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SODIUM FLUORIDE VACUTAINER - AN ALTERNATIVE TO EDTA FOR ESTIMATION OF GLYCATED HEMOGLOBIN.

Biochemistry

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ABSTRACT

INTRODUCTION: Glycated haemoglobin (HbA1c) has been used primarily as a marker to identify the average amount of plasma glucose concentration over prolonged period of time. As the average amount of plasma glucose increases, the fraction of glycated haemoglobin increases in a predictable way. HbA1c is nowadays used as a prognostic and diagnostic marker for glycemia control in Diabetes Mellitus patients. Most of the commercial kits for HbA1c estimation require whole blood to be collected in EDTA (ethylenediaminetetraacetic acid) anticoagulant, which needs collection of additional blood sample from the patients. If blood sample collected in sodium fluoride vial could be used to estimate blood glucose as well as glycated haemoglobin, collection of additional blood sample from the patient could be avoided.
AIMS AND OBJECTIVES: The present study was designed to determine the effect of common blood additives like sodium fluoride and EDTA on HbA1c level and also to see the variation in values of HbA1c for one week.
MATERIALS AND METHODS: Blood samples were collected in both EDTA and sodium fluoride vials from randomly selected patients of either sex. HbA1c was estimated using HPLC method.
RESULTS AND OBSERVATIONS: For all the samples, the glycated hemoglobin value of the samples collected in EDTA vials showed no significant differences with that of the same samples collected in Flouride vials. It was also observed that the glycated hemoglobin values did not alter significantly within 7 days of collection when stored at 2-8° C in both the vials.
CONCLUSION: The sodium flouride vial used for estimation of plasma glucose can also be used to estimate HbA1c as well without having to collect additional blood sample from the patients, as has to be done when using EDTA vial.

KEYWORDS:

INTRODUCTION

Diabetes mellitus has made its presence since time immemorial, and through the ages people have known it by various and diverse terminologies. Presently, the term diabetes mellitus is coined for a conglomeration of metabolic disorders of various etiologies characterized by chronic hyperglycemia and disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both [1].

Associated with serious complications of the eyes, kidneys, heart and blood vessels, and other organ systems, which may markedly impair quality of life and shorten the patient's lifespan, it is a massive, growing, silent epidemic that has the potential to cripple health services in all parts of the world.[2]

Many people are affected by diabetes worldwide and the number is rising steeply [3]. According to the recent update by the International Diabetes Federation (IDF) more than 382 million adults aged 20–79 years had diabetes in 2013. The South-East Asia (SEA) Region is estimated to have more than 72 million adults with diabetes. According to the 2013 estimates by the IDF, the highest prevalence in the SEA region is found in Mauritius (14.8%) followed by India (9.1%). [4].

Early diagnosis and efficient management of diabetes mellitus has been advocated by various associations and organizations. Among the battery of tests recommended, fasting plasma glucose (FPG), 2 hour oral glucose tolerance test (OGTT), random plasma glucose (RPG) and HbA1c are advocated. HbA1c is noteworthy because it can be used for both diagnosis of T2DM (bearing some exception), as well as in prognosis. Monitoring of glycated hemoglobin (HbA1c) levels has become more common and frequent, because it is considered a stable indicator providing an accurate measure of average glycemic control over the past three months.[5. 6]

Traditionally, HbA1c has been thought to represent average glycemia over the past 12 to 16 weeks [7]. In fact, glycation of hemoglobin occurs over the entire 120-day life span of the red blood cell [8] but within these 120 days recent glycemia has the largest influence on the HbA1c value [9]. Kinetic studies have revealed that glycemia in the recent past influences the Glycated Hemoglobin values more than the remote past [10]. Thus, mean blood glucose of past 1 month, 2 months and 3 months contributes 50%, 40% and 10% respectively to the final result. By mathematical modelling the $t_{1/2}$ of HbA1c is estimated to be 35.2 days [11]. This means that half of glycation seen during estimation has occurred in the previous 35.2 days. Thus, in theory, one patient with wildly fluctuating glucose concentrations could have the same HbA1c value as one whose glucose varies little throughout the day.

