

ORIGINAL RESEARCH

Medicine Science 2019;8(1):32-6

The effect of measurement uncertainty for HOMA-IR in assessment of insulin resistance**Mujgan Ercan¹, Aysen Caniklioglu¹, Esra Firat Oguz², Cigdem Yucel², Mehmet Ozcan³, Fatma Meric Yilmaz⁴, Sedat Abusoglu⁵, Canan Topcuoglu², Yusuf Bayrakceken³**¹Bozok University Faculty of Medicine, Department of Biochemistry, Yozgat, Turkey²Ankara Numune Training and Research Hospital, Biochemistry Laboratory, Ankara, Turkey³Hacettepe University School of Medicine, Department of Medical Biochemistry Ankara, Turkey⁴Yildirim Beyazit University, Faculty of Medicine, Department of Biochemistry, Ankara, Turkey⁵Selcuk University, Faculty of Medicine, Department of Biochemistry, Konya, Turkey

Received 13 June 2018; Accepted 26 July 2018

Available online 18.10.2018 with doi:10.5455/medscience.2018.07.8905

Copyright © 2019 by authors and Medicine Science Publishing Inc.

Abstract

In this study, it is aimed to estimate not just the uncertainty measurement for the parameters of glucose and insulin having role when calculating HOMA-IR, but also true positiveness and negativeness in terms of cut-off values. The evaluation of the acceptability of the uncertainty value was also among the goals of the study. The uncertainty measurement for glucose and insulin is calculated as uncertainty measurement of HOMA-IR based on Eurochem/CITAC Guide CG 4. The uncertainty measurement for glucose and insulin was estimated as 3.83% and 4.16%, respectively. The measurement uncertainty for the calculated test HOMA-IR was calculated with cut-off values as 27% (0.27). Based on the cut-off value for HOMA-IR of 2.5%, IR(+) and IR(-) patients were 155 and 265, respectively. If the uncertainty measurement for HOMA-IR was added to its calculation, the cut-off value was detected as 2.23-2.77%. We reported the measurement uncertainty of the calculated test HOMA-IR at 95 % CI as 0.27 between 2.23 - 2.77. Especially for the tests used for screening; determination of cut-off values as a range including measurement uncertainty instead of a single value will be more suitable. No data is found in the literature for the calculated test HOMA-IR up to date.

Keywords: HOMA-IR, insulin resistance, cut-off**Introduction**

Insulin resistance (IR) can be defined as a state in which a given concentration of insulin is associated with a subnormal glucose response in clinical practice. Insulin resistance is known to play a significant pathophysiological role in the development of diabetes, dyslipidemia, hypertension and cardiovascular diseases [1,2]. Prospective studies have shown that it is a strong risk predictor for the development of diabetes or cardiovascular diseases [3-5].

Accurate measurement of IR requires complex techniques that are expensive and time-consuming. Therefore, a number of surrogate indices of IR had been developed [3,6]. The most widely used formula is the homeostatic model of assessment-insulin resistance (HOMA-IR), which uses fasting insulin and glucose levels to calculate IR [7]. HOMA-IR was calculated by using the following

formula: $\text{HOMA-IR} = \frac{\text{fasting serum glucose (mg/dL)} \times \text{fasting serum insulin value (}\mu\text{U/mL)}}{405}$. HOMA-IR (the cut-off value for insulin resistance) ≥ 2.5 was identified as an indicator of insulin resistance [8].

In the clinical laboratory, all measurements are affected by some type of errors with different significance. The potential sources of errors are classified basically as preanalytical, analytical and postanalytical [9]. The quantitative expression of the error is a value termed the uncertainty of measurement (UM) [10,11]. The practical benefit of UM is to check if the patient's values correlate with quality goals or to compare the measured value of the same patient with the previous results or clinical decision value [12,13]. Clinicians mostly compare test results with reference values or previous values of the same patient. Laboratory results must be reliable and accurate for this reason, however, laboratory results include different type of errors in practice [9]. UM is important for accurately interpreting results, especially those that are close to the limit and are crucial for making a clinical decision [14]. Currently, few clinical laboratories present UM values in their reports. In

*Corresponding Author: Mujgan Ercan, Bozok University Faculty of Medicine, Department of Biochemistry, Yozgat, Turkey
E-mail: mujganercan@hotmail.com

the literature, measurement of uncertainty studies are limited in number. There are even fewer studies regarding hormone tests. Providing the clinician with the measurement uncertainty of the analysis will be of great importance in the treatment of the disease and patient's follow-up care. When calculating UM, it is important to calculate the standard uncertainty value including all uncertainty factors. Total uncertainty value is calculated by combining all standard uncertainty values, while the expanded uncertainty value is estimated by multiplying total uncertainty by a coverage factor ($k=1.96$). UM is the value that is added to the laboratory results as $\pm$ value representing the dispersion of the test result [9].

