Blood glucose level of plasma samples prepared from sodium fluoride and lithium heparin anticoagulants for diabetes mellitus diagnosis

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The aim of this study was to compare blood glucose levels, which were prepared from NaF and lithium heparin anticoagulants for diabetes mellitus (DM) screening test. DM-check up Clinic at Kutchap Hospital, Udonthani province collected the blood samples and filled sodium fluoride (NaF) and lithium heparin tubes (Greiner Bio-One, Austria) from 300 customers. Then, plasma was separated immediately from each blood sample tube by centrifugation and analyzed for blood glucose level by automatic analyzer, Cobas c111 automated chemistry analyzer (Roche Diagnostics GmbH, Mannhein, Germany). Four groups of samples were divided to the all sample group, normal group, prediabetic group and diabetic group according by American Diabetes Association. The result was presented as blood glucose levels of NaF plasma and lithium heparinized plasma in normal and prediabetic groups, which corresponded significantly ($p < 0.05$). This may imply that lithium heparin tube can replace NaF tube and not affect DM diagnosis when immediately analyzing blood glucose levels. In addition, lithium heparin tubes can collect blood samples for other biochemical tests. This may reduce turnaround time, cost, and mistakes from sample overload, and also labour spending on laboratory staff.

Key words: Diabetes mellitus, blood glucose, fasting blood sugar, sodium fluoride, lithium heparin.

INTRODUCTION

Clinical biochemical tests have been performed to diagnose, predict, and monitor disease for patients including annual health checkups for normal people. This investigation should provide beneficial data for the doctor to treat a patient. Sodium fluoride (NaF) is anticoagulant, which is added into blood specimens to inhibit the glycolysis of blood cells (Chan et al., 1989; Young and Bermes, 1999), and then the specimen undergoes centrifugation to separate plasma sample for fasting blood sugar (FBS) test. Recently, lithium heparin (LH) is often used for blood collection in clinical chemistry testing because plasma can be immediately separated from blood cells. Several laboratories used lithium heparin plasma for all routine biochemical testing including plasma glucose, especially emergency cases (Smith et al., 1987). Moreover, heparin usage trends to decrease spending in laboratory and medical check-up services in Thailand. Recently, lithium heparinized (LH) plasma samples have been used in urgent cases to simultaneously measure glucose and perform routine biochemical analytes in clinical chemistry testing. Shi and co-workers (2009) found that the rapid separation of a plasma sample from blood cells containing lithium heparin is better than using fluoride alone for blood glucose measurements. However, difference in anticoagulant usage for plasma preparation may affect the accuracy of the glucose measurement and lead to misinterpretation for diabetes mellitus diagnosis. For this
reason, the aim of this study was to compare fasting blood glucose concentration between NaF and lithium heparinized plasma from 300 customers who were admitted at DM-check up Clinic, Kutchap Hospital, Udonthani province.

MATERIALS AND METHODS

Subjects

DM-check up Clinic, Kutchap Hospital, Udonthani province collected blood samples and filled sodium fluoride (NaF) and lithium heparin tubes (Greiner Bio-One, Austria) from 300 customers. The health data demographic was based on their medical history and a physical examination. All subjects gave written consent, and the study protocol was approved by the Institutional Review Board for Research Ethic of Kutchap hospital, Udonthani, Thailand.

Specimen collection and handling

All samples were obtained from venous blood. For each sample, six milliliters of fasting blood samples was collected and then, divided to three milliliters of blood sample was drawn into NaF and the remaining was drawn into lithium heparinized plasma commercial tubes (Greiner Bio-One, 2002; Young and Bermes, 1994).

Blood glucose analysis

The samples were separated for plasma by centrifugation at 1,542 × g (~3,000 rpm)/5 min (room temperature) and interval time of plasma separation was 2 h for NaF treated tubes and Li heparin treated tubes (Thompat et al., 2011). Each obtained plasma sample was measured for blood glucose level, using Cobas c111 automated chemistry analyzer (Roche Diagnostics GmbH, Mannheim, Germany) in vitro test kits for the qualitative test of human serum and plasma using two levels of control material. Analyses were used and performed following procedures from the manufacturer. The room temperature ranged from 26 to 28°C under the air condition control at Laboratories of Clinical Pathology Unit, Kutchap Hospital, Udonthani, Thailand. The internal quality control for the automatic analyzer was performed using two levels of control materials purchased from manufacturer to calculate standard deviation (SD) and coefficient variance (%CV) of glucose.

