Journal of Cardiovascular Disease Research

ISSN:0975 -3583,0976-2833 VOL 15, ISSUE 03, 2024

Original research article

COMPARATIVE STUDY OF DIFFERENT ANTICOAGULANT VACUTAINERS ON ESTIMATION OF HBA_1C LEVELS

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Abstract

Introduction

The routine testing and monitoring includes the parameters such as FBS, PBBS, HbA_1C. Currently the vaccutainers or collection tubes for FBS, PBBS, RBS contains Grey capped sodium Fluoride, which inhibits glycolysis there by preventing glucose from utilization on long standing, and HbA1C is being collected in EDTA containing vacutainer with purple cap. Very few studies have done a comparison of HbA1C values by High performance liquid chromatography method using the sample collected in fluoride Grey cap and EDTA purple cap vaccutainers.

The Study aims to compare locally made vaccutainers for the analysis of HbA1C.

Materials and Methods: Blood samples were collected in EDTA and Sodium fluoride vacutainers were analyzed in D10 HPLC auto analyzer.

Results: There was no significant variation in HbA1C levels by using both EDTA and Sodium Fluoride vacutainers in study group with low, normal & high levels of HbA1C in all study group participants by using same Sodium Fluoride vacutainer. So same vacutainer can be used for both blood Sugar & HbA1C estimations, to decrease the cost burden, turnaround time, manpower, and sample quantity to patient.

Conclusions: HbA1C assay is regularly done by using EDTA vacutainer as per manufacturer's recommendations. Present study proved that Glycated HbA1c assay can also be done by using Sodium fluoride vacutainer, which is used for estimation of FBS, PLBS & RBS, so, a single vacutainer can be used for both Blood Sugar and HbA1C estimations.

ISSN:0975 -3583,0976-2833 VOL 15, ISSUE 03, 2024

Keywords: HbA1C, FBS, PLBS, RBS, EDTA, Sodium Fluoride.

Introduction

Diabetes is a chronic, metabolic disease characterized by elevated levels of blood glucose (or blood sugar), which leads over time to serious damage to the heart, blood vessels, eyes, kidneys, and nerves. The most common is type 2 diabetes, usually in adults, which occurs when the body becomes resistant to insulin or doesn't make enough insulin. In the past 3 decades the prevalence of type 2 diabetes has risen dramatically in countries of all income levels [1]. HbA1c can be used as a diagnostic test for diabetes providing that stringent quality assurance tests are in place and assays are standardized to criteria aligned to the international reference values, and there are no conditions present which preclude its accurate measurement [2]. Glycated hemoglobin (HbA1c) was initially identified as an "unusual" hemoglobin in patients with diabetes over 40 years ago [3]. It established a validated relationship between A1C and average glucose across a range of diabetes types and patient populations [4]. HbA1c was introduced into clinical use in the 1980s and subsequently has become a cornerstone of clinical practice [5].

Owing in large part to the inconvenience of measuring fasting plasma glucose levels or performing an OGTT, and day-to-day variability in glucose, an alternative to glucose measurements for the diagnosis of diabetes has long been sought. HbA1c has now been recommended by an International Committee and by the ADA as a means to diagnose diabetes [6]. Glycated Hb measurement is the best index of long-term control of blood glucose levels, and diagnosis of Diabetes mellitus [7]. Many people identified as having diabetes based on HbA1c will not have diabetes by direct glucose measurement and vice versa [2].

HbA1C assay by Ion Exchange Chromatography

HbA1c has lower isoelectric point and migrates faster than other Hb components. We can inspect chromograms for Hb variants. Measurements with great precision [2].

Table 1: Patient preparation and processing of blood sample

	Glucose	HbA1c
Patient preparation prior to collection of blood	Stringent requirements if measured for diagnostic purposes.	None.
Processing of blood	Stringent requirements for rapid processing, separation and storage of plasma or serum minimally at 4 °C.	Avoid conditions for more than 12 hr at temperatures >23 C.
		Otherwise keep at 4 °C (Stability minimally 1 week).

