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Original Research Article

Can Oxalate-Fluoride Vacutainers Be Used for Measurement of Co-Requested HbA1c along With Blood Glucose? - A Hospital Based Study in Northern India

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ABSTRACT

Introduction: Glycated haemoglobin (HbA1c) has been primarily used as a prognostic marker to identify average amount of plasma glucose concentration over the past 12 to 16 weeks. HbA1c is increasingly being used as diagnostic marker in assessment of diabetes mellitus patients. Mostly, the available commercial kits for HbA1c estimation require whole blood to be collected in EDTA (ethylenediaminetetraacetic acid) anticoagulant. This necessitates additional blood sample collection for glucose estimation in fluoride/potassium oxalate vial, in case of both HbA1c and blood sugar test requisition from same patient. This study was designed to determine the effect of EDTA and fluoride/potassium oxalate anticoagulants on HbA1c estimation and also to observe the variation in values of HbA1c after one-week storage at -20^0 C.

Methods: Blood samples were collected in both EDTA and fluoride/potassium oxalate vials from 280 randomly selected patients of either sex. The estimation of HbA1c was done using Latex agglutination inhibition method.

Results: There were no significant changes in HbA1c values between EDTA and fluoride/oxalate vacutainers estimated on same day as well as after seven days of sample storage at -20^0 C. The two methods, using different anticoagulants, were found to be comparable on Bland-Altman plot comparison.

Conclusions: The fluoride/oxalate vacutainer used for estimation of plasma glucose can also be used for HbA1c estimation. It can also be accounted that the avoidance of an additional vacutainer will definitely improve patient compliance and reduce the cost of resources.

Keywords: Glycated hemoglobin, HbA1c, Anticoagulant, EDTA, Fluoride/oxalate vacutainer

INTRODUCTION

Diabetes mellitus is one of the most important public health challenges of the 21st century contributing over 1.5 million deaths a year. [1] It is a growing challenge in India with estimated 8.7% diabetic population in the age group of 20 and 70 years. [2]

The proper glycemic control of the diabetic patient is a key component in the global strategy to reduce the complications and deaths related to diabetes. The United Kingdom Prospective Diabetes Study (UKPDS) and the Diabetes Control and Complications Trial (DCCT) have shown a direct relation between good glycemic control, measured by the blood concentration of glycated hemoglobin (HbA1c), and the reduction in the overall risk of developing diabetes complications. [2,3]

The diagnosis of diabetes mellitus is classically based on FPG ≥126 mg/dl (7.0 mmol/L). Fasting is defined as no caloric intake for at least 8 hours. The 2-h Post prandial blood glucose is to be performed as described by the WHO, using a glucose load containing the equivalent of 75-gm anhydrous glucose dissolved in water and a value of ≥200 mg/dl (11.1 mmol/L) during OGTT is an indicator of the presence of diabetes mellitus. In the absence of unequivocal hyperglycemia, results should be confirmed by repeat testing. The HbA1c test is an addition in the diagnostic criteria which should be performed in a laboratory using a method that is NGSP certified and standardized to the DCCT assay. Individuals are classified based on HbA1c values as normal if HbA1c value is less than 5.6%, pre-diabetic 5.7-6.4% and diabetic if greater than 6.5%. [4]

HbA1c has several advantages over blood glucose measurements. HbA1c gives an integrated index of glycaemia over the entire 120-day lifespan of the red blood cell showing the cumulative effect of hyperglycaemia over the past 2-3 months. [5] Secondly, it is a relatively convenient test, not requiring the patient to be fasting and only using a single blood sample.

Further, for an oral glucose tolerance test (OGTT), extensive pre-test preparation, appropriate diet for 3 days before the test and overnight fasting is required. Moreover, it is time-consuming, taking at least 2 hours and has poor patient compliance. HbA1c, in contrast, is not affected by prandial status and has no diurnal rhythm, allowing measurement at any time of day. [6-7] Overall reproducibility of oral glucose tolerance testing is poor, in the order of 66% variability, which can result in inappropriate labels being given to patients. [8-11]

