



Can the Level of Glucose & Glycated Hemoglobin be Estimated from Same Container?

Souvik Konar¹, Amrita Karmakar², Soma Gupta³

¹MD (Biochemistry), Senior Resident, Department of Biochemistry, Nilratan Sircar Medical College & Hospital; ²MD (Biochemistry), Assistant Professor, Department of Biochemistry, Nilratan Sircar Medical College & Hospital; ³MD (Biochemistry), Professor & HOD, Department of Biochemistry, Nilratan Sircar Medical College & Hospital.

ABSTRACT

Context: Burden of diabetes mellitus is increasing gradually along with its complications. Nowadays along with fasting and post-prandial plasma glucose (FPG and PPPG), HbA1c is included in the diagnosis and assessing prognosis. The current practice is samples for HbA1c and FPG, PPPG are collected in different vials (EDTA and Fluoride vial respectively).

Aims: Present study was designed to find out whether any difference occurs or not in HbA1c values when collected in EDTA & fluoride vials & also to find out the stability of HbA1c in fluoride vials for five consecutive days.

Settings & Design: This case control study was conducted at service laboratory of Dept. of Biochemistry at NRS Medical College & Hospital

Methods & Material: Samples were collected from 107 subjects (44 male & 63 female) in both EDTA (E) & fluoride vials (F). Estimation of HbA1c was done from both the vials separately by Latex Turbidimetric and chromatography method (HPLC). 10 randomly chosen samples were collected and stored at 4°C in fluoride vials. HbA1c estimation was done from F vial for 3 consecutive days.

Statistical Analysis: The results were tabulated and analyzed using software.

Results: The HbA1c values from both EDTA & Fluoride vials were well correlated and no statistically significant differences ($p=0.69$) existed among E & F vials. ANOVA analysis showed no significant differences ($p=0.99$) among HbA1c level in Fluoride vial for 3 consecutive days.

Conclusions: Estimation of blood glucose level & HbA1c level can be done from the fluoride (F) vial without compromising the quality of reports.

Key Words: HbA1C estimation in Fluoride vial

Key Messages: Considering on huge number of samples estimated daily for glucose and HbA1C, the procedure of collecting blood in one container will decrease the total cost per patient.

INTRODUCTION

Prevalence of diabetes mellitus (DM) is increasing rapidly. In India estimated age adjusted prevalence of DM in adults is >9%. India stands second among top ten countries for number of adults with DM (1). In addition to the prognostic role of HbA1c, American Diabetes Association has recommended HbA1c for the diagnosis of Diabetes Mellitus (2)

Though fasting plasma glucose and HbA1C are advised simultaneously in almost all cases, the sample cannot be collected in same vial as HbA1c is stated to have high preanalytical stability (3) (4) when collected in EDTA vial rather than in traditional glucose vial. But no reason for it has been demonstrated. Same container is always preferred, so far time, cost and convenience is considered.

Corresponding Author:

Dr. Amrita Karmakar, 158, Barakhola, R K Pally, Mukundapur, Kolkata - 700099
Phone: 9830835373; Email: amritatua@gmail.com

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SUBJECTS AND METHODS

In this backdrop, this study was undertaken to find out whether any significant variation in HbA1c level occurs or not if the sample is estimated from the same vial which is used to estimate blood glucose level. It was also aimed to find out the stability of the level of HbA1c, when estimated from F vial.

The study was conducted in the service Laboratory of the Department of Biochemistry, Nil Ratan Sircar Medical College and Hospital, Kolkata. A total of one hundred seven subjects who were advised for blood glucose & HbA1c estimation were consecutively selected & included in this study. Individuals of both genders were included in this study. After obtaining proper consent, whole blood was drawn from the subjects and was divided separately in EDTA vial and Fluoride vial. A total of 10 samples collected in Fluoride vial were chosen randomly & were stored in 4 degree centigrade. The estimation was repeated for 3 consecutive days to find out the stability of HbA1c level.