Glycated hemoglobin is formed by a posttranslational, non-enzymatic, substrate-concentration dependent irreversible process of combination of aldehyde group of glucose and other hexoses with the amino-terminal valine of the β -chain of hemoglobin [12].

Its potential utility in diabetic care was first reported in 1985 World Health Organization report [13], and by 2010 all the major expert committees and associations across the globe including the American Diabetes Association (ADA) have recommended HbA1c for the diagnosis of Type 2 DM, besides its role in prognosis [14]. Since then the importance of HbA1c estimation in diabetes has increased manifold in recent years.

Most of the commercial kits for HbA1c estimation require sample to be collected in EDTA anticoagulant. This requires additional sample collection for the patient. Also most laboratories use fresh sample for estimation of HbA1c. As standardized methods for HbA1c estimation like high performance/pressure liquid chromatography (HPLC) etc. are not available in all the laboratories in India, so samples are to be stored and transported to a standardized laboratory for estimation. 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°C and for 1 year when stored at -70°C [15, 16].

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 (Ethylene-diamine-tetra-acetic acid) vacutainer. The purpose of collecting sample in

EDTA tube is to prepare hemolysate from the red blood cells. Blood sugar vacutainers contain sodium fluoride and potassium oxalate as anticoagulants which can also be used in preparation of hemolysate. The question that arises is why can we not 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, if any, in the values of HbA1c by using anticoagulants EDTA and sodium fluoride; and to verify the stability of HbA1c in the above vials when stored at 4°C separately, 7 days apart, by checking for any variation in HbA1c values, in context to our settings.

AIMS and OBJECTIVES

- To estimate HbA1c in blood samples collected in EDTA and sodium fluoride vial by HPLC method and observe the difference, if any, in the results.
- To observe the variation, if any, in the results of HbA1c daily in both the vials for one week after storage at 2-8^{o}C.

MATERIALS AND METHODS:

The study was carried out in the Advanced Clinical Biochemistry Laboratory of the Department of Biochemistry, Assam Medical College and Hospital, Dibrugarh. Fifteen randomly selected nondiabetic as well as diabetic individuals of either sex were enrolled for the study

Sample collection: Under all aseptic and antiseptic conditions, 5 ml whole blood was drawn from the subjects and collected separately in EDTA and sodium fluoride vial. Time of blood collection was noted.

Analysis: The samples were analysed on Day 1 and repeated till the 7th day. In between the samples were stored at 4°C. HbA1c was estimated after 2-3 hours of blood collection by cation exchange HPLC based D10 Analyser (BIORAD). Results were analysed statistically by GRAPH PAD PRISM software Version 5.0. P values less than 0.05 were considered significant.

RESULTS AND OBSERVATIONS:

FIGURE 1: Figure shows the comparison of glycated hemoglobin values of the study subjects in EDTA and Flouride vials on Day 1

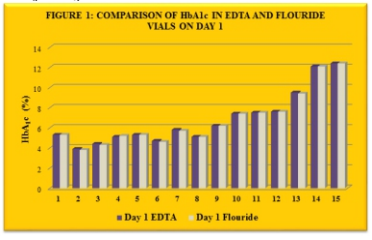


FIGURE 2: Figure shows the comparison of glycated hemoglobin values of the study subjects in EDTA and Flouride vials on Day 2

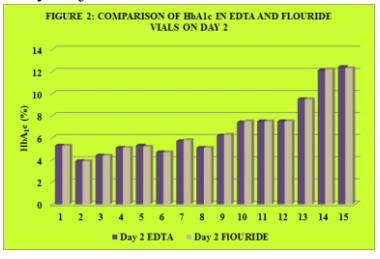


FIGURE 3: Figure shows the comparison of glycated hemoglobin values of the study subjects in EDTA and Flouride vials on Day 3