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When there is hyperglycemia protein in the body undergoes glycation by non-enzymatic process.

Glucose forms Schiff base with N-terminal amino group of proteins. This is reversible. Later Amadori rearrangement takes place to form ketoamines when they form irreversible. The overall reaction is called millard reaction. When once attached, glucose is not removed from hemoglobin. Therefore, it remains inside the erythrocyte, throughout the life span of RBC (120 days). The HbA1c levels reveal the mean glucose level over the previous 10-12 weeks. It is unaffected by recent food intake or recent changes in blood glucose levels [8].

Glycated hemoglobin/HbA1c is always considered as a stable indicator of glycemia for the preceding three months [9]. Its potential utility in diabetic care was first reported in 1985 WHO (T) report, and by 2010 all the major expert committee and association across the globe including the ADA has recommended HbA1c for the diagnosis of Type 2 DM, besides its role in prognosis [10]. So, the importance of HbA1c estimation in diabetes has increased manifold in recent years. Most of the commercial kits for HbA1c estimations done in EDTA vacutainers.

The normal adult hemoglobin HbA consists of 2 α and 2 β chains (α2 & β2) and make up to 97% of normal adult hemoglobin. By the process of post translational modification, HbA is modified into minor types- HbA1a, HbA1b, HbA1c, of which HbA1c is the abundant and is formed by a non-enzymatic glycation process and occurs in two steps reversible binding as an aldimine/Schiff base and irreversible binding through amadori rearrangement to form a ketoamine linkage [11]. The process of post-translational modification of HbA to HbA1c occurs at a slow rate throughout the lifespan of RBC. In 1976, HbA1c assay is proposed for the first time as a biomarker [12]. In recent years, globally India has emerged as one of the epicenters of diabetic mellitus pandemic with more than 69.2 million diabetic individuals in a total population of 1339.2 million accounting for an incidence of 5.2% [13]. Glycosylated hemoglobin (HbA1c) and fasting blood glucose are the two standard biochemical tests that are used for the diagnosis and therapeutic management of diabetes. Physicians are tailoring the diabetes therapy based on HbA1c levels in type 2 diabetes with HbA1c levels between 7.0-7.4% for monotherapy (metformin preferably), HbA1c between 8.0-8.4% for dual therapy and HbA1c between 9.0-9.4% for triple therapy [14]. Poor glycemic control was reported to increase incidence of severe hypoglycemia, diabetic ketoacidosis, and increase risk for micro-vascular complications such as nephropathy and neuropathy in type 1 diabetes [15]. Fasting blood glucose >172 mg/dl and HbA1C>9.0% were shown to be associated with microalbuminuria [16]. In view of the pivotal role of these two biochemical tests as surrogate markers for diabetic related complications, frequent monitoring is mandatory.

Sodium fluoride and potassium oxalate were the conventional anticoagulants for glucose vacutainer where in sodium fluoride acts as inhibitor of glycolytic enzymes.

Ethylenediaminetetraacetic acid (EDTA) strongly and irreversibly chelates (binds) calcium ions, preventing blood from clotting. EDTA is a polyprotic acid containing four carboxylic acid groups and two amine groups with lone-pair electrons that chelate calcium and several other metal ions. Calcium is necessary for a wide range of enzyme reactions of the coagulation cascade and its removal irreversibly prevents blood clotting

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within the collection tube [17].

