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LETTER TO EDITOR / EDİTÖRE MEKTUP

Estimation of glycated hemoglobin sample in sodium fluoride vacutainer: A better option

Sodyum floridly içinde kan alımı glikolize hemoglobin örneği tahmini: Daha iyi bir seçenek

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ABSTRACT

Glycated hemoglobin (HbA1c) nowadays used as a prognostic and diagnostic marker for glycaemic control in patients with diabetes mellitus. For estimation of HbA1c, preferred sample type is whole blood collected in vacutainer containing ethylenediaminetetraacetic acid (EDTA) as anticoagulant. Sodium fluoride tubes can also be used as an alternative for estimation of HbA1c. Hence, by using fluoride vacutainer we can analyze both blood sugar and glycated hemoglobin. *J Clin Exp Invest 2014; 5 (2): 336-338*

Key words: Diabetes, glycated hemoglobin, sodium fluoride

ÖZET

Glikolize hemoglobin (HbA1c) diabetes mellituslu hastalarda şimdilerde bir öngördürücü ve tanısal belirteç olarak kullanılmaktadır. HbA1c ölçümü için tercih edilen örnek alımı EDTA içeren tam kan örneğidir. Sodyum florid tüpleri de HbA1c tayini için alternative bir yöntem olarak kullanılabilir. Böylece sodium florlü tüpler ile hem kan şekeri hem de glikolize hemoglobin ölçülebilir.

Anahtar kelimeler: Diyabet, glikolize hemoglobin, sodium florid

Dear Sir,

Glycated hemoglobin (HbA1c) was first identified in 1968 as novel hemoglobin associated with diabetes and later in 1977 it was recognized as potential indicator of glycaemic control [1]. Since then it is used for monitoring glycaemic control in patients with diabetes mellitus. Few years back, American Diabetes Association (ADA) and World Health Organization (WHO) recommended that the criteria for diagnosis of diabetes mellitus should be HbA1c level above 6.5% [2,3].

A number of methods are available for estimation of HbA1c, e.g. like Immunoassay, High Pressure Liquid Chromatography (HPLC), Affinity Chromatography etc. Among these techniques HPLC is recommended as the gold standard for estimation of glycated hemoglobin. However, in all the techniques the sample type used for the estimation of HbA1c is whole blood in EDTA (Ethylenediaminetetraacetic acid) vacutainer. The purpose of collecting sample in EDTA tube is to prepare hemolysate from the red blood cells. Blood sugar vacutainer contain sodium fluoride and potassium oxalate as an anticoagulant which can also be used in preparation of hemolysate. The question arises why we cannot use the same vacutainer for estimation of HbA1c, will there be any effect on the values? And if there is no difference in the HbA1c values of EDTA and sodium fluoride/ potassium oxalate tube, why should we use two different tubes (fluoride and EDTA) for estimation of blood sugar and HbA1c? The aim of our study was to observe the difference in the values of HbA1c if any, by using anticoagulants EDTA and sodium fluoride/ potassium oxalate.

The study was carried out in Department of Biochemistry, King George's Medical University, Lucknow Uttar Pradesh (India) after approval from institutional ethical committee. We collected blood sample from 12 patients in EDTA and sodium fluoride/ potassium oxalate vacutainer for estimation

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of HbA1c by nephelometry method. This method utilizes the interaction of antigen and antibody to directly determine the HbA1c in whole blood. Total hemoglobin and HbA1c have the same non- specific absorption rate to latex particle. When mouse antihuman HbA1c monoclonal is added, latex HbA1c-mouse antihuman HbA1c antibody complex is formed. Agglutination occurs when goat anti-mouse IgG polyclonal antibody interacts with the monoclonal antibody. The amount of agglutination is proportional to the amount of HbA1c absorbed onto the surface of latex particle which was measured by using autoanalyzer MISPA i (Agappe, India). Table 1 shows comparison of HbA1c values estimated in EDTA and sodium fluoride/ potassium oxalate tubes by nephelometry method. The SPSS 16.0 (Chicago Inc. USA) version is used for statistical analysis. Unpaired t test is used to compare the results and it indicates that the two groups are not statistically different from each other (p value 0.96) as shown in Table 1.

Table1. HbA1c results in EDTA and sodium fluoride/ potassium oxalate vacutainer by nephelometry method

HbA1c value in EDTA vacutainer	HbA1c value in Fluoride vacutainer
3.9	3.9
4.2	4.1
4.8	4.9
5.6	5.7
6.4	6.4
6.8	6.7
6.8	6.8
8.0	8.2
9.0	9.1
9.2	9.3
11.6	11.7
11.8	11.9

Unpaired t-test, p = 0.96

Then we used the standard method recommended by Diabetes Control and Complication Trial (DCCT) and national Glycohemoglobin Standardization Program (NGSP) i.e High Pressure Liquid Chromatography (HPLC) for estimation of HbA1c in 12 samples from different patients. We collected samples from 12 patients in EDTA and sodium fluoride/ potassium oxalate vacutainer and estimated HbA1c by using Bio Rad D10 (Hercules, California) analyzer based on HPLC method. In these samples, 8 samples collected in fluoride tube were not adequate hence they were diluted by diluent in the ratio recommended by the manufacturer. Table 2 shows that p value is 0.99 hence there is no difference in two groups.

Table 2. HbA1c results in EDTA and sodium fluoride/ potassium oxalate vacutainer by HPLC method

HbA1c value in EDTA vacutainer	HbA1c value in Fluoride vacutainer
5.4	5.5
5.5	5.5
5.8	5.8
6.0	6.0
6.0	6.1
6.2	6.3
7.0	7.0
9.6	9.8
11.3	11.1
11.8	11.5
12.1	12.2
12.2	12.2

Unpaired t-test, p = 0.99

Samples ranging from a non-diabetic adult to the patients with very poor glycaemic control i.e 5.5% - 12.2% were included in this concept. We used nephelometry method for assessing the difference in the values of samples collected in EDTA and sodium fluoride vacutainer and also confirmed our observation by standard HPLC technique. As shown in table 1 and 2 we compared the HbA1c values by using standard anticoagulant EDTA with sodium fluoride/ potassium oxalate and there was no significant difference in the results. In 2012 Mailankot M etal and Sharma B etal in 2013 observed no significant changes in HbA1c values collected in different tubes [4,5]. This aspect of using sodium fluoride/ potassium oxalate anticoagulant for estimation of glycated hemoglobin is not much studied and very few data is available. Hence we can conclude that HbA1c can also be estimated in sodium fluoride/ potassium oxalate vacutainer. It will be more convenient for the phlebotomist and laboratory technician to use same vacutainer for both the investigation i.e blood sugar and glycated hemoglobin. It will also be more cost effective and friendly for the patients.

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