Within-subject biological variation of HbA1c is in the order of 3.6%, compared with 5.7% for fasting plasma glucose and 16.7% for the 2-hour post-OGTT value. Analytical precision for HbA1c now approaches that for glucose, with intra- and between-laboratory analytic variability in the order of 2.5%. Unlike plasma glucose, HbA1c has high pre-analytical stability (one week at 4 °C). [11]

Mostly HbA1c estimation require sample to be collected in EDTA anticoagulant as usually specified by the manufacturer, which requires additional sample collection for blood glucose estimation in fluoride/potassium oxalate vial. Sample in EDTA tube is used to prepare hemolysate from the red blood cells. Blood sugar vacutainers contain sodium fluoride and potassium oxalate as anticoagulants which, incidentally, can also be used in preparation of hemolysate.

Further, most laboratories emphasize on freshly collected sample for estimation of HbA1c. Standardized methods for HbA1c estimation like high performance/pressure liquid chromatography (HPLC) etc. are not available in all the laboratories in India, so samples need to be stored and transported to a standardized laboratory for estimation. [12-13] Thus, the sample requires storage until it is being analyzed. It is said that HbA1c has high pre-analytical stability and is stable for 1 week when stored at 4^0 C and for 1 year when stored at -70^0 C. [12,13]

Thus, the question that arises is that whether we can use the same sodium fluoride/potassium oxalate vacutainer for the estimation of both plasma glucose as well as HbA1c? Also, can this sample be stored without any significant effect on HbA1c values? Hence, our aim in this study was to estimate HbA1c in blood samples collected in EDTA and sodium fluoride/potassium oxalate vacutainers by latex agglutination inhibition method and observe for any significant difference in the results. Further, we also observed any variation in the results of HbA1c in both types of vacutainers after one-week storage at -20^0 C.

MATERIALS AND METHODS

This was a hospital based comparative study to see the effect of anticoagulant on the estimation of HbA1c. The study was performed in the clinical

biochemistry laboratory of Vardhman Mahavir Medical College & Safdarjung Hospital, a tertiary care hospital located in New Delhi, India. Ethical clearance for the project was obtained from the institutional review board committee. After written informed consent, blood samples were collected in the EDTA and fluoride/ potassium oxalate vacutainers for estimation of HbA1c.

Our study population included 280 randomly selected patients, irrespective of age and gender, who came to the Clinical Biochemistry Laboratory for blood sugar and HbA1c estimation. These individuals were included in the study, irrespective of their diabetic status (i.e. non-diabetic, controlled or uncontrolled diabetic) or duration of diabetes. Thus, samples ranging from a non-diabetic adult to the patients with very poor glycaemic control will be included in this study. According to the ADVIA Chemistry, HbA1c method gives accurate and precise results for a range of total hemoglobin varying between 7g/dl and 23g/dl. Hence, this method is not suitable for patients with severe anemia and polycythemia. All the vials used for estimation were BD (Becton, Dickinson and Company) vacutainers manufactured by Becton- Dickinson, India. Under aseptic and antiseptic conditions, 3 ml of venous blood was collected in each sodium EDTA and sodium fluoride/potassium oxalate vacutainers.

The concentration of HbA1c and the concentration of total haemoglobin were measured and ratio is reported as percentage HbA1c.The method for percentage HbA1c used 4 reagents: Hemoglobin denaturant reagent, total hemoglobin reagent, HbA1c antibody reagent (R1), and HbA1c agglutinator reagent (R2). Initially in a pre-treatment step, the whole blood sample was mixed with the hemoglobin denaturant reagent (1:41 dilution) and incubated for 10 minutes at room temperature. The red blood cells were lysed and the hemoglobin chain was hydrolyzed by the protease present in the reagent. For the measurement of total hemoglobin, the total hemoglobin reagent was used. The method was based on the conversion of all haemoglobin derivatives into alkaline hematin in an alkaline solution of a non-ionic detergent. Fasting blood sugar levels were also determined using GOD-POD method in the autoanalyser.

The HbA1c estimation in both types of vacutainers, was done on ADVIA 2400 chemistry autoanalyzer by using ADVIA Chemistry, Siemens Kit. After the estimation of HbA1c, both types of vacutainers were stored at -20^0 C for a week. The HbA1c values were again estimated. The data was collected for further analysis.

