African Journal of Advanced Pure and Applied Sciences (AJAPAS)
Online ISSN: 2957-644X
Volume 4, Issue 4, 2025
Page No: 823-829
Website: *https://aaasjournals.com/index.php/ajapas/index*
ISI 2025: 1.126 **SJIFactor 2024: 6.752** **معامل التأثير العربي: 1.62**

Comparison of Hemoglobin A1c Results measured by three different Assay Methods in relation with Age and Hemoglobin Impacts

Najat M. H. Mohammed [1 *], Eman Ahamed Almaky [2]
[1] Medical Laboratory Department, Faculty of Health Sciences, Omer AL-Mukhtar University, EL-Bieda, Libya
[2] General Orientation Department, Faculty of Health sciences, Omer AL-Mukhtar University, EL-Bieda, Libya

مقارنة نتائج الهيموغلوبين السكري (*HbA1c*) المقاسة بثلاث طرائق فحص مختلفة وعلاقتها بالعمر وتأثيرات الهيموغلوبين

نجاة محمد حمد محمد [1*]، ايمان محمد المكي [2]
[1] قسم المختبرات الطبية، كلية العلوم الصحية، جامعة عمر المختار، البيضاء، ليبيا
[2] القسم العام، كلية العلوم الصحية، جامعة عمر المختار، البيضاء، ليبيا

[*]Corresponding author: najat.mohammed@omu.edu.ly

Received: October 16, 2025	Accepted: December 20, 2025	Published: December 25, 2025

Abstract
Hemoglobin A1c is a reliable index of glycemic control reflecting the mean blood glucose levels over the past three months and provides a sensitive predictor of developing risk of diabetes related complications. However, the accuracy of HbA1c measuring methods can be adversely affected by various assay techniques. This study is aimed to compare HbA1c results by three various assay methods in the group of medical laboratories in EL-Bieda City. EDTA blood collected from 36 diabetic patients were used to measure HbA1c by using three different assays including immunoassay technique, Boronate affinity and high-performance liquid chromatography at three medical centers; in EL-Bieda medical center, Ajabal-ALkdar diabetic and AL-Burj medical centers respectively. Hematology analyzer was used for complete blood picture (Hb, WBC and PLTs). Plasma was separated from so¬dium fluoride tubes for measuring fasting blood glucose levels (FBG). All data gathered were analyzed by R software version 3 using Correlation test, T-test and expressed as mean, median, minimum and maximum with p value is significant if less than 0.05. Results showed that there was no significant difference in glycated hemoglobin (HBA1c) measured by three protocols as p value is larger than 0.05 (p value =0.41), as well as Persons coefficient of correlation revealed that HbA1c and Hemoglobin (Hb) are not significantly correlated since the p-value is larger than the significance level alpha. (R= 0.083, p=0.41). A significant correlation was found between HbA1c and age (R= 0.2 and p-value =0.05). Additionally, the results demonstrated positive correlation between fasting blood glucose levels (FBG) and HbA1c with a p-value of 1.8e-09 (R=0.55), which is less than the significance level alpha. It can be concluded that with using various operators principles for estimating HbA1c, there was no obvious variation between the results obtained indicating the acceptable accuracy of analytical performance.

Keywords: Hemoglobin A1C, Diabetes mellitus, Boronate affinity, High-performance liquid chromatography.

الملخص
يُعدّ الهيموغلوبين السكري (HbA1c) مؤشرًا موثوقًا للتحكّم السكري، إذ يعكس متوسط مستويات الغلوكوز في الدم خلال الأشهر الثلاثة الماضية، ويوفّر أداة حساسة للتنبؤ بخطر حدوث مضاعفات مرتبطة بمرض السكري. ومع ذلك، قد تتأثر دقة طرق قياس HbA1c باختلاف تقنيات الفحص المستخدمة.

