Clin. Lab. 2022;68:XXX-XXX

ORIGINAL ARTICLE

Influence of RBC Indices on HbA1c Measurement by Capillary Electrophoresis and HPLC Methods

Yi-Jie Juang [1], Hsin-Mian Wu [1], Ya-Wen Cheng [1], Hock-Liew Eng [2], Tsun-Mei Lin [3, 4]

[1] *Department of Laboratory Medicine, E-DA Hospital, Kaohsiung, Taiwan*
[2] *Department of Pathology, Kaohsiung Chang Gung Memorial Hospital, Chang Gung University College of Medicine, Kaohsiung, Taiwan*
[3] *Department of Medical Research, E-DA Hospital, Kaohsiung, Taiwan*
[4] *Department of Medical Laboratory Science, I-Shou University, Kaohsiung, Taiwan*

SUMMARY

Background: **HbA1c is the gold standard of diabetic surveys to monitor the long-term glycemic control. Anemia is cited as a major confounder to HbA1c analysis; however, the effect of RBC indices influences on HbA1c analysis is not known. The aim of this study is to compare ion-exchange high-performance liquid chromatography, and capillary electrophoresis to evaluate the influence of RBC parameters on HbA1c values in anemia patients.**
Methods: **Erythrocyte parameters were collected from the 307 randomly selected specimens from the Hematology division. HbA1c was measured on the same specimen using Tosoh G8 and Capillarys 2 Flex Piercing on the same day.**
Results: **There is acceptable concordance between the results of capillary electrophoresis and HPLC methods ($R^2 = 0.953$, $p < 0.001$). However, significant differences in HbA1c value between the two assay methods were obtained in the patients with abnormal RBC indices ($p < 0.001$).**
Conclusions: **Our results demonstrated HbA1c differences were significantly different in the patients with low Hb (≤ 8 g/dL) and high RDW-CV (≥ 13.7%). It is suggested that in the analysis of HbA1c level in anemia patients, simultaneous testing for hemoglobin level is needed. In addition, development of a new reference value of HBA1c for patients with severe anemia should be considered.**
(Clin. Lab. 2022;68:xx-xx. DOI: 10.7754/Clin.Lab.2021.211053)

Correspondence:
Professor Tsun-Mei Lin, MT, PhD
Department of Medical Research
E-DA Hospital
No. 1, Yi-Da Road
Jiao-Su Village
Yan-Chan District
Kaohsiung City 82445
Taiwan
Phone: + 886 7-6151100 ext 5063
Fax: + 886 7-6155352
Email: ltmei@mail.ncku.edu.tw

Professor Hock-Liew Eng, MD
Department of Pathology
Kaohsiung Chang Gung Memorial Hospital
Chang Gung University College of Medicine
No. 123, Dapi Road
Niaosong District
Kaohsiung City 833
Taiwan
Phone: + 886 7-7317123 ext 2578
Fax: + 886 7-7333198
Email: eng6166@ms8.hinet.net

KEY WORDS

HbA1c, high-performance liquid chromatography, capillary electrophoresis, anemia, RBC indices

Manuscript accepted May 3, 2021

INTRODUCTION

Glycosylated hemoglobin (HbA1c) has a glucose residue specially attached to the N-terminal valine residue of one or both HbA beta chains. Red blood cells (RBC) are freely permeable to the plasma glucose molecules; therefore, HbA1c level is directly proportional to average blood glucose concentration over the previous 4 weeks to 3 months or the average lifespan of the erythrocyte [1]. However, factors besides mean blood glucose appear to affect HbA1c levels by a variety of genetic, physiological, hematological, and illness-related factors [2]. Depending on the type of anemia, it may be associated with a rapid turnover of erythrocytes, resulting in lower HbA1c levels, or alternatively, slower turnover of erythrocytes, resulting in increased glycation of hemoglobin (Hb) and, consecutively, higher HbA1c levels [3]. Therefore, anemia is cited as a major confounder to HbA1c analysis; however, the effect of RBC indices and to what degree they influence HbA1c analysis in a laboratory is still unknown. The use of HbA1c for the diagnosis of diabetes is now widely recommended despite limitations to its use.

Accurate HbA1c results are essential for monitoring and diagnosis of diabetic patients. Presently, the methods most commonly used to quantify HbA1c are capillary zone electrophoresis or ion-exchange high-performance liquid chromatography (HPLC). Both methods separate and determine HbA1c from other Hb fractions based on charge or mass differences and have the advantage of showing the presence of hemoglobin variants which is important for the analysis of HbA1c results [4,5]. As different methods for HbA1c determination exhibit different characteristics and performances, the reliability and comparability of different methods used to measure HbA1c and their potential interchangeability represents a key feature in a clinical laboratory [6,7]. Therefore, the aim of this study was to compare Tosoh G8 HPLC Analyzer and Sebia Capillary 2 Flex Piercing (Cap 2FP) instrument to evaluate the correlation in patients with various hemoglobin content and RBC indices.