Statistical Methods - The results were statistically analysed by graph pad prism version 6. Bland- Altman plot comparison was used to evaluate the agreement between the two methods. P value <0.05 was considered to be significant.

RESULTS

The study included 280 patients of which 103 were found to be nondiabetic, 77 were pre-diabetic and 100 patients were diabetic according to diagnostic criteria based upon fasting blood sugar levels.

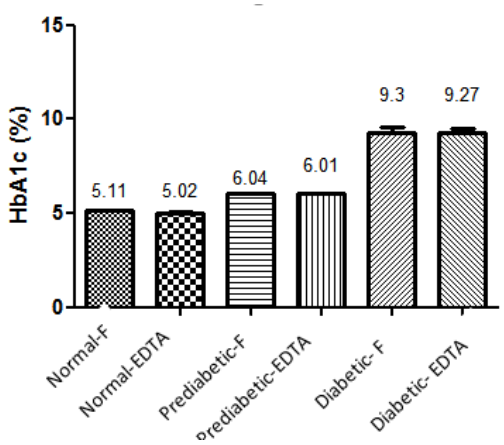

Fig. 1: Bar diagram showing HbA1c values in percentage in fluoride/potassium oxalate and EDTA vials.
Normal F: mean of Glycated hemoglobin level in fluoride/potassium oxalate vials in non-diabetic 103 individuals
Normal EDTA: mean of Glycated hemoglobin level in EDTA vials in non-diabetic 103 individuals
Pre-diabetic F: mean of Glycated hemoglobin level in fluoride/potassium oxalate vials in 77 pre-diabetic individuals
Pre-diabetic EDTA: mean of Glycated hemoglobin level in EDTA vials in 77 pre-diabetic individuals
Diabetic F: mean of Glycated hemoglobin level in fluoride/potassium oxalate vials in diabetic 100 individuals
Diabetic EDTA: mean of Glycated hemoglobin level in EDTA vials in diabetic 100 individuals

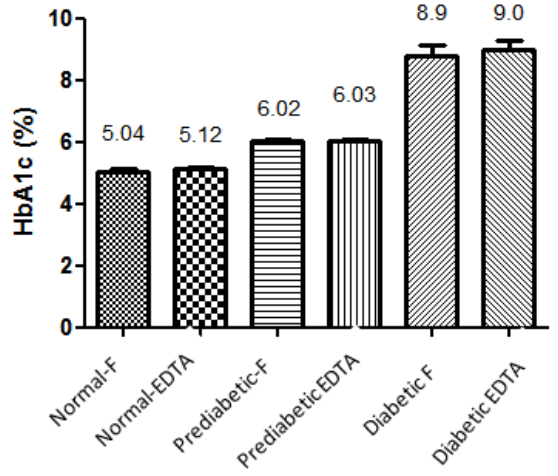

Fig. 2: Bar diagram showing HbA1c values in percentage in fluoride/potassium oxalate and EDTA vials after 7days storage.

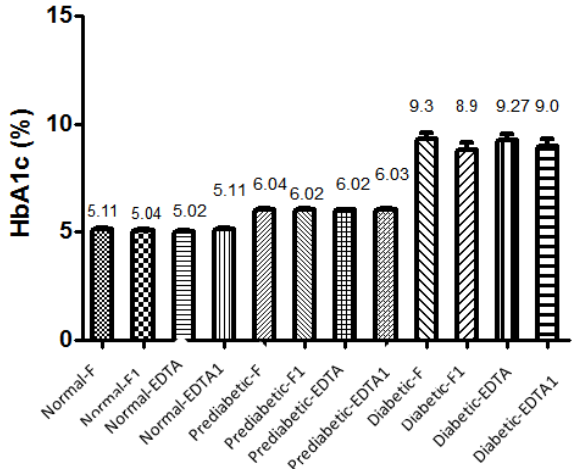

Fig. 3: Bar diagram showing mean HbA1c values in percentage in fluoride/potassium oxalate and EDTA vials on day of collection and after 7days storage.

Normal F: mean of Glycated hemoglobin level in fluoride/potassium oxalate vials in non-diabetic 103 individuals

Normal F1: mean of Glycated hemoglobin level in fluoride/potassium oxalate vials in non-diabetic 103 individuals after 7days storage.

