DOI: 10.7860/JCDR/2022/55585.16564

Original Article

Biochemistry Section

Efficacy of Sodium Fluoride as an Anticoagulant in the Estimation of Glycated Haemoglobin in Diabetic Patients: An Alternative to EDTA

GAYATHRI KINI[1], ANURAG YADAV[2], RAMLINGA REDDY[3], MALATHI MALA[4], NANDAKUMAR KUMAR L GOLLA[5], GOLLA YADAV ANMOL MANASWINI[6]

ABSTRACT

Introduction: Vacutainer used for collecting blood sample for plasma glucose estimation contains sodium fluoride (NaF) as an additive and for Glycated Haemoglobin (HbA1c) an Ethylenediamine Tetraacetic Acid (EDTA) tube. This necessitates additional blood to be collected for testing from the same patient at same period of time.

Aim: To evaluate difference in the HbA1c values in blood samples collected in EDTA and NaF coated vacutainers.

Materials and Methods: This cross-sectional study was conducted at Biochemistry section of central laboratory, Father Muller Medical College and Hospital, Mangalore, Karnataka, India, for the duration of two months (June to July 2018). The samples drawn for fasting plasma glucose estimation in NaF vacutainer and for HbA1c estimation in EDTA sample of same patients was included in present study. The data were entered in the statistical software Statistical Package for the Social Sciences (SPSS) version 23.0.

Results: Samples from 140 patients whose blood had been drawn for fasting plasma glucose levels and estimation of HbA1c in which 80 subjects were males and 60 females with mean age of 54.4±13.6 years were included in the present study. There were no significant changes in the mean levels of HbA1c in EDTA and NaF tubes (8.61±1.93 and 8.64±1.93, respectively).

Conclusion: Current results exclude the absolute necessity for the blood collection in EDTA vacutainers for HbA1c estimation.

Keywords: Diabetes mellitus, Ethylenediamine tetraacetic acid, Fasting plasma glucose

INTRODUCTION

Diabetes mellitus is a group of metabolic disorders due to absolute or relative insulin deficiency resulting in a condition called hyperglycaemia. Chronic hyperglycaemia is associated with long-term damage, dysfunction and failure of different organs especially eyes, kidneys, nerves and heart [1]. There is a rapid increase in the prevalence of diabetes mellitus globally approaching epidemic proportions. According to the World Diabetes Atlas, India has around 51 million people with diabetes and has been designated as the diabetes capital of the world [2,3]. There are an estimated 285 million people currently with diabetes worldwide and this number is likely to increase to 438 million by the year 2030 [4]. Wild S et al., have predicted a similar two fold escalation in the prevalence of diabetes in the world as a whole, with a maximum increase in India afflicting upto 79.4 million individuals [5]. Hence diabetes is a major healthcare problem in India.

To diagnose and monitor the treatment efficiency, fasting plasma glucose and 2nd hour plasma glucose levels, oral Glucose Tolerance Test (GTT) and HbA1c are commonly estimated in the laboratory. Vacutainer used for collecting blood sample for Fasting Blood Glucose (FBS) and Post Prandial Blood Sugar (PPBS), and GTT contains NaF as additive and potassium oxalate as the anticoagulant. American Diabetes Association (ADA) and World Health Organisation (WHO) recommended that criteria for diagnosis of diabetes mellitus should be HbA1c level equal or above 6.5% [1].

For estimation of HbA1c, techniques such as immunoassay, High Performance Liquid Chromatography (HPLC), affinity chromatography are used. Of these, HPLC has been recommended as the gold standard [6]. For all these methods, blood is collected in an EDTA vacutainer. EDTA is used to prepare haemolysate from red blood cells. NaF can also act as a haemolysing agent [6-8]. This can help in negating the necessity of drawing a second blood sample from a patient who has already given a sample for estimation of fasting blood glucose. There are limited studies conducted to rule out any interference between the types of vacutainer with HbA1c estimation [7,8]. This study aimed to evaluate if there was any difference in the HbA1c values in blood samples collected in EDTA and NaF coated vacutainers.

MATERIALS AND METHODS

This cross-sectional study was conducted at Biochemistry section of central laboratory, Father Muller Medical College and Hospital, Mangalore, Karnataka, India, for the duration of two months (June to July 2018). The ethics clearance from the Institutional Ethics Committee (IEC) was obtained prior to start of study (FMMCIEC/CCM/371/2018).

Sample size calculation: The study included the samples drawn fasting plasma glucose estimation in NaF vacutainer and for HbA1c estimation in EDTA sample at same period of time. The sample size was calculated using the SPPS sample-power calculator for the power of >80% and p-value <0.05 which was estimated as 119 minimum number, study included total of 140 samples.

Inclusion criteria: The study included the samples from diabetic patients coming for routine health check-up at the endocrine and general medicine Out Patient Department (OPD) and hence informed consent wasn't required.

