Research Article

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Evaluation of biological variations in glucose and glycated hemoglobin levels in healthy individuals

Sağlıklı Kişilerde Glukoz ve Glike Hemoglobinin Biyolojik Varyasyonlarının Değerlendirilmesi

Objective: In this study, we firstly aimed to determine components of biological variations (BVCs) in levels of glucose and glycated hemoglobin (HbA1c) in detail based on guidance from relevant organizations and experts. We also investigated whether reference intervals for both analytes were useful for evaluations, particularly consecutive test results.

Methods: The study group consisted of 36 healthy volunteers. Samples were collected from each individual 4 times every 2 weeks for 45 days. All samples were assayed in duplicate within a single run. Finally, we estimated BVCs and the analytical performance specifications of both analytes.

Results: Our results were fairly compatible with current biological variations (BVs) in both analytes reported in a database. It was calculated as within biological variation (CVI) = 4.2% and between-subject variation (CVG) = 5.3% for glucose while calculating as CVI = 1.7% and CVG = 4.5% for HbA1c. According to these results, the index of individuality (II) of glucose was higher than 0.6 while HbA1c’s II was lower than this value.

Conclusion: We thought that guidelines from relevant international organizations should be followed to standardize the study design and to appropriately calculate BVCs for any analyte in BV studies. Finally, reference change value should be used to evaluate meaningful differences in HbA1c levels instead of reference interval.

Keywords: Biological variation; Glucose; Glycated hemoglobin; The index of individuality; Reference change value; Analytical performance specification.

Özet

Giriş ve amaç: Biz bu çalışmada öncelikle ilgili kuruluşlar ve uzmanların rehberliğinde glukoz ve glike hemoglobin (HbA1c)’nin biyolojik varyasyon komponenti (BVK)’lerini detaylı olarak tespit etmeyi amaçladık. Ayrıca bu analitiklere ait referans aralıklarının özellikle arıslaştı test sonuçları değerlendirme için yararlı olup olmadığını araştırdık.

Yöntem ve gereçler: Çalışma grubu 36 sağlıklı kan örnekleri olarak seçildi. Kan örnekleri her biri 2 haftada bir olmak üzere 45 gün boyunca toplanmıştır. Tüm örnekler tek seferde olmak üzere iki kez ölçüldü. Son olarak, her analitikler için referans aralıkları hesaplandı.

Sonuçlar: Gösterilen sonuçlar, her iki analizin gösterdiği varyasyonlarla uyumlu olarak kabul edildi. Glukozun varyasyonu (CVI) = 4.2% ve bireysel varyasyon (CVG) = 5.3% olarak hesaplandı. HbA1c için ise, CVI = 1.7% ve CVG = 4.5% olarak hesaplandı. Bu sonuçlara göre, glukozun bireysel indisindeki 0.6’dan büyük iken, HbA1c’nin bireysel indisindeki bu değerden daha küçüktü.
Introduction

Most clinical laboratory tests are commonly requested by clinicians for various clinical situations, including diagnosis, monitoring, assessment of risk factors and screening [1]. Glucose and glycated hemoglobin (HbA1c) levels are also assessed for similar purposes. In this context, plasma glucose criteria, either the fasting plasma glucose (FPG) levels or the 2-h value in the 75-g oral glucose tolerance test have been used to diagnose diabetes mellitus (DM) for several years. An International Expert Committee that included representatives of the American Diabetes Association (ADA), the International Diabetes Federation (IDF), and the European Association for the Study of Diabetes (EASD) recommended that both the glucose test and the HbA1c test should be used to diagnose DM, and the ADA adopted this criterion in 2010 [2–4]. Moreover, numerous diabetes organizations have defined the range of HbA1c levels and FPG levels to predict the progression to DM [5].

Techniques used to monitor glucose and HbA1c levels are important tools in assessing DM management, since poor glycemic control over time has been specifically linked to the development and progression of microvascular DM complications [6]. But, it sometimes is really difficult for clinicians to decide upon in some cases, although most tests have various clinical decision limits. Alterations observed in consecutive test results may be clinically significant, even if these results are within their reference intervals. This condition may also related to inherent variation [expressed as the coefficient of variation (CV)], which consist of the variations in pre-analytical (CVp), analytical (CVa) and within-subject biological variation (CVw).