تهدف هذه الدراسة إلى مقارنة نتائج HbA1c باستخدام ثلاث طرائق تحليل مختلفة في مجموعة من المختبرات الطبية بمدينة البيضاء. تم جمع دم بمواد مانعة للتجلط (EDTA) من 36 مريضًا سكريًا لقياس HbA1c بثلاث تقنيات هي: التحليل المناعي، وتقنية الارتباط بالبورونات(Boronate affinity) ، والكروماتوغرافيا السائلة عالية الأداء(HPLC) ، وذلك في ثلاثة مراكز طبية: المركز الطبي البيضاء، مركز الجبل الأخضر للسكري، ومركز البرج الطبي. استُخدم محلل أمراض الدم لإجراء صورة الدم الكاملةHb) ، WBC، PLTs).كما فُصلت البلازما من أنابيب فلوريد الصوديوم لقياس سكر الدم الصائم.(FBG)
تم تحليل جميع البيانات باستخدام برنامج R الإصدار 3 عبر اختبار الارتباط واختبارT ، وعُرضت النتائج على شكل متوسطات ووسيط وقيم صغرى وكبرى، مع اعتبار قيمة الاحتمال (p) دالة إحصائيًا إذا كانت أقل من 0.05. أظهرت النتائج عدم وجود فروق ذات دلالة إحصائية في HbA1c بين الطرائق الثلاث .(p=0.41) كما بيّن معامل ارتباط بيرسون عدم وجود ارتباط دال بين HbA1c والهيموغلوبين الكلي(R=0.083) (Hb) ، .(p=0.41)وُجد ارتباط دال بين HbA1c والعمر(R=0.2) ، .(p=0.05)إضافةً إلى ذلك، أظهرت النتائج ارتباطًا إيجابيًا بين سكر الدم الصائم (FBG) و HbA1c (R=0.55، .(p=1.8e-09
تُستنتَج من ذلك عدم وجود تباين واضح بين نتائج HbA1c رغم اختلاف مبادئ القياس، بما يشير إلى دقة مقبولة في الأداء التحليلي للطرائق المختبرة.

الكلمات المفتاحية: الهيموغلوبين السكري HbA1c، داء السكري، ارتباط البورونات، الكروماتوغرافيا السائلة عالية الأداء (HPLC)

Introduction

Hemoglobin A1c is defined as hemoglobin that is irreversibly glycated at one or both N-terminal valines of the beta chains, enabling it to be used as an index of glycemic control and assist for appropriate treatment of diabetic patients. Glycated hemoglobin reflects glycemic history over the past 3 months [1,2,3], since erythrocytes have an average lifespan of 120 days [4]. According to the American Diabetes Association (ADA) guidelines, the level of HbA1c should be ≥ 6.5% as diagnostic threshold in diabetics patients [4,5]. Elevated glycated hemoglobin in diabetics patients is inversely related to microvasculature and body organs health [6]. Thus, it is really essential to obtain an accurate and reliable analytical procedure of HbA1C essay. However, its measurements can be influenced by various essay principles or by presence of other pathological disorders, and even by age and ethnicity [3,4,7]. Normal adult human hemoglobin is tetrameric protein consisting of alpha chain and beta chain in duplicate (HbA α2β2) [8,9,10]. HbA is usually have ability to bind to the blood glucose at a special sequence of amino acids producing glycated forms (HbA1c) [11,12]. According to ‘‘International Federation of Clinical Chemistry working group (IFCC) ’’, Any point of mutation of globin chain of hemoglobin molecule will result in production of hemoglobin variants or unstable forms of hemoglobin such as HbE, HbD, HbS and HbSC which surely can impact on HbA1c accuracy measures [13,14]. It has been identified around 1000 of hemoglobin variants with majority of them experiencing no clinical symptoms. 1% deviation in HbA1c indicates 1.4 – 1.9 mmol/L elevation in the blood glucose level. Thus, presence of a clinically silent hemoglobin will probably cause falsely high or low HbA1c level which in turn lead to inappropriate managements to diabetic peoples. Recently, there different assays have developed to measure HbA1c ranging from immunoassay to boronate affinity and more accurately High-performance Liquid Chromatography (HPLC) [13,15]. HPLC technique is based on ion exchange method which separate the protein fractions throughout a column depends on the differences in the polarity. The HbA1c measurement is based on the ratio of the HbA1c peak area to the total hemoglobin peak areas [16]. With this technique any genetic hemoglobin variants can be easily detected which in turn identify possible effects could occurred on HbA1c estimation [10,13]. Boronate Affinity Chromatography depends on using a column coated with boronic acid particles which are attached to cis-diol group of glycated hemoglobin [1,16,17]. This method determines total glycated Hb, either HbA1c or other Glycated forms at any site rather than N terminal Valine. While, in immunoassays method, requires hemolyzing samples firstly to separate glycated from non-glycated and then adding monoclonal antibodies to recognize and attach to the N-terminal glycosylated valine structure on the β-chain. Immune-complexes is measured by turbidimetric assay [13,16,18]. Some previous studies had reported that a single sample result was varied from 4.0-8.1% by several different assay procedures, [19] which absolutely will have a serious impact on the clinical patient status that is why the aim of the current study was to compare the HbA1c results by three various assay methods in a group of medical laboratories in EL-Bieda City and determine if there are any significant differences between the results obtained.