Exclusion criteria: Samples which are insufficient or haemolysed and drawn at different interval of time were excluded.

Study Procedure

Data collection methodology: Fasting sample of 2 mL blood in NaF and EDTA vacutainers were collected. The HbA1c in fluoride and EDTA tubes estimated after mixing the sample 6-8 times by inverse before estimation. The samples were analysed for HbA1c in both types of samples by using the Bio-Rad Variant Turbo-II instrument based on chromatographic separation of the analytes by ion-exchange HPLC technique [9].

Tool: Instruments used: i) A three minute short program that allows the area percent determination of HbA1c using the Bio-Rad Variant Turbo-II instrument. ii) Dual program HbA1c Calibration/Diluent set.

iii) Pipettes. The Bio-Rad Variant Turbo II haemoglobin testing system uses the ion-exchange HPLC technique with a dual pump and buffer gradient to provide the well defined HbA1c peak separation that helps to deliver a good HbA1c result. The lab uses the Variant II Turbo HbA1c Kit-2.0 with interchangeable kit components for long shelf life, which provides 2500 test per kit with one calibration per cartridge. The reagent replacements included cartridge, prefilter, Buffer #1, Buffer #2, Buffer #3 (if used), and wash/diluent replacements.

Quality control: Once the cartridge had been calibrated, quality controls were run as follows:

Sample #1 Diabetes Control level 1

Sample #2 Diabetes Control level 2

STATISTICAL ANALYSIS

The data were entered in the statistical software SPSS version 23 institution licensed, and results were presented as Mean±Standard Deviation (SD). The mean difference of HbA1c between the groups was tested using student's t-test, a p-value <0.05 were considered statistically significant. Strength of association between the measurements was analysed by Pearson's correlation and the linear regression to derive the equation and model fit R^2.

RESULTS

Samples from 140 patients whose blood had been drawn for fasting blood glucose levels and estimation of HbA1c were included in the study. QC value during the analysis of the patient samples for the study; was 5.5% for Bio-Rad level 1 control and 9.5% for the Bio-Rad level 2 controls. The gender and mean age is shown in [Table/Fig-1].

Parameter		N (%)
Gender	Male	80 (57.1)
	Female	60 (42.9)
Age (years)	Mean±SD	54.4±13.6

[Table/Fig-1]: Demographic details of the patients included in study.

This study showed no significant changes in the measured levels of HbA1c when it was measured using vacutainers containing different anticoagulants; EDTA and NaF. Approximately 95% of HbA1c test requests were accompanied by the blood sugar testing request in same patient, drawn at same time [Table/Fig-2].

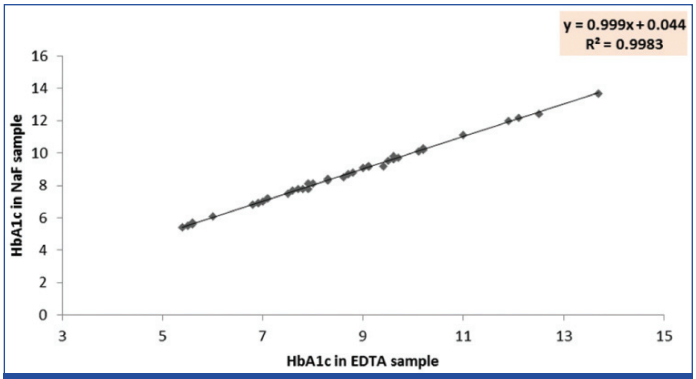
HbA1c	EDTA specimen	NaF specimen	p-value
	8.61±1.93	8.64±1.93	0.936 NS

[Table/Fig-2]: Showing the mean difference between the HbA1c measured in EDTA and NaF specimen using student t-test.
Result showing no statistical significance between the mean level of HbA1c measured in the EDTA specimen and NaF specimen. NS: Not significant, p-value <0.05 Significant

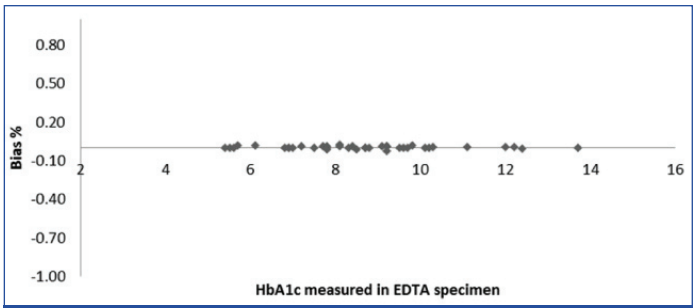
The correlation between the HbA1c measured in two different vacutainers, showed very strong strength of association between the levels with r=0.999, p<0.001. [Table/Fig-3] shows linear regression to assess the strength of association between the measured levels of HbA1c in both the vacutainers, and it showed an equation of y=0.999x+0.044 and R^2=0.998 with a strong association between the levels measured and model of fit. Difference between the measurements in both the tubes was assessed as the mean bias% which is 0.004% [Table/Fig-4]. The overall testing method accuracy and precision was acceptable during present study.