Between-subject biological variation (CVb) defined as the differences between the set points of different individuals is another component of biological variation (BV) as well as CVw. The CVb/CVw ratio is also used to calculate the index of individuality (II) which determines whether population-based reference interval for any analyte are useful. Moreover, the reference change value (RCV) derived from BV data (BVD) is also particularly helpful for the clinician to decide whether the changes in values for any analyte between consecutive tests are significant for patient health [7].

However, in the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) 1st Strategic Conference, doubts about the quality of BVD in current clinical use were raised, and its utility was suggested to be limited [8]. Indeed, existing BVD exhibit marked heterogeneity in terms of study design, as well as uncertainty in the calculations [9]. In particular, the EFLM has continued to work to overcome the challenges in these issues and endeavor to expand reliable BVD through the Working Group on BV (WG-BV) [10, 11]. In this context, the BV database (BV-DB) that were previously prepared by Ricos and colleagues [12] were recently updated by gathering data from new publications that have been continuously expanded, updated and published online. In addition, analytical performance specifications (APS) for imprecision (%), bias (B%) and total error (TE%) of various analytes calculated from BVD were also published on the same website [13]. Moreover, Bartlett and colleagues [9] have recently published a critical appraisal checklist to enable the standardized assessment of papers on BV in the name of the EFLM WG-BV. Perich and colleagues [14] have also reported criteria used to evaluate the reliability of the BV-DB.

Consequently, our initial goal was to estimate BVD for both analytes in detail based on the guidance of relevant organizations and experts. The other goal was to guide to the clinician to make decision about reference interval or RCV should be used for both analytes while managing the DM process.

Materials and methods

Systematic search strategy and participants’ characteristics

The study was conducted at two centers. Working samples were measured in the Biochemistry Department of Haseki Hospital after they were collected from Haseki Training and Research Hospital and Istanbul Training and Research Hospital. Thirty-six healthy volunteer subjects (19 males and 17 females between 20 and 45 years of age) participated in this study. Volunteers were all selected from the laboratory staff and their friends or relatives. The study
was approved by the Ethics Committee of Haseki Hospital. All procedures were conducted according to institutional ethical and legal standards.

**Exclusion criteria**

We excluded subjects with a history of chronic diseases, including DM, coronary artery disease, hypertension, anemia, thyroid diseases, hepatic diseases, kidney disease and autoimmune diseases. We also excluded subjects with a family history of thalassemia syndrome and other hemoglobinopathies; a known carrier state for the hepatitis B virus, hepatitis C virus, or human immunodeficiency virus; a history serious illness during the previous 4 weeks or blood donation in the previous 3 months; irregular fasting glucose levels; a past history of psychiatric and/or personality disorders; and subjects who were taking any medication, vitamin supplements, tobacco or alcohol. None of the women were pregnant, breastfeeding, or within 1 year of childbirth. Moreover, subjects with a diagnosis of malignant or benign chronic disease prior to the study were also excluded. The subjects maintained their usual lifestyle during this study. The information was obtained by recording patients’ answers during face-to-face interviews, and we did not perform any additional analysis for this purpose [8].

**Sampling and analytical procedures**

Samples, including serum and whole blood, were consecutively collected from each individual 4 times every 2 weeks for 45 days. All samples were collected between 8.00 and 10.00 in the morning from subjects who had not performed morning exercises, had fasted for 8–12 h fast and had rested for 5 min in a seated position by the same phlebotomist at each hospital to minimize sources of pre-analytical variation [7, 8].

Venous blood samples, which is specimen used to measure glucose levels, were collected via antecubital vein puncture. Samples were collected in tubes containing clot activator gel (BD Vacutainer® Plastic SST™ II Advance tube, BD Diagnostics, Franklin Lakes, NJ, USA, Cat. No. 3679545 mL). Serum samples were incubated at room temperature for 30 min and then centrifuged at 3000 g for 10 min at 25°C (room temperature). Whole blood samples, which is the specimen used to measure HbA_1c levels, were collected in tubes containing potassium EDTA (BD Vacutainer® Plastic K_EDTA tube, BD Diagnostics, Franklin Lakes, NJ, USA, Cat. No. 368857, 3 mL) and then centrifuged at 3000 g for 10 min at 4°C. All samples were aliquoted and stored at −80°C until analysis [8].

