Research Article

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Biological variation of glycated albumin, glucose and albumin in healthy Turkish subjects

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Abstract

Objectives: Biological variation (BV) in laboratory tests can be defined as the variation in analyte concentration over time within and between individuals. Glycated albumin (GA) is a ketoamine which is used in the short-term monitoring of diabetes. The aim of this research was to determine BV of GA, glucose, and albumin under a well-designed and standardized protocol.

Methods: Blood samples were collected weekly from 21 healthy subjects (10 males, 11 females) for four consecutive weeks. Samples were analyzed using enzymatic methods in duplicate. After subjected to outlier and normality tests, variables as the within-subject biologic coefficient of variation (CV_I) and between-subject biologic coefficient of variation (CV_G), the index of individuality (II), and reference change value (RCV) were calculated.

Results: Analytical coefficient of variation (CV_A) was 3.5, 1.78, and 2.9%, for GA, glucose and albumin, respectively. The estimates for CV_I and CV_G: GA: 4.1%, 6.3%; glucose: 3.8%, 4.8%; albumin: 3.5%, 4%. RCVs and IIs were: 15%, 0.60; 12%, 0.79; 13%, 0.9 for GA, glucose and albumin, respectively.

Conclusions: The BV data of GA derived from this study might be applied to understand routine test results better and establish the quality standards for the analyte.

Keywords: between-subject variation; biological variation; glycated albumin; reference change values; within-subject variation.

ÖZ

Amaç: Laboratuvar testlerinde biyolojik değişkenlik (BV), bireyler içinde ve arasında zaman içinde analit konsantrasyonunda meydana gelen değişim olarak tanımlanabilir. Glike albümin (GA), diyabetin kısa süreli izlenmesinde kullanılan bir ketoamindir. Bu araştırmanın amacı, iyi tasarlanmış ve standartlaştırılmış bir protokol altında GA, glikoz ve albümin BV'sini belirlenmesidir.

Gereç ve Yöntem: 21 sağlıklı kişiden (10 erkek, 11 kadın) ardışık dört hafta boyunca haftalık kan örnekleri alındı. Numuneler, enzimatik yöntemler kullanılarak iki tekrar olacak şekilde analiz edildi. Aykırı değer ve normalite testlerine tabi tutulduktan sonra, birey içi biyolojik değişkenlik katsayısı (CVI) ve bireyler arası arası biyolojik değişkenlik katsayısı (CVG), bireysellik indeksi (II) ve referans değişim değeri (RCV) olarak değişkenler hesaplandı.

Bulgular: Analitik varyasyon katsayısı (CVA) GA, glikoz ve albümin için sırasıyla %3.5, %1.78 ve %2.9 idi. CVI ve CVG için hesaplamalar, sırasıyla: GA: %4.1, %6.3; glikoz: %3.8, %4.8; albümin:%3.5, %4 idi. RCV'ler ve II'ler GA, glikoz ve albümin için sırasıyla: %15, 0.60; %12, 0.79; %13, 0.90 idi.

Sonuç: Bu çalışmadan elde edilen GA'e ait BV verilerinin, rutin test sonuçlarının daha iyi anlamlandırılması ve analit için kalite standartlarının oluşturulması amacıyla kullanılabileceğini öngörmekteyiz.

Introduction

Biological variation (BV) can be defined as the inherent concentration fluctuations of a measurand around a homeostatic set point. BV is a key factor while interpreting the results of an analysis correctly. The two main components of biological variability are intra-individual variability (CV_I), and inter-individual variability (CV_G). Factors such as age, change over the lifespan, gender, ethnicity, geographical location of residence, and seasonal influences have an effect on the BV values. On the other hand, the index of individuality (II) is a variable that is

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derived from the comparison of the BV of an analyte within an individual over time, to that value of between all population, and determines the validity level of the reference intervals for that measurand [1].

