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Evaluation of Manual Hb A_{1C} Determination Methods in Diabetic Patients with Sickle Cell Trait

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Background and Aim

HbA1c is an irreversible non-enzymatic glycation at one or both NH2- terminal valines of the β –chain. Sickle hemoglobin, or HbS, has a valine for glutamic acid substitution at position 6 of the β chain. The prevalence of sickle cell trait reported to be 2.5% in Basrah city. The hemoglobin A1C (A1C) test can lead to false outcomes resulting in over- treatment or under-treatment of diabetes in people with inherited hemoglobin variants.

This study focus on the evaluation of sickle cell trait effect on HbA1c measurement by the minicolumn ion exchange resin methods and compare them with Biorad Variant (HPLC method).the one of methods certified by national glycohemoglobin standardization program (NGSP)

Materials and Methods

60 diabetic patients identified with sickle cell trait were recruited in this study .Blood sample collect in EDTA tubes and were assessed for hemoglobin A1c using 3 methods commercially available and most fequently used for routine investigations. Stanbio Glycohemoglobin (Pre- Fil®), HUMAN Glycohemglobin HbA1-Test (Fast Ion Exchange Resin Separation Method) ,and the Bio-Rad VARIANT Hemoglobin A1c (HPLC) method

Results

Mean age group for patient were 39.6 yrs with their mean blood glucose level of about 203 mg/dl , the average glycohemoglobin was 9.1% measured by Biorad Variant, compare to 7.57% and 7.99% for both Stanbio method and Human Method respectively, which show statistically significant difference ($p<0.05$). It also had been found that average difference between both manual methods (|Stanbio and Human) and Automated method (biorad variant

HPLC) about (1.5, 1.15) . These difference show a significant statistical correlation with hemoglobin S level with r =0.856; p value<0.001.

Conclusion

This study demonstrate the significant difference in Glycated hemoglobin measurements between different methods commercially available in local markets which had been shown to give false negative results in sickle trait patients when compared to those methods standardized by NGSP system. And recommend that such methods to be avoided in patient with sickle cell trait.

Introduction

HbA1c is defined as irreversible non-enzymatic glycation at one or both NH2-terminal valines of the β-chain.[1] Hb A1c is the major fraction, constituting approximately 80% of Hb A1[2]. Glycated hemoglobin (gHb), measured as HbA1c, is used to evaluate long-term control of diabetes mellitus and is directly related to the risk for complications of diabetes. Depending on the method of determination used, the concentration of HbA1c is approximately 4–6% in healthy non diabetic subjects.[3]

The hemoglobin A1C (A1C) test can lead to false outcomes resulting in over-treatment or under-treatment of diabetes in people with inherited hemoglobin variants[4]. Sickle hemoglobin (HbS), a hemoglobin variant, has a valine for glutamic acid substitution at position 6 of the β chain. The prevalence of variant HbS has been demonstrated to be as high as one-third of all diabetic patients undergoing testing.[5] The prevalence of sickle cell trait report to be 6.48 % in Basrah city.[6]

More than 20 methods for the determination of gHb are commercially available to clinical laboratories. These methods measure gHb based on its physical, chemical, or antibody-recognized characteristics. Structural variants and chemical derivatives of hemoglobin (Hb) interfere with many methods.[5]

Common cation-exchange and HPLC systems include the Tosoh A_{1C} 2.21 and the Bio-Rad Variant. Diamat, and Diastat systems.[7] These method were Certified by National Glycohemoglobin Standardization Program (NGSP) as having documented traceability to the Diabetes Control and Complications Trial Reference Method and they had less interference from hemoglobin variants[8].

Manual HbA1c determination methods commercially available based on ion –exchange resin (negatively charge) packed in minicolumn and had a great affinity for positively charged hemoglobin. Sample with gHb (less positively charge) will elute first and then detected by spectrophotometer

This study focus on the evaluation of sickle cell trait effect on gHBs measured by the minicolumn ion exchange resin and compare it with method certified by NGSP use ion exchange HPLC.