Material and methods

The present study comprised of 36 Libyan diabetic patients with different age groups (10-80 years) and both genders are involved, subjects were fasted for 10–12 hours before standard phlebotomy and blood samples were collected in potassium EDTA tubes to measure hemoglobin (Hb), platelets (PLTs), white blood cells (WBCs) and

HbA1c levels, and we used also sodium fluoride tubes for measuring fasting blood glucose (FBS). HBA1c assay was measured by immunoassay technique using (Eruba Mannheim XL200 analyzer) and Boronate affinity using (HemCue HbA1c 501system) and high performance liquid chromatography on HLC 723 analyzer at different medical centers; in EL-Bieda medical center, diabetes aljabal AL.akhdar center and AL-Burj medical centers (respectively). All samples were measured in comparison and on the same day of collection and by the same laboratory staffs to avoid inter-essay variations. Hematological parameters (Hb, WBC and PLTs) were analyzed by Erba H560 hematology analyzer. The samples were taken in the period of two months in 2023. All data of the study population were analyzed by R software version 3 2.3 (eye holes) using Correlation test, T-test and expressed as mean, median, minimum and maximum with p value is significant if less than 0.05.

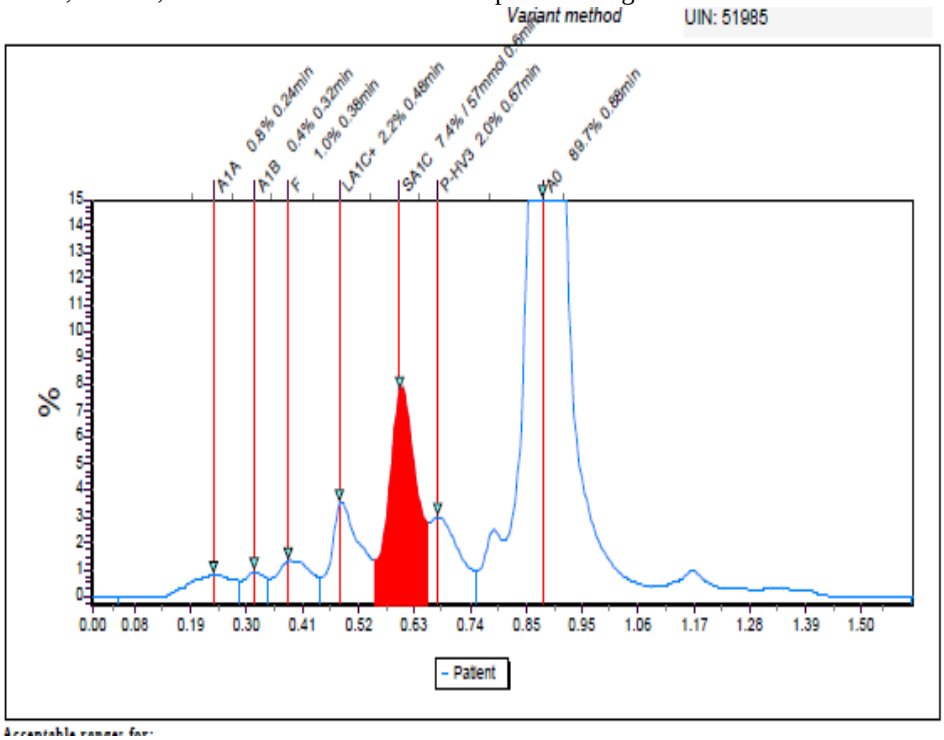

Figure 1. Normal Chromatograph of Hemoglobin by HLC 723 analyzer showing HbA1c peak elution(red).

Results and Discussion

Analysis of our data showed that from total of 36 samples involved in this study, there were 17 (47%) females and 19 (53%) males, age group was found between 10-80 years with mean age 52 years, mean fasting of blood glucose levels in patients was found to be 170mg/dl, and other hematological parameters were also analyzed and presented in terms of minimum, maximum and mean as presented in Table 1.