Presently, diabetes mellitus (DM) is a major public health problem worldwide [2]. Glycated Albumin (GA), a ketoamine formed by the non-enzymatic glycation of serum albumin reflects the short-term average blood glucose concentration during the lifetime of either total plasma proteins or albumin (2–3 weeks). GA might be an alternative to hemoglobin A1c (HbA1c) in the diagnosis and monitoring of diabetes since it is not affected by erythrocyte lifespan, anemia, and other structural hemoglobin variants, thus serving as an indicator of glycemic control in conditions such as anemia, pregnancy, postprandial hyperglycemia and chronic kidney diseases [3].

EFLM Working Group on Biological Variation (WG-BV) and the Task Group for the Biological Variation Database (TG-BVD) have developed a critical appraisal list for the evaluation of BV studies, determining variables including minimum and maximum CV_I and CV_G values for a variety of analytes [4].

In today's laboratory medicine, the establishment of quality standards, and a better understanding of the factors that might interfere with the test results are essential. Since analytical variations and fluctuations between test results are expected outcomes, a brief determination of the level of various factors on analytical performance would yield a more convenient evaluation process for those variables by laboratory specialists and physicians.

Thus, the purpose of this study was to generate scientific data by determining BV and analytical performance specifications (APS) in terms of CV_A, CV_I, CV_G, and II for GA in order to evaluate its efficiency as a monitoring tool in the management of DM.

Materials and methods

Study population

The study was conducted at the Clinical Chemistry Laboratory of Turkish Ministry of Health, Istanbul Education and Research Hospital. Twenty-one healthy Caucasian volunteers who met the criteria specified in the reference interval determination survey form in accordance with the Clinical and Laboratory Standards Institute (CLSI) C28-A3 standards (10 males and 11 females, aged between 20 and 45) were enrolled in this study [5]. According to this guideline, preanalytical factors for consideration included subject preparation (diet, pharmacological agents, routine drug use, sampling time, physical activity, resting prior to sample collection, stress), specimen collection (environmental conditions, body posture, specimen type, specimen collection site, blood flow, sampling equipment, technique), and specimen handling (transport, clotting, separation of serum, storage, preparation). In addition, menstrual cycles and the use of hormone-containing contraceptives were questioned for female participants.

The subjects who did not meet the criteria specified in the survey form were excluded as recommended by the CLSI C28-A3 standards. Individuals were questioned for medications, vitamin supplements, tobacco or alcohol use, vegetarian diet for exclusion criteria, and subjects with a body mass index >30.0 kg/m^2 were also excluded in order to avoid the possible effects of those variables on BV of the analytes of interest.

Prior to the study, the health status of the included participants was determined and confirmed on the basis of medical history, complete blood count with differential, microscopic evaluation of the peripheral blood smear, and routine clinical chemistry panel.

All volunteers signed the informed consent form. The study was approved by the local Ethics Committee of Turkish Ministry of Health, Istanbul Education and Research Hospital. All procedures were conducted in strict conformity with corporate ethical and legal standards.

Sample collection and handling

Venous blood samples were collected by the same phlebotomist on a weekly basis for a duration of 4 weeks (November to December 2018) at 08:45–09:30 on the same day of the week. Blood samples were collected in tubes containing clot activator gel tubes (BD vacutainer SST™II Advance Plus Blood Collection Tubes). Samples after 8–12 h fasting were drawn from the volunteers in the resting condition, and subjects were asked to stay without a morning exercise prior to sampling. Serum samples were incubated at room temperature for 20 min for clot activation and then centrifuged at 3,000 *g* for 10 min and the serum was stored immediately after the centrifugation at −80 °C until the day of analyses.

To calculate the CV_A, we prepared a pool of sera according to the EP 7A-2 protocol published by CLSI [6]. Twenty fresh samples, of which laboratory test results were within the reference interval for all variables were used for sera pool preparation, and the samples were analyzed in duplicate for 10 consecutive days. The intralaboratory variance was calculated using the sum of the within and intermediate variances as described by the CLSI document EP15-A3 [7].

Analytical measurements

Since GA is formed by glucose and albumin, BVs of glucose and albumin were also calculated in the context of this study.

