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Original Article

The importance of measuring the uncertainty of HbA1c analysis

Hakan Ayyildiz, Mehmet Kalayci

Department of Medical Biochemistry, Elazig Fethi Sekin City Hospital, Elazig, Turkey

Abstract

Aim: HbA1c, which reflects blood glucose levels in the last two to three months, has a significant role in the diagnosis, treatment and follow-up of diabetic patients. Measurement uncertainty is defined as the range of possible values, which comprises the measured level. This study aims at showing how to calculate the measurement uncertainty of HbA1c and informing clinicians on its significance for the efficient diagnosis, treatment and follow-up.

Materials and Methods: The measurement uncertainty calculating model was used to determine the measurement uncertainty of HbA1c. This model comprises six steps and is constructed depending on European Accreditation Guidelines, described in Nordest Guidelines, Eurolab Technic Report, and ISO/DTS 21748 Guidebook. The HbA1c analysis was chromatographically studied with Trinity Biotech Premier Hb9210 analyzer. Besides internal and external quality control data of HbA1c tests done in Elazig City Hospital in the months between January 2019 and September 2019, data analyses were done to calculate the measurement uncertainty by using the HbA1c test results.

Results: The measurement uncertainty of HbA1c was calculated as HbA1c±4.27% using a confidence interval of 95%. These results, were found to be lower than the total allowable error, determined by international organizations. Based on our results, the cut-off value of HbA1c of 6.5% had a measurement uncertainty between 6.2% and 6.8%.

Conclusion: We consider that this study will guide the laboratory specialists, concerned with the suggestion that "medical laboratories are required to calculate the measurement uncertainty for quantitative results." Furthermore, reporting the measurement uncertainty with test results will supply clinicians with information about measurement quality and contribute to creating awareness on this issue.

Keywords: HbA1c; laboratory; measurement uncertainty

INTRODUCTION

As a result of increasing standardization studies in recent years worldwide, it has been stated that HbA1c could be used as a diagnostic criterion of diabetes mellitus. The ADA Clinical Practical Guidelines and NGSP suggest using HbA1c with a cutoff of ≥ 6.5% to diagnose diabetes (1). Furthermore, HbA1c measurement is suggested in diabetic patients with stable glycemic control twice a year, and four times a year in patients who change treatment or cannot meet glycemic targets (2).

In America, HbA1c measurement methods are to be certified by the National Glycohemoglobin Standardization Program (NGSP) and reported results to be calibrated using the golden standard High-Performance Liquid Chromatography (HPLC) (3).

Measurement uncertainty is a parameter describing the distribution of all values which may influence the measured value and alter analysis content and is associated with the measurement result (4). According to internationally approved approaches, clinical laboratories are recommended to have procedures that can predict the uncertainty of test results (5).

Because of complicated formulas and components, a variety of guidelines have been prepared for the calculation of measurement uncertainty. However, there are no accepted standards to date (6). The Nordtest handbook is one of the guidelines prepared to render the calculation of measurement uncertainty more understandable (7).

Uncertainty sources are generally classified in the manner that they may influence pre-analytical, analytical, and post-analytical steps in laboratory medicine. As for the calculation of measurement uncertainty, it often focuses on analytical processes and presents the uncertainty to the last user (clinician and laboratory specialist)

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Corresponding Author: Mehmet Kalayci, Department of Medical Biochemistry, Elazig Fethi Sekin City Hospital, Elazig, Turkey
E-mail: dr_mehmetkalayci@msn.com

numerically. The factors present in the analytical phase and influence uncertainty may be exemplified as follows: calibration process, the accuracy of the values identified by the calibrator, and the changeability of calibrators and reference materials, factors related to the sample (matrix effects, interference constructor factors), lot differences in reagents, product calibrators and reference materials, differences among device users (technicians), equipment variability (pipettes, device maintenance), and environmental variability (temperature, moisture, vibration, voltage) (8).

This study aims at calculating the measurement uncertainty of HbA1c test, which has a significant role in the diagnosis and treatment of diabetes, and is recommended in diabetic patients at least twice a year regardless of their glycemic control, and showing the effects of the uncertainty, which may be essential in the analytical phase for clinicians.

MATERIALS and METHODS

The measurement uncertainty calculation model described in the Nordtest handbook was used in the present study. HbA1c analysis was studied chromatographically with the Trinity Biotech Premier Hb9210 analyzer. Internal and external quality control data of HbA1c tests performed in Elazig Fethi Sekin City hospital between January 2019 and September 2019 were used in the calculation of measurement uncertainty. Furthermore, data analyses were conducted using the results of HbA1c tests, performed between January 2019 and September 2019.

1-Coefficients of variation (CV%) of low, normal, and high level-control materials were used to calculate the within-lab reproducibility (uRw).