Each sample collected from the study participants was assayed in duplicate within a single run [15, 16]. All samples were studied on the same day by the same laboratory staff to exclude interassay variation. Samples were incubated at room temperature until they dissolved and then studied. Before measuring the levels of these analytes, both analytical systems were calibrated, and then two control materials (normal and pathological) were assayed in duplicate in each single run, according to the manufacturer’s established validation range. The same lots of calibrators, reagents and quality-control materials were used throughout the experiment used to determine the levels of the two analytes [16, 17].

Glucose levels were measured spectrophotometrically using the hexokinase-G6PD method that was recommended as one of two reference methods by the Joint Committee for Traceability in Laboratory Medicine [18] in an AU2700 biochemical autoanalyzer (Beckman Coulter, Inc., USA). HbA_1c levels were measured using the HPLC (ion exchange) method that was approved by The International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) in July 2001 [19] in an ADAMS™ Alc HbA-8180V autoanalyzer (ARKRAY Co. Ltd., Inc., Kyoto, Japan).

**Statistical analysis and calculations**

We used Excel 2016 (Microsoft, WA, USA), Excel XLSTAT 2016 (Addinsoft, New York, NY, USA) and SPSS 21 (IBM, New York, NY, USA) for the statistical analyses. We firstly determined whether the glucose and HbA_1c levels exhibited a normal “Gaussian” distribution using the “Shapiro-Wilk test”, and separately applied the test to compare data within-subject and between-subject, respectively. Natural logarithmic (ln) transformation was performed all HbA_1c data prior to the analysis, since the data did not exhibit a Gaussian distribution. The Shapiro-Wilk test was again used to verify the normality of the HbA_1c data, and we then used the Dixon-Reed criterion to detect outliers in mean between-subject values for both analytes. Meanwhile, Cochran’s C test was used to exclude outliers from among within-subject values, including duplicate measurements. We also analyzed the homogeneity of the variances using the “Bartlett test”, which was applied to all data, including replicate measurements, after removing data for outliers [16].

Analytical (SD_a), intra-individual (SD_i), inter-individual (SD_d) standard deviations were calculated using a nested ANOVA design, and then these results
were converted to coefficients of variance component (CV_x, CV_p, CV_g, and CV_t, respectively). In addition, we also calculated RCV and II for both analytes [7, 15]. The calculated SDs for glucose levels were finally converted them to CV values using the formula $[CV = (SD/mean) \times 100]$ [7]. SDs for HbA1c levels that had undergone log transformation were later converted to CV values using the formula [20].

We calculated RCV values for both analytes via using the following formula [7, 21]:

$$RCV = 2^{1/2} \times z \times (CV_x^2 + CV_g^2)^{1/2}.$$ 

We also calculated the II values for both analytes using the following equation [7]:

$$II = CV_t / CV_g.$$ 

We finally defined desirable APS for I%, B% and TE% using our BV data for both analytes and the following formulas [22]:

$$I% = 0.5 CV_t$$

$$B% = 0.25 (CV_x^2 + CV_g^2)^{1/2}$$

$$TE% = I% \times 1.65 + B%.$$ 

**Results**

The data from 33 subjects were statistically evaluation after three subjects with IFG were excluded from the study. Glucose levels were normally distributed, according to the results of the normality test ($p=0.063$). After outlier tests, one dataset from five subjects (together with repeated measurements) was removed the study and we then confirmed whether the data were normally distributed by performing the normality test again ($p=0.069$).

It was applied In transformation to all HbA1c data because they did not exhibit a normal distribution ($p=0.001$). After transformation, the data exhibited a normal distribution ($p=0.053$), and we then removed one dataset from four subjects (including repeated measurements) and all data for one subject from the study after performing the outlier test. Finally, we performed a statistical evaluation on HbA1c data from 32 subjects.

We graphically presented the glucose and HbA1c data obtained after completing all statistical procedures below (Figures 1 and 2).