MATERIALS AND METHODS

Sample collection

This study comprised 307 randomly selected samples from the subjects who were at the clinical laboratory between June and August 2018 for routine hematological assay and HbA1c analysis.

Blood samples were obtained through venipuncture into 2.0 mL BD Vacutainer® Hemogard tubes with K2-EDTA (Becton Dickinson, Plymouth, UK). Complete blood counts were analyzed in fresh blood, and leftover samples were used to measure the HbA1c levels. HbA1c levels were measured with two different methods and were completed within 8 hours following blood sampling.

Erythrocyte indices

The levels of hemoglobin (Hb), mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), hematocrit (Hct), red blood cell distribution width (RDW), and red blood cell count were measured by the automated counter Sysmex XE-9000 (Sysmex Co., Kobe, Japan) according to the manufacturer's instructions.

Analyzers for HbA1c

The Sebia Capillary 2 Flex Piercing instrument (Sebia Cap2FP) (Sebia, Evry Cedex, France) is an automated analyzer for the quantitative analysis of proteins with fast separation and good resolution. The CAPILLARYS HbA1c kit is based on the capillary zone electrophoresis principle, where hemoglobin fractions are separated by their charge in an alkaline buffer (pH 9.4). The instrument has capillaries functioning in parallel allowing 8 simultaneous analyses for hemoglobin quantification from the whole blood sample. Then, 18 µL of the whole-blood sample is automatically diluted (1/6) with a hemolyzing solution and is injected at the anodic end of the capillary by hydrodynamic injection. A high-voltage protein separation is then performed and direct detection of the hemoglobin is made at the cathodic end of the capillary at 415 nm.

Tosoh G8 Analyzer (Tosoh Bioscience, San Francisco, CA, USA) is a fully automatic HPLC analyzer for the fast determination of HbA1c and hemoglobin variants. Separation is achieved by non-porous ion-exchange HPLC for rapid, accurate, and precise separation of the stable form of HbA1c from other hemoglobin fractions. Approximately 3 µL of whole blood is automatically diluted (1/200) with hemolysis buffer and washing solution in the dilution port. Next, the diluted sample is injected onto the column, and the separated fractions are continuously monitored by the diode detector system at 415 and 510 nm.

Statistical analysis

Statistical analysis was performed using SPSS software (Version 22.0; IBM SPSS Statistics for Windows; IBM Corp., Armonk, NY, USA). Data are expressed as mean ± standard deviation. The *t*-test was used to compare differences between the groups. Intergroup comparisons were performed using one-way ANOVA for continuous variables. Pearson's correlation and multiple regression analysis were performed to assess the correlation between RBC indices and HbA1c differences between Tosoh G8 and Sebia Cap2FP analyzers. The p-values of < 0.05 ($p < 0.05$) was considered to be statistically significant.

RESULTS

During the study period, a total of 307 patients (53.1% males) with mean age 60.85 (range 15 - 98) participated in the study. The baseline demographic and hematological parameters obtained from the patients are displayed in Supplemental Data Table S1. Of the 307 patients, 238 were anemic with Hb < 10 g/dL, including 26 macrocytic with MCV > 100 fL and 42 microcytic with MCV < 80 fL.

All samples were simultaneously analyzed with Tosoh G8 HPLC and Sebia Cap2FP to detect HbA1c values. The HbA1c values obtained using the G8 system were significantly correlated with Cap2FP (R^2 = 0.953, Pearson's correlation coefficient r = 0.976, p < 0.001). Linear regression analysis of the data from the comparison between the Sebia analyzer and the Tosoh G8 (Figure 1A) showed a slope of 0.963 (95% confidence interval: 0.939 to 0.987) and an intercept of 0.106 (95% confidence interval: -0.044 to 0.257), without significant deviation from linearity. The Bland-Altman plots of differences between Tosoh G8 and Sebia Cap2FP are shown in Figure 1B. The mean difference (± 1.96SD) was 2.00% (-7.29 to 11.29 %). The Tosoh G8 value is on average about 2% higher than the Sebia Cap2FP value.

The World Health Organization (WHO) and American Diabetes Association (ADA) have both advocated the use of HbA1c for the diagnosis of diabetes at a value of "6.5%" [8]. Based on Figure 1A, the samples were demonstrated to have more obvious relative differences between the two assay methods when the HbA1c value was within normal ranges (< 6.5%). It is probable that in non-diabetic people the main cause of the difference between the two analyzers is also related to the other parameters of the blood sample. We sought to compare RBC indices where there is an interference for HbA1c detection with Tosoh G8 and Sebia Cap2FP. A Pearson product-moment-correlation coefficient (Pearson's correlation) was calculated to assess the correlation of HbA1c differences between Tosoh G8 and Sebia Cap2FP and RBC parameters. Correlation analysis revealed that HbA1c differences between Tosoh G8 and Sebia Cap2FP significantly correlated with RBC count (r = -0.204, p = 0.0003), Hb (r = -0.0.348, p < 0.001), Hct (r = -0.333, p < 0.0001), MCV (r = -0.184, p = 0.001), and MCH (r = -0.176, p = 0.002) and RDW-CV (r = 0.207, p = 0.0003) as shown in Table 1. However, after multivariable regression analysis, only MCV, MCHC, and MCV/MCH were strongly inversely correlated with HbA1c differences between Tosoh G8 and Sebia Cap2FP analysis (β = -3.22, p = 0.001; β = -2.59, p < 0.0001; β = -1.19, p = 0.001), and a positive correlation was seen between HbA1c differences and MCH (β = 3.89, p = 0.0001) as shown in Table 2.