Normal EDTA: mean of Glycated hemoglobin level in EDTA vials in non-diabetic 103 individuals

Normal EDTA1: mean of Glycated hemoglobin level in EDTA vials in non-diabetic 103 individuals after 7 days of storage.

Pre-diabetic F: mean of Glycated hemoglobin level in fluoride/potassium oxalate vials in 77 pre-diabetic individuals.

Pre-diabetic F1: mean of Glycated hemoglobin level in Fluoride/potassium oxalate vials in 77 pre-diabetic individuals after 7 days of storage.

Pre-diabetic EDTA: mean of Glycated hemoglobin level in EDTA vials in 77 pre-diabetic individuals

Pre-diabetic EDTA1: mean of Glycated hemoglobin level in EDTA vials in 77 pre-diabetic individuals after 7 days of storage

Diabetic F: mean of Glycated hemoglobin level in fluoride/potassium oxalate vials in diabetic 100 individuals

Diabetic F1: mean of Glycated hemoglobin level in fluoride/potassium oxalate vials in diabetic 100 individuals after 7 days of storage

Diabetic EDTA: mean of Glycated hemoglobin level in EDTA vials in diabetic 100 individuals

Diabetic EDTA1: mean of Glycated hemoglobin level in EDTA vials in diabetic 100 individuals after 7 days of storage

The mean HbA1c values in fluoride/potassium oxalate vacutainers were 5.1% in nondiabetic, 6.04% in pre-diabetic, and 9.3% in diabetic individuals respectively. The corresponding values were 5.02% in nondiabetic, 6.02% in pre-diabetic, and 9.27% in diabetic individuals as estimated in EDTA vacutainers (Fig. 1). Similarly, after storage for 7 days at -20^0 C, the mean HbA1c values in fluoride/ potassium oxalate vacutainers were 5.04% in nondiabetics, 6.02% in pre-diabetics and 8.9% in diabetic individuals. The corresponding values were 5.12% in nondiabetics, 6.03% in pre-diabetics and 9% in diabetic individuals estimated in EDTA vacutainers (Fig. 2). There was no significant change in HbA1c values even after seven days of storage of samples at -20^0 C (Fig. 3). Further, on Bland-Altman plot comparison between two methods, using different anticoagulant for the estimation of HbA1c, mean difference remained between 1.96SD over the range of HbA1c indicating two methods were comparable (Fig. 4A,4B,4C).

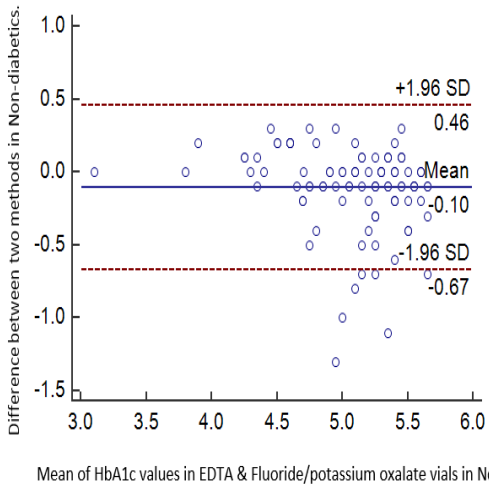

Fig. 4A: Bland- Altman Plot comparison showing mean of HbA1c in Non- diabetic individuals by two methods using fluoride/potassium oxalate and EDTA vacutainers plotted versus difference between two methods.

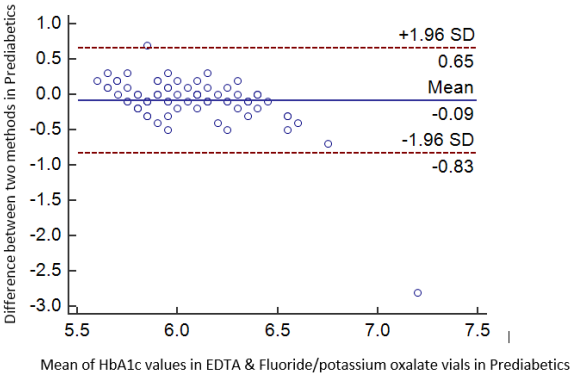

Fig. 4B: Bland- Altman Plot comparison showing mean of HbA1c in pre-diabetics by two methods using fluoride/potassium oxalate and EDTA vials plotted versus difference between two methods.

