Furthermore, CCC found a poor concordance in 4 out of 7 laboratories (laboratory 1 (IE-HPLC), four (S-CER), six (IE-HPLC) and seven (S-CER), (0.84, 0.59, 0.65 and 0.50, respectively); a moderate concordance in 2 out of 7 [three (IE-HPLC) and five (TI)], and a significant but not almost perfect concordance (CCC= 0.95) only in laboratory 2 (I-CER).

Table III shows the CV and CCC of the local laboratories when the participants were divided into two groups, selected by an HbA1c cutoff value of 8.0% in the reference laboratory. There was not a trend to better agreement of any local clinical laboratory in HbA1c values higher or lower than 8.0% in the reference laboratory. In values lower or equal than 8.0% in the reference laboratory, the CCC decreased in all three laboratories that had a better performance as a whole. The CCC of laboratory three remained in the same category (moderate concordance) but laboratory 2 and 5 showed a worse performance (two from significant to moderate concor-

Table II
COEFFICIENT OF VARIATION AND CONCORDANCE CORRELATION COEFFICIENT OF THE CLINICAL LABORATORIES (N= 38). MONTERREY, MEXICO, APRIL-AUGUST 2012

	Range		Mean ± SD	CV	CCC
	Min.	Max.			
1	5.30	11.90	8.54 ± 1.68	16.69	0.84
2	5.20	10.90	8.14 ± 1.49	18.33	0.95
3	5.00	12.00	8.34 ± 1.69	20.31	0.94
4	6.30	11.40	8.76 ± 1.34	15.28	0.59
5	5.20	10.70	8.01 ± 1.44	18.02	0.93
6	3.80	10.70	7.14 ± 1.60	22.41	0.65
7	3.90	14.20	7.67 ± 2.33	30.35	0.50
8*	5.00	10.70	8.13 ± 1.50	18.47	

* Reference laboratory

CV= coefficient of variation
CCC= concordance correlation coefficient

Table III
COEFFICIENT OF VARIATION (CV) AND CONCORDANCE CORRELATION COEFFICIENT (CCC) OF THE PARTICIPANTS WITH AN HBA1C VALUE LESS THAN OR EQUAL TO 8% (N= 18) OR GREATER THAN 8% (N= 20) IN THE REFERENCE LABORATORY MEASUREMENT. MONTERREY, MEXICO, APRIL-AUGUST 2012

	HbA1c ≤ 8.0%		HbA1c > 8.0%	
Lab	CV %	CCC	CV %	CCC
1	14.11	0.88	9.00	0.49
2	15.22	0.91	7.15	0.80
3	15.89	0.90	9.55	0.77
4	13.73	0.20	12.52	0.50
5	13.63	0.86	8.50	0.79
6	21.24	0.46	13.40	0.24
7	37.06	0.35	19.41	0.01
8*	13.89			7.88

* Reference laboratory

dance, and five from moderate to poor concordance). In HbA1c values higher than 8.0% the concordance with the reference laboratory was worse. No laboratory showed excellent, significant or moderate concordance. Laboratories that had a better performance as a whole (2, 3 and 5) all had poor concordance. The CCC of HbA1c values reported by the three local laboratories that used the IE-HPLC technique (laboratories 1, 3 and 5) was also analyzed. Laboratory 3 had a moderate concordance (CCC=0.91), and laboratory 1 and 6 had a poor concordance (CCC= 0.83 and 0.60, respectively).

The agreement assessment between the local and the reference laboratory was also analyzed by the Bland & Altman method. Laboratories 2, 3 and 5 had the best agreement again (Table IV). As a whole, laboratory 2 showed the best agreement again with a means difference of −0.01 (95% confidence interval 0.64 to -0.66). Laboratories 4, 6 and 7 had unacceptable HbA1c agreement values.

