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 glycaemia 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 haemoglobin value of the samples collected in EDTA vials showed no significant differences with that of the same samples collected in Fluoride 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 fluoride 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: Glycated hemoglobin, Stability of HbA1c, Sodium fluoride.
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°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.
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 Fluoride vial respectively (Day 1, Day 2, Day 3, Day 4 and Day 7)**

<table>
<thead>
<tr>
<th>PATIENT SAMPLE</th>
<th>HbA1C (%) IN EDTA DAY 1 – 7 (MEAN ± SD)</th>
<th>HbA1C (%) IN FLUORIDE DAY 1 – 7 (MEAN ± SD)</th>
<th>p VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>NON DIABETIC 1</td>
<td>5.4 ± 0.01</td>
<td>5.38 ± 0.08</td>
<td>0.74</td>
</tr>
<tr>
<td>NON DIABETIC 2</td>
<td>5.76 ± 0.05</td>
<td>5.78 ± 0.04</td>
<td>0.55</td>
</tr>
<tr>
<td>NON DIABETIC 3</td>
<td>3.98 ± 0.13</td>
<td>3.88 ± 0.04</td>
<td>0.14</td>
</tr>
<tr>
<td>NON DIABETIC 4</td>
<td>4.36 ± 0.05</td>
<td>4.36 ± 0.05</td>
<td>1.00</td>
</tr>
<tr>
<td>NON DIABETIC 5</td>
<td>5.18 ± 0.06</td>
<td>5.22 ± 0.08</td>
<td>0.47</td>
</tr>
<tr>
<td>NON DIABETIC 6</td>
<td>5.32 ± 0.04</td>
<td>5.3 ± 0.07</td>
<td>0.61</td>
</tr>
<tr>
<td>NON DIABETIC 7</td>
<td>4.68 ± 0.04</td>
<td>4.66 ± 0.05</td>
<td>0.55</td>
</tr>
<tr>
<td>NON DIABETIC 8</td>
<td>5.8 ± 0.10</td>
<td>5.78 ± 0.04</td>
<td>0.69</td>
</tr>
</tbody>
</table>

As was evident from Table 1, samples of each non-diabetic patient were estimated for HbA1c in EDTA and Fluoride 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 Fluoride vial respectively (Day 1, Day 2, Day 3, Day 4 and Day 7)**

<table>
<thead>
<tr>
<th>PATIENT SAMPLE</th>
<th>HbA1C (%) IN EDTA DAY 1 – 7 (MEAN ± SD)</th>
<th>HbA1C (%) IN FLUORIDE DAY 1 – 7 (MEAN ± SD)</th>
<th>p VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>DIABETIC 1</td>
<td>6.84 ± 0.05</td>
<td>6.84 ± 0.05</td>
<td>1.00</td>
</tr>
<tr>
<td>DIABETIC 2</td>
<td>7.50 ± 1.00</td>
<td>7.52 ± 0.08</td>
<td>0.74</td>
</tr>
<tr>
<td>DIABETIC 3</td>
<td>7.48 ± 0.04</td>
<td>7.44 ± 0.05</td>
<td>0.24</td>
</tr>
<tr>
<td>DIABETIC 4</td>
<td>7.64 ± 0.11</td>
<td>7.52 ± 0.04</td>
<td>0.06</td>
</tr>
<tr>
<td>DIABETIC 5</td>
<td>9.46 ± 0.05</td>
<td>9.44 ± 0.05</td>
<td>0.58</td>
</tr>
<tr>
<td>DIABETIC 6</td>
<td>12.18 ± 0.08</td>
<td>12.14 ± 0.05</td>
<td>0.39</td>
</tr>
<tr>
<td>DIABETIC 7</td>
<td>12.36 ± 0.05</td>
<td>12.30 ± 0.07</td>
<td>0.17</td>
</tr>
</tbody>
</table>

As shown in Table 2, samples from each diabetic patient were estimated for HbA1c in vials containing EDTA and Fluoride 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 fluoride 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 fluoride 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 fluoride 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.

**REFERENCES:**