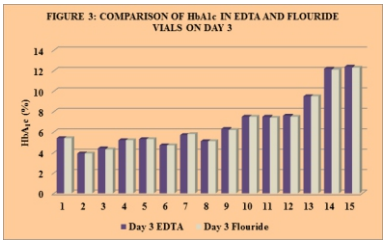


FIGURE 4: Figure shows the comparison of glycated hemoglobin values of the study subjects in EDTA and Flouride vials on Day 4

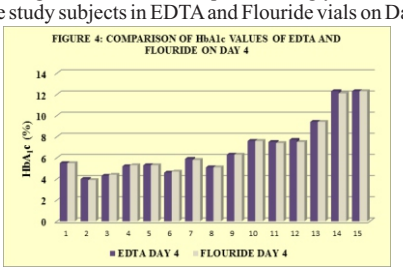
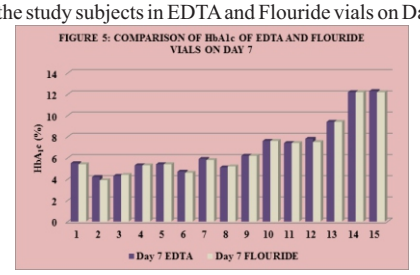
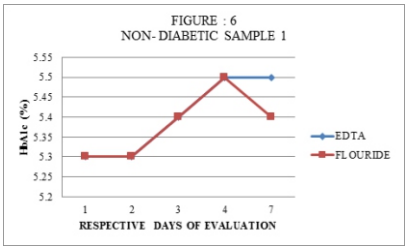
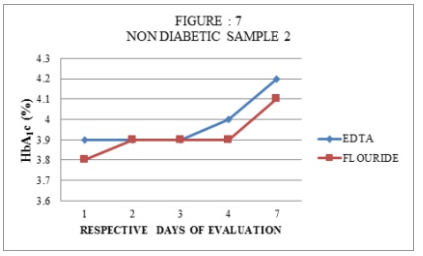


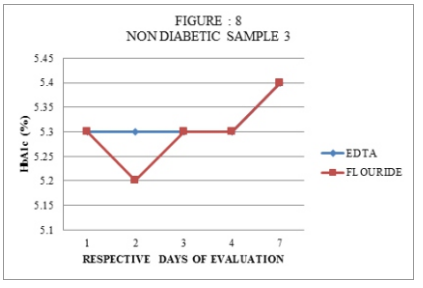
FIGURE 5: Figure shows the comparison of glycated hemoglobin values of the study subjects in EDTA and Flouride vials on Day 7



FIGURES 6 - 8: Figures depicting the comparison of glycated haemoglobin values in EDTA and fluoride vials of 3 non-diabetic individuals.







FIGURES 9 - 11: Figure showing the comparison of glycated haemoglobin values in EDTA and fluoride vials of 3 diabetic individuals.

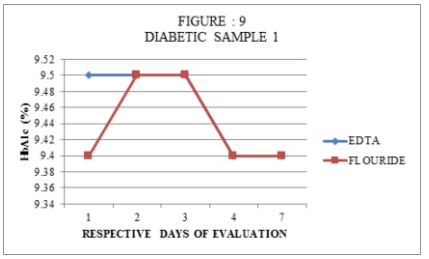


FIGURE : 10
DIABETIC SAMPLE 2

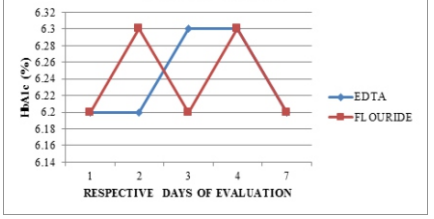
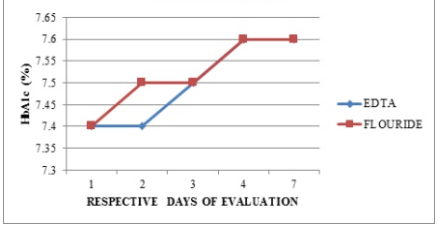


FIGURE : 11
DIABETIC SAMPLE 3



As indicated by Figures 6 to 11, no significant change in the HbA1c values were observed in the vials containing EDTA and fluoride respectively. Moreover the stability of HbA1c was not found to be altered in the vials containing the two different anticoagulants, when stored at 4°C for 7 days.