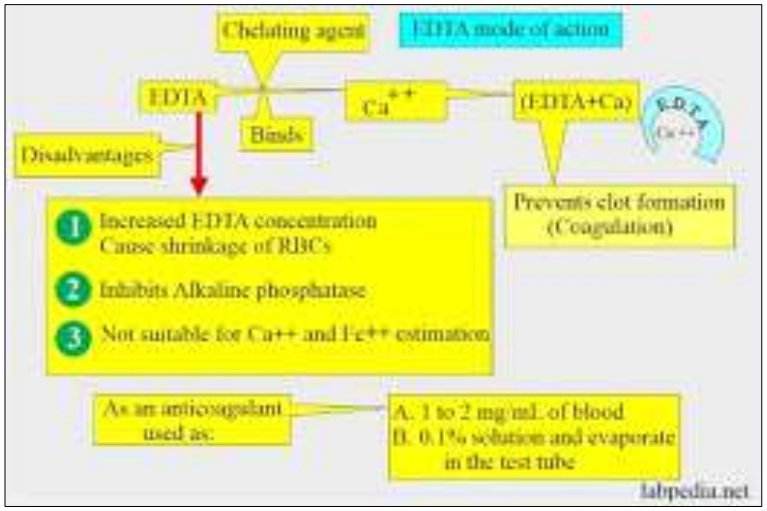

Fig 1: Anticoagulant mechanism of EDTA, NaF

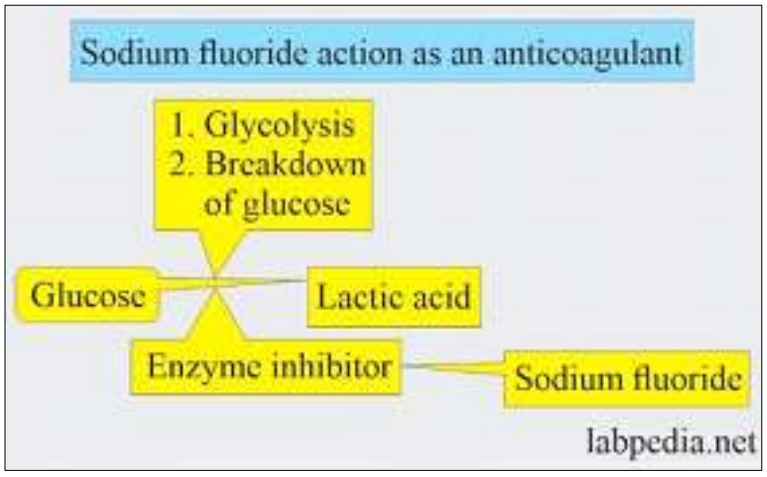

Fig 2: Anticoagulant mechanism of EDTA, NaF

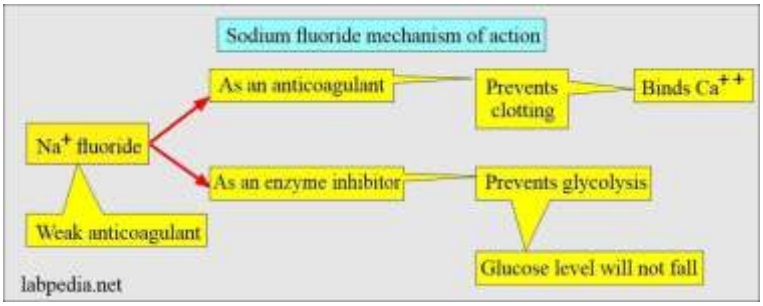

Fig 3: NaF mechanism of action

Many laboratories are using anticoagulant EDTA which is specified by the kit manufacturer for HbA1C assay. So, patients need to give sample in two vacutainers for Glucose & HbA1C assay. EDTA anticoagulant is used for hemolysate preparation from

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Red Blood Cells. Incidentally we can also use Sodium Fluoride & potassium Oxalate anticoagulant containing vacutainer also for preparation of hemolysate from Red Blood Cells.

Now our question is can we use Sodium Fluoride for measurement of both Blood Glucose and hbA1C. Is there any deviation of HbA1C values in these two types of anticoagulants used? If there is no significant difference in values, why should we use two vacutainers. So as our aim of study was to observe any difference in estimated values of HbA1c in both type vacutainers that is Sodium Fluoride, Potassium Oxalate and EDTA by HPLC method. Among all the methods HPLC method is considered as gold standard for assessing HbA1C.