In this study, it is aimed to estimate not just the measurement of uncertainty for the parameters of glucose and insulin when calculating HOMA-IR, but also true positiveness and negativeness in terms of cut-off values. The evaluation of the acceptability of the uncertainty value was also among the goals of the study.

Material and Methods

The HOMA-IR results of 420 subjects admitted to Endocrinology Clinic in Bozok University, Faculty of Medicine were evaluated retrospectively for three months period between September and November 2017. Blood samples were collected into yellow capped gel containing tubes (5 mL BD Vacutainer SST II advance, Becton, Dickinson and Company Franklin Lakes, NJ, USA). Measurement of glucose and insulin were carried out with Abbott Architect ci8200 autoanalyser. Glucose levels were measured using hexokinase method, while insulin levels were estimated by chemiluminescence immunoassay technique. $\text{HOMA-IR} \geq 2.5$ was used as the cut-off value by our endocrinologists. Patients with fasting blood glucose ≥ 126 mg/dL were not included in the study. The UM for glucose and insulin were calculated by using internal and external quality (one world accuracy) control data. In this study, we used the Eurochem/CITAC Guide CG 4 to calculate the UM of HOMA-IR.

$$\text{uCVglucose} = \sqrt{((x1)^2 + (x2)^2)/2}$$

$$\text{uCVinsulin} = \sqrt{((y1)^2 + (y2)^2)/2}$$

x1 and y1: uncertainty of internal quality control

x2 and y2: uncertainty of external quality control

For routine use, homeostatic model of assessment-insulin resistance (HOMA-IR) is derived as follows:

$$\text{HOMA-IR} = \text{fasting serum glucose (mg/dL)} \times \text{fasting serum insulin (}\mu\text{U/mL)} / 405.$$

If the calculation is done by multiplication or division, the squares of the CVs are summed and the square roots are taken (15).

$$\text{uCVglucose} = \sqrt{((x1)^2 + (x2)^2)/2}$$

$$\text{uCVinsulin} = \sqrt{((y1)^2 + (y2)^2)/2}$$

$$\text{uCV HOMA-IR} = \sqrt{(\text{uCVglucose})^2 + (\text{uCVinsulin})^2}$$

$\text{SD HOMA-IR} = \text{uCV HOMA-IR} \times \text{HOMA-IR}$ (The cut-off value for HOMA-IR=2.5)

The expanded uncertainty of HOMA-IR;

$$\text{U HOMA-IR} = \text{SD HOMA-IR} \times 1.96 (k)$$

Measurand (analyte)=value $\pm$ U

U is the expanded uncertainty of HOMA-IR

The study was approved by the Ethical Committee of Bozok University, Faculty of Medicine (Ref number.2017/58).

Results

Mean age of 420 subjects (M=74, F=346) included in the study was 37 ± 10 years. In accordance to the routinely used cut-off value (≥ 2.5) in our hospital, 265 participants were IR negative and 155 were IR positive. UM of the internal quality control for insulin and glucose tests in our laboratory were calculated as 5.8%, 4.28%, respectively, while external quality control measurements for these tests were estimated as 1.03% and 3.32%, respectively (Table 2 and 3). The measurement uncertainty for the calculated test HOMA-IR was calculated with cut-off values as 0.14 ($(5.66 \times 2.5)/100$). Expanded HOMA-IR value was calculated as $(0.14 \times 1.96) \times 0.27$ (Table 3).

Our test result according to expanded measurement uncertainty of HOMA-IR is 2.5 ± 0.27 . In 95% of confidence interval, our real value is between 2.23-2.77. At this maximum error rate, the limit values were determined as 2.5 for 0 error rate, 2.23 for -0.27 error rate and 2.77 for +0.27 error rate (Table 4).

