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Original Article / Orijinal Araştırma

Comparison of Three Different EDTA Tubes for HbA1c Measurement

HbA1c Ölçümü için Üç Farklı EDTA'lı Tüpün Karşılaştırılması

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ABSTRACT

Objective: In clinical laboratories, it is aimed to minimize the sources of error in pre- and postanalytical processes along with the analytical process. The most common errors are pre-analytical process errors, which are frequently encountered during sample collection. For quality and reliable test results, the validation of sampled tubes is important and helps reduce pre-analytical errors. This study aimed to compare the HbA1c results of the K2-EDTA BD Vacutainer tube that we currently use for HbA1c measurement in our laboratory and two different brands of EDTA tubes from another manufacturer.

Methods: Blood was drawn simultaneously from 29 patients with diabetes mellitus, 20 with prediabetes, and 29 apparently healthy individuals into three different EDTA tubes (K2-EDTA BD Vacutainer, K2-EDTA Samplix, and K3-EDTA Vacuette). The bias% was calculated for clinical significance and evaluated according to the CLIA bias% target (1.5%).

Results: The median (interquartile range) values of HbA1c levels of K2-EDTA BD Vacutainer, K2-EDTA Samplix, and K3-EDTA Vacuette tubes were the same and were 6.0% (5.5-7.6). There was no statistically significant difference between the HbA1c results of the BD Vacutainer, Samplix, and Vacuette tubes (p=0.980). The calculated bias% values were <0.3%.

Conclusion: There was no clinically or statistically significant difference between the HbA1c results of K2-EDTA BD Vacutainer, K2-EDTA Samplix, and K3-EDTA Vacuette tubes, and they were observed to meet the performance target. We believe that these 3 different brands of tubes can be used simultaneously and safely interchangeably, regardless of the distinction between K2-EDTA and K3-EDTA in HbA1c measurement.

Keywords: HbA1c, pre-analytical error, K2-EDTA, K3-EDTA

ÖZ

Amaç: Klinik laboratuvarlarda analitik süreçle birlikte preanalitik ve postanalitik süreçlerde de hata kaynaklarının en aza indirilmesi amaçlanır. En yaygın hatalar preanalitik süreç hatalarıdır ve numune toplama sırasında sıklıkla karşılaşılır. Kaliteli ve güvenilir test sonuçları için numune alınan tüplerin doğrulanması önemlidir ve preanalitik hataların azaltılmasına yardımcı olur. Halihazırda laboratuvarımızda HbA1c ölçümü için kullandığımız K2-EDTA BD Vacutainer tüp ile başka bir üreticinin iki farklı marka EDTA tüpünün HbA1c sonuçlarının karşılaştırılması amaçlandı.

Yöntem: Diabetes mellituslu 29 hasta, prediyabetli 20 hasta ve görünürde sağlıklı 29 kişiden eş zamanlı olarak üç farklı EDTA tüpüne (K2-EDTA BD Vacutainer, K2-EDTA Samplix ve K3-EDTA Vacuette) kan alındı. Bias %'si klinik anlamlılık açısından hesaplandı ve CLIA % bias hedefine (%1,5) göre değerlendirildi.

Bulgular: K2-EDTA BD Vacutainer, K2-EDTA Samplix ve K3-EDTA Vacuette tüplerinin HbA1c düzeylerinin ortanca (çeyrekler arası aralık) değerleri aynıydı ve %6,0 (5,5-7,6) idi. BD Vacutainer, Samplix ve Vacuette tüplerinin HbA1c sonuçları arasında istatistiksel olarak anlamlı fark yoktu (p=0,980). Hesaplanan % bias değerleri <%0,3 idi.

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Sonuç: K2-EDTA BD Vacutainer, K2-EDTA Samplix ve K3-EDTA Vacuette tüplerinin HbA1c sonuçları arasında klinik ve istatistiksel olarak anlamlı fark bulunmadı ve performans hedefini karşıladıkları görüldü. HbA1c ölçümünde K2-EDTA ve K3-EDTA ayrımı gözetmeksizin bu 3 farklı marka tüpün eş zamanlı ve güvenle birbirinin yerine kullanılabileceğini düşünüyoruz.