Table 1: Represents descriptive statistical data of diabetic peoples in terms of minimum, median, mean and maximum.

	Ag (years)	**Hb (g/dl)**	**WBC (x103µ/L)**	**PLT (x10^9/L)**	**FBG (mg/dl)**
Minimum	10	6.90	2.10	147	77
Median	54	13.3	6.44	250	147
Mean	52	12.44	7.90	240	170
Maximum	80	20.30	18.5	360	415

The aim of the current study was to demonstrate if there is any analytical variation in the HbA1C results, there were no pronounce changes between three different analytical methods as p value is larger than 0.05 (p =0.41) as shown in **(Fig. 2)**. Similar to a study conducted in Tripoli showed that results obtained from two different devices were equivalents [18,20]. This indicating a reliable analytical performance and ensure that there is no variation in the analysis could possibly affect on patient's glucose monitoring.

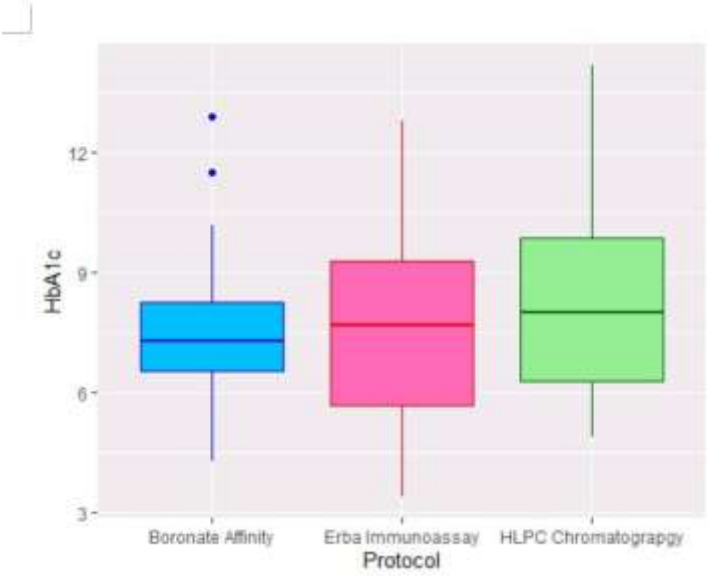

Figure 2. Illustrates the difference in HbA1c results by different assay methods , represented by boxplot.

Persons coefficient of correlation revealed that HbA1c and hemoglobin values are not significantly correlated since the p-value is larger than the significance level alpha = 0.05. (R= 0.083, p=0.41) (**Fig.3**), which is consistent with the study carried by Alap Christy in 2013 who confirmed no significant relation between HBA1c and hemoglobin amount. According to Tunisian study, discovered the presence of mutated hemoglobin in some diabetic's people which had been influenced on the reliability of HbA1c assay as the best biological marker of glycemic state [18,20,21,22]. The existence of hemoglobin variants could possibly limit HbA1c validation and interfere with assay accuracy. While, using high performance chromatography can assist in detecting any mutation in hemoglobin molecules which is certified by National glycohemoglobin standardization program (NSGP) [23]. HbA1c is a sensitive predictor for progressing of diabetes related complications and thus maintaining HbA1c will certainly reduce the risk of cardiovascular diseases and subsequent lower the incidence rate of retinopathy and neuropathy therefore, the accurate and precise assay methods are essential issue [19,24]. Furthermore, reduced of body iron stores have been linked to elevate glycated hemoglobin [26,27]. Thus, diabetic patients should be regularly check for Iron deficiency anemia (IDA) and physician pay attention for falsely elevation of glycated hemoglobin due to iron stores depletion [28].