DISCUSSION

The HbA1c is an established index for average blood glucose level and is the recommended method for diagnosis of diabetes and monitoring glycaemic control [9]. Prolonged hyperglycaemia results in the production of dominant HbA1c formed by the non enzymatic binding of circulating glucose to N-terminal valine of haemoglobin β-chain. The binding is directly proportional to blood glucose levels. Hence greater levels of HbA1c is a reflection of the degree of elevated



[Table/Fig-3]: Showing a significant association between the variables with the R^2=0.998& the level of HbA1c measures in NaF is comparable with EDTA with equation of y=0.999x+0.044.



[Table/Fig-4]: Showing a very small bias % between the levels of HbA1c in NaF compared with the EDTA specimen, with mean bias % of 0.004.

blood glucose level [10]. Measurement of HbA1c concentration helps distinguish between reactive and diabetic hyperglycaemia and can safely be requested from the original glucose tube [11].

The level of fasting plasma glucose reflects the real-time glycaemic position of the patient at the time of sample collection, whereas the HbA1c concentration indicates the glycaemic status over the last 90-120 days. Both these concentrations have clinical relevance in diabetes monitoring because they provide complementary information. Simultaneous requests of HbA1c and plasma glucose levels are common, the results may cause alteration of the therapeutic regime [11].

HbA1c is an important laboratory test not only for diagnosis but also is an invaluable tool for monitoring longitudinal glycaemic control and evaluate quality of care. Thus it is useful in setting definite treatment goals and management decisions. Awareness of these and successful standardisation of HbA1c testing has led to considerable improvement in the comparability of results [12].

Using same vacutainer for two tests in turn reduces the cost of HbA1c test, which undoubtedly is a costly test in India. In present study, HbA1c was estimated in 140 blood samples collected in NaF tubes and the values compared with the results obtained in EDTA samples to determine if there was any variations in HbA1C level. Venous blood samples from suspected diabetes and confirmed diabetic patients were collected in commercially available EDTA, and NaF tubes (BD Ltd) as per the manufacturer's instructions. HbA1c was estimated using Bio-Rad variant II turbo cation exchange HPLC analyser. No significant changes in the HbA1C values were observed between the samples taken in different tubes. Similar results were revealed in study conducted by Mailankot M et al., who conducted on four samples of patients compared in the EDTA, heparin, citrate and fluoride tubes, which were comparable [7]. Other studies also concluded that HbA1c levels were not affected by the type of anticoagulant used [7-9,11].

Similarly, study by Kalita S et al., reported no significant difference in HbA1C value collected in EDTA vials and fluoride vials [13]. In concordance Kumawat R et al., documented 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°C [14]. To strengthen similar findings, Konar S et al., documented a good correlation of HbA1c from both EDTA and fluoride vials and no statistical significant difference in the mean levels HbA1c in EDTA (6.2±1.8) and fluoride vials (6.1±1.9) [15].

For routine blood investigations in the laboratory, different anticoagulants/additives are required during blood collection based on the test requested. The commercially available kits for HbA1c estimation by the HPLC/Immunoturbidometric method require blood samples to be collected in EDTA tubes. Therefore, an additional blood sample has to be usually collected from the patient [7]. Routinely, simultaneous request for HbA1c and plasma glucose measurement are common.

A high prevalence of diabetes in India with its large population would further drain the overburdened healthcare system [2,3]. Maximum utilization of available resources and ways to diagnose the condition in a cost-effective manner is thus the need of the hour, which can be achieved by cutting the cost of an additional EDTA tube for HbA1c when same can be analysed in the NaF tubes. Moreover, it would be a patient and phlebotomist friendly measure avoiding the need of collecting a second blood sample. Furthermore, if on testing for fasting/random blood glucose level it is detected that there is hyperglycaemia, HbA1c can be assessed from the same NaF tube instead of calling back the patient for another sample collection when indicated especially since HbA1c is not affected by fasting/non fasting status of the patient.

Limitation(s)

The present study had some limitations, which included, the single centric study, with limited number of samples tested. The study tested HbA1c estimation in two types of vacutainer by HPLC technique. The study can be expanded to various type of sample tubes and also different method of HbA1c estimation which include capillary method, electrophoresis, immunoturbidometric method.