The linear regression curves show RBC, Hb, Hct, MCV, and MCH decreased gradually with the increase of HbA1c differences between Tosoh G8 and Sebia Cap2FP analysis (r = -0.193, p = 0.0007; r = -0. 371, p < 0.0001; r = -0.332, p < 0.0001; r = -0.203, p = 0.0004; r = -0.226, p < 0.0001; respectively). Only RDW-CV increased slightly with HbA1c differences (r = 0.257, p < 0.0001) as shown in Supplemental Data Figure S1.

The subgroups were categorized into quartiles with HbA1c differences between Tosoh G8 and Sebia Cap2FP values, RBC, Hb, and Hct were decreased gradually and significantly, as the differences of Tosoh G8 and Sebia Cap2FP value increased (p = 0.0004, p < 0.0001, p < 0.0001, respectively) as shown in Table 3. Based on the results of our analysis, there were no significant differences in age, gender, and MCV between different subgroups stratified by HbA1c differences of Tosoh G8 and Sebia Cap2FP values. In addition, MCH, MCHC, MCV/MCH, and RDW-CV also showed significant differences between subgroups (p = 0.032, p = 0.003, p = 0.004, and p = 0.002, respectively).

The distribution of HbA1c differences of Tosoh G8 and Sebia Cap2FP across quartiles of RBC parameters is presented in Table 4. In the subgroups with available RBC parameters, HbA1c analysis differences were strongly inversely associated with Hb, Hct, and MCHC (p < 0.0001, respectively). By contrast, RDW-CV was positively associated with HbA1c differences of Tosoh G8 and Sebia Cap2FP (p < 0.0001). In other words, when the values of RBC indices, Hb, Hct, and MCHC, are low or RDW-CV is high, the HbA1c measurement in the instruments may be unstable and unreliable. We further find the cutoff value of RBC parameters to compare the association of HbA1c differences with two analyzers used in our study. All the samples were separated into two groups according the cutoff of RBC parameters (Hb = 8 g/dL, Hct = 25.4%, MCHC = 31.6 g/dL, and RDW-CV = 13.7%), HbA1c differences between Tosoh G8 and Sebia Cap2FP were demonstrated to have statistical significance between the groups (Supplemental Data Figure S2).

Based on our data, the comparisons between the results of HbA1c obtained with Tosoh G8 and Sebia Cap 2FP were reanalyzed separately according to the cutoff of Hb or RDW-CV. When the cutoff of Hb is 8 g/dL, the HbA1c values obtained using the G8 system were significantly correlated with Cap2FP. Linear regression analysis of the data from the comparison between the Sebia analyzer and the Tosoh G8 is shown in Figure 2A and 2C with slopes of 0.969 and 0.949 and intercepts of -0.014 and 0.287, without any significant deviation from linearity. The Bland-Altman plots of differences between Tosoh G8 and Sebia Cap2FP analyzers are shown in Figure 2B and 2D. When the cutoff of Hb at ≤ 8 g/dL, the mean difference (± 1.96SD) was 3.55% (-4.86 to 11.96%) (Figure 2B); however, the mean difference (± 1.96SD) was 0.18% (-6.89 to 7.26%) in the group of Hb cutoff at Hb > 8 g/dL (Figure 2D). In addition, the samples were analyzed based on RDW-CV cutoff at 13.7%, the HbA1c values obtained using the G8 system all were significantly correlated with Cap2FP. Linear regression analysis of the data from the

Table 1. Pearson's correlation of RBC parameters with HbA1c differences between Tosoh G8 and Sebia Cap2FP.

RBC parameters	Pearson r	p
RBC (10^6/μL)	-0.204	0.0003
Hb (g/dL)	-0.348	< 0.0001
Hct (%)	-0.333	< 0.0001
MCV (fL)	-0.184	0.001
MCH (pg/cell)	-0.176	0.002
MCHC (g/dL)	-0.100	0.082
MCV/MCH	0.074	0.195
RDW-CV (%)	0.207	0.0003

Table 2. Multivariable Linear Regression analysis of RBC parameters with HbA1c differences between Tosoh G8 and Sebia Cap2FP.