Materials and Methods

This is a cross sectional observational study. A total of 200 subjects have given consent for participating in the study at Gandhi Medical College, Secunderabad, Telangana. Out of 200, 5 participants have dropped due to their personal reasons and these werenot included in the study. So, the study continued with 195 sample size only. The study has been approved by the Institutional ethical committee (Ref no: IEC/GMC/2019/03/36). Informed consent was obtained from all the study participants. Inclusion Criteria is all the samples received to the lab in both vacutainers EDTA, Sodium Fluoride.

Exclusion Criteria is insufficient quantity, hemolyzed, clotted, icteric, hyper lipidemic samples.

Whole venous blood samples were collected in NaF and EDTA anticoagulant added two separate vacutainers from each participant. The assessment of HbA1c was done by ion exchange high performance liquid chromatography (HPLC) using Bio-Rad D-10.

The results obtained were entered in tabular columns in MS Excel sheet under relevant headings. The data obtained is further statistically analyzed using IBM SPSS Statistics 20 software. Spearmen rank correlation coefficient (r) was performed to compare HbA1c levels. Paired t-test was done between HbA1c levels of both the vacutainer samples.

Results

A total of 195 samples of blood have been analysed for the levels of HbA1c from vacutainers with EDTA and NaF anticoagulants separately. Upon further gender wise analysis of the data, it has been found that out of 195 total sample size, 135 were females and 60 were males as shown in table-2 below.

Table 2: Gender wise frequency distribution

			Sex		
		Frequency	**Percent**	**Valid Percent**	**Cumulative Percent**
Valid	F	135	69.2	69.2	69.2
	M	60	30.8	30.8	100.0
	Total	195	100.0	100.0	

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The gender wise frequency distribution is shown in Figure-4 below.

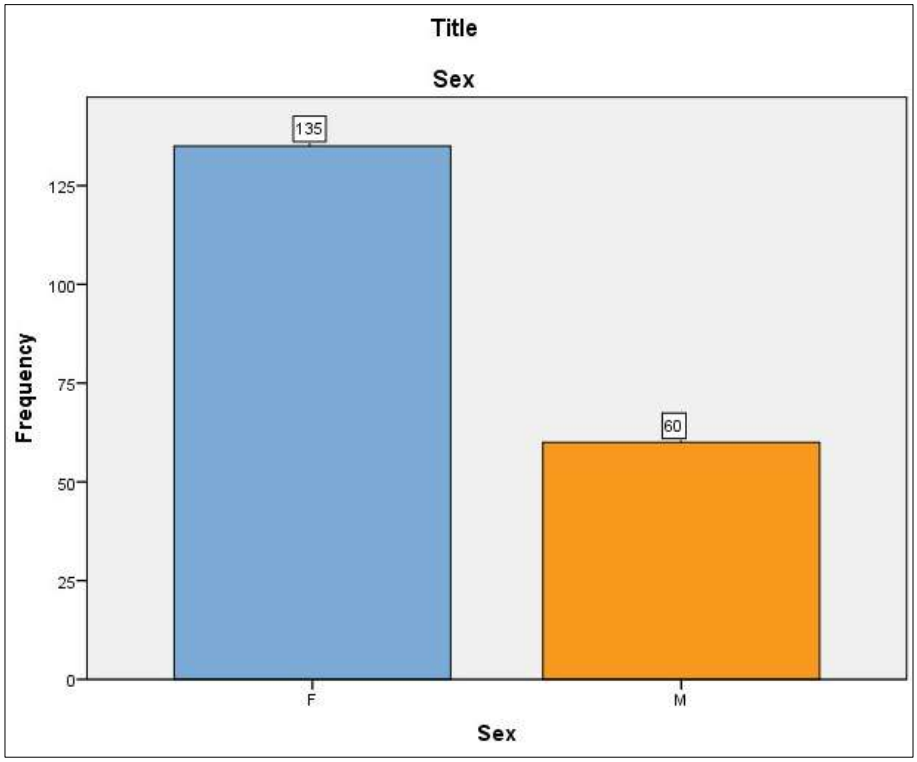

Fig 4: The gender wise frequency distribution

The frequency distribution of HbA1c estimated through EDTA vacutainer is shown in figure-5 below.