The levels of GA, albumin and glucose were measured in duplicate within a single run on AU5800 Series Clinical Chemistry Analyzers (Beckman Coulter Inc., Brea, CA, USA) using an enzymatic assay for GA (Diazyme Laboratories, Inc., CA, USA), and spectrophotometrically for glucose and albumin, using the original assay kits of Beckman Coulter. Internal quality control materials are provided by Beckman Coulter (Beckman Coulter Ireland Inc. Co. Clare, Ireland, REF: ODC0004) for glucose and albumin assays, and the Diazyme Control set (REF: DZB112B-CON) samples were used for GA. Expected mean and Standard deviation (SD) values set by the manufacturer were used for the evaluation of the internal quality control results. The target values for internal quality control materials are shown in Table 1. We used standard deviation index (SDI)

values of glucose and albumin provided by Randox quality control (Randox Laboratories Limited. Crumlin, United Kingdom) following the analysis of external quality control samples tested in our laboratory to evaluate the accuracy. An SDI value of ≤±1.99 was set as an acceptable limit.

Statistical analysis

Excel 2013 (Microsoft, WA, USA), Excel XLSTAT 2019 (Addinsoft, New York, USA), SPSS 18 (IBM, New York, USA) and Analyse-it (Analyse-it Software, Leeds, UK) were used for the statistical analyses. Initially, we assessed the outliers and distribution of the measured results. The Cochran's C test among within-subject values and Dixon-Reed criterion for between-subject values were employed in order to detect the outliers. The normality of the data distribution was checked using the Kolmogorov-Smirnov test. In case of an abnormal distribution, the data were log-transformed and a Shapiro Wilk analysis was applied.

After outlier tests and the exclusion of the outlier values, glucose had a normal distribution pattern (p=0.089). Normal distribution was confirmed after log transformation for albumin and GA (p>0.1, and p=0.089, respectively).

The coefficient of variations (CV_A, CV_I, CV_G) were obtained by converting standard deviations, which were calculated using Nested ANOVA.

The "index of individuality" for each analyte was determined based on the formula: $II=CV_I/CV_G$ [8].

We calculated analytical performance specifications as follows:

$$I\% = 0.5 \times CV_I$$

$$B\% = 0.25 \times \left(CV_I^2 + CV_G^2\right)^{1/2}$$

$TE\% = I\% \times 1.65 + B\%$, where I% represents imprecision; B% represents bias; TE% represents total error; CV_I represents the within-subject biological coefficient of variation and CV_G represents the between-subject biological coefficient of variation [1].

Analysis of the variance component values were calculated according to the CLSI EP15-A3 guideline since we employed an automated analyzer platform using the manufacturer's reagents to calculate within laboratory precisions [7].

Results

The individual mean and non-parametric interval values (95% CI) for GA, glucose and albumin are shown in Figure 1.

The number of excluded and analyzed samples, BV components, and II for GA, albumin, and glucose are shown in Table 2. The degree of freedom was found to be 20 for the Nested ANOVA analyses. We also calculated imprecision, bias, and total error for each procedure. The results are shown in Table 3. The within-laboratory precision values of the analytes measured in this study were 6.72, 1.95, 4.514 µmol/L for GA, glucose, and albumin, respectively.

Our monthly CV, SD and mean values of two levels of internal quality control, SDI of external quality control values for the sampling period are also shown in Table 3. We could not provide an SDI value for GA since we are not registered in any external quality program for this measurand.

Discussion

Although a large number of studies have been made on the BV calculations of glucose and albumin, a limited number of studies are available on GA. In the context of our study, we performed analytical performance calculations for glucose and albumin due to their close relationship in the process of GA formation [2].

In our study, we compared our results with the results from another study performed on the Chinese population [9]. They estimated CV_I as 1.23%, CV_G as 4.67% and CV_A as 0.43% for GA which were lower than our CV values. Therefore, their findings for APS's of GA exhibited better analytical performance characteristics compared to our data (TE%; 2.22 vs. 5.20%, Bias %; 1.21 vs. 1.80 %, I%; 0.62 vs. 2.05%). Similarly, in their regional BV study, Yang et al. observed that BV of white blood cells and red bloods cell in the Chinese population was lower than those of Caucasians, suggesting the employment of several population-related variables resulting in the differences between their study group and ours [10]. Besides, Liang et al. used a different brand of GA assay than we used, which might have an effect on the calculated results. They also did not

Table 1: Our monthly coefficient of Variation (CV), Standard deviation (SD) and mean values of two levels of internal quality control, SDI (standard deviation index) of external quality control samples (Values of February 2019).