TABLE 1: Table showing the comparison of glycated hemoglobin values of nondiabetic patients in EDTA and Flouride vial respectively (Day 1, Day 2, Day 3, Day 4 and Day 7)

PATIENT SAMPLE	HbA1C (%) IN EDTA DAY 1 – 7 (MEAN ± SD)	HbA1C (%) IN FLOURIDE DAY 1 – 7 (MEAN ± SD)	p VALUE
NON DIABETIC 1	5.4 ± 0.01	5.38 ± 0.08	0.74
NON DIABETIC 2	5.76 ± 0.05	5.78 ± 0.04	0.55
NON DIABETIC 3	3.98 ± 0.13	3.88 ± 0.04	0.14
NON DIABETIC 4	4.36 ± 0.05	4.36 ± 0.05	1.00
NON DIABETIC 5	5.18 ± 0.08	5.22 ± 0.08	0.47
NON DIABETIC 6	5.32 ± 0.04	5.3 ± 0.07	0.61
NON DIABETIC 7	4.68 ± 0.04	4.66 ± 0.05	0.55
NON DIABETIC 8	5.8 ± 0.10	5.78 ± 0.04	0.69
[P > 0.05 - not significant] [P < 0.05 - significant] [P < 0.001 – highly significant]			

As was evident from Table 1, samples of each non-diabetic patient were estimated for HbA1c in EDTA and Flouride vials on Day 1, Day 2, Day 3, Day 4 and Day 7 respectively. No statistically significant difference was observed in the HbA1c values of either vial.

TABLE 2: Table showing the comparison of glycated hemoglobin values of diabetic patients in EDTA and Flouride vial respectively (Day 1, Day 2, Day 3, Day 4 and Day 7)

PATIENT SAMPLE	HbA1C (%) IN EDTA DAY 1 – 7 (MEAN ± SD)	HbA1C (%) IN FLOURIDE DAY 1 – 7 (MEAN ± SD)	p VALUE
DIABETIC 1	6.84 ± 0.05	6.84 ± 0.05	1.00
DIABETIC 2	7.50 ± 1.00	7.52 ± 0.08	0.74
DIABETIC 3	7.48 ± 0.04	7.44 ± 0.05	0.24
DIABETIC 4	7.64 ± 0.11	7.52 ± 0.04	0.06
DIABETIC 5	9.46 ± 0.05	9.44 ± 0.05	0.58
DIABETIC 6	12.18 ± 0.08	12.14 ± 0.05	0.39
DIABETIC 7	12.36 ± 0.05	12.30 ± 0.07	0.17
[P > 0.05 - not significant] [P < 0.05 - significant] [P < 0.001 – highly significant]			

As shown in Table 2, samples from each diabetic patient were estimated for HbA1c in vials containing EDTA and Flouride vials on Day 1, Day 2, Day 3, Day 4 and Day 7 respectively. No statistically significant difference was observed in the HbA1c values of either vial.

The findings herein withdraw the need for a separate EDTA sample for HbA1c estimation as advocated by various commercial kits, and also ensure the stability of the collected sample for 7 days when stored in either EDTA or fluoride vials at 4°C.

CONCLUSION

In this study, we found that samples collected in EDTA and sodium fluoride vials showed no significant difference in the results when analysed by standard HPLC technique. Moreover the values of HbA1c do not vary significantly either in EDTA or flouride for one week. Our study indicates that sodium fluoride vial, which is used for blood glucose estimation could also be used for estimating glycated hemoglobin. Using flouride vial for HbA1c estimation would not only require lesser amount of blood to be collected from the patient, it would also be convenient for the patient as well as for the laboratory staffs (phlebotomist and laboratory technician. The study thus is a pointer towards the fact that in a developing country like ours, an innovative method of glycated hemoglobin estimation in flouride vials would reduce cost of consumables like vials, and also enable reliable estimation where the time-gap between transportation and running of the test is higher than expected.

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