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RESEARCH ARTICLE

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Evaluation of biological variation of glycated hemoglobin and glycated albumin in healthy Chinese subjects

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Background: Glycated hemoglobin (HbA1c) and glycated serum albumin (GSA) are used to evaluate the mean blood glucose levels. To ensure safe clinical application of HbA1c and GSA, reliable biological variation (BV) data are required. The aim of this research was to define the BV of HbA1c and GSA employing stringent rules.

Methods: Blood samples were drawn from 19 healthy subjects (10 females, nine males) once per week for 5 weeks. All samples were analyzed using enzymatic method for GSA and HPLC for HbA1c. The data were assessed for outliers, normality and variance homogeneity, and coefficient of variation (by ANOVA) for BV. Sex-stratified BV including within-subject (CV_I) and between-subject (CV_G) was defined for HbA1c and GSA.

Results: The following estimates for BV values for CV_I and CV_G, respectively, were GSA: 1.23% and 4.67%, Alb: 0.75% and 3.18%, and HbA1c: 0.12% and 2.91%. The RCV of GSA was 3.61%, and HbA1c was 1.41%. And the II was 0.26 for GSA, and 0.07 for HbA1c, both of them less than 0.6. According to the 95% CI, the CV_I of HbA1c was statistically different between females and males. And both the CV_G of HbA1c and GSA were statistically different between females and males.

Conclusion: All CV_I and CV_G estimates were lower than those reported in the online BV database. And there is a significant difference between males and females. Analytical performance specifications derived from BV of this research can be applied internationally.

KEYWORDS
between-subject variation, biological variation, glycated albumin, glycated hemoglobin, within-subject variation

1 | INTRODUCTION

The prevalence of diabetes among Chinese adults is as high as 11.6%, and about 113 million Chinese have type 2 diabetes mellitus (T2DM).[1] Glycated hemoglobin (HbA1c) plays an increasingly important role in the diagnosis and treatment of diabetes. However, the detection of HbA1c depends on the blood glucose concentration and the quality of red blood cells, including shortened red blood cell life, hemolytic anemia, renal anemia, and variant hemoglobin. HbA1c in such patients may not be measured accurately by high performance liquid chromatography (HPLC).[2] Glycated serum albumin (GSA) is a nonenzymatic glycation product of serum albumin upon reaction with glucose. It reflects the postprandial average blood glucose concentration and the average blood glucose level within the

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last 2-4 weeks. The GSA level is based on the percentage of glycosylated serum albumin from total serum albumin. When the HbA1c detection is affected by the amount and quality of red blood cells, GSA can more accurately reflect a patient's average blood glucose level than glycated hemoglobin. Therefore, the detection of GSA is an effective supplement to HbA1c as an indicator for the glucose levels in diabetic patients.

BV is widely used in medical laboratories,[3,4] including in the assessment of clinical significance of changes in continuous test results from the same individual by RCV, the applicability of population-based reference values through index of individuality (II), and evaluation of analytical performance. BV is highly beneficial for improving the laboratory quality management level. In 2014, the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) questioned the online BV data on the Westgard website about its effectiveness and reliability.[5,6] The EFLM BV working group then proposed the biological variation data critical appraisal checklist (BIVAC). This checklist is used to standardize operations for assessing existing biological variation studies and guiding future biological variation studies. Currently, based on the checklist, there are nine enzymes, creatinine, and 21 hematological parameters reported in EFLM. According to these papers, all the BV data are less than that reported on Westgard website.[7–9]

The BV data can confirm if patients treated with antihypertensive or insulin are close to normal levels, indicating that the condition is getting better and the development of complications such as cardiovascular disease or diabetes is unlikely.[10] Therefore, the establishment of effective BV can contribute to the management of chronic diseases such as diabetes. At present, there are many reports on the BV of HbA1c, but the results are contradictory.[11] And there is no report about the biological variation of GSA among the Chinese population. The current study measured the biological variation of HbA1c and GSA in 19 nondiabetic subjects through the standard protocol designed by EFLM.