Retrospective evaluation of HOMA-IR values of 420 subjects (according to cut-off value) revealed 265 results as negative, while 155 results as positive when the cut-off is defined as 2.5. When the maximum low error rate (-0.27) was accepted, 193 subjects were positive, 227 subjects were negative. When the maximum high error rate (+0.27) was accepted, 121 subjects were positive, while 299 were negative (Table 5).

Table 1. %CV values of internal quality control

	Glucose (n=128) Level1 control (mg/dL)	Glucose (n=128) Level2 control (mg/dL)	Glucose (n=128) Level3 control (mg/dL)	Insulin (n=91) Level1 control (μIU/mL)	Insulin (n=91) Level2 control (μIU/mL)	Insulin (n=91) Level3 control (μIU/mL)
Mean	45.36	123.67	285.74	15.06	46.93	113.39
SD	1.88	6.45	9.29	0.95	3.16	4.48
%CV	4.15	5.22	3.25	6.32	6.74	3.95
Rw	4.28			5.8		

SD: Standard deviation; %CV: (SD/Mean)*100; Rw: Laboratory reproducibility

$\text{Rw} = \sqrt{(\text{L1 control material \%CV})^2 + (\text{L2 control material \%CV})^2 + (\text{L3 control material \%CV})^2} / 3$

Table 2. External quality control within and between groups bias values and Standard uncertainty for glucose

EQA Samples	Glucose bias within group (deviation%)	Glucose biasEQA ² within group	Glucose bias between groups (deviation%)	Glucose biasEQA ² between groups
1	-3.6	12.96	-6.2	38.44
2	-9.5	90.25	-7.3	53.29
3	-3.4	11.56	-5.7	32.49
Uncertainty value %	4.68		0.11	
RMS bias	3.32			
RMS bias: $\sqrt{[(\text{glucose bias within group})^2 + (\text{glucose bias between groups})^2]}/2$				

Table 3. External quality control within and between groups bias values and Standard uncertainty for insulin

EQA Samples	Insulin bias within group (deviation%)	Insulin biasEQA ² within group	Insulin bias between groups (deviation%)	Insulin biasEQA ² between groups
1	-0.93	0.8649	4.55	20.7025
2	-2.44	5.9536	7.45	55.5025
3	2.01	4.0401	11.56	133.6336
Uncertainty value %	0.34		1.42	
RMS bias	1.03			
RMS bias: $\sqrt{[(\text{insulin bias within group})^2 + (\text{glucose bias between groups})^2]}/2$				

Table 4. Calculation of uncertainty value

	Calculations	Uncertainty value %
UCV(glukoz)	$\sqrt{(Rw(\text{glucose})^2 + \text{RMS}(\text{glucose})^2)}/2$ $\sqrt{((4.28)^2 + (3.32)^2)}/2$	3.83
UCV(insulin)	$\sqrt{(Rw(\text{insulin})^2 + \text{RMS}(\text{insulin})^2)}/2$ $\sqrt{((5.8)^2 + (1.03)^2)}/2$	4.16
UCV(HOMA-IR)	$\sqrt{(\text{UCV}(\text{glucose})^2 + \text{UCV}(\text{insulin})^2)}$ $\sqrt{((3.83)^2 + (4.16)^2)}/2$	5.66
SD(HOMA-IR)	UCV(HOMA-IR) x 2.5/100 (5.66 x 2.5)/100	0.14
U(HOMA-IR)	SD(HOMA-IR) x k	
Expanded uncertainty (confidence interval 95%)	0.14 x 1.96	0.27

Table 5. Error rates for 0.27 uncertainty, cut-off values and values of true positive and true negative