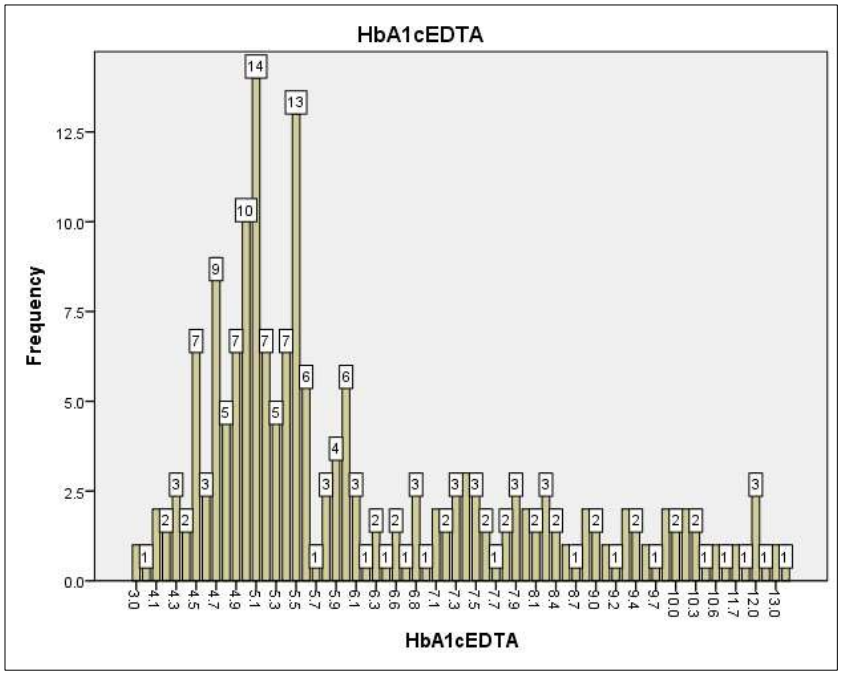

Fig 5: The frequency distribution of HbA1c estimated through EDTA

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The frequency distribution of HbA1c estimated through NaF vacutainer is shown in Fig 6 below.

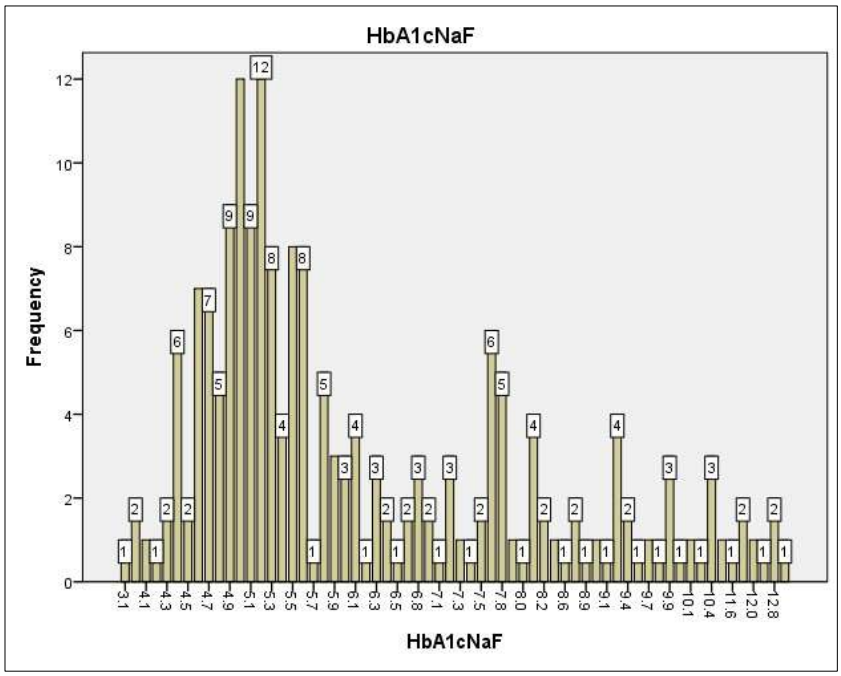

Fig 6: The frequency distribution of HbA1c estimated through NaF

The analysis of the data as per the age has shown that the mean age was 39.21 years as shown in table-3 below.