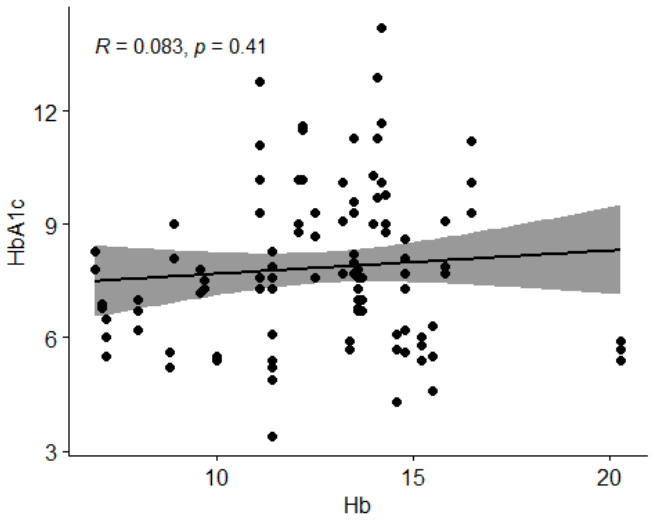

Figure 3. Shows correlation between HA1c and levels of hemoglobin of participants

Regarding to the relation of HbA1c with age of patients, there was a significant correlation between the age of diabetic patients and the raised of HbA1c level as the p-value was 0.05 which is equal to the significance level alpha (R= 0.2 and p-value =0.05) (**Fig. 4**). Thus older patients are more likely to have elevated glycohemoglobin than others, this findings in accordance with the previous works who said that glycated hemoglobin increased nearly by 1mmol/mol per decades [3,7,23,28].

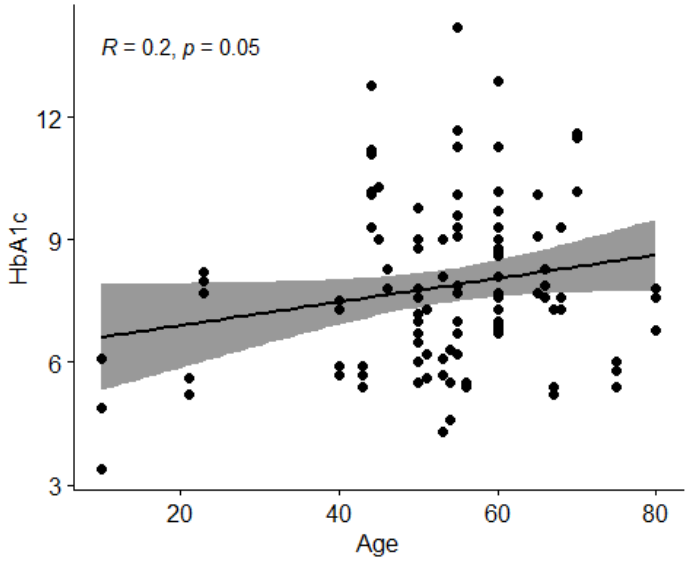

Figure 4. Relationship between HbA1C and the age which showed a positive correlation (R=0.2,p=0.05).

The results demonstrated that there was positive correlation between levels of fasting blood glucose (FBG) and HbA1C with a p-value of 1.8e-09 which is less than the significance level alpha = 0.05 and correlation coefficient of (R=0.55). **(Fig.5)**, this explained that fluctuation of blood glucose levels during a month will be absolutely observed through HbA1c result reflecting past three months period, Shariq et al had proven that there was direct proportion between blood glucose levels and HbA1c thus more glucose in the blood means increased chance to attached to hemoglobin molecule and subsequent increased of HbA1c levels [4,29,30].

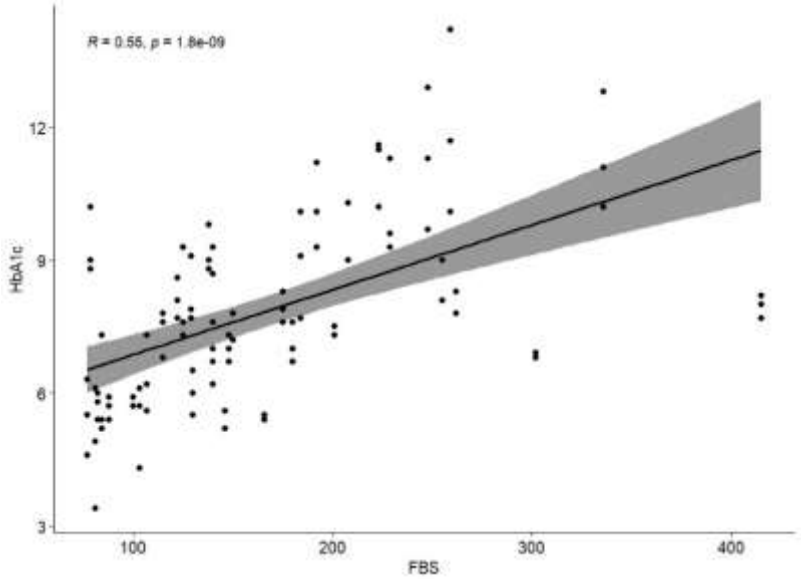

Figure 5. Illustrates the correlation between HbA1c and levels of fasting blood glucose of diabetic's patients

Conclusion

It can be concluded that comparison of HbA1c results by different assay methods demonstrated that there was no variation between the results obtained indicating the accuracy of analytical performance. However, in the recent era there are different factors attributed to the laboratory analysis could have great influence on patient's wellbeing involving pre-analytical and post analytical errors.