Variables	β	Adjusted β	95% CI	p
RBC (10^6/μL)	0.49	0.10	-4.20, 5.18	ns
Hb (g/dL)	-0.14	-0.08	-4.69, 4.40	ns
Hct (%)	-0.24	-0.38	-1.98, 1.51	ns
MCV (fL)	-1.45	-3.22	-2.29, -0.6	0.001
MCH (pg/cell)	4.24	3.89	1.82, 6.66	0.001
MCHC (g/dL)	-6.14	-2.59	-9.11, -3.17	0.000
MCV/MCH	-25.19	-1.19	-40.34, -10.04	0.001
RDW-CV (%)	-0.003	-0.005	-0.07, 0.06	ns

comparison between the Sebia analyzer and the Tosoh G8 as shown in Figure 2E and 2G with slopes of 0.954 and 0.991 and intercepts of 0.122 and 0.051, without any significant deviation from linearity. The Bland-Altman plots of differences between Tosoh G8 and Sebia Cap2FP analyzers are shown in Figure 2F and 2H. When RDW-CV range ≤ 13.7%, the mean difference (± 1.96SD) was 0.13% (-7.61 to 7.86%) (Figure 2F); however, the mean difference (± 1.96SD) was 2.61% (-5.95 to 11.17%) in the group of RDW-CV range of > 13.7% as shown in Figure 2H.

DISCUSSION

HbA1c level has an important role for the clinical diagnosis and treatment of diabetes [9,10]. RBC indices, used to diagnose the cause of anemia, have been found to affect the detection of HbA1c [3,11]. We hypothesized that RBC indices could underlie analytical disparity of HbA1c by Tosoh G8 and Sebia Cap 2FP analytical methods. Our results are in concurrence with the previous report, the two assay methods, capillary electrophoresis (CE), and HPLC methods, have acceptable concordant results (R^2 = 0.953, p < 0.001) [12-14]. However, significant differences of HbA1c between the two assays were shown to be inversely associated with Hb, Hct, and MCHC (p < 0.0001, respectively). By contrast, RDW-CV was positively associated with HbA1c differences of Tosoh G8 and Sebia Cap2FP methods (p < 0.0001). In other words, when the values of Hb, Hct, and MCHC are low or RDW-CV is high, the HbA1c measurement in these two methods may be unstable and unreliable. Our results showed HbA1c from capillary electrophoresis and HPLC methods were significantly different in the patients with low Hb (≤ 8 g/dL) and high RDW-CV (≥ 13.7%).

A systemic review suggested that HbA1c should be seriously considered in patients who had abnormalities in RBC indices [3]. Koga et al. studied the relationship between RBC indices and HbA1c in premenopausal women [15]. They found that MCV and MCHC were negatively associated with HbA1c. In addition, RBC's life span is a known physiological variable influencing HbA1c. There is evidence to show a linear increase in HbA1c as the mean RBC age increases *in vivo* [16]. Both

Table 3. Baseline demographic and RBC parameters characteristics with quartiles of HbA1c differences between Tosoh G8 and Sebia Cap2FP.

		Q1 (n = 77)	Q2 (n = 78)	Q3 (n = 76)	Q4 (n = 76)	p
Gender	Male (%)	51.95	60.26	48.68	51.32	0.308
	Female (%)	48.05	39.74	51.32	48.68	0.967
Age (years)		58.96 ± 18.74	60.26 ± 16.34	62.75 ± 18.11	61.49 ± 19.04	0.603
RBC (10^6/µL)		3.49 ± 1.07	3.36 ± 1.04	3.08 ± 0.98	2.86 ± 0.81	0.0004
Hb (g/dL)		10.10 ± 3.08	9.50 ± 2.61	8.60 ± 2.26	7.47 ± 1.28	< 0.0001
Hct (%)		31.03 ± 8.70	29.05 ± 7.88	26.43 ± 7.02	23.79 ± 4.72	< 0.0001
MCV (fL)		90.29 ± 9.94	88.08 ± 10.70	87.41 ± 9.26	85.83 ± 13.25	0.086
MCH (pg/cell)		29.33 ± 4.10	28.86 ± 4.01	28.60 ± 3.95	27.30 ± 5.40	0.032
MCHC (g/dL)		32.41 ± 2.11	32.71 ± 1.48	32.62 ± 1.75	31.62 ± 2.51	0.003
MCV/MCH		3.10 ± 0.26	3.06 ± 0.15	3.08 ± 0.18	3.19 ± 0.28	0.004
RDW-CV (%)		16.91 ± 14.33	15.92 ± 3.99	16.01 ± 2.77	17.38 ± 3.35	0.002

Table 4. Distribution of HbA1c differences between Tosoh G8 and Sebia Cap2FP analysis in relation to quartiles (Q1-Q4) of RBC parameters.