Materials and Methods

Sample Collection

60 diabetic patients identified with sickle cell trait were recruited in this study. they have been identified to have Sickle cell trait using Biorad Variant I. Blood Sample collect in EDTA tubes and were assessed for hemoglobin A1c using 3 methods commercially available and most frequently used for routine investigations. Two manual methods: Stanbio Glycohemoglobin (Pre- Fil®) supplied by Stanbio Laboratories, USA, and Glycohemglobin HbA1-Test (Fast Ion Exchange Resin Separation Method) supplied by HUMAN, Wiesbaden, Germany. Both these methods depended on the same principle in which whole blood is mixed with a lysing reagent containing a detergent and borate ions. Elimination of the labile Schiff's base is thus achieved during the hemolysis. The hemolysate is then mixed for 5 minutes with a weekly binding cation exchange resin. During this time HbA0 bind to the resin. A special resin separator is used to remove the resin from the supernatant fluid which contains the HbA1. The glycohemoglobin percentage of total hemoglobin is determined by measuring the absorbance of the glycohemoglobin and of the total hemoglobin fraction at 415 nm in comparison with a standard glycohemoglobin preparation carried through the test procedure. The result expressed as HbA1 and then converted to HbA1c by using of conversion equation. The result from the above two methods where compared with third method method using Biorad variant I HbA1c program based on ion –exchange high performance liquid chromatography (HPLC).

Preceding analysis, a simple preparation of the patient sample is required to hemolyze the blood and remove labile A1c. Samples are first diluted with hemolysis reagent and then incubated at 18-28 °C for a minimum of 30 minutes. Prepared samples are automatically injected into the analytical cartridge, where the hemoglobins are separated based on their ionic interactions with the material. The separated hemoglobin then passes through the flow cell of the filter photometer, where changes in the absorbance (415 nm) are measured; background variations are corrected by an

additional filter at 690 nm. A built-in integrator performs reduction of raw data collected from each analysis. A calibrator is analyzed with each run for adjustment of the calculation parameters for determination of HbA1c. A chromatogram (graph) of the changes in the absorbance is plotted versus the retention time. Each chromatogram printout is accompanied by a report identifying each peak detected, plus the relative percent and retention times of each peak.

Statistical Analysis

Statistical analysis of the data was performed using the Statistical Package for Social Sciences (SPSS) for Windows 15.0 (SPSS Inc., Chicago, IL) (t-test, Pearson correlation, and ANOVA tests). Values of $p < 0.05$ were considered to be significant.

Results

60 diabetic patients found to have sickle cell trait on the examination for routine checkup for glycemic control, their demographic data had been shown in **Table1** Mean age group for patient were 39.6 yrs. with their mean random blood glucose level of about 203 mg/dl, there average glycohemoglobin (Hb A1c) were 9.1 % and average hemoglobin S level for our study were 4.9 %.

Table 1	
	Mean ± SD
Patient's No.	60
Age	39.6 (±14.2) yrs
Random Blood Glucose	203 (±110) mg/dl
Hb A_{1c} Biorad Variant	9.1 % (±2.5)
Hb A_{1c} Human Method	7.57% (± 1.5)
Hb A_{1c} Stanbio Method	7.95% (±1.66)
Hb S*	4.9 % (±1.35)

**Hb S obtained by biorad variant method*

It had been shown that there was significant difference between the mean hemoglobin A1c by Biorad Variant I and hemoglobin A1c by human method with p value <0.05. There was also significant difference between the mean hemoglobin A1c by Biorad Variant I and hemoglobin A1c by Stanbio method with p value <0.05. But there were no significant differences between human method and Stanbio method for HbA1c.(table 2,3)

Table 2

	Biorad Variant II (HPLC)	Human (Fast Ion Exchange Resin)	Stanbio (Fast Ion Exchange Resin)	P value
Hb A1c	9.1 (±2.55)	7.57% (± 1.5)	7.95% (±1.66)	.001

Table 3

	Human (Fast Ion Exchange Resin)	Stanbio (Fast Ion Exchange Resin)	P value
Hb A1c	7.57% (± 1.5)	7.95% (±1.66)	.205

Table 4 show the variance Hb A1c between the Biorad Variant I method and the other two methods and level of Hb S in patients. It show there the mean differences between Biorad Variant I and Human method is around 1.5 % and mean difference between Biorad Variant and Stanbio method 1.15 %.

