Research Article

The evaluation of measurement uncertainty of HbA1c and its effect on clinical decision levels

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Abstract

Objectives: The prevalence of diabetes mellitus (DM) is increasing all over the world. Hemoglobin A1c (HbA1c) is one of the diagnostic tests for DM. But is the HbA1c analysis result accurate and absolute? Uncertainty of measurement is a quality parameter of measurement results, and characterizes the dispersion of the values attributed to a measured quantity. The aim of this study was to estimate the measurement uncertainty (MU) of HbA1c and retrospectively re-evaluate patient results with respect to the estimation of uncertainty and to suggest a solution for results that are close to cut-off level.

Methods: The results of 10212 patients who had an HbA1c analysis performed in our laboratory in 2016 were retrospectively reviewed. The HbA1c levels were measured using a high performance liquid chromatography method. The uncertainty of measurement of the serum HbA1c level was estimated according to the Eurachem/Co-operation on Traceability in Analytical Chemistry Guide CG 4.

Results: The measurement uncertainty (95% confidence interval) of HbA1c was estimated to be ±4.6%. When measurement uncertainty was taken into account, the acceptable range for the 6.5% value typically used to diagnose DM was between 6.2% and 6.8%. It was observed that the results of 1555 patients were affected by uncertainty values.

Conclusion: Medical laboratories must produce the necessary data and analytical results in order to achieve the correct interpretation and use of the results. A test result is not sufficiently powerful without an assessment of its reliability. The interpretation of values that are close to cut-off levels may change when evaluated with the uncertainty of measurement. Therefore, reporting HbA1c analysis results with the estimation of the MU is important to illustrate the true limits and the level of confidence.

Keywords: Diabetes mellitus, hemoglobin A1c, measurement uncertainty

The objective of a measurement is to determine an estimate of the true value of the measurand. The measurement uncertainty (MU) is a parameter associated with the result of a measurement that characterizes the dispersion of the values that could reasonably be attributed to the measurand [1]. The MU is used as a quantitative measure of accuracy. With the adoption of the International Organization for Standardization (ISO) Medical Laboratories–Particular Requirements for Quality and Competence standard (ISO 15189), laboratories are required to provide estimates of the MU for all quantitative test results [2]. Recent developments include the requirement in ISO 17025 and the ISO 15189 accreditation standards that laboratories provide the MU of the results and the calculation of total allowable error (TAE) [3]. The MU estimates an interval of values within which the "true" value of a measured analyte lies, with a stated level of confidence [4]. Inclusion of the MU is the appropriate approach for meaningfully comparing measurement results with reference values [5].

Diabetes mellitus (DM) is a complex and chronic disorder, the prevalence of which has been increasing all over the world. Tests for DM screening and diagnosis are available. Diabetes may be diagnosed based on plasma glucose criteria, either the fasting plasma glucose (FPG) or the 2-hour plasma glucose (2-h PG) value after a 75-g oral glucose tolerance test (OGTT), or hemoglobin A1C (HbA1c) criteria [6]. DM may be diagnosed based on a value of FPG ≥126 mg/dL or plasma glucose ≥200 mg/dL 2 hours after a 75-g glucose load or an HbA1c ≥6.5% [6]. HbA1c testing should be performed using a method that is standardized and certified by the National Glycohemoglobin Standardization Program (NGSP) [7]. The HbA1c test has advantages compared with the FPG and OGTT, including greater convenience (fasting not required), and greater preanalytical stability [7]. An HbA1c of 48 mmol/mol (6.5%) is recommended as the cut-off point for diagnosing diabetes [8]. The American Diabetes Association has suggested 5.7% to 6.4% as the high-risk range for diabetes (prediabetes) [7]. Studies have also shown a strong relationship between HbA1c and the risk for the development and progression of complications of DM [9].

The aim of this study was to estimate the MU for HbA1c and re-evaluate patient results with respect to the estimation of MU, and to suggest a solution for results that are close to the cut-off level.

Materials and Methods

The study was conducted at Ankara Polatlı Public Hospital. The records of 10212 patients whose HbA1c level was tested between January 2016 and December 2016 were retrospectively reviewed.

High performance liquid chromatography method

The high performance liquid chromatography (HPLC) method (Premier Hb9210 autoanalyzer, Trinity Biotech plc, Bray, Ireland) was used to determine HbA1c values in fresh human whole blood samples per the manufacturer's instructions using original commercial kits.

