



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

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

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### 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.

## المخلص

يُعد الهيموغلوبين السكري (HbA1c) مؤشرًا موثوقًا للتحكم السكري، إذ يعكس متوسط مستويات الجلوكوز في الدم خلال الأشهر الثلاثة الماضية، ويوفر 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 الكاملة (Hb)، 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 واختبار T، وعُرضت النتائج على شكل متوسطات ووسيط وقيم صغرى وكبرى، مع اعتبار قيمة الاحتمال (p) دالة إحصائية إذا كانت أقل من 0.05. أظهرت النتائج عدم وجود فروق ذات دلالة إحصائية في HbA1c بين الطرائق الثلاث. (p=0.41) كما بين معامل ارتباط بيرسون عدم وجود ارتباط دال بين HbA1c والهيموغلوبين الكلي (Hb) (R=0.083). (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) شُتنتج من ذلك عدم وجود تباين واضح بين نتائج HbA1c رغم اختلاف مبادئ القياس، بما يشير إلى دقة مقبولة في الأداء التحليلي للطرائق المختبرة.

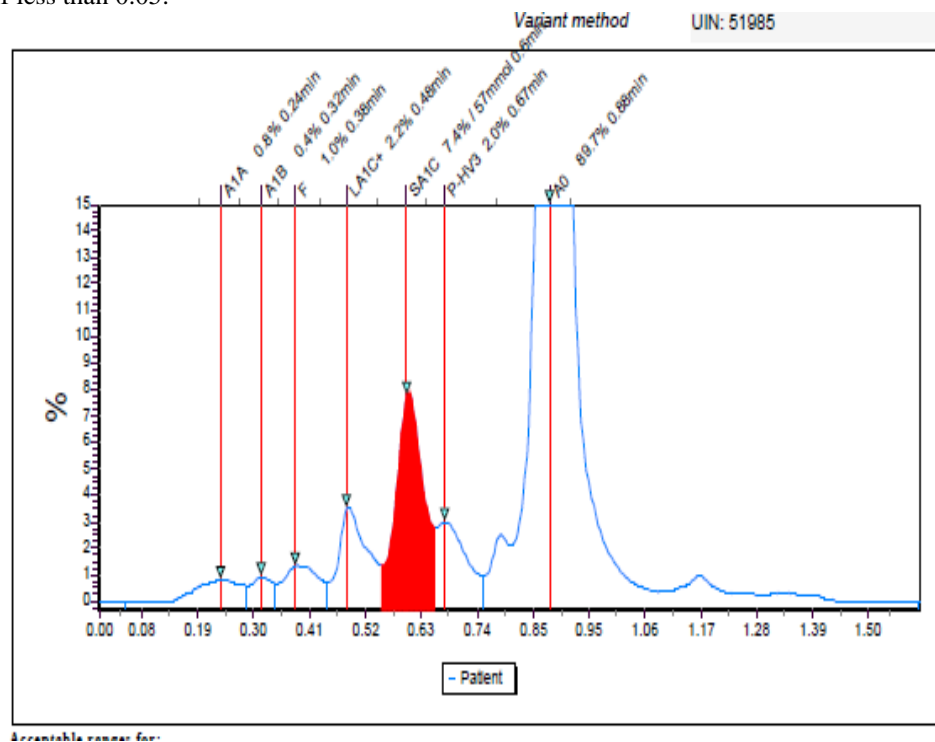
**الكلمات المفتاحية:** الهيموغلوبين السكري HbA1c، داء السكري، ارتباط البورونات، الكروماتوغرافيا السائلة عالية الأداء (HPLC)

## Introduction

Hemoglobin A1c is defined as hemoglobin that is irreversibly glycosylated 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. Glycosylated 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  $\geq 6.5\%$  as diagnostic threshold in diabetics patients [4,5]. Elevated glycosylated 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 assay. However, its measurements can be influenced by various assay 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  $\alpha_2\beta_2$ ) [8,9,10]. HbA is usually have ability to bind to the blood glucose at a special sequence of amino acids producing glycosylated 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 glycosylated hemoglobin [1,16,17]. This method determines total glycosylated Hb, either HbA1c or other Glycosylated forms at any site rather than N terminal Valine. While, in immunoassays method, requires hemolyzing samples firstly to separate glycosylated from non-glycosylated and then adding monoclonal antibodies to recognize and attach to the N-terminal glycosylated valine structure on the  $\beta$ -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-assay variations. Hematological parameters (Hb, WBC and PLTs) were analyzed by Erba H560 hematology analyzer. 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 Chromatogram of Hemoglobin by HLC 723 analyzer showing HbA1c peak elution(red).

