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Original Research Article

Comparison of glycated hempglobin with HPLC and capillary electrophoresis

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ABSTRACT

Background: Hemoglobin A1c, also called A1c or glycated hemoglobin, is hemoglobin with glucose attached. The A1c test evaluates the average amount of glucose in the blood over the last 2 to 3 months. The higher the level of glucose in the blood, the more glycated hemoglobin is formed. Once the glucose binds to the hemoglobin, it remains there for the life of the red blood cell – normally about 120 days. The predominant form of glycated hemoglobin is referred to as A1c. Testing of HbA1c levels via capillary electrophoresis is a relatively new but well-validated method that separates A1c and other Hb fractions via charge difference at high voltage using electro-osmotic flow. This method can be useful in patients who possess such variant hemoglobins because it has a longer runtime, leading to better resolution.
Methods: We have processed random samples coming to our laboratory for HbA1C analysis on both the analyzers Biorad D 10 (HPLC method) and Sebia Flex piercing (Capillary Electrophoresis).
Results: The value of t is 0.056748 for paired 't' test. The value of p is 0.954819. The result is not significant at $p \leq 0.05$. There is no significant difference between the results obtained from both the equipment.
Conclusions: From this study, it is concluded that the results obtained after testing samples in Sebia Flex Piercing II and Biorad D10 are comparable and there is no significant difference in the results obtained. The advantage of of using Sebia is detection of underlying hemoglobinopathies is easier and can serve as passive surveillance in population that will provide additional information for multidisciplinary approach of treatment. Whereas Biorad D10 has benefit of shorter testing time and is cost effective.

Keywords: Capillary electrophoresis, Electro-osmotic, Glycated hemoglobin, HPLC

INTRODUCTION

Hemoglobin A1c, also called A1c or glycated hemoglobin, is hemoglobin with glucose attached. The A1c test evaluates the average amount of glucose in the blood over the last 2 to 3 months by measuring the percentage of glycated (glycosylated) hemoglobin.[1-4]

Hemoglobin is an oxygen-transporting protein found inside red blood cells (RBCs). There are several types of normal hemoglobin, but the predominant form - about 95-98% - is hemoglobin A. As glucose circulates in the blood, some of it spontaneously binds to hemoglobin A.

The higher the level of glucose in the blood, the more glycated hemoglobin is formed. Once the glucose binds to the hemoglobin, it remains there for the life of the red blood cell – normally about 120 days. The predominant form of glycated hemoglobin is referred to as A1c. A1c is produced on a daily basis and slowly cleared from the blood as older RBCs die and younger RBCs (with non-glycated hemoglobin) take their place.

A1c assay methods can broadly be categorized into 2 types: those based on charge and those based on structure. HPLC and electrophoresis are methodologies of the first type, whereas immunoassay, boronate affinity chromatography, and mass spectrometry are of the latter category. In clinical laboratories, HPLC and immunoassay are the most commonly used methods to measure A1c.[5] Testing of HbA1c levels via capillary electrophoresis is a relatively new but well-validated method that separates A1c and other Hb fractions via charge difference at high voltage using electro-osmotic flow. This method can be useful in patients who possess such variant hemoglobin because it has a longer runtime, leading to better resolution. Capillary zone electrophoresis is very precise and accurate for A1c estimation. It is not prone to common interferences and has the ability to detect several hemoglobin variants.[6]

Here in Our study we have done a comparative study of HbA1C analysis by HPLC method using Biorad D10 and capillary electrophoresis method using Sebia Flex piercing.

METHODS

We have processed random samples coming to our laboratory for HbA1C analysis on both the analyzers Biorad D 10 (HPLC method) and Sebia Flex piercing (Capillary Electrophoresis). Strict Quality assurance protocols were followed using third party QC material. Level 1 and level 2 QC run daily during the period of analysis.

The D-10 Hemoglobin A1c Program utilizes principles of ion-exchange high-performance liquid chromatography (HPLC). The samples are automatically diluted on the D-10 and injected into the analytical cartridge. The D-10 delivers a programmed buffer gradient of increasing ionic strength to the cartridge, where the hemoglobins are separated based on their ionic interactions with the cartridge material. The separated hemoglobins then pass through the flow cell of the filter photometer, where changes in the absorbance at 415 nm are measured.