	CV		SD		Mean		SDI
	Level 1	Level 2	Level 1	Level 2	Level 1	Level 2	
Glycated albumin, µmol/L	8.90%	4.24%	28.32	29.47	318.12	695.50	NA
Glucose, mmol/L	2.66%	2.38%	0.14	0.312	5.26	13.09	0.27
Albumin, µmol/L	2.66%	1.56%	9.02	10.53	338.54	672.58	−0.20

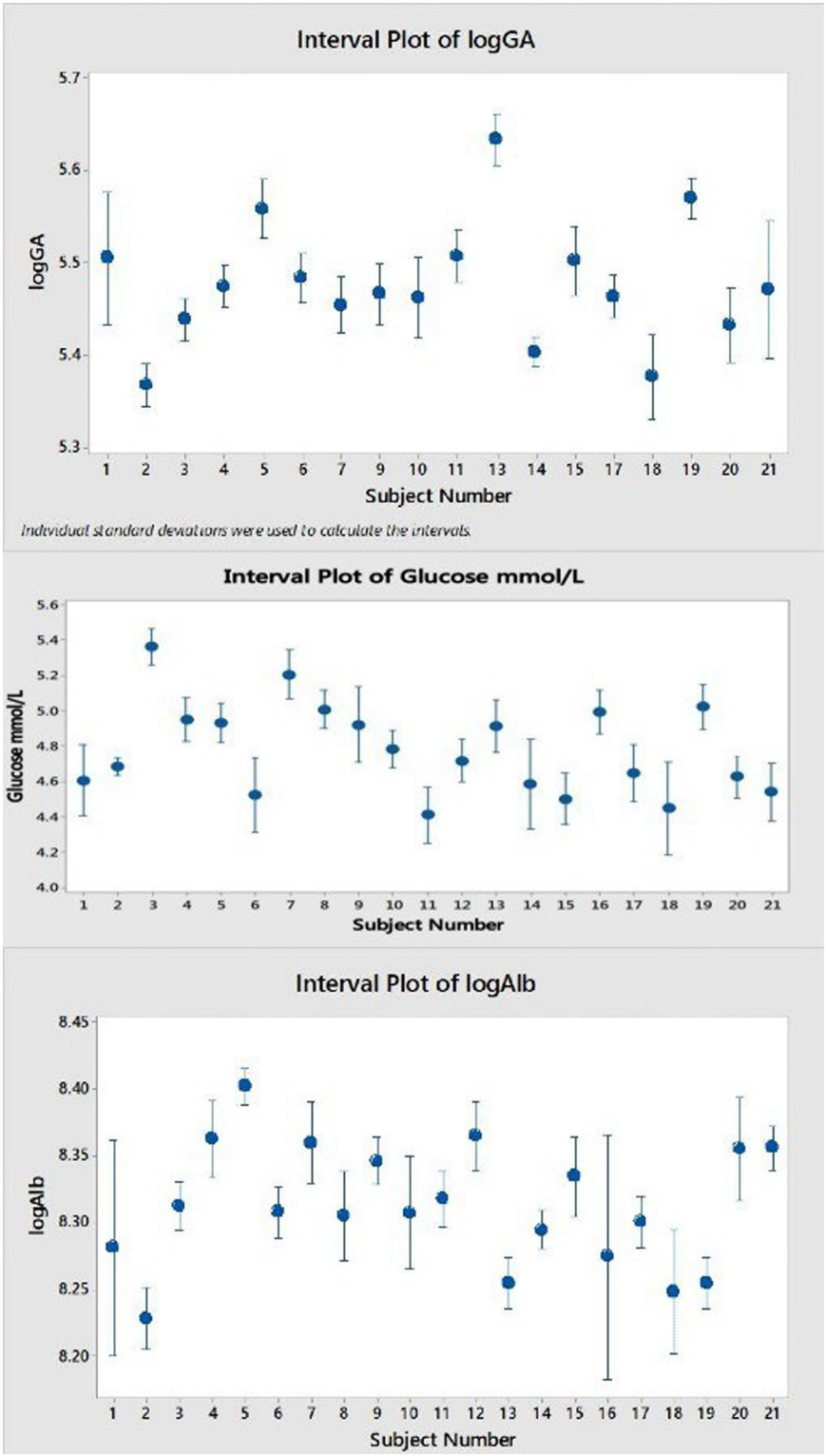

Figure 1: Individual mean and range values for glycated albumin, albumin and glucose in healthy subjects.
Log-transformed values used for glycated albumin and albumin.

state which month of the year their study was conducted since some alterations are possible due to the seasonal variances.

In their studies, Montagnana et al. and Liang et al. presented data on the estimated CV_A and CV_I values for GA and albumin, which were lower than our findings, whereas CV_G for GA in Montagnana et al.'s study was higher than our results [9, 11]. However, the estimated within- and between-subject BVs of glucose and GA are questionable despite their relatively longer duration of the study period

Table 2: The CV, and estimated RCV and II values of Glycated Albumin, Glucose and Albumin.

	Glycated albumin	Glucose	Albumin
Excluded/analyzed samples (number)[a]	22/146	11/157	8/160
CVG, %	6.30 (4.50–9.80)	4.80 (3.40–7.20)	4.00 (2.80–6.10)
Online database*	Not determined	3.4–12.1	2.2–6.3
Montagnana data**	10.60	Not determined	2.90
Liang data***	4.67	Not determined	3.18 (2.59–3.46)
CV_I, %	4.10 (3.30–5.30)	3.80 (3.20–4.70)	3.50 (2.90–4.40)
Online database*	Not determined	2.70–10.80	2.20–3.90
Montagnana data**	2.10	Not determined	2.30
Liang data***	1.23	Not determined	0.75 (0.72–0.83)
CV_A, %	3.57 (2.70–6.20)	1.78 (1.20–3.00)	2.90 (2.20–4.80)
Online database*	Not determined	Not determined	Not determined