2-Uncertainty of external quality assessment (uEQA) and relative uncertainty of calibration (uCref) values were used in the calculation of Ubias, a component of the uncertainty. Bias values obtained through external quality control were used in the calculation of Bias.

3-uCref is defined as an uncertainty component obtained by calculating the true or expected value obtained using the certified reference material or external quality control. The uCref value was calculated using CV%s obtained using the external quality control data for each parameter, and the number of laboratories using the same method and device.

4-All of the uncertainty values were transformed into standard uncertainty [u(Bias)].

5-The composed standard uncertainty (Uc) was calculated using all standard uncertainty components.

6-The composed standard uncertainty value was multiplied with k factor to calculate an expanded uncertainty value (U) (k is approximately 2, 95% reliability interval) (Figure 1).

Expanded uncertainty value (U) was evaluated using total error limits allowed by Westgard (% TEa). The measurement of the samples collected from the patients may be considered as essential in the real world and impossible to be acquainted with the "real" value of the measurement. Both uncertainty and TEa are related to the monitorability of measurement. The target of the TEa concept should be "the comparability of laboratory results."

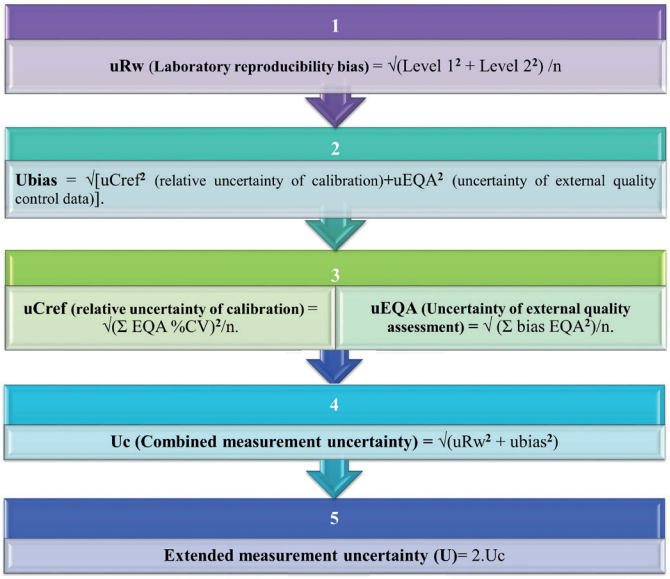

Figure 1. The calculation of measurement uncertainty

RESULTS

Measurement uncertainty of HbA1c was calculated as HbA1c±4.27% within a 95% confidence interval. These results were found to be lower than the total allowable error determined by international institutions (CLIA, RILIBAK, Fraser rules).

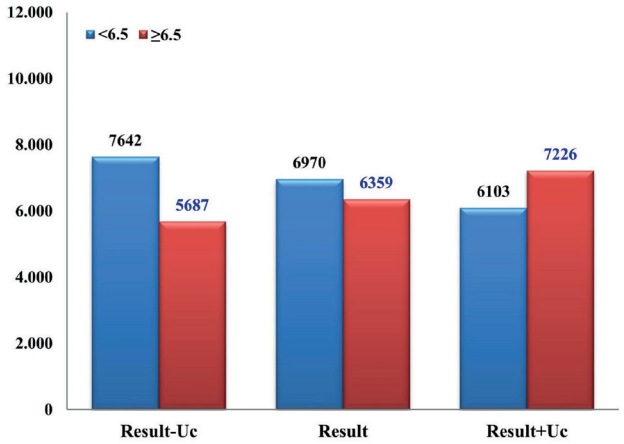

Figure 2. The analysis of HbA1c test results, studied between the dates of January-September 2019 in terms of the measurement uncertainty

Between January and September 2019, 13329 HbA1c results from our hospital were analyzed with the calculated uncertainty value. It was observed that 672 (5%) HbA1c results remained in the grey zone when the negative uncertainty value was calculated, whereas 867 (6.5%) results stayed in the grey zone for the diagnosis of diabetes mellitus (Figure 2). Overall, 1539 results with

an, HbA1c value between 6.2 and 6.8 (6.2 ≤ HbA1c < 6.8) remained in the grey zone (Figure 3). When the calculated uncertainty value was higher, this number increased more. As the uncertainty value of every laboratory is different from each other, the grey zones of the parameters, evaluated using cut-offs such as HbA1c should be determined through uncertainty.