Compliance with ethical standards

Disclosure of conflict of interest

The authors declare that they have no conflict of interest.

References

[1] Braga F, Dolci A, Mosca A, & Panteghini M. (2010). Biological variability of glycated hemoglobin. Clinica chimica acta; international journal of clinical chemistry, 411(21-22), 1606–1610.

[2] Cembrowski G, Mei J, Guérin, R, Cervinski AM., McCudden C. (2020). Derivation of real metrics of long term patient and analytical variation of three hemoglobin A1c assays demonstrates both borderline and highly acceptable analytical performance.

[3] Ma, Q, Liu, H, Xiang, G, Shan, W, & Xing, W. (2016). Association between glycated hemoglobin A1c levels with age and gender in Chinese adults with no prior diagnosis of diabetes mellitus. *Biomedical reports*, *4*(6), 737-740.

[4] Sherwani SI, Khan HA., Ekhzaimy A, Masood A, & Sakharkar MK. (2016). Significance of HbA1c Test in Diagnosis and Prognosis of Diabetic Patients. Biomarker insights, 11, 95–104

[5] Coskun C, Inal B, Emre H Baz S, GumusA, Sonmez, D, Orhan B, Duz M, Serin E & Koldas M. (2018). Evaluation of biological variations in glucose and glycated hemoglobin levels in healthy individuals. Turkish Journal of Biochemistry, 43(5), 495-50.

[6] Ćorić, J., Kučukalić, E., Hasanefendic, B., Bodulović, A., Mujic, J., & Panjeta, M. (2015). Comparison of Hemoglobin A1c assay performance on two different commercial systems. *Journal of Health Sciences*, *5*(1), 11-14.

[7] Ravikumar, P, Bhansali, A, Walia, R, Shanmugasundar, G, & Ravikiran, M. (2011). Alterations in HbA1c with advancing age in subjects with normal glucose tolerance: Chandigarh Urban Diabetes Study (CUDS). *Diabetic Medicine*, *28*(5), 590-594

[8] Katwal PC, Jirjees S, Htun Z M, Aldawudi I, & Khan S. (2020). The Effect of Anemia and the Goal of Optimal HbA1c Control in Diabetes and Non-Diabetes. Cureus, 12(6), e8431.

[9] Ockerman A. (2022). Clinical and analytical interferences with HbA1c assays. Uz,Leuven.pp.1-17

[10] Clarke GM, & Higgins TN. (2000). Laboratory investigation of hemoglobinopathies and thalassemias: review and update. Clinical chemistry, 46(8 Pt 2), 1284–1290

[11] Chen J, Diesburg-Stanwood A, Bodor G, & Rasouli N. (2016). Led Astray by Hemoglobin A1c: A Case of Misdiagnosis of Diabetes by Falsely Elevated Hemoglobin A1c. Journal of investigative medicine high impact case reports, 4(1), 2324709616628549.

[12] Nasir NM, Thevarajah M, & Yean CY. (2010). Hemoglobin variants detected by hemoglobin A1c (HbA1c) analysis and the effects on HbA1c measurements. International journal of diabetes in developing countries, 30(2), 86–90.

[13] Little RR, & Roberts W L. (2009). A review of variant hemoglobins interfering with hemoglobin A1c measurement. Journal of diabetes science and technology, 3(3), 446–451.

[14] Klonoff DC. (2020). Hemoglobinopathies and Hemoglobin A1c in Diabetes Mellitus. Journal of diabetes science and technology, 14(1), 3–7.

[15] Lenters-Westra E, & Slingerland R.J. (2010). Six of eight hemoglobin A1c point-of-care instruments do not meet the general accepted analytical performance criteria. Clinical chemistry, 56(1), 44–52.