We calculated all SD values for both analytes using a nested ANOVA design after testing the homogeneity of variances in glucose ($p=0.945$) and HbA1c levels ($p=0.226$). In addition, we calculated RCV and II for glucose and HbA1c levels (Table 1).
Table 1: Reference change values, the index of individuality and the coefficient of variation of between-subject, within-subject, analytical and total for glucose and HbA1c levels.

| Analytes   | CV<sub>WS</sub> | CV<sub>I</sub> | CV<sub>W</sub> | CV<sub>T</sub> | RCV | II |
|------------|-----------------|----------------|---------------|---------------|-----|----|
| Glucose    | 5.3 (4.0–7.3)   | 4.2 (3.7–4.9)  | 1.1           | 6.9           | 12.0| 0.8|
| HbA1c %    | 4.5 (3.4–6.2)   | 1.7 (1.4–2.0)  | 1.3           | 5.0           | 5.9 | 0.37|

CV<sub>WS</sub>, Coefficient of variation of between-subject levels; CV<sub>I</sub>, coefficient of variation of within-subject levels; CV<sub>W</sub>, coefficient of variation of analytical levels; RCV, reference change value; II, index of individuality. CV<sub>WS</sub> and CV<sub>I</sub> were given together with their confidence interval values.

Table 2: APS for imprecision, bias and total error derived from our BV data compared with the desirable specifications reported in an online database.

| Analytes   | APS derived from our BV data | APS reported in an online database* |
|------------|-----------------------------|-------------------------------------|
|            | I% | B% | TE% | I% | B% | TE% |
| Glucose    | 2.1 | 1.7 | 5.2 | 2.8 | 2.3 | 7.0 |
| HbA1c %    | 0.9 | 1.2 | 2.7 | 0.9 | 1.5 | 3.0 |

APS, Analytical performance specification; BV, biological variation; I%, imprecision; B%, bias; TE%, total error. *https://www.westgard.com/biodatabase1.htm [23].

Finally, we obtained desirable APS for I%, B% and TE% for both analytes and compared these values with the desirable specifications reported in an online database (Table 2) [23].

**Discussion**

In this study, we firstly analyzed each sample in duplicate to estimate analytic variation for both analytes, since this procedure was reported as the best approach in studies by some experts [15, 24]. The CV<sub>WS</sub> value for glucose was 1.1%. This result was slightly lower than the results from other studies [21, 25]. We used the reference method approved by IFCC and reference material supplied by manufacturers; in addition, we clearly defined the analytical system used to measure glucose levels in this study. In contrast, previous studies did not completely explain which methods and analytical systems were used to measure glucose levels, and the reference materials used to calibration the glucose measurement methods were not defined [12, 21, 25]. However, notations of the use of commercial methods with validated calibrators are important, since some commercial systems used for glucose measurements may produce non-traceable values because of a lack of validated calibrations [20]. The CV<sub>WS</sub> for HbA1c was 1.3%. This value was similar to the values reported in studies that used the ion exchange method to measure HbA1c levels [17, 26–28], but was also less than the values reported in several studies that used different methods [29, 30].

Moreover, within-subject and between subject variations calculated for glucose in this study were also a slightly lower than the values reported in the study by Ricos et al. [12, 23] and published online on the Westgard website, whereas they were fairly consistent with the results presented in another study of Ricos et al. [21] and a study by Fraser et al. [25]. Moreover, we observed that components of biological variation regarding HbA1c calculated by ours were consistent with the results reported in some studies [17, 30, 31], but were different from the results obtained other studies [28, 32].

We observed that between-subject variation in HbA1c was much more higher than within-subject variation in HbA1c. Therefore, the II value of HbA1c were more less than 0.6. Whereas II value of glucose was higher than this value. There are serious similarities between reference interval and RCV in terms of those of production and use even though the requirements for delivery and characterisation of the latter have been clearly identified by the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) and more recently in guidance issued by the Clinical and Laboratory Standards Institute (CLSI) [9]. As a consequence, it may be thought that reference interval for HbA1c are also not useful in evaluating HbA1c results and therefore the RCV for HbA1c may be used to assess meaningful difference in HbA1c levels from consecutive tests in the clinic. As mentioned previous studies, we also thought that it may be required very different HbA1c target values to obtain the same overall glycemic status while management of patient with DM according to glucose [32]. On the other hand, it should be also considered the current clinical practice guidelines for the DM management as well as our suggestions in this issue.