Table 1. Demographic data of customers who joined in this study (N = 300).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>60±11 (16-87)</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
</tr>
<tr>
<td>- Male</td>
<td>80</td>
</tr>
<tr>
<td>- Female</td>
<td>220</td>
</tr>
<tr>
<td>DM</td>
<td>202</td>
</tr>
<tr>
<td>Hypertension</td>
<td>13</td>
</tr>
<tr>
<td>DM with hypertension</td>
<td>85</td>
</tr>
</tbody>
</table>

Table 2. Numbers of normal, prediabetic and diabetic customers who were screened for DM (N = 300).

<table>
<thead>
<tr>
<th>Sample group</th>
<th>FBG (mg/dL)</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>70-110</td>
<td>48</td>
</tr>
<tr>
<td>Prediabetic</td>
<td>&gt; 110 and &lt; 126</td>
<td>62</td>
</tr>
<tr>
<td>Diabetic</td>
<td>&gt; 126 mg/dL</td>
<td>190</td>
</tr>
</tbody>
</table>

Data analysis

Statistic analysis was performed using the SPSS computer program version 11.0 (SPSS, Chicago, IL). The Kolmogorov-Smirnov test was statistically used for normal distribution test and paired t-test statically tested for difference between the blood glucose level of NaF and lithium heparinized plasma after 8 to 12 h fasting among 4 groups: all sample group, normal group (fasting blood glucose = 70 to 110 mg/dL), prediabetic group or impaired glucose tolerance (fasting blood glucose > 110 mg/dL and < 126 mg/dL) and diabetic group (fasting blood glucose >126 mg/dL) according to the American Diabetes Association diagnostic criteria (American Diabetes Association, 2007). In the case of non normal distribution data, we used non parametric test, Wilcoxon signed rank test. The analysis of differences was judged by using α < 0.05 (two-tailed) as the significant statistic.

RESULTS

The demographic data of healthy volunteers who joined in this study is shown in Table 1. The parameters included age, gender, and clinically diagnosis including diabetes mellitus, hypertension and diabetes mellitus with hypertension. The diagnosis of clinical customers into the normal group (fasting blood glucose = 70 to 110 mg/dL), the prediabetic group or impaired glucose tolerance (fasting blood glucose > 110 mg/dL and < 126 mg/dL) and the diabetic group (fasting blood glucose > 126 mg/dL) was done according to the blood glucose level after 8 to 12 h fasting accorded to American Diabetes Association diagnostic criteria (Table 2). The fasting blood glucose level of NaF and lithium heparinized in the all sample groups and diabetic were not normally distributed, whereas, normal group and prediabetic group was normally distributed after tested by Kolmogorov-Smirnov test (data not shown). Then, we tested statically different between fasting blood glucose level of NaF and lithium heparinized plasma by Wilcoxon signed rank test for the all sample group and diabetic group, and paired t-test for normal group and prediabetic group. Blood glucose levels in NaF and lithium heparinized plasma were statically compared for each group, the all sample groups (NaF1/Haparin1), the normal group (NaF2/Haparin2), the prediabetic group (NaF3/Haparin3) and the diabetic group (NaF4/Haparin4). We found that blood glucose levels in NaF and lithium heparinized plasma from normal group (NaF2/Haparin2) and
DISCUSSION

Loss of glucose from sample containers is a serious and underappreciated problem. Decreases in glucose concentrations in whole blood ex vivo are due to glycolysis. The rate of glycolysis—reported to average 5 to 7%/h [approximately 0.6 mmol/L (10 mg/dL)] varies with the glucose concentration, temperature, leukocyte count, and other factors. Such decreases in glucose concentration will lead to missed diabetes diagnoses in the large proportion of the population who have glucose concentrations near the cut-points for diagnosis of diabetes.

This study demonstrated that blood glucose levels in NaF and lithium heparinized plasma from normal and prediabetic group were corresponded (p < 0.05) when prepared by NaF and lithium-heparin anticoagulants. This implies that lithium heparin tube can replace NaF tube and not affect DM diagnosis when immediately analyzed for blood glucose level. Because the physicians and medical technologists need to “cut-off” or separate normal from abnormal, then correlating blood glucose levels in NaF and lithium heparinized plasma and noting normal group is important. This is also important in prediabetic or impaired glucose tolerant persons to intervene with food consumption and life style. In addition, lithium heparinized plasma can be appropriated for other biological analysts more than NaF plasma, which analyses only blood glucose. Use of lithium heparin tube can save cost, reduce turnaround time (TAT) and decrease mistakes labelling of multiple blood collection tubes.

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REFERENCES