RBC parameters	Q1	Q2	Q3	Q4	p
RBC	2.88 ± 4.44 *	2.52 ± 4.34	1.87 ± 4.59	0.69 ± 5.33	0.023
Hb	3.55 ± 5.29	3.28 ± 4.13	1.52 ± 4.70	-0.71 ± 3.48	< 0.0001
Hct	3.61 ± 4.55	2.90 ± 4.17	2.08 ± 4.91	-0.70 ± 4.21	< 0.0001
MCV	3.21 ± 5.38	1.76 ± 4.88	1.65 ± 4.16	1.35 ± 4.29	0.069
MCH	3.55 ± 5.51	1.27 ± 4.72	1.22 ± 3.95	1.96 ± 4.35	0.007
MCHC	3.41 ± 5.78	1.53 ± 4.42	0.68 ± 3.99	2.33 ± 4.13	< 0.0001
MCV/MCH	2.12 ± 4.15	0.40 ± 4.01	2.26 ± 4.65	3.38 ± 5.76	0.002
RDW-CV	0.13 ± 3.94	2.15 ± 4.39	2.24 ± 4.50	3.68 ± 5.48	< 0.0001

*** Data were shown in percentage (mean ± SD).**

MCH and MCV decrease linearly during the lifespan of RBC. Previous reports suggested a positive correlation between HbA1c and RDW-CV because diabetic subjects have anisocytosis caused by inefficient erythropoiesis or destruction [17,18].

An efficient and reliable method is required for the measurement of glycated Hb in the management of diabetes mellitus. In previous reports, there was good concordance between the results of capillary electrophoresis and the HPLC method [12-14]. In our study, Sebia Cap2FP and Tosoh G8 HPLC methods revealed acceptable precision and good accuracy, but a significant difference was obtained between the mean levels of both methods, especially in patients with anemia (Hb < 8 g/dL). The Bland-Altman plot showed a mean absolute difference of 2.0% HbA1c. This means that the Tosoh G8 method measures on average 2% higher than the Sebia method. This bias is significant, it seems to be related to lower concentrations of HbA1c (< 6.5%). But regression analysis of the Tosoh G8-Sebia methods showed minor differences as it did not encompass zero. However, the difference was very small with no relative systematic difference which is not clinically relevant. The studies of Wu et al. have also shown that the HbA1c results obtained with the Tosoh G8 and Sebia methods are in good agreement using the Bland-Altman plots [13]. In the Klingenberg et al. study, the HPLC method also showed slightly higher results [12]. The aim of this study is to compare HbA1c analysis by the Sebia capillary electrophoresis and Tosoh G8 HPLC to evaluate the correlation in patients with different RBC indices. Based on our data, HbA1c alone might not be suitable for the diagnosis of diabetes in subjects with severe anemia. As shown in the previous reports, the patients with low Hb would have significantly lower HbA1c values than those with normal Hb [9,12,13]. Our

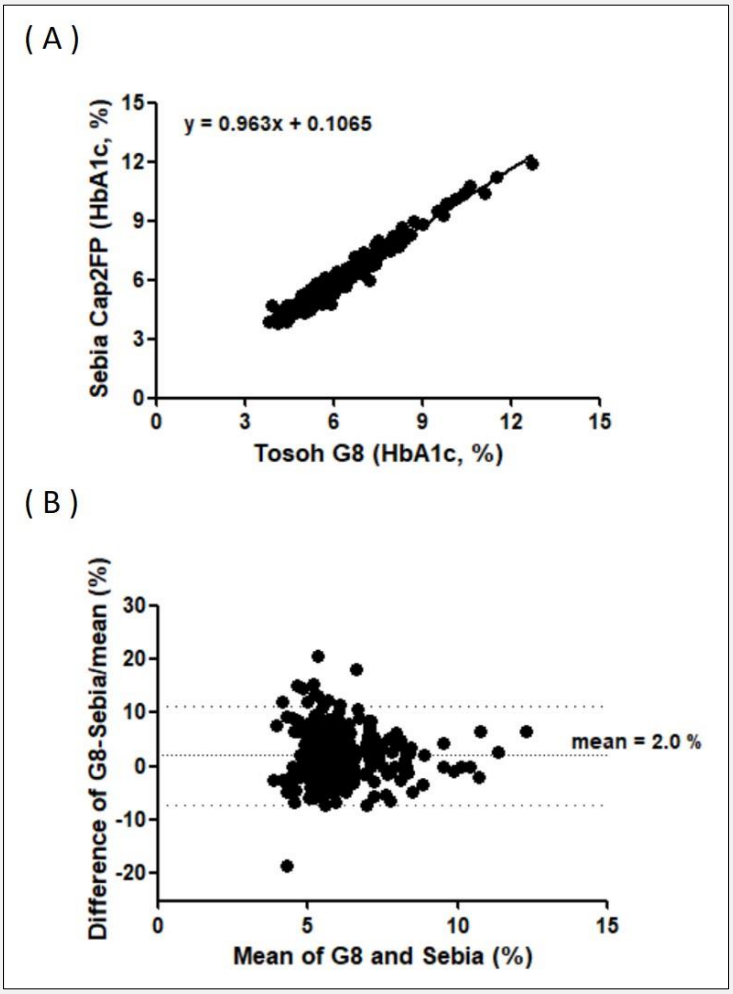

Figure 1. Comparison between the results of HbA1c obtained with Tosoh G8 and Sebia Cap 2FP. (A) Correlation plot with linear regression line (r = 0.976, p = 0.0001). (B) Bland-Altman difference plot comparing HbA1c results between two analyzers.