Precision study of the HbA1c assay was performed according to the Clinical and Laboratory Standards Institute (CLSI; formerly NCCLS) EP5A protocol [10] by estimating within-run and total standard deviation, and by calculating the coefficient of variation (CV%). Both normal and abnormal level internal quality control materials were used for the determination of precision (Table 1).

Estimation of measurement uncertainty

The HbA1c analysis results were re-evaluated with the estimation of MU. Internal and external quality control results were used to calculate the MU according to the Eurachem/Co-operation on Traceability in Analytical Chemistry (CITAC) Guide CG 4 [1].

The formulation of uncertainty is explained below.

The calculation of uncertainty of within-laboratory reproducibility (uRW) used the Trinity Biotech control level 1 CV%

Table 1. The precision data of the hemoglobin A1c assay obtained from normal and abnormal internal quality control samples

	Normal level	Abnormal level
A. Within-run precision		
Number of data points	80	80
Total mean	5.4	11.0
Within-run SD	0.112	0.094
Within-run CV%	2.08	0.85
B. Between-run precision		
Number of data points	80	80
Total mean	5.4	11.0
Between-run SD	0.011	0.158
Between-run CV%	0.21	1.44
C. Between-day precision		
Number of data points	80	80
Total mean	5.4	11.0
Between-day SD	0.182	0.176
Between-day CV%	3.37	1.61
D. Total Precision		
Number of data points	80	80
Total mean	5.4	11.0
Total SD	0.214	0.255
Total precision CV%	3.97	2.32

CV: Coefficient variation

(CV1) and level 2 CV% (CV2) for a month (Table 2).

uRW: $\sqrt{[(CV1^2 + CV2^2)/2]}$

The second step was to calculate the uncertainty of the bias (ubias). The Randox International Quality Assessment Scheme external quality control results of 8 months were used. The root mean squares of biases (RMS bias) and the uncertainty component from the certified or nominal value (ucref) were calculated. External quality control bias results were used to calculate RMS bias (Table 2).

RMSbias : $\sqrt{[(\Sigma bias \text{ (external quality control)}^2/N]}$

(N: external quality control number)

The external quality control result mean CV% (sR) and laboratory number were used to calculate the ucref (Table 2).

ucref: $(sR/\sqrt{n})$

Standard ubias was calculated according to a formula.

ubias: $\sqrt{[(RMSbias)^2+(ucref)^2]}$

The combined uncertainty was determined.

(u)= $\sqrt{\{[(uRW)^2+(ubias)^2]/2\}}$

And finally, the expanded uncertainty (U) was calculated using the standard uncertainty (uc).

U=k*uc.

k: coverage factor(for 95% level of confidence [CI], k=2)

U results were compared with the TAE for the HbA1c test.

Table 2. The measurement of uncertainty for hemoglobin A1c

Internal quality control, CV% and uRw values	Internal quality control level 1 (mean and CV%)	5.42- 2%
	Internal quality control level 2 (mean and CV%)	11.04- 1.29%
	uRW	1.68
External quality control, RMS bias, CV%, n and ucref values	RMSbias	1.53
	CV%	2.61
	n	55
	Ucref	0.35
Standard, combined and expanded uncertainty values	Standard uncertainty (ubias)	1.57
	Combined uncertainty (u)	2.3
	Expanded uncertainty	4.6

CV: Coefficient variation; RMS bias: Root mean squares of biases; Ucref: Uncertainty component from the certified or nominal value; URw: Uncertainty of within-laboratory reproducibility

Results

The precision data is provided in Table 1. In the precision study, the within-run precision was 2.08% CV, the between-run precision was 0.21% CV, the between-day precision was 3.37% CV, and the total precision was 3.97% CV for the normal level sample. The within-run precision was 0.85% CV, the between-run precision was 1.44%, the between-day precision was 1.61% CV, and the total precision was 2.32% CV for the abnormal level sample. The HbA1c assay showed that the within-run precision and total precision results were within the current NGSP requirement of ≤4%.