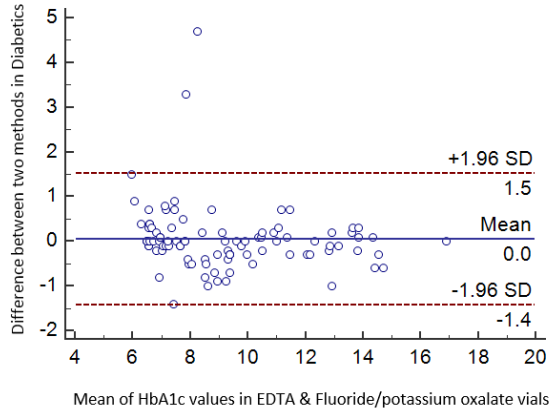

Fig. 4C: Bland- Altman Plot comparison showing mean of HbA1c in Diabetics individuals by two methods using fluoride/potassium oxalate and EDTA vials plotted versus difference of two methods.

DISCUSSION

Diabetes has been gradually and globally imposing a large economic burden on the national healthcare system of developed and developing nations. Recently, it was estimated that there were 171 million people in the world with diabetes in the year 2000 and this is projected to increase to 366 million by 2030.

HbA1c has been recommended by American Diabetic Association (ADA) as one of the tools to diagnose diabetes. [14] HbA1c is not only a useful biomarker of long-term glycaemic control but also a good predictor of lipid profile. [15] Hence, monitoring of glycaemic control using HbA1c could have additional benefits of identifying diabetes patients who are at a greater risk of cardiovascular complications.

Very often, the patients come to the diagnostic laboratory for simultaneous estimation of both blood sugar level as well as HbA1c. Most HbA1c estimation kits demand blood collection in EDTA tubes which often requires collection of additional blood. Considering this aspect of additional sample collection, the present study was designed to determine the effect of fluoride/potassium oxalate additive on HbA1c levels. There was no significant change in the HbA1c values in normal, pre-diabetic and diabetic individuals estimated in the vacutainers containing different anticoagulant (fluoride/potassium oxalate and EDTA) which was in accordance with the findings of Mailankot et al. [16]

Some previous studies with limited sample size have proved that there is no significant change in the HbA1c values estimated in the vials containing different anticoagulants. [12,13,17] A recent study by Singh B et al also shows that there is no difference in HbA1c measurements in sodium fluoride/potassium oxalate and EDTA vials.

Moreover, in the present study, the stability of HbA1c was not found to be altered in the vials containing different anticoagulants when stored at -20^0 C for 7 days which was in accordance with previous findings. [18-22] These findings eliminate the need for a separate EDTA sample for HbA1c estimation as advocated by various commercial kits, and it also ensures the stability of the collected sample for 7 days when stored at -20^0 C. Also, Bland Altman Plot comparison shows no significant differences when plotted against the average of HbA1c values obtained by two methods using different anticoagulants.

CONCLUSION

There was no significant difference in the HbA1c values in samples collected in fluoride/potassium oxalate and EDTA tubes at the time of initial estimation and after 7 days of collection under proper storage (-20^0 C). The results reveal that selections of either of these blood collection tubes do not affect HbA1c estimation. These findings justify the use of standard blood sugar vacutainers containing sodium fluoride and

potassium oxalate as anticoagulants for estimation of both glucose and HbA1c for diagnosis as well as monitoring of diabetes. Using a single vacutainer for blood glucose and HbA1c investigations, which are often co-requested, would not only require lesser amount of blood but also be convenient for the patient, which in turn, can improve patient compliance as well as reduce pre-analytical errors. It can also be accounted that the avoidance of an additional vacutainer for HbA1c testing, which indisputably adds towards a better diabetic care, will definitely reduce the cost of resources and turnaround time. Further, in a developing country like ours, where the time gap between the collection and analysis of sample is often higher than expected in the setting of remote areas, the present study points towards a reliable estimation on storage of HbA1c samples.

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Author contributions: All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission.
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