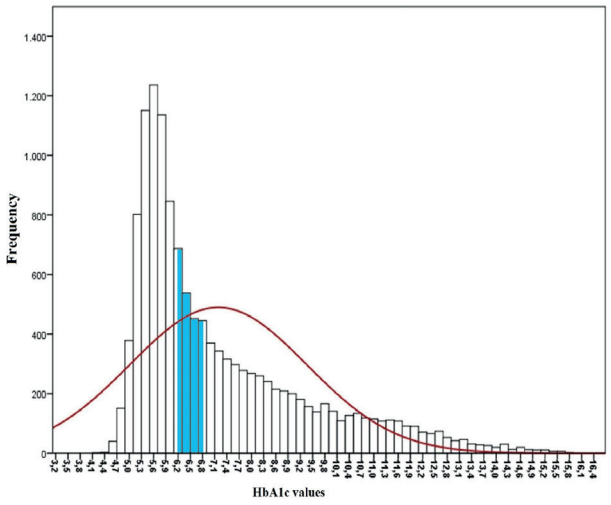

Figure 3. HbA1c results in the grey zone between the dates of January-September 2019

DISCUSSION

Laboratories use some methods such as boronate affinity, HPLC, immunoassay, immunoturbidimetric, column chromatographic, and turbidimetric inhibition to measure HbA1C (9). Almost all of these methods are used in our country. Therefore, it is inevitable to encounter result/device/method-based differences using the same sample among laboratories.

A 1% change in HbA1c enables us to make predictions about substantial complications (10). The similarity of results from laboratories (regardless of the methods used for measurement) is significant as much as the compatibility of HbA1c measurements from the same laboratory in the management of diabetes. The only exception to this condition is the hemoglobin variant (11). The uncertainty value presented in a patient's report or to a clinician is important for the comparison of the results among laboratories. This parameter should be required in current approaches as it influences clinical evaluation and makes comparability more objective.

In one of the studies carried out by Senturk et al., measurement uncertainty was added to the results of 729 patients. They noted an alteration in the cannabis value of 161 patients and the opiate decision value of 6 patients (12). Moreover, Ustundag et al., examined ethanol levels of 1034 drivers in emergency laboratories and determined the calculated expanded uncertainty to be 19.74%. Therefore, they stated that the results of blood ethanol tests which were close to legal limits should be reported within the 95% confidence interval and a confidence interval, covering real ethanol concentration (13).

Tekce et al., calculated measurement uncertainty for serum creatinine by using the Nordtest handbook with regards to its effects on the diagnosis of acute renal damage. They stated that measurement uncertainty is a significant factor for serum creatinine in the correct diagnosis of acute renal damage (14).

The measurement uncertainty of HbA1c was calculated as HbA1c ± 4.27% with a 95% confidence interval in this study. Considering that the cutoff value of HbA1c was 6.5% at the calculated uncertainty, there is a possibility to make a change in the clinical decision cutoff. Figure 3 shows that uncertainty value has an impact on the cutoff of HbA1c (6.5%). The ± 4.27% uncertainty value indicated in the figure results in a variable cutoff. This interval is between 6.2% and 6.8%. As indicated in the histogram, 11.6% of the patients (−Uc;672, +Uc;867, Total;1539) were classified in the "grey zone." These values indicate that uncertainty measurement tends to be an obligation in the test in which cutoff is applied (Troponin, βhCG, PSA, etc.). Considering positive uncertainty, the cutoff for clinical decision is 6.8% instead of 6.5% influencing the clinical decision in 867 patients.

The calculation of measurement uncertainty as defined by the Nordest handbook uses the data of quality control and validation studies as the other measurement uncertainty calculations do. The main aim of this calculation is to present common, understandable, and practical applications to users so that they can make this calculation more easily (7). Therefore, regarding the tests with critical values helping with the diagnosis of a disease, it is substantial to inform clinicians about measurement uncertainty together with the results (15). It seems necessary to form a test-based consensus by calculating the uncertainty of all tests and to note the uncertainty in test-based patient reports.

It is important to do comparative studies of laboratories for harmonization and standardization in laboratory medicine. Data acquired with a specific analyte reagent and examined through different techniques in different laboratories should be used interchangeably (comparable/substitutable). Uncertainty value is essential as it reflects at least a part of the analytical processes of a sample, and its calculation will base harmonization studies on more solid ground, that is, it will make the clinical decision process more efficient.

CONCLUSION

Although, in our study, the measured uncertainty value of HbA1c is found to be lower than TEa% values of institutions including CLIA88, RiLiBAK, and Fraser, both the number of patients in the grey zone and their change in results close to cutoff values were significant. Therefore, we consider that the uncertainty in the results of tests particularly with cutoff values should be calculated and shared with clinicians. We think that results presented with the uncertainty value will influence clinical decisions and that the uncertainty value reported with reference values

should be used as a decision-making tool in laboratory medicine in the near future. In conclusion, presenting laboratory reports of tests evaluated with cutoff values along with the expression of cutoff or grey zone in the form of ***"test result ± uncertainty value"*** could have positive impacts on clinical decisions.

Conflict of interest : The authors declare that they have no competing interest.

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