HbA1c level was estimated from both vials separately by Latex Turbidimetric method and HPLC method. The underlying principle of latex turbidimetric method (5) is that it utilizes antigen-antibody interaction to directly determine the HbA1c in whole blood. Total hemoglobin and HbA1c have the same unspecific absorption rate to latex particles. When mouse antihuman HbA1c monoclonal antibody is added, latex-HbA1c-mouse anti human HbA1c antibody complex is formed. Agglutination is formed when goat anti-mouse IgG polyclonal antibody interacts with the monoclonal antibody. The amount of agglutination is proportional to the amount of HbA1c absorbed on to the surface of latex particles. HbA1c estimation done by Hb-Vario Analyser uses cation exchange high performance liquid chromatography (HPLC) in conjunction with gradient elution to separate hemoglobin subtypes and variants from hemolysed whole blood. The separated hemoglobin fractions are monitored by absorption of light at 415 nm. A software program analyses the chromatogram and reports are generated. (6) The results were tabulated and statistically analyzed. The significance of difference in the level of HbA1c was found out using Student's t test ($p < 0.05$ was considered as statistically significant). The correlation of the two methods was found out by calculating Pearson's Correlation Coefficient. The levels of HbA1c done for three consecutive days were compared by ANOVA test to find out whether any statistically significant difference exists among the groups or not.

RESULTS

Among the total of 107 subjects involved in the study, 44 were male & 63 were female. Most of the study population belonged to 51-85 year age group. The age group ranged from 14 years to 85 years.

Table 1 shows comparison of HbA1c level in study subjects done from two different vials (E & F). The analysis shows that no significant difference exists among the two values ($p > 0.05$). The two methods were well correlated positively as calculated by Correlation Coefficient, which was found to be 0.9 (Fig. 1).

Table 2 shows the result of ANOVA test done to compare the level of HbA1c for 3 consecutive days. The result shows that no statistically significant difference exists among any group ($p = 0.905$, which is > 0.05).

DISCUSSION

This study was undertaken to find out whether any variation in HbA1c level occurs or not if the sample is estimated from E vial & from F vial. It was found that no significant difference (p value-0.69 in E; 0.83 in F vial) was seen among the values of HbA1c when HbA1c estimation was done from two different (E & F) vials by HPLC and Latex agglutination method. The result of this study is in accordance with the results of the study done by Mailankot M et al (4). Mailankot M et al in their study found no significant changes between the samples taken in different tubes. They excluded the absolute necessity for blood collection in EDTA tubes for HbA1c estimation. Sharma B et al (3) in their study concluded that unnecessary extra sample collection for HbA1c estimation can be avoided which can improve patient compliance & avoidance of extra vial for HbA1c can reduce the cost of HbA1c test.

This study was also conducted to find out the stability of HbA1c level when HbA1c estimation was done from F vial for at least three consecutive days when the samples were stored at 4 degree centigrade. It was found that no significant difference existed when HbA1c estimation was done only from the F vial for 3 consecutive days by latex agglutination method days (p value- 0.99) and HPLC method (p value- 0.99). The comparison could not be extended beyond three days as sample in F vial was getting clotted and could not be run by HPLC method.

India is considered as the diabetic capital of the world. For diagnosis of Diabetes Mellitus, American Diabetes Association has recommended HbA1c estimation. Among Indians, proper diabetic screening is needed but for awareness of diabetes, often the adequate manpower is lacking. Moreover, HbA1c test is a costly test & for testing HbA1c using a separate E vial further increases cost. Usually, HbA1c estimation is done on the same day of blood collection but blood collection in a different E vial also requires more sample volume.

Hence, considering these facts our study is very promising when it comes to estimation of HbA1c. Our study does not encourage the need for extra E vial for estimation of HbA1c.

HbA1c can be tested in the same vial which is used to estimate blood sugar (F vial).