Anahtar Kelimeler: HbA1c, preanalitik hata, K2-EDTA, K3-EDTA

INTRODUCTION

The HbA1c test is used in the diagnosis of diabetes mellitus and in the follow-up of treatment.[1,2] HbA1c values have important advantages such that they do not vary from day to day like glucose, and more importantly, fasting is not required as in glucose measurement. Because the importance of early diagnosis and treatment cannot be ignored, these advantages are extremely important.[1] The clinical use of the HbA1c test should be supported by standardized results; that is, it should be accurate and comparable between different methods and clinical laboratories using these methods.[3] Therefore, test manufacturers should use analytical systems that provide results that are traceable to high-level references and that can provide analytical targets for measurement uncertainties in clinical applications of the test.[4]

In the quality system of clinical laboratories, reliability must be ensured in pre- and postanalytical activities and in the analytical phase. Because pre-analytical errors are the most frequently observed errors, their control is even more important.[5] One of the pre-analytical variables is the sample collection process. Blood samples are the most commonly used sample type in clinical laboratories, and various factors affect sample quality. One of these factors is the performance of the blood tubes.[6]

Anticoagulants, separating gels, and clot activators in blood collection tubes can affect the quality of samples and the accuracy and precision of laboratory tests. Separating gels in blood collection tubes may adsorb the analytes in the sample or tube additives may alter the analyte stability. Because of these interactions with blood samples, blood collection tubes can adversely affect the accuracy of laboratory test results as a potential source of pre-analytical errors in laboratory tests. Understanding the complex interactions between blood collection tubes and blood samples is necessary to obtain accurate laboratory test results.[7]

HbA1c measurement is usually performed in whole blood anticoagulated with ethylenediaminetetraacetic acid (EDTA). EDTA is found in blood tubes as dipotassium, tripotassium, and sodium EDTA salt. It has been accepted that the choice of EDTA salt type may affect the accuracy of cell counting and sizing.[8,9] Although the preferred tube for HbA1c measurement is K2-EDTA, there are also laboratories that use K3-EDTA. We aimed to compare the HbA1c results of the K2-EDTA BD Vacutainer tube, which we use for HbA1c measurement in our laboratory, with those of two different brands of EDTA tubes from another manufacturer.

METHODS

The study included 29 patients with diabetes mellitus, 20 patients with prediabetes, and 29 apparently healthy volunteers who were followed up in the Clinic of Endocrinology and Metabolic Diseases of University of Health Sciences Türkiye, İzmir Tepecik Training and Research Hospital. Ethics committee approval of the study was obtained from the Ethics Committee of University of Health Sciences Türkiye, İzmir Tepecik Training and Research Hospital (dated: 23.11.2022 and numbered: 2022/17/1). In accordance with the principles of the Declaration of Helsinki, written informed consent was obtained from each volunteer.

Blood samples were collected by the same bloodletting specialist according to the recommendations of the Clinical and Laboratory Standards Institute.[10] Venous blood samples were taken from the patients between 08:00 and 10:00 in the morning after at least 8 hours of fasting, using a 21-gauge needle (Becton Dickinson and Company Vacutainer® Eclipse blood collection needle, 21Gx 1-1/4 (0.8x32 mm), NJ USA).For the measurement of HbA1c, blood samples were taken simultaneously with the K2-EDTA BD Vacutainer (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) tube that we currently use in our laboratory, into the K2-EDTA Samplix (Greiner Bio-one GmbH Kremsmünster, Austria) and K3-EDTA Vacuette (Greiner Biosterone GmbH) tubes, without any order. All three tubes in our study contained spray-dried EDTA. After blood collection, the tubes were inverted 8 to 10 times according to the manufacturer's recommendation. HbA1c levels were analyzed within 1 h by ion exchange high-performance liquid chromatography (HPLC) using a BIO-RAD Variant II instrument (Bio-Rad Laboratories, Marnes-la-Coquette, France).