results demonstrated that abnormalities of RBC indices are a considerable confounder in the analysis of HbA1c. Especially, Hb ≤ 8 g/dL or RDW-CV ≥ 13.6% values would show significant differences for HbA1c detection by different methods. The key questions that are still to be answered are whether anemia and erythrocyte abnormalities will have a significant impact on HbA1c analysis by different analytical instruments in the general population.

The value of HbA1c is affected by three main factors: 1) the Hb content of reticulocytes when they are released from the bone marrow, 2) the mean age of RBCs in the circulation, and 3) the Hb glycation rate [19,20]. Hyperglycemia has different effects on the RBC indices. The effects may be extended to include glycation of Hb, reduced RBC deformability, and lifespan [1,17]. The exact degree of significant changes in RBC indices under the diagnostic value of HbA1c of 6.5% is not known yet nor the degree of severity of anemia. RDW measures the heterogeneity of the volume of RBCs (variation in cells size) that may help to differentiate between some types of anemia [21]. In our study, the anemia participants recruited showed a significant positive correlation between HbA1c differences of Tosoh G8 and Sebia Cap 2FP and RDW-CV.

There are many pathological conditions other than hyperglycemia that can interfere with HbA1c values. A low level of HbA1c may be seen in conditions that shorten the lifespan of RBCs, irrespective of the method of the assay used. Anemia can increase the turnover of RBCs and lower HbA1c values due to blood loss, hemolysis, hemoglobinopathies, and red blood cell disor-

