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Original Article

Effects of Anticoagulants on Fasting Blood Glucose of Diabetics and Non-Diabetics Individuals, as well as Random Blood Glucose of Apparently Healthy Individuals, Determined by One Touch Ultra Glucometer

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Abstract

In our present study, the effects of anticoagulants on the stability of blood glucose concentration were carried out using the one touch ultra glucometer. Fasting blood samples were gotten from twenty (20) diabetic and twenty (20) non-diabetic patients, and Random blood samples from twenty (20) apparently normal individuals. Our results showed that the rate at which the blood glucose decreases with time vary with specific anticoagulants. It was noticed that random blood glucose in Lithium Heparin, EDTA and Fluoride Oxalate decreased at a mean value of 7.85mg/dl, 8.1mg/dl and 2.7mg/dl every 30 minutes respectively. Also, it was noticed that fasting blood glucose in Lithium heparin, EDTA and Fluoride oxalate decreased at mean percentage values of 5.9%, 6.0% and 5.3% for diabetic blood samples. While, it decreased at mean percentage values of 24.6%, 10.9% and 5.0% respectively for non-diabetic blood samples. It was also observed that irrespective of the anticoagulant used, the random and fasting blood glucose significantly (p < 0.05) decreased steadily compared to the value before storage. These showed that irrespective of the specimen type, time of collection or type of anticoagulant used, the concentration of blood glucose remained unstable during storage. It is therefore suggested that, to obtain a reliable result, analysis for blood glucose concentrations should be carried out immediately after collection of specimen or within the shortest possible time after storage in an anticoagulant.

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Key Words: Anticoagulants, Fasting Blood Glucose, Random Blood Glucose, EDTA, Lithium Heparin, Fluoride Oxalate and Glucometer.

INTRODUCTION

Glucose can be measured in whole blood, serum or plasma (Richard, 2001). Collection of blood specimen for measurements of blood glucose level should be done on the day and time requested. This is because collection times are related to food intake, insulin treatment or both. Fasting specimen refers to blood collected after a period of fasting or no food intake, while Post-prandial specimen is blood collected usually 2 hours after a meal and Random specimen, blood sample collected at any time regardless of food consumption (Ochei and Kolhatkar, 2005). There are basically two different methods of determining glucose levels. They include the chemical method and the enzymatic method. The chemical method exploits the non specific reducing property of glucose in reactions with an indicator substance which concomitantly changes color on its reduction. While the enzymatic method, which is more specific for glucose, employs enzymes such as glucose oxidase and hexokinase (Louie, et. al., 2002). The enzymatic method has reached an advanced stage where the enzymes could be immobilized in electronic machines or devices for easier and faster analysis. The glucometer is one of such instruments which has gained wide acceptance in this part of the world. The use of glucometer for blood glucose level determination is the fastest method used in the laboratories. It is also used for self monitoring of blood glucose (SMBG) (Chernow et. al., 1996). The glucometer makes use of the enzyme glucose oxidase, impregnated in a strip.

Blood coagulates by the transformation of soluble fibrinogen into insoluble fibrin. Anticoagulants are compounds that help prevent the clotting (coagulation) of blood. Glucose estimation using plasma or whole blood requires the use of an anticoagulant. When blood is shed or collected, the cells do not die immediately. They continue to metabolize and use up glucose as a source of energy, via the glycolytic process. Glucose thus disappears from whole blood on standing over a period of time. Glycolysis can be
prevented with an enzyme inhibitor. The commonest inhibitor for this purpose is sodium fluoride which is usually used in conjunction with an anticoagulant potassium oxalate. Fluoride actually inhibits the enzyme enolase which is found in the metabolic pathway of glucose and has little effect on glucose oxidase and peroxidase enzymes. It also inhibits bacterial growth (Lawrence, et al., 2008). Other widely used anticoagulants are EDTA (Ethylene Diamine Tetra-acetate) and Heparin. When EDTA is added to blood samples, it chelates the calcium needed for blood clotting thereby preventing the formation of fibrin. It forms an insoluble calcium salt by chelation. Heparin acts by forming a complex with anti-thrombin, this complex inhibits factor X (stuart prower) and thrombin which is required in the formation of fibrin. In this study, three widely used anticoagulants (Lithium heparin, EDTA and Fluoride oxalate) were used to collect blood samples from diabetic and non-diabetic, as well as apparently normal individuals, with a view to determining the changes in blood glucose (Fasting and Random) due to the effects of the anticoagulants, using the glucometer.

MATERIALS AND METHODS
Sample type: Fasting blood samples were gotten from twenty (20) diabetic and twenty (20) non-diabetic patients, and Random blood samples from twenty (20) apparently normal individuals at Igbinedion University Teaching Hospital (IUTH).

Anticoagulants: The three (3) anticoagulants used were Fluoride oxalate, EDTA and Lithium Heparin.

Sample collection: About 6 mL of the subjects’ blood were collected via venipuncture and put into three sample bottles (2 mL in each) containing the three anticoagulants; Fluoride oxalate, EDTA and Heparin. The blood samples were then centrifuged and the plasma separated from the blood cells.

Glucose determination: The concentrations of glucose in the plasma were determined immediately after collection using the One Touch Ultra Glucometer. The procedure was repeated at every 30 minutes interval for 3 hours.

RESULTS

TABLE 1: Effects of some anticoagulants on fasting blood glucose of some apparently healthy individuals.