We also determined that the desirable analytical performance specification obtained in this study for both analytes were fairly consistent with the desirable specifications reported in an online database [23], with the exception that I% for HbA1c shows minimum analytical performance (CV<sub>WS</sub> < 0.75 CV<sub>I</sub>), as reported in other studies [26, 27]. However, several studies using the same methodology achieved desirable performance [28, 33]. The studies that used a different methodology to measure HbA1c levels generally displayed a worse analytical performance than our methodology [17, 29, 30, 31, 34]. On the other hand,
according to the study by Braga and colleagues [35], HPLC systems with ion-exchange columns has more specificity for HbA\textsubscript{1c} than many methodologies, but still did not exhibit good performance in terms of analytical specificity, according to the IFCC reference measurement procedure.

Previous studies had various levels of quality in terms of study design and substantial uncertainty in terms of estimating BVD, because accepted international standards for these issues have not been available. However, some international organizations, such as the IFCC, EFLM and CLSI have made an effort to standardize the BVD studies [9]. As a result, we performed this study according to the recommendations of these organizations as well as relevant experts.

Regarding the limitations of our work, we could have collected more than four samples for both analytes because their consecutive measurements obtained samples which were collected over a longer period time interval may better reflects variables in their blood levels.

**Conclusion**

We thought that the guidelines of some relevant international organizations, such as the EFLM, should be published to standardize the study design used in BV studies and to calculate BVD using an appropriate method. In addition, we suggested that the RCV should be used to assess meaningful difference in consecutive results of HbA\textsubscript{1c} instead of reference interval.