Clinical impact of the disagreement in HbA1c results

Classification of the differences in the HbA1c results in each patient between the reference laboratory and each local laboratory is shown in Table V. The HbA1c results were classified into four categories of range dissimilarity: 1) equal or less than ± 0.5%, 2) ± 0.6 to 1.0%, 3) ± 1.1 to 2.0% and 4) greater than ± 2%. Laboratory 2 had the best plus/minus percent fluctuation equal or less than 0.5% in the HbA1c result (92%, 35 out of 38 cases). Laboratories 3 and 5 had 33 and 32 out of 38 cases, 87 and 84%, respectively. Laboratories 4, 6 and 7 had this category in less than a third of the cases (31, 29 and 29%, respectively). Laboratories 2 and 5 had any case with a plus/minus fluctuation in HbA1c greater than 1.0%. Laboratories 4, 6 and 7 had a plus/minus fluctuation in HbA1c greater than 1.0% in 32, 45 and 54% of the cases, respectively. A fluctuation greater than plus/minus 2.0% in HbA1c occurred in 11% (4 out of 38), 16% (6 out of 38) and 21% (8 out of 38) of the cases, respectively. Most cases in laboratory 4 had an HbA1c result above the result (greater than 1.0%) of the reference laboratory. The

Table IV
AGREEMENT OF THE HEMOGLOBIN A1C RESULTS BETWEEN THE LOCAL AND THE REFERENCE LABORATORY BY THE BLAND & ALTMAN METHOD (21) (N=38). MONTERREY, MEXICO, APRIL-AUGUST 2012

Laboratory	Means Difference	Confidence Interval 95% (CI95%) Upper limit	Lower limit
1	-0.41	0.67	-1.49
2	-0.01	0.64	-0.66
3	-0.21	0.54	-0.96
4	-0.60	1.70	-2.90
5	0.12	0.90	-0.65
6	0.98	2.91	-0.94
7	0.50	4.20	-3.30

Table V
Variation ranges of HbA1c results on the local clinical laboratory when compared to the reference laboratory. Monterrey, Mexico, April-August 2012

Laboratory	*HbA1c ± variation range*			
	≤ 0.5%	*0.6-1.0%*	*1.1-2.0%*	*≥ 2.0%*
Laboratory 1				
+ n=	24	8	1	1
- n=	3	1	0	0
Total [n, (%)]	[27, (71)]	[9, (23.7)]	[1, (2.6)]	[1, (2.6)]
Laboratory 2				
+ n=	12	1	0	0
- n=	23	2	0	0
Total [n, (%)]	[35, (92.1)]	[3, (7.9)]		
Laboratory 3				
+ n=	21	4	1	0
- n=	12	0	0	0
Total [n, (%)]	[33, (86.8)]	[4, (10.5)]	[1, (2.6)]	
Laboratory 4				
+ n=	3	10	8	3
- n=	9	4	0	1
Total [n, (%)]	[12, (31.5)]	[14, (36.9)]	[8, (21.1)]	[4, (10.5)]
Laboratory 5				
+ n=	8	3	0	0
- n=	24	3	0	0
Total [n, (%)]	[32, (84.2)]	[6, (18.8)]		
Laboratory 6				
+ n=	2	2	0	0
- n=	9	8	11	6
Total [n, (%)]	[11, (29)]	[10, (26.2)]	[11, (29)]	[6, (15.8)]
Laboratory 7				
+ n=	5	1	3	3
- n=	6	6	9	5
Total [n, (%)]	[11, (29)]	[7, (18.4)]	[12, (31.6)]	[8, (21)]

(+)= a higher (positive) HbA1c value in the local laboratory when contrasted to the reference laboratory
(-)= a lower (negative) HbA1c value in the local laboratory when contrasted to the reference laboratory

opposite was found in laboratory 6 and 7, which had an HbA1c result below the value (greater than 1.0%) of the reference laboratory.

Discussion

In our study, blood samples from 38 participants sent to seven of the most important local laboratories in a metropolitan area in a large city in Mexico and to one reference laboratory showed that 4 out of 7 laboratories had a poor concordance or agreement by different statistical methods and analytical procedures. This lack of agreement resulted in a greater lack of precision or reliability with higher HbA1c values when population was divided into cases above or below 8.0%. These findings reveal a huge problem in diabetes management in our large population, that could be present in many other communities or countries in daily clinical practice,

since the characteristics of our study design is illustrative of a real-life type 2 diabetes patient when HbA1c is measured. Many clinical situations are well-recognized causes of misleading values of HbA1c.[1] Variations in the lifespan of the erythrocyte and the molecular structure of hemoglobin, chronic renal failure, medications, ethnicity, aging, etc., are some of the most common etiologies. The physician should take all these situations into consideration in day-to-day clinical practice of type 1 and type 2 diabetes patients when assessing the quality of chronic glycemic control by an HbA1c measurement. Nevertheless, incorrect HbA1c values, in a wide error range, due to problems in laboratory measurements are not easy to suspect and could lead the physician to make a mistake in the classification of the quality of glycemic control and the decision-making process on pharmacological management in patients with diabetes.