Table 4
Differences between Hb A1c determined by Biorad Variant I and other methods group by Hb S levels

HB S %	Biorad Variant I– Human method	Biorad Variant I – Stanbio method
5.2 (±1.16)*	1.5 (±1.9)	1.15 (±1.8)

When correlate this level of difference with Hb S level, a Significant statistical correlation found with hemoglobin S level with r =0.856 ; p value<0.001 , r=0.826,p value <0.001 for Human method and Stanbio method respectively***. (Fig 1,2)***

Figure 1

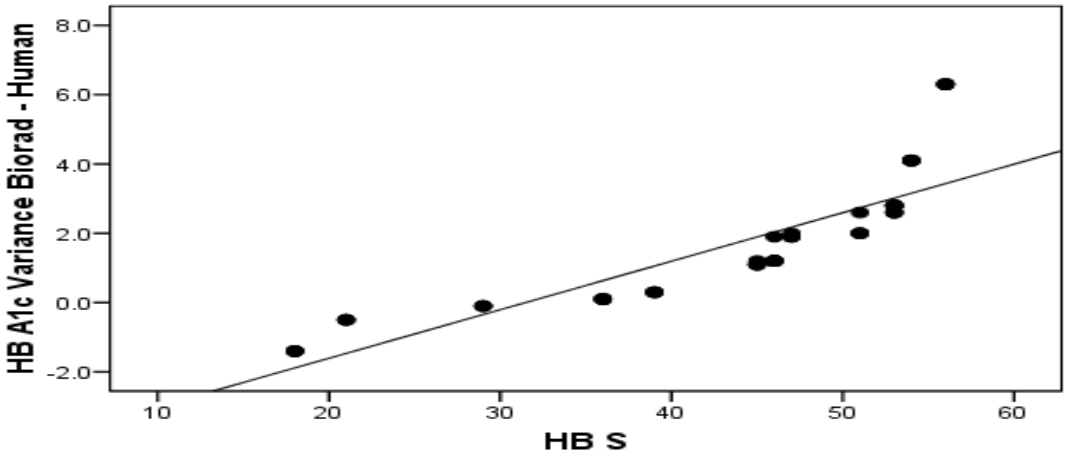
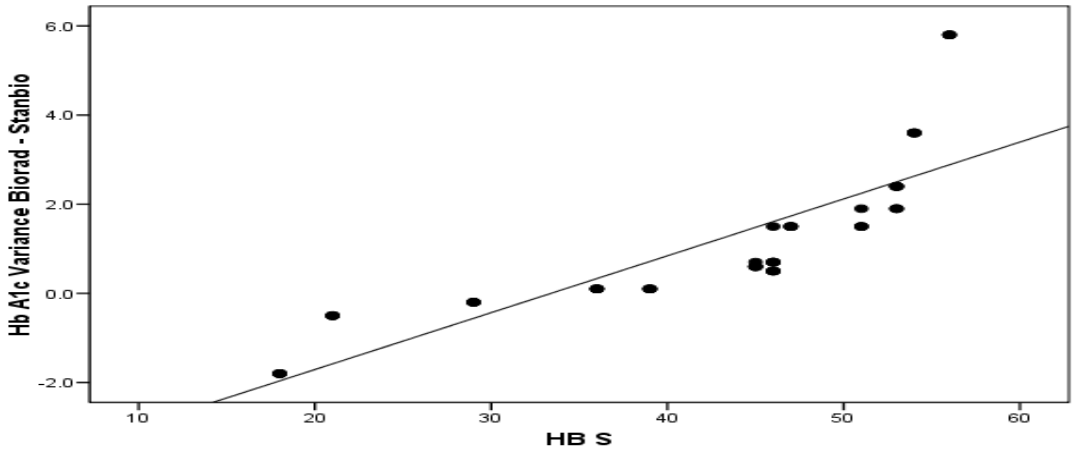


Figure2



Discussion

The prevalence of variant HbS has been demonstrated to be as high as one-third of all diabetic patients undergoing testing.[9] The prevalence of Hb S ranges from 2.5% in the City Centre up to 16% in Abu al-Khasib district[10,11.] It had been found that there is increase in frequency of Hb S to in city centre due to immigration.[6] According to American Diabetes Association (ADA) officially recommends HbA1c testing for the diagnosis and monitoring of diabetes for that reason accurate measurement of Hb