**Conflict of interest statement:** The authors declare there are no conflicts of interest.

**References**

1. Fischbach FT, Dunning MB, Surenna H, Kogut H, Gibbons T, Ewan H, editors. Diagnostic testing: A manual of laboratory and diagnostic test, 8th ed. China: Woters Kluwer Health, Lippincott Williams@Wilkins Press, 2009:1–52.
2. International Expert Committee. International Expert Committee report on the role of the A\textsubscript{1c} assay in the diagnosis of diabetes. Diabetes Care 2009;32:1327–34.
3. American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care 2010;33:62–9.
4. Yalcin H, Toprak B, Colak A. The independent relationship between hemoglobin A\textsubscript{1c} and homeostasis model assessment of insulin resistance in non-diabetic subjects. Turk J Biochem 2017;42:31–6.
5. Cefalu WT, editor in chief. Classification and diagnosis of diabetes. Standards of medical care in diabetes. Diabetes Care 2016;39 (Suppl. 1):S13–22.
6. The Diabetes Control and Complications Trial Research Group. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. N Engl J Med 1993;329:977–86.
7. Fraser CG, editor. Biological variation: from principles to practice. Washington, DC: AACC Press, 2001.
8. Carobene A, Strollo M, Jonker N, Bartia G, Bartlett WA, Sandberg S, et al. Sample collections from healthy volunteers for biological variation estimates’ update: a new project undertaken by the Working Group on Biological Variation established by the European Federation of Clinical Chemistry and Laboratory Medicine. Clin Chim Lab Med 2016;54:4599–608.
9. Bartlett WA, Braga F, Carobene A, Coskun A, Prusa R, Fernandez-Calle P, et al. A checklist for critical appraisal of studies of biological variation. Clin Chim Lab Med 2015;53:879–85.
10. Carobene A. Reliability of biological variation data available in an online database: need for improvement. Clin Chim Lab Med 2015;53:871–7.
11. Available from: http://www.efcclm.org/index.php/wg-biological-variation.html. Last accessed June 2017.
12. Ricos C, Alvarez V, Cava F, Garcia-Lario JV, Hernandez A, Jimenez CV. Current databases on biological variation: pros, cons and progress. Scand J Clin Lab Invest 1999;59:491–500.
13. Ricos C, Alvarez V, Cava F, Garcia-Lario JV, Hernandez A, Jimenez CV, et al. Desirable specification for total error, imprecision, and bias, derived from intra- and inter-individual biologic variation. The update. Available from: https://www.westgard.com/biodatabase1.htm (Last accessed May, 2017).
14. Perich C, Minchinel J, Ricos C, Fernandez-Calle P, Alvarez V, Domenech MV, et al. Biological variation database: structure and criteria used for generation and update. Clin Chim Lab Med 2015;53:299–305.
15. Fraser CG, Harris EK. Generation and application of data on biological variation in clinical chemistry. Crit Rev Clin Lab Sci 1989;27:409–37.
16. Carobene A, Raraas T, Salvik UD, Sylte MS, Sandberg S, Guerra E, et al. Biological variation estimates obtained from 91 healthy study participants for 9 enzymes in serum. Clin Chem 2017;63:1–10.
17. Ucar F, Erden G, Ginis Z, Ozturk G, Sezer S, Gurler M, et al. Estimation of biological variation and reference change value of glycated hemoglobin (HbA\textsubscript{1c}) when two analytical methods are used. Clin Biochem 2013;46:1548–53.
18. Chang YX, Ou L, LanZhen W, GuoBing X. Trueness assessment for serum glucose measurement using commercial systems through the preparation of commutable reference materials. Ann Lab Med 2012;32:243–9.
19. Maes M, Momen K, Hendrickx D, Peeters D, D’Hondt P, Ranjan R, et al. Components of biological variation, including seasonality, in blood concentrations of TSH, TT3, FT4, PRL, cortisol and testosterone in healthy volunteers. Clin Endocrinol 1997;46:587–98.
20. Fokkema MR, Herrmann Z, Muskiet FA, Moecks J. Reference change values for brain natriuretic peptides revisited. Clin Chem 2006;52:1602–3.
21. Ricos C, Cava F, Garcia-Lario JV, Hernandez A, Iglesias N, Jimenez CV, et al. The reference change value: a proposal to interpret laboratory reports in serial testing based on biological variation. Scand J Clin Lab Invest 2004;64:175–84.
22. Marshall WJ, Bangert SK, editors. The acquisition of biochemical data. Clinical biochemistry metabolic and clinical aspects, 2nd ed. China: Churchill Livingstone Elsevier Limited Press, 2008:7–16.
23. https://www.westgard.com/biodatabase1.htm Desirable Specifications for Total Error, Imprecision, and Bias, derived from intra- and inter-individual biologic variation (Last accessed: June 2017).
24. Young DS, Harris EK, Cotlove E. Biological and analytic components of variation in long term studies of serum constituents in normal subjects. IV. Results of a study designed to eliminate longterm analytic deviations. Clin Chem 1971;17:403–10.
25. Fraser CG, Cummings S, Wilkinson SP, Neville RG, Knox JD, Ho O, et al. Biological variability of 26 clinical chemistry analytes in elderly people. Clin Chem 1989;35:783–6.
26. Kolatkar NS, Cembrowski GS, Callahan PL, Etzwiler D. Intensive diabetes management requires very precise testing of glycohemoglobin. Clin Chem 1994;40:1608–10.
27. Garde AH, Hansen AM, Skovgaard LT, Christensen J. Seasonal and biological variation of blood concentrations of total cholesterol, dehydroepiandrosterone sulfate, hemoglobin A1c, IgA, prolactin, and free testosterone in healthy women. Clin Chem 2000;46:551–9.
28. Desmeules P, Cousineau J, Allard P. Biological variation of glycated haemoglobin in a paediatric population and its application to calculation of significant change between results. Ann Clin Biochem 2010;47:35–8.
29. Phillipou G, Phillips PJ. Intraindividual variation of glycohemoglobin: implications for interpretation and analytical goals. Clin Chem 1993;39:2305–8.
30. Trápé J, Aliart M, Brunet M, Dern E, Abadal E, Queraltó JM. Reference change value for HbA1c in patients with type 2 diabetes mellitus. Clin Chem Lab Med 2000;38:1283–7.
31. Godsland IF. Intra-individual variation: significant changes in parameters of lipid and carbohydrate metabolism in the individual and intra-individual variation in different test populations. Ann Clin Biochem 1985;22:618–24.
32. Rohlfing C, Wiedmeyer HM, Little R, Grotz V, Tennill A, England J, et al. Biological variation of glycohemoglobin. Clin Chem 2002;48:1116–8.
33. Howey JE, Bennet WM, Browning MC, Jung R, Fraser C. Clinical utility of assays of glycosylated haemoglobin and serum fructoseamine compared: use of data on biological variation. Diabet Med 1989;6:793–6.
34. Carlsen S, Petersen PH, Skeie S, Skadberg Ø, Sandberg S. Within-subject biological variation of glucose and HbA1c in healthy persons and in type 1 diabetes patients. Clin Chem Lab Med 2011;49:1501–7.
35. Braga F, Dolci A, Mosca A, Panteghini M. Biological variability of glycated hemoglobin. Clinica Chimica Acta 2010;411:1606–10.