[16] Lynn Bry, Philip C Chen, David B Sacks. Effects of Hemoglobin Variants and Chemically Modified Derivatives on Assays for Glycohemoglobin, Clinical Chemistry, Volume 47, Issue 2, 1 February 2001, Pages 153–163

[17] Yadav N, & Kumar Mandal A. (2023). Interference of hemoglobin variants in HbA1c quantification. Clinica chimica acta; international journal of clinical chemistry, 539, 55–65

[18] Bouzid K, Bahlous A, Ferjani W, Kalai E, Ducrocq R., Ben Mami F, & Abdelmoula J. (2012). Advantage of HbA1c assay by HPLC D-10 versus cobas integra 400 in a population carrier for HbS and HbC. Clinical laboratory, 58(7-8), 821–828

[19] Al-Awkally NA, Ghriba I M, Eljamay SM, Alabeedi R.M, Ali M.A, Awad SM, Mousaay WO, Al-Awkally NM, & Ghareeb K.S. (2022). The Role of HbA1c as a Screening and Diagnostic Test for Diabetes Mellitus in Benghazi City. African Journal of Advanced Pure and Applied Sciences (AJAPAS), 1(1), 5–11.

[20] Mongia SK, Little, RR, Rohlfing CL, Hanson S, Roberts R F, Owen WE, D'Costa, MA., Reyes, CA., Luzzi, VI, & Roberts WL. (2008). Effects of hemoglobin C and S traits on the results of 14 commercial glycated hemoglobin assays. American journal of clinical pathology, 130(1), 136–140

[21] Klonoff DC. (2020). Hemoglobinopathies and Hemoglobin A1c in Diabetes Mellitus. Journal of diabetes science and technology, 14(1), 3–7

[22] Ahlam Ali Basheer. (2023). Effect of Iron Deficiency Anemia on HbA1c Levels in Non-Diabetic Females in Brack Alshatti. AlQalam Journal of Medical and Applied Sciences, 476–481. Retrieved from

[23] Ockerman A. (2022). Clinical and analytical interferences with HbA1c assays. Uz,Leuven.pp.1-17

[24] Pratumvinit B, Reesukumal K, Hanyongyuth, SWangchaijaroenkit S, Pooliam J, Kost GJ., Kamkang P, & Loh TP. (2019). Hemoglobin A1c Levels Are Slightly but Significantly Lower in Normoglycemic Subjects With the Hemoglobin E Phenotype. Annals of laboratory medicine, 39(2), 209–213.

[25] Wu, L, Lin, H, Gao, J, Li, X, Xia, M, Wang, D,& Gao, X. (2017). Effect of age on the diagnostic efficiency of HbA1c for diabetes in a Chinese middle-aged and elderly population: The Shanghai Changfeng Study. *PloS one*, *12*(9), e0184607.

[26] Lorenzo-Medina M, De-La-Iglesia S, Ropero P, Nogueira-Salgueiro, P, & Santana-Benitez J. (2014). Effects of hemoglobin variants on hemoglobin a1c values measured using a high-performance liquid chromatography method. Journal of diabetes science and technology, 8(6), 1168–1176

[27]. Torrejón M J, Ortíz-Cabrera NV, M Nieto J, González Fernández FA, Villegas A, Martínez R, & Ropero P. (2017). Hb Moncloa: A new variant of haemoglobin that interferes in the quantification of Hb A1c. Clinical biochemistry, 50(9), 521–524

[28] Christy AL, Manjrekar, PA, Babu R.P, Hegde A, & Rukmini MS. (2014). Influence of iron deficiency anemia on hemoglobin A1c levels in diabetic individuals with controlled plasma glucose levels. Iranian biomedical journal, 18(2), 88–93.

[29] Zechmeister B, Erden, T, Kreutzig B, Weber M., Joly P, Erdmann J, Brockmann-Hönig C, Fischer A, & Eidizadeh A. (2022). Analytical interference of 33 different hemoglobin variants on HbA1c measurements comparing high-performance liquid chromatography with whole blood enzymatic assay: A multi-center study. Clinica chimica acta; international journal of clinical chemistry, 531, 145–151.

[30] Xu J, Zhong Z, & Deng Y. (2021). Unexpected HbA1c results in the presence of three rare hemoglobin variants. Scandinavian journal of clinical and laboratory investigation, 81(1), 59–64.