2 | MATERIALS AND METHODS

2.1 | Materials

The research enrolled 19 healthy subjects, including nine males and 10 females, both of the average age 26 years old. All volunteers provided signed informed consents. The inclusion criteria included no familial diabetes, no thalassemia syndrome, or other hemoglobinopathy, females had regular menstrual cycles and did not use hormonal contraceptives, all subjects did not take drugs or smoked, fasting glucose <6.0 mmol/L, body mass index (BMI) <30.0 kg/m^2. Fasting venous blood (fasting 8-10 h) was collected from the subjects on alternate Mondays. The blood was collected between 9:00 and 9:30 AM, from a subject who first spent ten minutes sitting quietly, by a professional nurse using siliconized vacuum tube (BD Vacutainer®, New Jersey) for GSA and EDTA3K (BD Vacutainer®) for HbA1c. The samples were centrifuged at 3000 *g* for 10 minutes at 22°C to separate the serum. The serum and whole blood were stored separately at −80°C until further use.

2.2 | Methods

This study received approval of the West China Hospital's Ethical Review Committee (NO. 228-2015). All the volunteers were signed the informed consent.

We used an enzymatic assay kit (Lucica®GA-L, Tokyo, Japan) to test the GSA levels and HPLC (TOHOSH®, Tokyo, Japan) to test the HbA1c levels. All samples were tested five times.

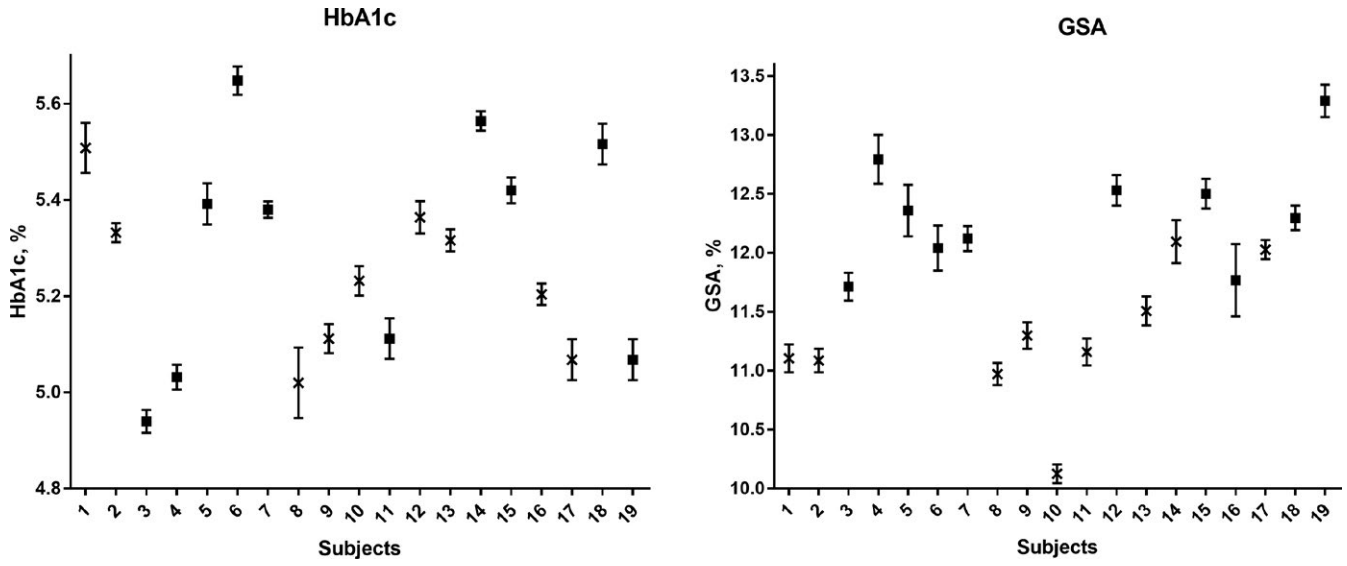

FIGURE 1 The average and 95% confidence interval of HbA1c and GSA in 19 healthy Chinese individuals. × represents males, and ■ represents females

2.3 | Statistical analysis

We assessed the outliers and the distribution of the data. Both the parameters are without outliers, according to Fraser and Harris. The coefficient of variation (CV) was analyzed using ANOVA. The difference between the male and female groups was compared using 95 CI%. If there was no significant difference between male and females, CV_I was the same in all test subjects. The CV_A, CV_I, and CV_G were calculated using nested ANOVA 95% CI.