Montagnana data**	1.70	Not determined	2.30
Liang data***	0.43	Not determined	1.67 (1.49–1.76)
II	0.65	0.79	0.87
Online database*	Not determined	Not determined	Not determined
Montagnana data**	Not determined	Not determined	Not determined
Liang data***	0.26	Not determined	0.24

CV_G, between-subject biologic variation; CV_I, within-subject biologic variation; CV_A, Analytical variation; RCV, Reference change value; II, index of individuality CV_I, CV_G and CV_A are given with their 95% confidence interval for our results and where available. The values between parentheses represent 95% confidence interval (CI) for the CV_I, CV_G and CV_A values. [a]Excluded samples were the outliers for each variable. *https://biologicalvariation.eu. **Montagnana M, Paleari R, Danese E, et al. Evaluation of biological variation of glycated Albumin (GA) and fructosamine in healthy subjects. Clin Chim Acta 2013;423:1–4. ***Liang L, He H, Zeng Y, et al. Evaluation of biological variation of glycated hemoglobin and glycated Albumin in healthy Chinese subjects. J Clin Lab Anal 2019;33:e22715.

Table 3: Values calculated and reported on online database. APS for Imprecision, Bias and Total Error.

	Glycated albumin	Glucose	Albumin
APS derived from our research			
I%	2.05	1.90	1.75
B%	1.80	1.50	1.32
TE%	5.20	4.60	4.20
APS reported by published data #1*			
I%[a]	1.03 (0.51–1.54)	Not determined	1.13 (0.57–1.70)
B%[b]	2.70 (1.35–4.05)	Not determined	0.93 (0.46–1.39)
TE%[c]	6.09 (3.05–9.14)	Not determined	4.67 (2.33–7.00)
APS reported by published data #2**			
I%	0.62	Not determined	0.38
B%	1.21	Not determined	0.82
TE%	2.22	Not determined	1.44