The D-10 software performs reduction of raw data collected from each analysis. Two-level calibration is used for quantitation of the HbA1c values. A sample report and a chromatogram are generated for each sample. The A1c peak is shaded. This area is calculated using an exponentially modified Gaussian (EMG) algorithm that excludes the labile A1c and carbamylated peak areas from the A1c peak area.[7]

The Sebia CapillaryFlex Piercing II instrument uses the principle of capillary electrophoresis in free solution. With this technique, charged molecules are separated by their electrophoretic mobility in an alkaline buffer with a specific pH. Separation also occurs according to the electrolyte pH and electro osmotic flow. The Sebia Capillary Flex Piercing II instrument has silica capillaries functioning in parallel allowing simultaneous analyses for HbA1c quantification from whole blood sample.[8] A sample dilution with hemolysing solution is prepared and injected by aspiration at the anodic end of the capillary. A high voltage protein separation is then performed and direct detection of the hemoglobins is made at the cathodic end of the capillary at 415 nm, which is the absorbance wave length specific to hemoglobins. Before each run, the capillaries are washed with a wash solution and prepared for the next analysis with buffer.[8]

Direct detection provides accurate relative quantification of individual hemoglobin A1c fraction. In addition, the high resolution of CAPILLARYS Hb A1c procedure allows the quantification of HbA1c, and particularly, even in the presence of labile.

HbA1c, carbamylated and acetylated hemoglobins, and major hemoglobin variants.

RESULTS

The results obtained are analyzed and tabulated. The data is analyzed usingtwo tail paired‘t’ test. After analyzing the data for results obtained from Sebia CapillaryFlex Piercing II and Biorad D10, it is found that there is no significant difference in results obtained from both the instruments. The p value is 0.954819. Results of statistical analysis are as shown in Table. 1.

Table 1: Difference scores calculations.

Mean: 0.00
$\mu = 0$
$S2 = SS/df = 27.77/(157-1) = 0.18$
$S2M = S2/N = 0.18/157 = 0.00$
$SM = \sqrt{S2M} = \sqrt{0.00} = 0.03$
T-value calculation
$t = (M - \mu)/SM = (0.00 - 0)/0.03 = 0.06$
The value of *t* is 0.056748. The value of *p* is 0.954819. The result is not significant at $p \leq 0.05$.

DISCUSSION

The availability of the hemoglobin A1c test has enhanced diabetic care and its measurement has become an integral part in the management of diabetes. Also, the relationship between the improved glycemic control and risk of diabetic complications has been established.[9, 10]

In present study, we have studied two methods of HBa1C comparison based on two different principles Sebia CapillaryFlex Piercing II and Biorad D10 HPLC. The data obtained shows that there is no significant difference between these two method analytical performances. Results obtained are comparable. The mean and standard deviation HbA1C levels of 157 subjects included in our study was 7.343±1.75% and 7.345±1.71% with Sebia and Biorad respectively. ($P \leq 0.05$). In 83 of the samples D10 values were found to be higher than Sebia and in 73 of

the samples Sebia values are found to be higher than D10. In 1 case the value was same in both the analyzer. As per NGSP data Sebia Capillary Flex Piercing II has does not shows any interference in HbA1C analysis for HbC Trait as well as HbF upto concentration of 15%, whereas Biorad D 10 short programme that we used has proven interference for HbC trait, as well as HbF upto 10% concentration.

Table 2: Interference for various substances.

Parameter	Levels to which interference is not detected	
	D10	Sebia
Icterus	20 mg/dL,	25.6 mg/dL
Lipemia, triglyceride	5680 mg/dL,	1120 g/dL
Hemoglobin F concentrations	up to 10%	up to 15%
Labile A1c (LA1c/CHb-1) concentrations	up to 4%	≤10.5%
Carbamylated hemoglobin (LA1c/CHb-2) concentrations	up to 3.5%	≤ 8.1%

Considering these facts Sebia Capillary Flex Piercing II proves to have superior performance in presence of additional hemoglobin fractions.[11-15] The linearity for Sebia Flex Piercing II is upto 16.4% and limit of detection is 4.6% (Kit Insert) which slightly lower than Biorad D10 for which the linearity is upto 18.5% as well as limit of detection is 3.8%. (Kit Insert) The run time for individual sample in Sebia Flex Piercing II is 9 minute whereas in Biorad D10 is 3 min. From Table 2, it is seen that the except lipemia (Triglyceride) Sebia Flex Piercing II is prone to less interference as compared to Bio Rad D 10. Both the equipment was thoroughly analysed for precision and accuracy and CV was compared for normal as well as high value samples.