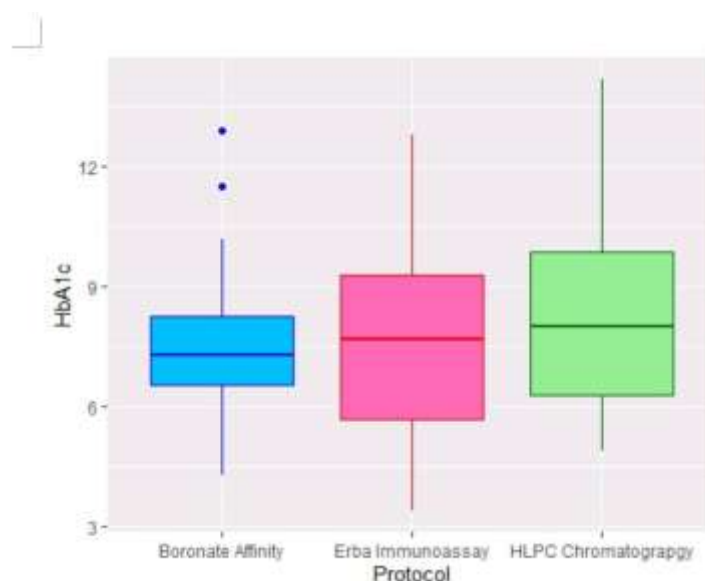
## Results and Discussion

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.

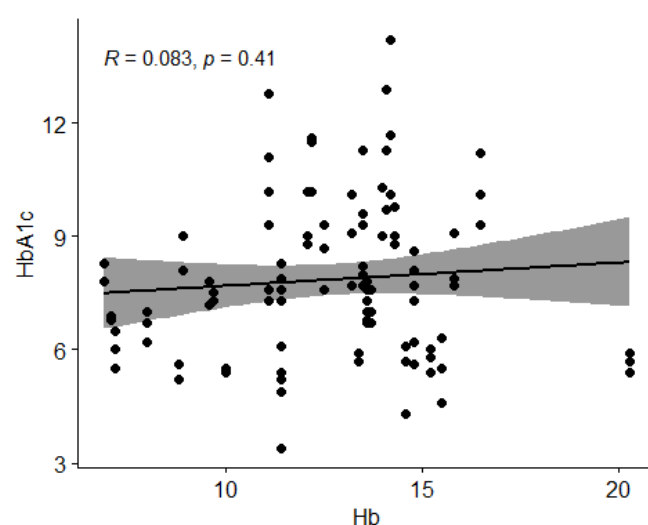
	Ag (years)	Hb (g/dl)	WBC (x103 $\mu$ /L)	PLT (x10 <sup>9</sup> /L)	FBG (mg/dl)
Minimum	10	6.90	2.10	147	77
Median	54	13.3	6.44	250	147
Mean	52	12.44	7.90	240	170
Maximum	80	20.30	18.5	360	415

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.



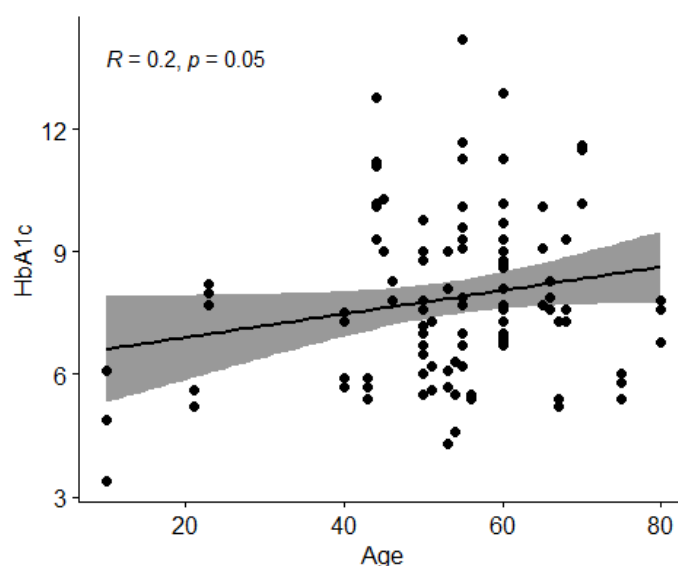
**Figure 2.** Illustrates the difference in HbA1c results by different assay methods , represented by boxplot.

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].



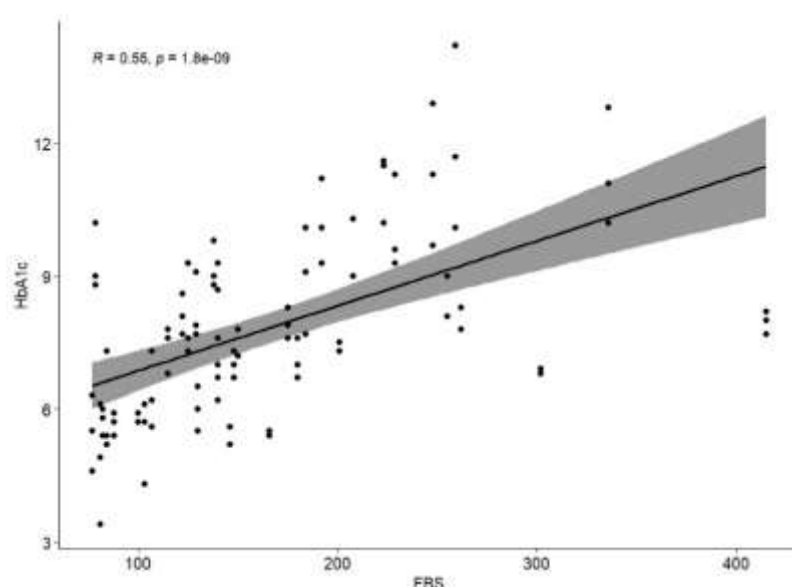
**Figure 3.** Shows correlation between HA1c and levels of hemoglobin of participants

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  $p\text{-value} = 0.05$ ) (**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.

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