Statistical Analysis

Statistical analyses were performed using MedCalc Statistical Software version 20.115 (MedCalc Software Ltd, Ostend, Belgium) and IBM Statistical Package for the Social Sciences version 20.0 (IBM Co., Armonk, NY, USA). The conformity of the variables to the normal distribution was investigated using the Shapiro-Wilk test. Data are shown as median (interquartile range) because they did not show a

normal distribution. The Pearson chi-square test was used to compare the genders of the groups, the Kruskal-Wallis H test was used to compare the ages, and the Friedman test was used to compare the HbA1c results. For clinical significance, bias% values were calculated using the formula bias%=mean value of the evaluated tube mean value of the compared tube/mean value of the compared tube ×100. According to CLIA, the HbA1c bias% performance target was determined as <1.5%.[11] P<0.05 was considered statistically significant. The intertube comparison was made by Passing-Bablok regression analysis and visualized by the Bland-Altman plot.

RESULTS

The median (interquartile range) age of 78 individuals (51 women and 27 men) included in the study was determined as 49 (41-57) years. Gender and age did not differ between groups (p=0.858, p=0.803, respectively) (Table 1). The median (interquartile range) values of HbA1c levels in the K2-EDTA BD Vacutainer, K2-EDTA Samplix, and K3-EDTA Vacuette tubes were the same and were 6.0% (5.5-7.6). There was no statistically significant difference between the HbA1c results of the BD Vacutainer, Samplix, and Vacuette tubes (p=0.980). The calculated bias% values were <0.3%, and when the performance target was evaluated according to 1.5%, it was determined that the HbA1c parameter did not show a clinically significant difference between the tubes (Table 2). The regression equation of the K2-EDTA BD Vacutainer and K2-EDTA Samplix tubes showed a slope of 1.010 and an intercept of (Figure 1). The regression equation of the K2-EDTA BD Vacutainer and K3-EDTA Vacuette tubes showed a slope value of 0.999 and an intercept value of -0.105 (Figure 2).

DISCUSSION

While HbA1c provides a reliable measurement of long-term glycemia, it is also considered to be the test of choice in the monitoring and management of diabetes because it has a good correlation with the risk of complications of diabetes.[12] Although fasting blood glucose is an excellent test for "instant" glucose levels, it does not provide detailed information on how glucose levels change over time. The HbA1c test is a marker of average glucose levels over a period of approximately 3 months. Contrary to popular belief, HbA1c is used for the diagnosis, management, and monitoring of type 1 and type 2 diabetes.[13] It is also very important to reduce the errors that may occur in a parameter that is important in the diagnosis and treatment of diabetes. In our study, the HbA1c results of K2-EDTA BD Vacutainer, K2-EDTA Samplix, and K3-EDTA Vacuette tubes were found to be similar, and there was no statistically or clinically significant difference between the tubes.

In the study by Mailankot et al.[14], in which the effects of EDTA, sodium citrate, heparin, and fluoride tubes on HbA1c levels were investigated, HbA1c analysis was performed within 3 h in blood samples taken from four healthy men and two diabetic patients. No significant variation was observed between tubes in HbA1c results, and although it is recommended to use tubes with EDTA in the HbA1c kit package inserts, it was concluded that there is no harm in using other anticoagulant tubes. In the study by Chakraborty et al.[15], 104 inpatients and/or outpatients who were diabetic or screened for glycemic disorders were included to investigate the usability of EDTA, EDTA-fluoride, and buffered citrate tubes for HbA1c measurement. HbA1c levels can be measured equally well using EDTA, EDTA-fluoride, and citrate-buffered EDTA-fluoride tubes in the Bio-Rad D10 cation exchange HPLC device (Bio-Rad Laboratories, Hercules, CA, USA).