A1c in this population is recommended.[12] Many ion – exchange chromatographic methods available for determination of Hb A1c, two of them commercially available using manual method and a third one using HPLC ion exchange method (Biorad Variant). It had been shown that Biorad Variant ion exchange HPLC had no interference from HB AS level and other hemoglobinopathies.[13]

This study compared Biorad variant result with those of two manual method which also depend on ion exchange method but had shown significantly lower result compared to that of biorad . this significant underestimate of HbA1c could be explained by the inability of minicolumn to separate Hb A_0 and Hb S_0 from each other.[14]

The glycation of Hb S and Hb A occur at the same rate and since the manual resin separator only elute Hb A1 from the all other Hb in the sample[15], this could be another attribute to false low result of Hb A1c by the manual methods compared with automated biorad method. In conclusion, accurate measurement of Hb A1c is mandatory, and since high prevalence of Hb S present in our city, a traceable method by Diabetes Control and Complications Trial Reference and standardized by NGSP so be adopted to avoid any interference from variant hemoglobin .

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تقييم الطرق اليدوية لقياس خضاب الدم السكري في مرضى السكري الحاملين لصفه فقر الدم المنجلي

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الخلاصة

خضاب الدم السكري ناتج عن ارتباط نهائي غير انزيمي بين السكر و احدى الأطراف الامينية في سلسة بيتا المكونه للهيموكلوبين . ينتج خضاب الدم المنجلي عن استبدال الحامض الاميني الفالين بال كلوتامك اسد في سلسلة بيتا. ينتشر صفة الدم المنجلي بنسبة ٢.٥ % في مدينه البصرة . لذا فان قياس خضاب الدم السكري لدى هذه الشريحة يمكن ان يؤدي إلى نتائج زائفه مما ادي إلى الإفراط في المعالجة أو المعالجة الناقصة لمرض السكري في الناس مع المتغيرات الهيموغلوبين الموروثة.
لذا ركزت هذه الدراسه على تقيم الطرائق المعتدة في العدد اليدوية لقياس خضاب الدم السكري و مقارنتها مع الطرق الممكنه و المعتدة عالميا

المواد والأساليب

ستون مريضا السكري الذين تم التعرف عليهم بسمات الخلية المنجلية في هذه الدراسة. جمع عينه من الدم في أنابيب EDTA وتم تقييمها c١A باستخدام ٣ طرق متاحه تجاريا و المستخدمة في العمل اليومي . ستانبيو غليكوهيموغلوبين (قبل الفي®) ، الهيومن جليكوهيلوبين HbA١--اختبار (سريعة الايونيه تبادل الراتنج طريقه الفصل) ، و مقارنتها مع الطريقة الاللية باستخدام جهاز Biorad variant

النتائج

متوسط العمر للمريض كانت الفئة ٣٩.٦ سنه مع مستوي الجلوكوز في الدم من حوالي ٢٠٣ ملغ/dl ، وكان معدل الهيموغلوبين الوسطي ٩.١ ٪ قياسها المتغير الحيوي ، مقارنه إلى ٧.٥٧ ٪ و ٧.٩٩ ٪ لكل من طريقه ستانبيو وطريقه هيومن علي التوالي ، والتي تظهر فرق كبير من الناحية الاحصائيه ($p < ٠.٠٥$). كما تم العثور علي ان متوسط الفرق بين كل من الأساليب اليدوية (| ستانبيو وهيومن) والطريقة الالية (Biorad Variant) حول (١.٥ ، ١.١٥). هذه الفرق تظهر ارتباطا إحصائيا كبيرا مع مستوي الهيموغلوبين بمقدار $r = ٠.٨٥٦$ ؛ قيمه $p < ٠.٠٠١$.

الختام

وتبين هذه الدراسة الفرق الكبير في قياسات الهيموغلوبين بالسكر بين الطرق المختلفة المتاحة تجاريا في الأسواق المحلية والتي تبين انها تعطي نتائج سلبيه زائفه في المرضي الذين يعانون من سمات المنجل عند مقارنتها بتلك الأساليب موحده بواسطة نظام التخطيط الاستراتيجي المشترك. ونوصي بتجنب هذه الأساليب في المريض مع سمه الخلية المنجلية.