Table 3: Precision study for Sebia Flex Piercing II and Bior Rad D 10

	D 10		Sebia	
	Normal patient	Diabetic patient	Normal patient	Diabetic patient
Within Run (%CV)	0.78	0.46	0.97	1.1
Between Day (%CV)	0.68	0.99	0.1	0.56
Between Run (%CV)	0.52	0.53	0.32	0.87
Total Precision (%CV)	1.16	1.22	1.06	1.15

From Table 3 it is found that, the CV% variation for D 10 Sebia is comparable for within run, between day and between run.The added advantage of Sebia Flex Piercing II is that it can detect hidden asymptomatic additional hemoglobin fraction. This can provide additional information for multidisciplinary treatment approach. As well as can be used as passive surveillance of hemoglobinopathies in society.

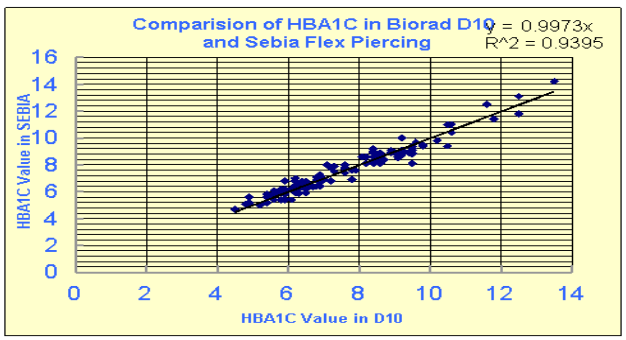

Figure 1: Correlation of HBA1C results obtained by sebia flex peiercing II and biorad D 10.

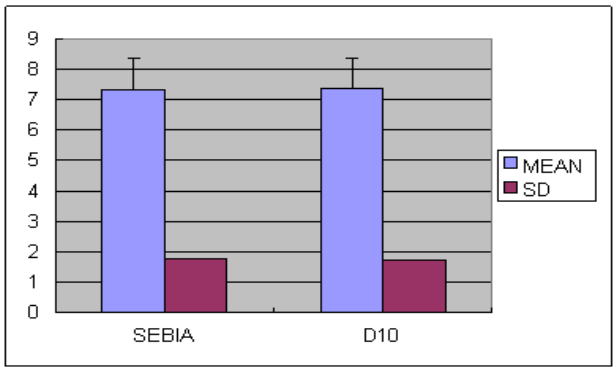

Figure 2: Comparison of mean HBA1C results by Sebia flex peiercing II and biorad D 10.

The graphs are easy to understand and can be screened and differentiated by even a technical grade staff with minimum training.

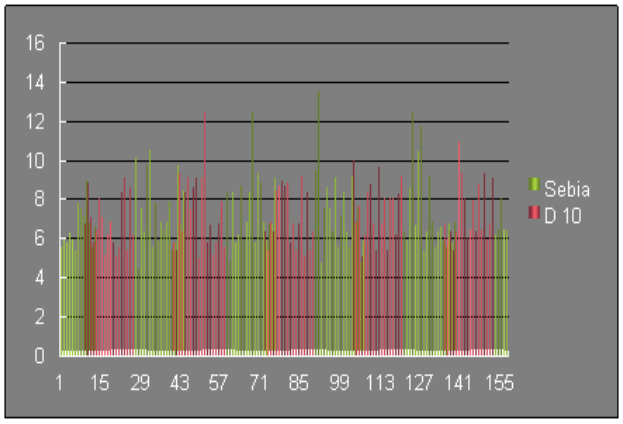

Figure 3: Sample by sample comparison of HBA1C results by sebia flex peiercing II and Biorad D 10.

The interference with various hemoglobins can be identified and additional value can be added to routine HbA1C reports on incidental detection of abnormal hemoglobin fraction which will prove of importance of patients care and further advice can be given accordingly.

CONCLUSION

From this study, it is concluded that the results obtained after testing samples in Sebia Flex Piercing II and Biorad D10 are comparable and there is no significant difference in the results obtained. The advantage of of using Sebia is detection of underlying hemoglobinopathies is easier and can serve as passive surveillance in population that will provide additional information for multidisciplinary approach of treatment. Whereas Biorad D10 has benefit of shorter testing time and is cost effective.

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Ethical approval: The study was approved by the Institutional Ethics Committee

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