Mistake rate	Cut off	IR+	IR-
0	2.5	155	265
-0.27	2.23	186	234
+0.27	2.77	126	294

Discussion

Uncertainty of Measurement (UM) parameter, associated with the result of measurement, that characterizes the dispersion of the values that could reasonably be attributed to the measured (the quantity intended to be measured) [16]. UM provides quantitative estimates of the level of confidence that a laboratory has in its analytical precision of test results. Therefore, it represents the expected variability in a laboratory result if the test is repeated a second time. Both imprecision and bias are taken into account. Hence, it is a measure of precision to which biological variation and a confidence level (coverage probability based on normal distribution) have been applied. UM is reported in standard

deviation (SD) units or relative SD expressed as the coefficient of variation (%CV) [15,16]. Errors near values of clinical decision limits have gain much importance lately. These errors are mainly caused by measurement uncertainty [17]. These errors may cause increase in medical expenses, malpractices and sometimes even life threatening situations for patients [18]. For these reasons, reporting of patient results with UMs is much important in tests with critical values which help clinical diagnosis. However, consensus have not been reached up to date on this subject. The determination of UM for calculated tests according to clinical decision concentration or cut-off value will be helpful. In the present study, we reported the measurement uncertainty of the calculated test HOMA-IR at 95% CI as 0.27 between 2.23-2.77 which takes attention to consideration

of UM results in determination of cut-off values. Especially for the tests used for screening, determination of cut-off values as a range including measurement uncertainty instead of a single value will be more suitable.

Internal and external quality control data for glucose and insulin were used in this study for determination of uncertainty. Internal quality control data reflects the precision of the method used, while external quality control data gives an objective evaluation of laboratory performance [19,20]. Any research related to the calculated test as HOMA-IR have not been conducted in the literature yet. The aim of this study is to detect the lower limit for a reporting range of HOMA-IR formula in order to determine UM for clinical decision limits and cut-off values to lower the rate of false negatives. In our study, low limit of the cut-off value with UM was calculated as 2.23 and 7% (25 cases) false negative ratio was detected, which may underestimate true diagnosis.

The primary goal of medical laboratories is to produce and report high quality, accurate reproducible results. Each laboratory should evaluate their analysis routines, compare their performance characteristics with other laboratories and periodically update their performance characteristics as it will be affected by time, new chemicals and equipment's [21,22]. The standardization problems in insulin test is currently persistent. That's why UM (from systematic and random errors) of each measured parameter should be calculated to detect 95% CI and acceptability in terms of Westgard database should be questioned.

In our study, the measurement uncertainty of glucose in HOMA-IR calculation is 3.83% and expanded uncertainty is 7.66%. Our data seems to exceed total acceptable error limits (TEa%) give in Westgard biological database criteria (6.96%) [23], however it is in concordance with CLIA 88 (10%) and Fraser (7.9%) criteria [24]. Ozlem et al. detected measurement uncertainty of glucose as 7.26% in their study. UM value detected in our data is 7.66% which was higher than those in the study conducted by Ozlem et al. [25]. In a similar study was found measurement uncertainty of glucose as 7.38% [26]. UM for insulin was found as 4.16% and expanded uncertainty was found as 8.32% in our study. These values are within the TEa (32%) of Westgard [23]. There are limited number of studies for UMs of calculated tests. In addition, to our knowledge any studies have not been carried out for UMs of both insulin and HOMA-IR.

Conclusion

As a result, when evaluating insulin resistance calculation, consideration of glucose and insulin measurement uncertainties may be helpful in clinical decision for not only the management of treatment, but also overcoming misdiagnosis. Notification of the clinicians for UM is of great importance.

Competing interests

The authors declare that they have no competing interest

Financial Disclosure

The financial support for this study was provided by the investigators themselves.

Ethical approval

Before the study, permissions were obtained from local ethical committee.