Table 1. Demographic data of the participants

	Control (n=29)	Prediabetes (n=20)	Diabetes mellitus (n=29)	p value
Gender (female/male)	18/11	13/7	20/9	0.858
Age (year)	49 (42-57)*	51 (40-58)*	49 (38-54)*	0.803

*Median (interquartile range)

Table 2. Comparison between K2-EDTA BD Vacutainer, K2-EDTA Samplix, and K3-EDTA Vacuette tubes for the HbA1c test

	Median (interquartile range)			Bias (%)			Desirablebias (%)	p value
Parameter (unit)	**BD Vacutainer**	**Samplix**	**Vacuette**	**BD Vacutainer-Samplix**	**BD Vacutainer-Vacuette**	**Samplix-Vacuette**		
HbA1c (%)	6.0 (5.5-7.6)	6.0 (5.5-7.6)	6.0 (5.5-7.6)	0.19	0.15	0.04	1.5	0.980
HbA1c (mmol/mol)	42.0 (37.0-60.3)	42.0 (36.8-60.3)	42.0 (36.8-60.0)	0.28	0.28	0	1.5	

In the study by Abdullateef and Saleh[16], HbA1c results in different anticoagulant materials (EDTA, lithium heparin, and sodium citrate) and methods (HPLC, ion exchange chromatography) were compared. The study consists of two parts. In the first part, the effect of different anticoagulant additives on HbA1c levels was investigated with 40 patients with diabetes. In the second part, the HbA1c results obtained by twusingdifferent methods in another group of 40 patients with diabetes were compared. There was no statistically significant difference between the HbA1c levels obtained using different anticoagulant materials and methods. Researchers have stated that lithium heparin or sodium citrate tubes can be used as an alternative to the EDTA tube in the measurement of HbA1c and that the ion exchange chromatography method can be one of the main methods for the analysis of HbA1c.

In the study by Ahn et al.[6], blood samples were drawn from 100 outpatients in a BD Vacutainer tube containing spray-dried K2-EDTA and an Improvacuter tube containing liquid K3-EDTA. There was no statistical difference in the HbA1c results of the tubes in which 17 hematological analytes, HbA1c, and erythrocyte sedimentation rates were analyzed. In the study by Vrtaric et al.[17], HbA1c was measured in K2-EDTA and K3-EDTA tubes in 45 outpatients. Both tubes are branded Greiner Vacuette and coated with spray-dried EDTA. No clinically or statistically significant difference was found in the HbA1c results of K2-EDTA and K3-EDTA tubes, and it has been reported that they can be safely used interchangeably in HbA1c analysis.

Similar to the studies, there was no statistically and clinically significant difference in HbA1c levels between the tubes in our study. We think that our study may contribute to the literature as it includes control, prediabetic, and diabetic patients, unlike previous studies for HbA1c.