The CV = 1/2 × CV_I,
bias (B) = 0.25 × (CV_I^2 + CV_G^2) × 0.5,
TE = 1.65 × CV + B.

The reference change value (RCV) and index of individuality (II) were calculated using the formula given by Fraser and Harris:

$$RCV = \sqrt{2} \times Z \times \sqrt{CV_I^2 + CV_A^2},$$

$$II = \sqrt{CV_I^2 + CV_A^2}/CV_G$$

All the data analyses were performed using SPSS 22.0 (IBM Corp., Armonk, NY, USA) and SAS 9.4 (SAS Institute Inc., Cary, NC, USA).

3 | RESULTS

The mean and 95% CI of HbA1c and GSA are shown in Figure 1. The mean of GSA for males was less than females statistically different. The mean levels of albumin were significantly higher in males than females. And there was no difference between males and females of HbA1c (Table 1).

The BV data for all subjects, males alone, and females alone are shown in Table 1. BV estimates were compared with those reported in the Westgard online database. The CV_A used intra-assay variation to minimize variation. Both of the estimated CV_I and CV_G were less than the online database.

We also calculated the variation for males alone and females. The CV_I of GSA and Alb was closed between males and females, but the CV_G for males larger than females statistically different. The CV_I and CV_G of HbA1c for males were less than females significantly different.

The analytic performance specification (APS) for imprecision, bias, and total error is derived from the BV data shown in Table 2 and compared with the desirable specifications reported in the Westgard database. Based on the BV data, the reference change value (RCV) of GSA and HbA1c was 3.16% and 1.41%, respectively. The index of individuality (II) of GSA and HbA1c was 0.26% and the 0.07, respectively. Both of the II were less than 0.6 (Table 2).

4 | DISCUSSION

The mean levels of GSA and Alb in males and females were significantly different, so the reference intervals should be separated.

TABLE 1 Biological variation of glycated serum albumin (GSA) and HbA1c between males and females

		Number of subjects	Total number of results	Mean value (95% CI)	CV_A (95% CI), %	CV_I (95% CI), %	CV_G (95% CI), %	Online BV database	
								CV_I	CV_G
HbA1c, %	All subjects	19	475	5.29 (5.25~5.34)	0.42 (0.39~0.46)	0.29 (0.26~0.34)	4.32 (4.27~4.55)	1.9	5.7
	Males	9	225	5.26 (5.21~5.30)		0.12 (0.09~0.18)	2.91 (2.27~3.4)	–	–
	Females	10	250	5.34 (5.25~5.40)		0.32 (0.28~0.41)[a]	5.16 (4.84~5.52)[a]	–	–
GSA, %	All subjects	19	475	11.83 (11.68~11.99)	0.43 (0.40~0.51)	1.23 (1.19~1.26)	4.67 (3.77~4.82)	5.2	10.3
	Males	9	225	11.26 (11.07~11.44)		1.24 (1.17~1.29)	4.07 (3.59~4.74)	–	–
	Females	10	250	12.35 (12.20~12.51)[a]		1.11 (0.69~1.32)	1.57 (1.19~2.4)[a]	–	–
Alb, g/dL	All subjects	19	475	4.88 (4.83~4.93)	1.67 (1.49~1.76)	0.75 (0.72~0.83)	3.18 (2.59~3.46)	3.2	4.75
	Males	9	225	5.04 (5.00~8.08)		1.24 (1.17~1.29)	4.07 (3.59~4.74)	–	–
	Females	10	250	4.73 (4.67~4.80)[a]		1.01 (0.64~1.35)	2.76 (2.22~3.61)[a]	–	–

[a]Compared with males, there was statistical significance.