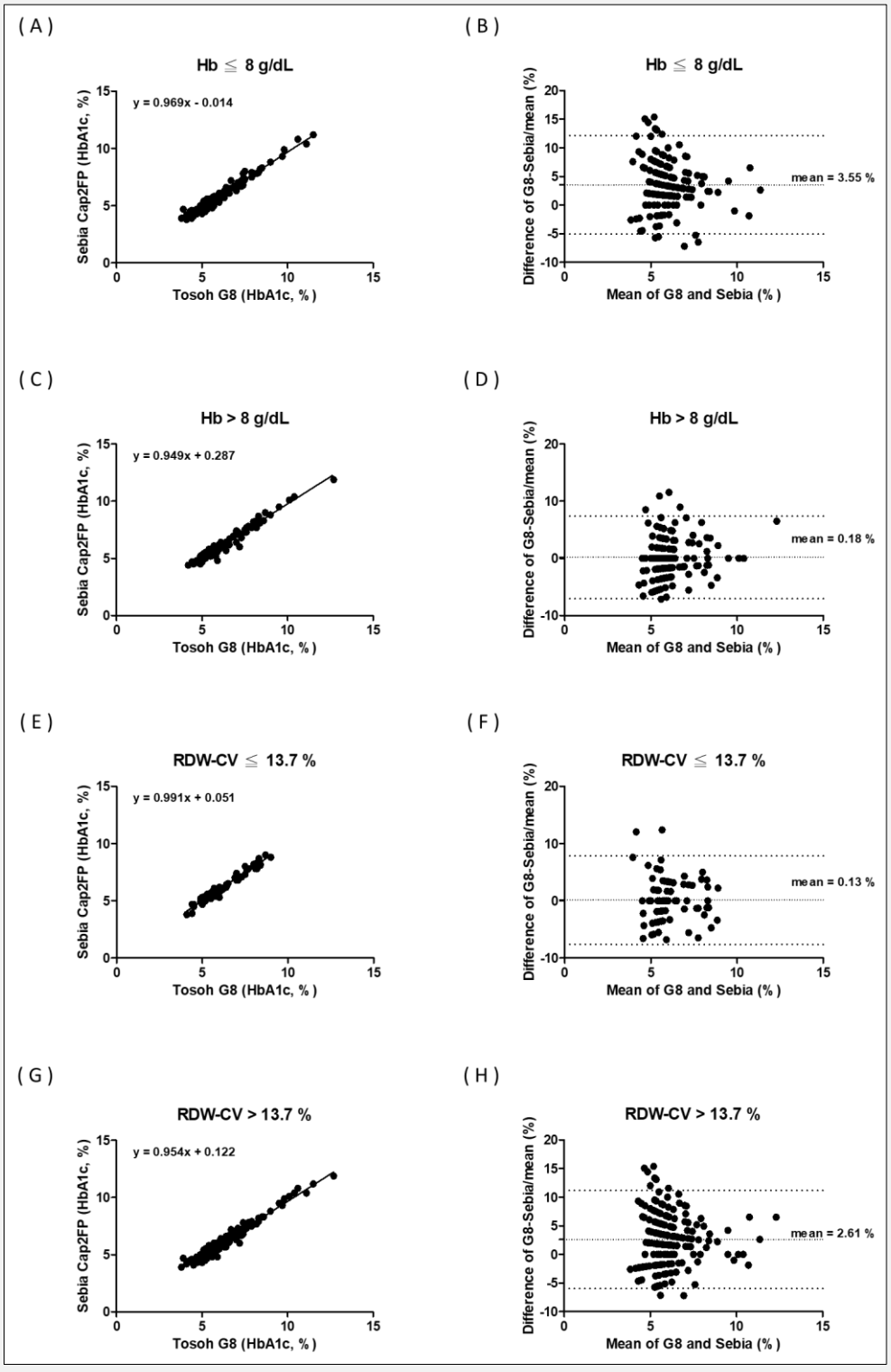

Figure 2. Comparison between the results of HbA1c obtained with Tosoh G8 and Sebia Cap 2FP in (A) (B) Hb ≤ 8 g/dL (n = 161), (C) (D) Hb > 8 g/dL (n = 146), (E) (F) RDW-CV ≤ 13.7% (n = 81) and (G) (H) RDW-CV > 13.7% (n = 226) subgroups. (A) (C) (E) (G) Correlation plot with linear regression line (r = 0.957, p < 0.0001; r = 0.957, p < 0.0001; r = 0.962, p < 0.0001; r = 0.953, p < 0.0001; respectively). (B) (D) (F) (H) Bland-Altman difference plot comparing HbA1c results between two analyzers.

The dashed lines show the mean and the mean difference ± 1.96SD.

ders [22]. In contrast, chronic anemia caused by iron deficiency and nutritional defect would increase erythrocyte survival time and decreased RBC volume and hemoglobin level [23,24]. We demonstrated that the detection difference between the two methods may be related to RBC indices. Except for RDW-CV, when other RBC indices are below the cut-off value, the HbA1c differences between the two methods were increased. In other words, low levels of Hb, MCV, Hct, MCH, and MCHC and high RDW-CV may also make the HbA1c measurement more unstable. When Hb ≤ 8 dL or RDW-CV ≥ 13.7%, the difference of HbA1c value between Tosoh G8 and Sebia Cap 2FPs methods were significantly different. Therefore, we suggest that Hb ≤ 8 dL or RDW-CV ≥ 13.7%, HbA1c should be analyzed by two kinds of principle machines. It can prevent false bias. Therefore, to check the reliability and comparability among commonly used analyzers have become important.

In conclusion, our results demonstrated HbA1c from capillary electrophoresis and HPLC methods were significantly different in the patients with low Hb cut-off (≤ 8 g/dL) and high RDW-CV (≥ 13.7%). It is thus suggested that in the interpretation of HbA1c in patients with anemia, simultaneous testing for RBC parameters is needed, and development of a new reference value of HBA1c for patients with severe anemia should be considered.

Acknowledgment:
The authors thank Ms. I-Chun Tsai for providing technical assistance.

Ethical Approval:
This study was approved by the Ethics Committee of E-DA Hospital (EMRP-106-086).

Source of Funds:
This work was supported by funding from E-DA Hospital [EDAHC-107001].

Declaration of Interest:
No potential conflicts of interest relevant to this study are reported.

References:

1. Cohen RM, Franco RS, Khera PK, et al. Red cell life span heterogeneity in hematologically normal people is sufficient to alter HbA1c. Blood 2008;112:4284-91. (PMID: 18694998)

2. Cavagnolli G, Pimentel AL, Freitas PA, Gross JL, Camargo JL. Factors affecting A1C in non-diabetic individuals: Review and meta-analysis. Clin Chim Acta 2015;445:107-14. (PMID: 25818244)

3. English E, Idris I, Smith G, Dhatariya K, Kilpatrick ES, John WG. The effect of anaemia and abnormalities of erythrocyte indices on HbA1c analysis: a systematic review. Diabetologia 2015;58:1409-21. (PMID: 25994072)

4. Chapelle JP, Teixeira J, Maisin D, et al. Multicentre evaluation of the Tosoh HbA1c G8 analyser. Clin Chem Lab Med 2010;48: 365-71. (PMID: 20020820)

5. Jaisson S, Leroy N, Meurice J, Guillard E, Gillery P. First evaluation of Capillarys 2 Flex Piercing(R) (Sebia) as a new analyzer for HbA1c assay by capillary electrophoresis. Clin Chem Lab Med 2012;50:1769-75. (PMID: 23089707)

6. Jalali MT, Bavarsad SS, Hesam S, Afsharmanesh MR, Mohammadtaghvaei N. Assessing agreement between the three common clinical measurement methods of HbA1c. J Diabetes Metab Disord 2020;19:273-9. (PMID: 32550176)