The results of the uncertainty estimation of HbA1c in our study are presented in Table 2. An HbA1c of 6.5% is recommended as a cut-off value for diagnosing DM. We evaluated 10212 patients retrospectively. The HbA1c values were between 4% and 17.9%. In all, 4960 patients had a measured level of ≥6.5%. The MU (95% CI) for HbA1c was estimated at ±4.6%. When the MU was taken into account, the acceptable range for a value was between 6.2% and 6.8% (Fig. 1). With this frame of reference, results that were initially measured as between 6.5% and 6.8% might actually be less than 6.5%, and results that were between 6.2% and 6.4% might be greater than 6.5%. It was observed that the results of 1555 patients were affected by uncertainty values.
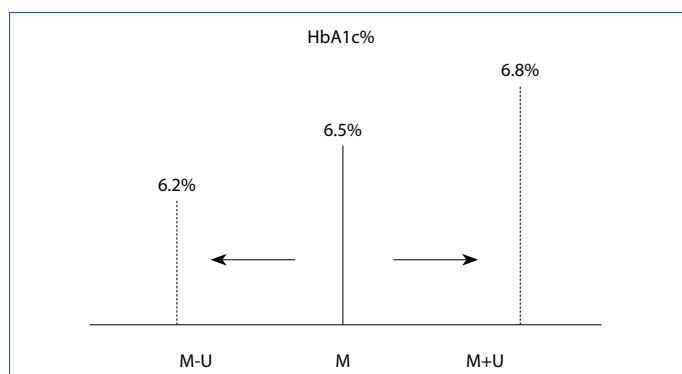

Figure 1. Diagnosing criteria for DM in a 4.6% uncertainty of HbA1C.

The TAE is ±6% according to the NGSP. The uncertainty of HbA1c was ±4.6% in our study.

Discussion

Measuring HbA1c to evaluate glycemic control in patients with DM is well established [11]. The NGSP program has achieved remarkable success regarding the imprecision of HbA1c measurement [12]. It is important that most laboratories have concluded that an imprecision goal between 2% and 4% is desirable for HbA1c [11]. The results of our research revealed a total precision of ≤4% for both normal and abnormal HbA1c samples.

Medical laboratories should produce the necessary data and analytical results in order to achieve the correct interpretation and use of the results. A test result is not sufficiently strong without an assessment of its reliability. The MU provides a quantitative estimate of the level of confidence that a laboratory has in the analytical precision of the test result, and therefore represents the expected variability in a laboratory result if the test is repeated a second time [12]. According to ISO 15189, the MU should be made available by the laboratory on request. Clinical decisions can be better evaluated knowing the MU of a test. Several studies have investigated the MU of different parameters since the importance was recognized [13-16]; however, to the best of our knowledge, this is the first study reporting the evaluation of the MU for HbA1c.

Some tests are evaluated against a cut-off value, such as glucose in a glucose tolerance test, cholesterol levels, and HbA1c measurements. For the HbA1c, a level of 6.5% is the clinical level used for a decision regarding a diabetes diagnosis. The MU of HbA1c at the level of 6.5% was ±4.6% in this study. With this point of view, taking the MU into account, the acceptable value was between 6.2% and 6.8%. Therefore, the decision needs to be made cautiously and interpreted with the knowledge that the cut-off has an associated measurement error margin.

In our study, 1555 patients were affected by uncertainty values. When clinicians know the uncertainty of a measurement

such as HbA1c, they may repeat the test at a different time before a diagnosis is made or they may follow up clinically or use another test for diagnosis. We used internal and external quality control results to calculate uncertainty according to Eurachem/CITAC recommendations. Internal and external quality controls help to assess within-laboratory reproducibility and bias (method and laboratory). The within-laboratory reproducibility includes repeatability for samples and variation from day-to-day. According to the International Vocabulary of Metrology, bias is defined as an estimate of the systematic error, where the systematic error is defined as a "component of measurement error that in replicate measurements remains constant or varies in a predictable manner" [17]. Bias variation may be evaluated using different samples over a time period using external quality control material. This quality control material provides essentially all of the data required for uncertainty estimation [18].

The uncertainty of an HbA1C value was below the TAE (±6%) in our laboratory. Medical laboratories must produce the necessary data and analytical results in order to achieve the correct interpretation and use of the results. A test result is not powerful enough without an assessment of its reliability. The interpretation of values that are close to cut-off levels may change when they evaluated with the MU. Therefore, reporting HbA1c analysis results with an estimation of the MU is important to demonstrate the true limits and the level of confidence. The limit values of HbA1c results (within the MU) requires careful follow-up.

According to the results of this study, it is important that clinicians should be aware of and consider the MU during the evaluation of HbA1c test results. The MU is still relatively new in the field of quantity measurement. We hope that MU can help clinicians and patients to better understand the accuracy of results and evaluate cut-off limits.

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