TABLE 2 APS derived from present research and online database

			APS derived from now research			APS derived from online database		
	RCV, %	II, %	Imprecision, %	Bias, %	Total allowable error, %	Imprecision, %	Bias, %	Total allowable error, %
GSA, %	3.61	0.26	0.62	1.21	2.22	2.6	2.9	7.2
Alb, g/dL	5.07	0.24	0.38	0.82	1.44	1.6	1.43	4.07
HbA1c, %	1.41	0.07	0.15	1.08	1.32	0.9	1.5	3.0

In this research, all estimated BV data were found to be less in comparison with that reported on the Westgard website. Especially for the BV of GSA, compared with the online data, it was decreased (CV_I: 1.23% vs 5.2%, CV_G: 4.67% vs 10.3%). The CV_I of Alb and HbA1c was also found to be lesser (Alb: 0.75% vs 3.2%, HbA1c: 0.29% vs 1.9%), and the CV_G was also reduced (Alb: 3.18% vs 4.75%, HbA1c: 4.32% vs 5.7%). These differences could be due to several reasons. First, BV varies based on multiple factors, including race, gender, age, growth and development, the economic level, occupation, living habits, and diet structure. Thus, the results obtained of different ethnic groups in different regions vary considerably. Yang reported that the BV of white blood cell and red blood cell in Chinese was less than in Caucasians. They further pointed out that it is necessary to estimate the BV suitable for a particular laboratory population.[12] Second, with the development of laboratory techniques, the sensitivity and specificity of different research methods using different analytical methods affect the design, sample collection, and statistics. Therefore, we employed the checklist proposed by EFLM to reduce variation and ensure reliable and effective data. In this research, we strictly controlled pre-analytical variability factors, such as sample collection time, status, and tourniquet using time, needle diameter, and tube mixing change. Third, there are few sources of biological variation data in the website. At present, the biological variation of GSA in the database is only from one study reported in 1993.[13] In the current study, the confidence intervals of GSA were less than that reported before (CV_I: 1.19%-1.26% and CV_G: 3.77%-4.82%).[14]

Calculating RCV, II, and analytical quality specifications based on BV data plays an important role in the laboratory. According to these results, if the difference between the two results of GSA measurement is more than 3.6% it is considered to be accompanied by changes in the disease condition, which may help the clinician to further diagnose or treat the condition. It should be noted that the RCV is based on the comparison of the single determination results of an individual with previous results from the same individual, rather than using a population reference interval. II is the ratio of CV_I and CV_G. When II is >1.4, the reference interval is suitable. When II is <0.6, the reference interval for assessing the results of continuous change is limited, because $CV_I < CV_G$, indicating that the analyte has a large individual characteristic and a wide reference interval. In this research, all the II values were <0.6, suggesting that the personalized reference interval based on BV is much more important than the reference interval based on population. Meanwhile, the II of GSA was greater than HbA1c, confirming that the reference interval of GSA is more valuable than HbA1c in the diagnosis and treatment of diabetes.

The well-demonstrated advantages of glycated albumin, compared with HbA1c, are that the changes are more significant and not affected by changes in erythrocyte quality.[15] Glycated albumin not only monitors glycemic levels but also has a good correlation with the development of diabetic complications including nephropathy and retinopathy, and can be considered as a marker for assessing atherosclerosis risk.[16] In addition, since GSA is the ratio of glycated albumin concentration to total serum albumin, different from fructosamine, GSA is not influenced by the concentration of albumin or other proteins.[17] Our research employed strict rules to estimate the BV data than the online website.[18] Determining specific analytical performance specifications based on the BV of each test helps to ensure the analytical performance of the laboratory to meet requirements and continuously improve. Moreover, it is beneficial to provide more accurate test results for clinicians.

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AUTHOR CONTRIBUTIONS

Liang Libo and Huang Hengjian research literature and conceived the study. He He involved in protocol and wrote the first draft. Zeng Yuping recruited the volunteers and analyzed the data. Zhang Mei gained the ethical approval and enrolled the volunteers. Wang Xia analyzed the data. Li Xiaoling and Liang Shanshan analyzed the samples, and An Zhenmei and Huang Hengjian approved the final version of the manuscript. All authors reviewed and edited the manuscript.

ETHICAL APPROVAL

Ethics Committee of West China Hospital (NO.228-2015).

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