CONCLUSION(S)

Current results excluded the absolute necessity for the blood collection in EDTA vacutainers for HbA1c estimation. Although the kit recommends the use of EDTA vacutainers, there is no harm in using the other anticoagulants like NaF tube which is used in estimating fasting glucose levels in diabetic patients on their follow-up. Using same vacutainer for two tests in turn reduces the cost of HbA1c test, which undoubtedly is a costly test in India.

REFERENCES

[1] Zvarova K, Zvarova Z, Callas PW, Malone-Rising D. New estimates of pre-diabetes and type 2 diabetes prevalence in Mexican Quintana Roo. Int J Diabetes Dev Ctries. 2013;33(1):08-12.
[2] Joshi SR, Parikh RM. India-diabetes capital of the world: Now heading towards hypertension. J Assoc Physicians India. 2007;55:323-24.
[3] Ramachandran A, Snehalatha C, Kapur A, Vijay V, Mohan V, Das AK, et al. High prevalence of diabetes and impaired glucose tolerance in India: National urban diabetes survey. Diabetologia. 2001;44(9):1094-101.
[4] Anjana RM, Ali MK, Pradeepa R, Deepa M, Datta M, Unnikrishnan R, et al. The need for obtaining accurate nationwide estimates of diabetes prevalence in India-rationale for a national study on diabetes. The Indian J Med Res. 2011;133(4):369.
[5] Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: Estimates for the year 2000 and projections for 2030. Diabetes Care. 2004;27(5):1047-53.
[6] Singh B, Singh K, Mahdi AA. Estimation of glycated hemoglobin sample in sodium fluoride vacutainer: A better option. J Clin Exp Invest. 2014;5(2):336-38.
[7] Mailankot M, Thomas T, Praveena P, Jacob J, Benjamin JR, Vasudevan DM. Various anticoagulants and fluoride do not affect HbA1C level. Indian J Clin Biochem. 2012;27(2):209.
[8] Sharma B, Sarmah D, Sonker P. Effect of different anticoagulants on HBA1C estimation and its stability. J Lab Physicians. 2013;5(02):143-44.
[9] Rohlfing CL, Little RR, Wiedmeyer HM, England JD, Madsen R, Harris MI, et al. Use of GHb (HbA1c) in screening for undiagnosed diabetes in the US population. Diabetes Care. 2000;23(2):187-91.
[10] Kilpatrick ES. Glycated haemoglobin in the year 2000. J Clin Pathol. 2000;53(5):335-39.
[11] Chakraborty S, Ghosh SS, Das A, Sawant P, Kallner A. Can Edta, Edta-fluoride, and buffered citrate tubes be used for measurement of HbA1c on the Bio-Rad D10? Clin Chem Lab Med. 2015;53(1):e5-8.
[12] Little RR, Rohlfing CL. The long and winding road to optimal HbA1c measurement. Clinica Chimica Acta. 2013;418(3):63-71.
[13] Kalita S, Deori R, Goswami R, Bhattacharyya K. Sodium fluoride vacutainer-An alternative to EDTA for estimation of glycated hemoglobin. Int J Sci Res. 2017;6(7):127-29.
[14] Kumawat R, Patel S, Saini V, Kaur C. Can oxalate-fluoride vacutainers be used for measurement of co-requested HbA1c along with blood glucose? - A hospital based study in Northern India. Int J Res Rev. 2019;6(6):287-93.
[15] Konar S, Karmakar A, Gupta S. Can the level of glucose & glycated hemoglobin be estimated from same container? Int J Cur Res Rev. 2020;12(8):01-04.

PARTICULARS OF CONTRIBUTORS:
1. Student, Father Muller Medical College and Hospital, Mangalore, Karnataka, India.
2. Assistant Professor, Department of Biochemistry, MNR Medical College and Hospital, Sangareddy, Telangana, India.
3. Associate Professor, Department of Biochemistry, SSIMS Davangere, Davangere, Karnataka, India.
4. Professor, Department of Biochemistry, Father Muller Medical College and Hospital, Mangalore, Karnataka, India.
5. Professor, Department of Physiology, MNR Medical College and Hospital, Sangareddy, Telangana, India.
6. Resident, Department of General Medicine, Malla Reddy Institute of Medical Sciences, Hyderabad, Telangana, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Anurag Yadav,
Sidhi Vinayaka Hospitals, H. No. 10-43, Plot No. 149, Road No. 5,
Sangareddy-502295, Telangana, India.
E-mail: yadav.anurag52@gmail.com

PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: Feb 12, 2022
- Manual Googling: Apr 22, 2022
- iThenticate Software: Apr 23, 2022 (18%)

ETYMOLOGY: Author Origin

AUTHOR DECLARATION:
- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? NA
- For any images presented appropriate consent has been obtained from the subjects. NA

Date of Submission: **Feb 10, 2022**
Date of Peer Review: **Mar 26, 2022**
Date of Acceptance: **Apr 25, 2022**
Date of Publishing: **Jul 01, 2022**