APS, Analytical performance specifications; I%, imprecision; B%, BIAS; TE%, Total error. *Montagnana M, Paleari R, Danese E, et al. Evaluation of biological variation of glycated Albumin (GA) and fructosamine in healthy subjects. Clin Chim Acta 2013;423:1–4. **Liang L, He H, Zeng Y, et al. Evaluation of biological variation of glycated hemoglobin and glycated Albumin in healthy Chinese subjects. J Clin Lab Anal 2019;33:e22715. [a, b, c]The value represents the desirable level whereas the values in the parentheses represent for optimal and minimal analytical goals.

and a higher number of analysis replications when compared to ours since GA is a relatively short-term biomarker, and glucose levels are highly variable between individuals.

In our research, it was found that all estimated BV components for each test were lower than those reported, except for the CV_I value provided by the EFLM database for albumin. Our calculation for CV_I of Albumin was 3.50%, whereas the reported ratio was 3.00% in the database [4]. We also found that, CV_I, CV_G, and APS of glucose were lower than the ratios presented in the online database, and those of other similar studies on BV of glucose [4, 12]. The index of individuality (II) and reference change value (RCV) for glucose was found as 0.79, and 15, respectively. These values prompted us to employ RCV values instead of a population-based reference interval for the interpretation of two consecutive results for glucose [13, 14].

A recent meta-analysis of BV studies on measurands including GA reported that the observed differences in published data might be a consequence of a series of variables including failure to identify outliers in some of the studies, heterogeneity of the groups, the lack of data on the total number of results implemented for the calculation of BV estimates in most studies. In addition, studies with subjects older than 75 years and diabetic individuals presented higher CVI values. The analytical methodology, race of the population of interest, total number of analyses, and the duration of the studies are among other factors that might influence the variability of presented studies [15].

In order to ensure the higher reliability of BV calculations, pre-analytical factors and external interferences need to be controlled closely. For this purpose, we tried to eliminate pre-analytical errors by taking systematic and controlled precautions in the light of relevant guidelines, and designed our study by following Braga and Fraser's recommendations step by step, and applied their endorsements to all stages [1, 16].

For BV studies, an accurate definition of the subject number and study duration is essential. We designed this study with 21 healthy volunteers (10 males and 11 females) considering two different sub-group settings based on gender. However, since the number of subjects in each group was the lowest desired number of subjects defined in the guidelines, and the reference intervals of GA and glucose do not depend on gender, we did not subgroup the participants in terms of gender [12, 17].

One essential limitation of our study is the lack of external quality assurance testing for the GA during the study period. However, to the best of our knowledge, there was not a commercial external quality control program available for GA in the time of study. Another limitation of our study is the lack of consensus regarding the study duration for BV analyses. In brief, the study duration and frequency of re-testing of the analyte are expected to be related to the clinical practice. In their experimental study on the half-life of endogenously formed ^{14}C GA in diabetic rabbits, Kallner et al. defined the half-life of GA as approximately 6 days, indicating ~70% shorter than that reported for rabbit serum albumin [18]. In our study, we established a study period of 4 weeks, and the experiments were performed within this time interval. However, this duration might be accepted as relatively short for a BV study.

In conclusion, we estimated that CV_G of GA with the method used in this study was lower than that reported in a previous study [11]. II (0.65) of GA was estimated close to 0.60 as defined by Fraser who reported that the traditional reference intervals may not be useful in the interpretation of patient results [1]. We suggest using the RCV value of GA, glucose, and albumin to evaluate the difference between two consecutive measurements of the same patient. We suggest that the calculations on the variables in this study would be useful for further studies. Additional research data with a higher number of participants with different patient groups such as those with chronic kidney disease, DM, and pregnant individuals are needed to define BV and APS of GA, besides a recently defined variable "minimal difference (MD)" which is based on detailed short-term and long-term (10 years) internal quality control data derived from glucose concentration measurements with low imprecision levels [19].

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Informed consent: Informed consent was obtained from all individuals included in this study.

Ethical approval: The study was approved by the local Ethics Committee of Turkish Ministry of Health, Istanbul Education and Research Hospital.

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