Mujgan Ercan ORCID: 0000-0002-9291-4197

Aysen Caniklioglu ORCID: 0000-0001-8128-6044

Esra Firat Oguz ORCID: 0000-0002-8147-5379

Cigdem Yucel ORCID: 0000-0003-2647-440X

Mehmet Ozcan ORCID: 0000-0002-1222-2802

Fatma Meric Yilmaz ORCID: 0000-0002-8252-5975

Sedat Abusoglu ORCID: 0000-0002-2984-0527

Canan Topcuoglu ORCID: 0000-0003-1479-1600

Yusuf Bayrakceken ORCID: 0000-0002-5652-8168

Reference

1. Tang Q, Li X, Song P, et al. Optimal cut-off values for the homeostasis model assessment of insulin resistance (HOMA-IR) and pre-diabetes screening: Developments in research and prospects for the future. *Drug Discov Ther.* 2015;9:380-5.
2. Magri CJ, Fava S, Galea J. Prediction of insulin resistance in type 2 diabetes mellitus using routinely available clinical parameters. *Diabetes Metab Syndr.* 2016;10 (Suppl 1):96-101.
3. Reaven GM. Pathophysiology of insulin resistance in human disease. *Physiol Rev.* 1995;75:473-86.
4. Eckel RH, Grundy SM, Zimmet PZ. The metabolic syndrome. *Lancet (London, England).* 2005;365:1415-28.
5. Lillioja S, Mott DM, Howard BV, et al. Impaired glucose tolerance as a disorder of insulin action. Longitudinal and cross-sectional studies in Pima Indians. *N Engl J Med.* 1988;318:1217-25.
6. Katz A, Nambi SS, Mather K, et al. Quantitative insulin sensitivity check index: a simple, accurate method for assessing insulin sensitivity in humans. *J Clin Endocrinol Metab.* 2000;85:2402-10.
7. Aregbesola A, Virtanen JK, Voutilainen S, et al. Serum ferritin and glucose homeostasis: change in the association by glycemic state. *Diabetes Metab Res Rev.* 2015;31:507-14.
8. Matthews DR, Hosker JP, Rudenski AS, et al. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia.* 1985;28:412-9.
9. Ellison S, Williams A. EURACHEM/CITAC Guide CG 4: Quantifying uncertainty in analytical measurement. 2012. pp. 4-6.
10. Matar G, Poggi B, Meley R, et al. Uncertainty in measurement for 43 biochemistry, immunoassay, and hemostasis routine analytes evaluated by a method using only external quality assessment data. *Clin Chem Lab Med.* 2015;53:1725-36.
11. Kin Tekce B, Tekce H, Aktas G, et al. The role of the uncertainty of measurement of serum creatinine concentrations in the diagnosis of acute kidney injury. *Ren Fail.* 2016;38:305-10.
12. Beck SC, Lock RJ. Uncertainty of measurement: an immunology laboratory perspective. *Ann Clin Biochem.* 2015;52(Pt 1):7-17.
13. Theodorsson E. Uncertainty in measurement and total error: tools for coping with diagnostic uncertainty. *Clin Lab Med.* 2017;37:15-34.
14. White GH, Farrance I, AACB Uncertainty of Measurement Working Group. Uncertainty of measurement in quantitative medical testing: a laboratory implementation guide. *Clin Biochem Rev.* 2004;25:1-24
15. Moses G, Crawford L. Traceability and measurement of uncertainty for medical laboratories. QMP-LS.OLA.2011. Canada (<http://www.qmpls.org>).
16. Farrance I, Frenkel R. Uncertainty of Measurement: A Review of the Rules for Calculating Uncertainty Components through Functional Relationships. *Clin Biochem Rev.* 2012;33:49-75.
17. Plebani M. Errors in clinical laboratories or errors in laboratory medicine? *Clin Chem Lab Med.* 2006;44:750-9
18. European Co-operation for Accreditation of Laboratories, EAL-G23, Edition 1, August. 1996
19. IFeinberg M, Boulanger B, Dewe W, et al. New advances in method validation and measurement uncertainty aimed at improving the quality of chemical data. *Anal Bioanal Chem.* 2004;380:502-14.

20. Adams TM. A2LA Guide for the Estimation of Measurement Uncertainty In Testing July 2002.
21. ISO 15189:2014. Medical laboratories requirements for quality and competence. 2nd ed. International organization for standards, Geneva, Switzerland, 2014
22. Kuwa K. Internal Quality Control and External Quality Assessment on POCT. Rinsho Byori. 2015;63:224-31.
23. Ricos C, Alvarez V, Cava F, et al. Current databases on biologic variation: pros, cons and progress. Scand J Clin Lab Invest. 1999;59:491-500.
24. Fraser CG. Biological variation: from principle to practice. 140 AACCPress, Washington. 2001
25. Öztürk O, Serdar M, Öztürk M, et al. Calculation of uncertainty for glucose: may it affect the diagnosis of gestational diabetes? Turk J Biochem. 2012;37:68-72
26. Chen H, Zhang L, Bi X, et al. Two evaluation budgets for the measurement uncertainty of glucose in clinical chemistry. Korean J Lab Med. 2011;31:167-171.