Table 3: Analysis of the data as per the age

Statistics		
Age		
N	Valid	195
	Missing	0
Mean		39.21
Std. Error of Mean		1.159
Std. Deviation		16.187

Upon sample statistical analysis, it was observed that the mean HbA1c value was 6.473±2.0847 and 6.462±2.0825 using EDTA and NaF anticoagulant respectively as shown it Table 4 below.

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Table 4: Paired samples statistics

Paired Samples Statistics					
		Mean	**N**	**Std. Deviation**	**Std. Error Mean**
Pair 1	HbA1cEDTA	6.473	195	2.0847	0.1493
	HbA1cNaF	6.462	195	2.0825	0.1491

Upon paired samples correlation, it was found that there was a positive correlation of 0.998 between the two estimation methods which was statistically significant as seen below Table 5.

Table 5: Paired samples correlations

Paired Samples Correlations				
		N	**Correlation**	**Sig.**
Pair 1	HbA1cEDTA & HbA1cNaF	195	0.998	0.000

When the samples are further statistically analysed for paired samples test, the HbA1c values estimated by the two methods were non-significant with a p-value of 0.173 as in the Table 6.

Table 6: Paired Samples Test

Paired Samples Test									
			Paired Differences				**t**	**DF**	**Sig. (2 tailed)**
		Mean	**Std. Deviation**	**Std. Error Mean**	**95% Confidence Interval of the Difference**				
					Lower	**Upper**			
Pair 1	HbA1cEDTA - HbA1cNaF	0.0113	0.1152	0.0083	-0.0050	0.0276	1.367	194	0.173

Discussion

Through this study we can confirm that the value of HbA1c estimated using different anticoagulants with a range HbA1c (4.8-16%) are not under the influence of the anticoagulant used. The mean difference between both the methods i.e. 0.173 is statistically insignificant, it will confirm that the result obtained are not having any significant impact on the value and its clinical significance. In support to other findings, there are few more studies related to comparision of HbA1c levels in sodium fluoride and EDTA vacutainers with results similar to our finding [18, 19].

Vrtaric A, *et al*. have reported no significant difference among K2-EDTA and K3-

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EDTA HbA1c estimation [20]. Another study by Sarmah D, *et al*. shown no change in the levels of HbA1c among fresh and stored whole blood upon analysis by K3-EDTA, Na-citrate, lithium-heparin and Na-fluoride/Na2 EDTA anticoagulant vials [21]. Further, Kumawat R, *et al*. also reported no significant change in HbA1c levels of samples with sodium fluoride/potassium oxalate and EDTA estimated immediately and after 7 days [22].

Our study concluded that there was no significant difference in the HbA1c values when it was measured in NaF/Na2 EDTA. As the day-by-day incidence of diabetes mellitus in India is increasing, the quantity of HbA1c test done are also significantly increasing. As the test for estimation of HbA1c levels is costlier comparatively, the sample collected for estimation of Blood glucose can be used for estimation of HbA1c levels also. If plasma glucose and HbA1c estimation if done with same sample, relatively it would reduce the cost associated with HbA1c testing.

Conclusion

The present study and previous other studies have reported that single glucose vacutainer is enough and can be safely castoff for HbA1c estimation, which is cost-effective in terms of laboratory perspective and in turn for patients also. Further studies may be done regarding comparison of other methods of HbA1c estimation like HPLC and non-HPLC based HbA1c assay.

Conflict of Interest: None.

Funding Support: Nil.

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