<table>
<thead>
<tr>
<th>ANTICOAGULANT</th>
<th>TIME (MINUTES)</th>
<th>30</th>
<th>60</th>
<th>90</th>
<th>120</th>
<th>150</th>
<th>180</th>
</tr>
</thead>
<tbody>
<tr>
<td>LITHIUM HEPARIN</td>
<td>±0.46</td>
<td>5.29</td>
<td>4.45</td>
<td>4.53</td>
<td>4.61</td>
<td>4.45</td>
<td>4.20</td>
</tr>
<tr>
<td>EDTA</td>
<td>±0.45</td>
<td>5.10</td>
<td>5.63</td>
<td>5.41</td>
<td>5.25</td>
<td>5.05</td>
<td></td>
</tr>
<tr>
<td>FLUORIDE OXALATE</td>
<td>±0.44</td>
<td>6.49</td>
<td>6.31</td>
<td>6.12</td>
<td>5.96</td>
<td>5.58</td>
<td>5.35</td>
</tr>
</tbody>
</table>

Random Blood Glucose at time zero (without anticoagulants) = 99.0 ± 2.89 mg/dl. Results are expressed as MEAN ± S.E.M, n = 20

TABLE 2: Effects of some anticoagulants on fasting blood glucose of some diabetic patients.

<table>
<thead>
<tr>
<th>ANTICOAGULANT</th>
<th>TIME (MINUTES)</th>
<th>30</th>
<th>60</th>
<th>90</th>
<th>120</th>
<th>150</th>
<th>180</th>
</tr>
</thead>
<tbody>
<tr>
<td>LITHIUM HEPARIN</td>
<td>±1.32</td>
<td>15.61</td>
<td>13.77</td>
<td>13.94</td>
<td>13.78</td>
<td>13.65</td>
<td>13.65</td>
</tr>
<tr>
<td>EDTA</td>
<td>±1.35</td>
<td>15.80</td>
<td>14.69</td>
<td>14.65</td>
<td>14.61</td>
<td>14.45</td>
<td>14.15</td>
</tr>
<tr>
<td>FLUORIDE OXALATE</td>
<td>±1.41</td>
<td>16.45</td>
<td>12.26</td>
<td>12.43</td>
<td>12.34</td>
<td>12.39</td>
<td>12.25</td>
</tr>
</tbody>
</table>

Fasting Blood Glucose at time zero (without anticoagulant) = 16.87 ± 0.94 mmol/L. Results are expressed as MEAN ± S.E.M, n = 20

TABLE 3: Effects of some anticoagulants on fasting blood glucose of some non-diabetic patients.

<table>
<thead>
<tr>
<th>ANTICOAGULANT</th>
<th>TIME (MINUTES)</th>
<th>30</th>
<th>60</th>
<th>90</th>
<th>120</th>
<th>150</th>
<th>180</th>
</tr>
</thead>
<tbody>
<tr>
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<td>5.29</td>
<td>4.45</td>
<td>4.53</td>
<td>4.61</td>
<td>4.45</td>
<td>4.20</td>
</tr>
<tr>
<td>EDTA</td>
<td>±0.45</td>
<td>5.10</td>
<td>5.63</td>
<td>5.41</td>
<td>5.25</td>
<td>5.05</td>
<td></td>
</tr>
<tr>
<td>FLUORIDE OXALATE</td>
<td>±0.44</td>
<td>6.49</td>
<td>6.31</td>
<td>6.12</td>
<td>5.96</td>
<td>5.58</td>
<td>5.35</td>
</tr>
</tbody>
</table>

Fasting Blood Glucose at time zero (without anticoagulants) = 6.89 ± 0.47 mmol/L. Results are expressed as MEAN ± S.E.M, n = 20

RESULTS AND DISCUSSION

The assay of blood glucose in samples stored in anticoagulants is a regular practice in this part of the world. When blood samples are collected they are stored in their native state by preserving them in different anticoagulants. Though their native state is preserved, the blood glucose when assayed in different anticoagulants, at different times, varies. In this study, an attempt was made to compare the changes in blood glucose level over three hours at intervals of thirty minutes using the one touch ultra glucose meter. From the results (Table 1), it was observed that the rate at which the blood glucose decreases with time vary with specific anticoagulants. It was noticed that random blood glucose in Lithium Heparin, EDTA and Fluoride Oxalate decreased at a mean value of 7.85mg/dl, 8.1mg/dl and 2.7mg/dl every 30 minutes respectively. With respect to the concentration of glucose before storage, this suggest that storage of blood using Fluoride Oxalate as an anticoagulant, tends to better preserve the glucose level over a long period of time. This may be due to the ability of fluoride ion to inhibit the activity of enolase, an enzyme in the glycolytic pathway, thereby slowing down the breakdown of glucose. It can also be observed that irrespective of the anticoagulant used, the random blood glucose significantly (p < 0.05) decreased steadily as compared to the value before storage. This actually shows that anticoagulants cannot stop, in totality, the breakdown of glucose. It can also be observed that anticoagulants do not prevent the breakdown of glucose with time, this suggest that the glucose concentration in the blood samples stored in lithium heparin tends to be comparatively more stable. As observed in Tables 1, Tables 2 and 3 also showed steady significant (p < 0.05) decrease in fasting blood glucose levels in the blood samples stored in all the anticoagulants under study. Thus, from our findings, it is obvious that irrespective of the specimen type, time of collection or type of anticoagulant, the concentration of blood glucose remained unstable during storage. It is
therefore suggested that analysis for blood glucose concentrations should be carried out immediately after collection of specimen or within the shortest possible time after storage in an anticoagulant, so as to obtain a reliable result.

CONCLUSION
From our findings, it is obvious that irrespective of the anticoagulant used, time of collection of specimen or type of specimen, the concentration of glucose is never stable. Thus, to get reliable results, glucose determination should be carried out immediately after collection of sample or within the shortest possible time.

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