For more than two decades, ADA, IDF, EASD, IFCC and representatives of different areas related to diabetes care have proposed global recommendations concerning HbA1c measurement methods and how the results should be informed.[13-19] Three conclusions have been the core in almost all these technical reports: 1) "all HbA1c test results should be standardized worldwide, including the reference system and the method used for reporting the results", 2) "the new IFCC reference system for HbA1c represents the only valid tool for obtaining standardized HbA1c measurements," and 3) "HbA1c results should be reported everywhere in IFCC units (mmol/mol) and derived NGSP units (%), using the IFCC-NGSP master equation". A recent paper has mentioned that the first recommendation has been completed worldwide.[23] Our results and other recent publications in different countries have recognized severe problems in this regard and their comments are in opposition to the statement of a completed globalization in HbA1c measurement.[24-26] Again, our evaluation, based on an experimental model of daily clinical practice, in an adequate sample size, has shown a critical issue concerning diabetes management by different statistical methods; a very poor concordance or agreement in HbA1c results was found in more than half of the participating laboratories. The robust clinical significance of our results would not change with involvement of more clinical laboratories or subjects. Our study was carried out in the largest clinical laboratories of the metropolitan area of a big community in Mexico. Some studies have revealed concordance with some of our findings.[27-30] A recent recommendation suggests that a 0.5% fluctuation range in HbA1c values is the acceptable limit for HbA1c measurement.[13] Our results show that almost 2/3 of the cases in 4 out of 7 of our participating laboratories reported an HbA1c error value greater than 1.0% above or below the reference value. This finding makes HbA1c measurement unacceptable in our population in a daily clinical practice. A limitation of our study is the lack of participation of other cities in Mexico; it is necessary to extend this study to other countries. It is likely that our medical scenario is still occurring in many places in the world with high diabetes prevalence. The lack of this valuable assessment tool makes it very difficult to follow international diabetes screening diagnosis and management recommendations.[8,9,31] It is clear that the recent recommendation of HbA1c measurement for diabetes screening and diagnosis is not conceivable to be applied in countries that share the same problematic uncovered by our study.[8] Diabetes screening and diagnosis were not an endpoint in our study but our results in a type 2 diabetes population clearly show that HbA1c measurements cannot be used for all these purposes. As a consequence, it would be urgent that health authorities in many countries linked to these international academic organizations in diabetes carry out an international certification procedure of clinical laboratories to obtain a license for standardized HbA1c measurement.

The third statement is that HbA1c should be reported in IFCC units and derived NGSP units.[13-19] IFCC units are not widely used by physicians on daily clinical practice and teaching institutions in our country. Patients are still less aware of the use of IFCC units. A technical report on HbA1c has mentioned that from January 2012, all scientific communications in prestigious journals were going to report HbA1c values in IFCC units and derived NGSP (%) units.[13] We carried out a critical review of the 20 highest impact factor journals on diabetes and some prestigious internal medicine journals on articles published during this year (2012) and found that 90% are still using NGSP (%) units for reporting HbA1c values. Most physicians related with diabetes care are not aware of the advantages of this new way to report HbA1c results but this issue increases the complexity of the implementation of strategies for HbA1c clinical interpretation and standardization all over the world.

In conclusion, in some locations there is still a serious problem in HbA1c measurements as a result of a lack of standardization of the methods in clinical routine laboratories. This situation makes it difficult to apply many important recommendations on diabetes patient management and, as a consequence, patients are at greater risk of developing chronic diabetes complications. In the case of diabetes screening or diagnosis, the unreliability and dispersion of our HbA1c findings makes this strategy a risk for under or over diagnosis of diabetes. All recommendations or guidelines related to

HbA1c use in clinical practice must be strong and clear, pointing out that they apply only with standardized laboratories. In daily clinical practice, however, physicians do not ask whether the HbA1c result comes from a standardized laboratory or not. It is necessary to carry out randomized studies to evaluate the reliability of the HbA1c results in everyday management of patients with diabetes, particularly in countries that are likely in the same situation as us. Once this situation has been solved, it could be valuable to go for implementation of HbA1c in IFCC units and other issues with a less valuable clinical relevance in day-to-day diabetes care.

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Declaration of conflict of interests. The authors declare that they have no conflict of interests.

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