7. Niclas R, Gunnar N, Aleksandra MH, et al. Good Agreement Between Hba1c Analyzed Using Capillary Electrophoresis, HPLC, Immunological and Enzymatic Methods. Journal of Diabetes, Metabolism and its Complications 2019;1:2-7. http://dx.doi.org/10.31487/j.JDMC.2019.01.03

8. Zemlin AE, Matsha TE, Hassan MS, Erasmus RT. HbA1c of 6.5% to diagnose diabetes mellitus -- does it work for us? -- the Bellville South Africa study. PLoS One 2011;6:e22558. (PMID: 21857932)

9. Christy AL, Manjrekar PA, Babu RP, Hegde A, Rukmini MS. Influence of iron deficiency anemia on hemoglobin A1c levels in diabetic individuals with controlled plasma glucose levels. Iran Biomed J 2014;18:88-93. (PMID: 24518549)

10. Sumathi K, Dilliraj G, Shanthi B, Kalai Selvi VS, Jamuna Rani A. Correlation between iron deficiency anemia and HbA1C levels in type 2 diabetes mellitus. Int J Clin Biochem Res 2020;7:400-2. http://dx.doi.org/10.18231/j.ijcbr.2020.085

11. Hamdan MA, Hempe JM, Velasco-Gonzalez C, Gomez R, Vargas A, Chalew S. Differences in Red Blood Cell Indices Do Not Explain Racial Disparity in Hemoglobin A1c in Children with Type 1 Diabetes. J Pediatr 2016;176:197-9. (PMID: 27156184)

12. Klingenberg O, Furuset T, Hestbraten CR, et al. HbA1c analysis by capillary electrophoresis - comparison with chromatography and an immunological method. Scand J Clin Lab Invest 2017;77: 458-64. (PMID: 28644050)

13. Wu X, Chao Y, Wan Z, et al. A comparative evaluation of the analytical performances of Capillarys 2 Flex Piercing, Tosoh HLC-723 G8, Premier Hb9210, and Roche Cobas c501 Tinaquant Gen 2 analyzers for HbA1c determination. Biochem Med (Zagreb) 2016;26:353-64. (PMID: 27812304)

14. Genc S, Gurdol F, Kanmaz-Ozer M, Ince N, Ozcelik F, Omer B. The Analytical Performances of Four Different Glycated Hemoglobin Methods. Med Chem 2014;4:501-5. http://dx.doi.org/10.4172/2161-0444.1000185

15. Koga M, Saito H, Mukai M, Matsumoto S, Kasayama S. Influence of iron metabolism indices on glycated haemoglobin but not glycated albumin levels in premenopausal women. Acta Diabetol 2010;47 Suppl 1:65-9. (PMID: 19404566)

16. Beltran Del Rio M, Tiwari M, Amodu LI, Cagliani J, Rodriguez Rilo HL. Glycated Hemoglobin, Plasma Glucose, and Erythrocyte Aging. J Diabetes Sci Technol 2016;10:1303-7. (PMID: 27422013)

17. Xiong XF, Yang Y, Chen X, et al. Red cell distribution width as a significant indicator of medication and prognosis in type 2 diabetic patients. Sci Rep 2017;7:2709. (PMID: 28578411)

18. Wang J, Zhang Y, Wan Y, Fan Z, Xu R. The Relationship between Red Blood Cell Distribution Width and Incident Diabetes in Chinese Adults: A Cohort Study. J Diabetes Res 2020;2020: 1623247. (PMID: 32185232)

19. Abass AE, Musa IR, Rayis DA, Adam I, Gasim IG. Glycated hemoglobin and red blood cell indices in non-diabetic pregnant women. Clin Pract 2017;7:999. (PMID: 29071062)

20. Gupta S, Jain U, Chauhan N. Laboratory Diagnosis of HbA1c: A Review. J Nanomed Res 2017;5:00120. http://medcraveonline.com/JNMR/JNMR-05-00120.pdf

21. Salvagno GL, Sanchis-Gomar F, Picanza A, Lippi G. Red blood cell distribution width: A simple parameter with multiple clinical applications. Crit Rev Clin Lab Sci 2015;52:86-105. (PMID: 25535770)

22. Radin MS. Pitfalls in hemoglobin A1c measurement: when results may be misleading. J Gen Intern Med 2014;29:388-94. (PMID: 24002631)

23. Chaparro CM, Suchdev PS. Anemia epidemiology, pathophysiology, and etiology in low- and middle-income countries. Ann N Y Acad Sci 2019;1450:15-31. (PMID: 31008520)

24. Le CH. The Prevalence of Anemia and Moderate-Severe Anemia in the US Population (NHANES 2003-2012). PLoS One 2016; 11:e0166635. (PMID: 27846276)

Additional material can be found online at:
http://supplementary.clin-lab-publications.com/211053/