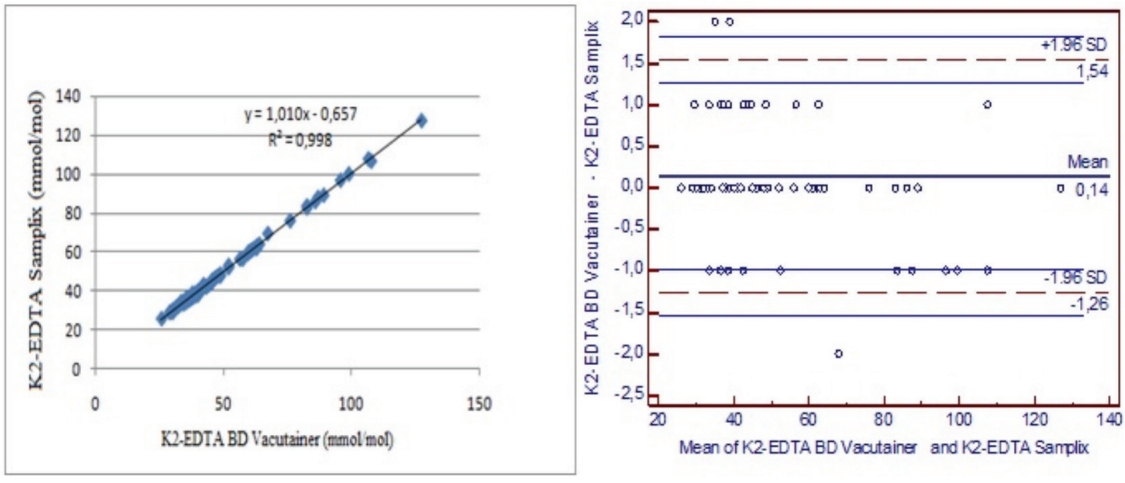

Figure 1. Passing-Bablok graph and Bland-Altman plot of K2-EDTA BD Vacutainer and K2-EDTA Samplix tubes

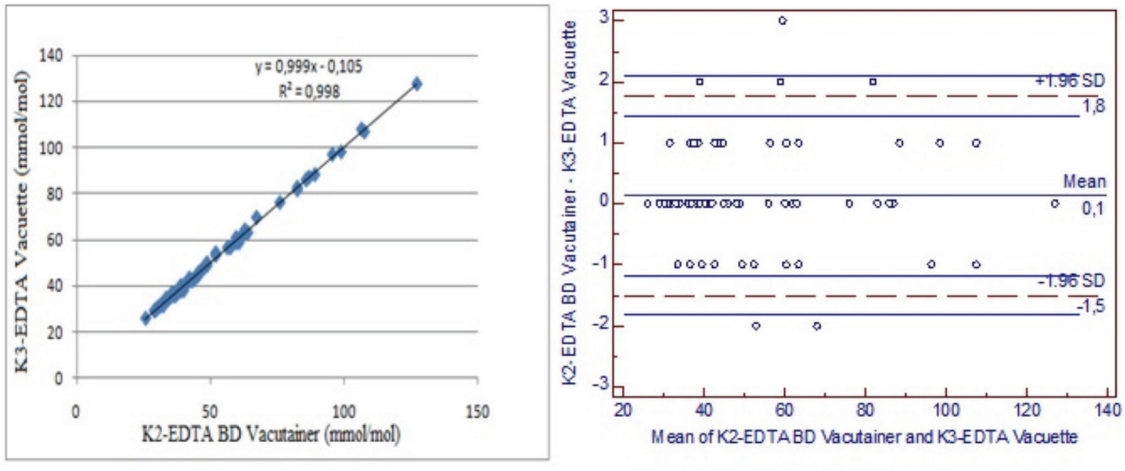

Figure 2. Passing-Bablok graph and Bland-Altman plot of the K2-EDTA BD vacuuminer and K3-EDTA vacuumette tubes

Study Limitation

A limitation of our study is that it was single-centred.

CONCLUSION

It was observed that the HbA1c results of the BD Vacutainer, Samplix, and Vacuette tubes were consistent and the performance targets were met. Coronavirus disease-2019 (COVID-19) has created a problem in the supply of blood collection tubes as well as affecting the supply chain of many products. Laboratories had to use different brands of tubes at the same time, especially during the COVID-19 period. In our study, it has been shown that these 3 different brands of tubes can be used safely simultaneously, regardless of K2-EDTA and K3-EDTA measurement in HbA1c measurement.

Ethics

Ethics Committee Approval: Ethics committee approval of the study was obtained from the Ethics Committee of University of Health Sciences Türkiye, İzmir Tepecik Training and Research Hospital (dated: 23.11.2022 and numbered: 2022/17/1).

Informed Consent: Written informed consent was obtained from each volunteer.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Concept: M.A., A.Ç., İ.K., M.M.D., H.Y.Y., Design: M.A., A.Ç., İ.K., M.T., M.K., Data Collection or Processing: M.A., M.T., M.K., M.M.D., H.Y.Y., Analysis or Interpretation: M.A., A.Ç., İ.K., M.T., M.K., Literature Search: M.A., M.T., M.K., M.M.D., H.Y.Y., Writing: M.A.

Conflict of Interest: No conflict of interest was declared by the authors.

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