So, estimation of HbA1c among Indians is very much needed. HbA1c should be used for diagnosis of Diabetes Mellitus in India because it can diagnose many cases which go undetected by routine blood sugar analysis. For establishing a baseline HbA1c level among Indians, a pan India study of HbA1c should be conducted based on which diagnosis & screening of diabetes could be predicted.

CONCLUSION

The study shows that HbA1c level is comparable significantly with standard practice even if it is done from vials used to estimate blood glucose level. Moreover, the concentration of HbA1c remains stable in F vials for at least 3 consecutive days, when stored in 4 degree centigrade. Usually the estimation of HbA1c is done on the same day of blood collection. Hence, the estimation of blood glucose level & HbA1c level can be done conveniently from the same vial without compromising the quality of reports.

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Table 1: Comparison of HbA1c level in study subjects done from two different vials by latex turbidimetric method.

HbA1c	Method	Mean	Standard Deviation	Student's t-test	P value
By EDTA vial	Latex Turbidimetric method	6.2	1.8	0.4	0.69
By Sodium Fluoride vial		6.1	1.9		
By EDTA vial	HPLC method	6.31	2.45	0.2	0.83
By Sodium Fluoride vial		6.46	2.54		

Table 2: ANOVA test to compare level of HbA1c done in fluoride vial for 3 consecutive days

HbA1C estimation	No of Subjects	Day1 (Mean ±SD)	Day2 (Mean ±SD)	Day3 (Mean ±SD)	(p Value)
Latex Agglutination Method	10	6.62±2.21	6.66±2.26	6.62±2.25	0.99
HPLC method	10	6.45±1.59	6.4±1.67	6.39±1.67	0.99

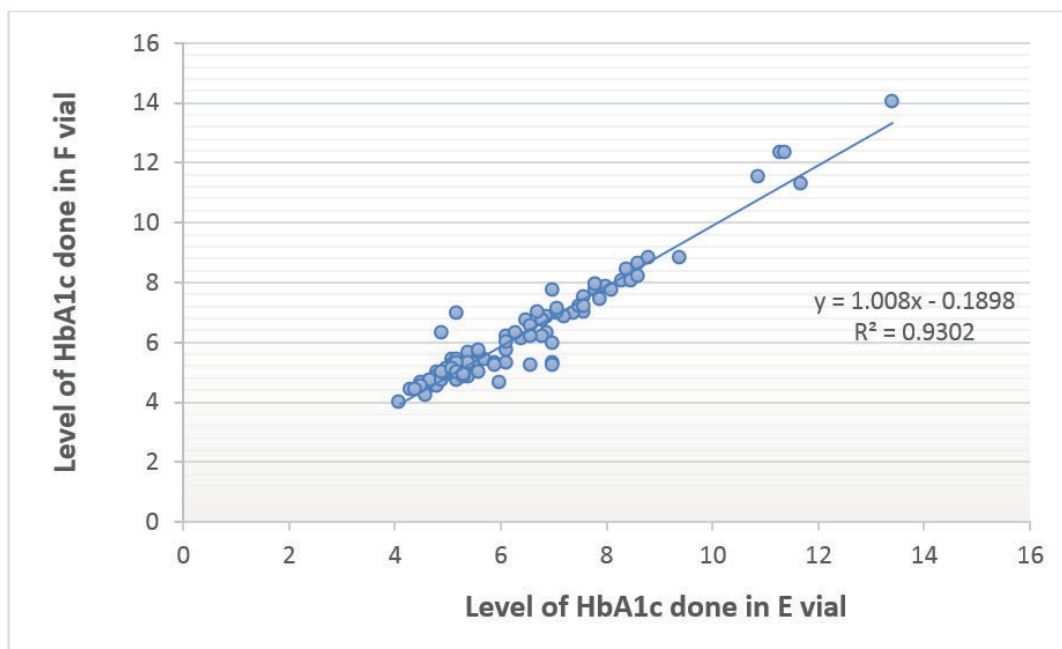


Figure 1: Correlation in level of HbA1c done from two different vials.