A study to compare the plasma glucose levels obtained in sodium fluoride and citrate buffer tubes at a tertiary care hospital in Punjab

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ABSTRACT

Objectives: Recent guidelines for estimation of glucose recommend the use of citrate buffer tubes to inhibit glycolysis if the sample cannot be cooled immediately and separated within 30 min. These tubes are currently not available in India. We prepared the citrate tubes and compared the glucose results obtained with sodium fluoride tubes. Methods: Random blood samples of 44 apparently healthy volunteers were collected in three pairs of citrate buffer and sodium fluoride tubes during September to October 2013. They were labeled as 0 h, 1 h and 2 h samples indicating a delay in centrifugation to separate plasma. Glucose was analyzed on the fully auto analyzer in duplicates using glucose oxidase-peroxidase method. Results: The mean glucose concentrations at 0 h in citrate tubes were 105.8 ± 19.5 mg/dl compared to 99.6 ± 18.3 mg/dl in sodium fluoride tube. There was statistically significant difference in the glucose levels measured in plasma separated from citrate buffer tube and sodium fluoride tube at 0 h, 1 h, and 2 h. The difference between citrate and sodium fluoride tube results ranged from 6.1 mg/dl at 0 h to 7.4 mg/dl at 2 h. Glucose levels decreased significantly at 2 h in both citrate and sodium fluoride tubes. Conclusion: There is a significant decrease in glucose levels in sodium fluoride tubes even with immediate separation of plasma. There is urgent need to standardize the preanalytical conditions for glucose estimation so that effective inhibition of glycolysis can be done.

Key words: Citrate buffer, glucose estimation, glycolysis inhibition, sodium fluoride
Submission: 24-09-2014 Accepted: 23-05-2015

INTRODUCTION

The most recent guidelines of American Association for Clinical Chemistry (AACC) and American Diabetes Association (ADA) for laboratory analysis in the diagnosis and management of diabetes mellitus recommend that to minimize glycolysis, the sample tube should be placed immediately in ice water slurry and plasma should be separated from the cells within 30 min. If that cannot be achieved, a tube containing a rapidly effective glycolysis inhibitor, such as citrate buffer, should be used for collecting the sample. Tubes with only enolase inhibitors, such as sodium fluoride, should not be relied on to prevent glycolysis.[1] This is a significant change from the 2002 guidelines of AACC and ADA for glucose estimation which recommended that plasma should be separated from cells within 60 min and if that could not be done, a tube containing glycolysis inhibitor such as sodium fluoride should be used for collection of sample.[2]

These changes in guidelines reflect upon the ineffectiveness of sodium fluoride as an anti-glycolytic agent.

Fluoride inhibits enolase, which is far downstream in the glycolytic pathway. Enzymes upstream of enolase remain active and continue to metabolize glucose until substrates are exhausted. The antiglycolytic action of fluoride is delayed...
for up to 4 h and has little or no effect on the rate of glycolysis during the first 1–2 h after blood is collected. Glucose levels can fall as much as 10 mg/dl during this period.[3]

Other preservatives used to prevent glycolysis, e.g., iodoacetate, which inhibits glyceraldehyde 3 phosphate dehydrogenase, also takes up to 3 h to become effective.[4] A more effective method of inhibiting glycolysis by acidification of blood was described by Uchida et al.[5] in 1988. Acidification inhibits hexokinase and phosphofructokinase enzymes that act early in the glycolytic pathway. Glycolysis is instantly inhibited in erythrocytes, leukocytes, and platelets when the blood pH is maintained between 5.3 and 5.9 with citrate buffer. The inhibitory effect of acidification is sustainable for approximately 8 h at 25°C.[5]

Though this method was available for more than two decades, it could not catch the attention of clinical biochemists till Gambino et al.[6] in 2009 rediscovered it when they published their research showing a significant difference in glucose results obtained using citrate buffer tubes compared to sodium fluoride tubes. When blood-collection tubes were stored for 2 h and 24 h at 37°C before plasma was separated from cells, the mean glucose concentration in blood collected in commercially available tubes containing acidification reagents had decreased by 0.3% (P < 0.33) and 1.2% (P < 0.05) respectively. In contrast, when sodium fluoride was the sole inhibiting agent and the tubes were stored at 37°C, the mean glucose concentration decreased by 4.5% after 2 h (P < 0.001) and by 7.0% after 24 h (P < 0.001). Glucose concentrations in heparinized plasma sample immersed immediately into slurry of ice and water and separated within 30 min was used as the reference concentration. Another recent study has reported the statistically significant difference in the glucose levels measured in citrate buffer tubes and sodium fluoride tube.[4]

In light of the clear scientific evidence and change in recommendations for sample collection for glucose estimation, our lab also wanted to implement the use of citrate buffer tubes. The tubes are being manufactured by Terumo Corporation; Tokyo, Japan to whom the acidification method patent was assigned in 1988. It is being marketed in Europe as Venosafe® glycemia tube. The exact composition of the contents of the tube has not been disclosed.[5] The tube is not currently available in India. This study was planned with the aim of preparing citrate buffer tubes and compares the plasma glucose results obtained in these tubes with those in sodium fluoride tube.

**Methods**

Citrate buffer tubes were prepared using the composition described by Uchida et al.[5] Analytical grade reagents of Loba Chemie were used to prepare citrate buffer. Citric acid, sodium citrate, disodium EDTA and sodium fluoride were used in the ratio of 3.4:1.6:4.8:0.2. The tube contained 10 mg/ml of this buffer as an antiglycolytic agent as described by the authors.[3] Sodium fluoride tubes were prepared by using potassium oxalate and sodium fluoride. The tube contained 2 mg/ml of sodium fluoride as glycolysis inhibitor.[7]

Random blood samples of 44 apparently healthy volunteers recruited among the hospital staffs were collected for the study during September to October 2013. The participants were informed about the subject and objective of the study and their consent was taken. The participants aged 20–58 years (average 31 years), were 22 males and 22 females.

For sample collection, 2–10 nonfasting volunteers were assembled in the clinical biochemistry laboratory at a time and blood was drawn from the antecubital vein.

The blood was put in three pairs of citrate buffer and sodium fluoride tubes labeled as 0 h, 1 h, and 2 h respectively. First pair of citrate buffer and sodium fluoride tube labeled as “0 h” was centrifuged within 3 min of draw and glucose analysis was completed within 30 min. Other two pairs of samples labeled as “1 h” and “2 h” were kept at room temperature (22–25°C) for 1 h and 2 h respectively. They were centrifuged after their respective wait period and glucose levels were analyzed.

The glucose levels were estimated in duplicates on Mindray BS 400 autoanalyzer by glucose oxidase-peroxidase method with reagents, calibrators, and controls from Shenzhen Mindray biomedical electronics. The CVs of the glucose assay as determined by the duplicate assays 1 h apart were 0.92–1.5% in 10 independent data sets.

The results obtained were arranged according to the type of glycolysis inhibitor tube used and time of glucose estimation. The means and standard deviations were calculated and paired t-test was used to calculate P values.

**Results**

A statistically significant difference in the glucose levels measured in plasma separated from citrate buffer tube and sodium fluoride tube at 0 h, 1 h, and 2 h was found in the study [Table 1]. The difference between citrate and sodium fluoride tube results ranged from 6.1 mg/dl at 0 h to 7.4 mg/dl at 2 h. The plasma separated from sodium fluoride tube within 3 min (0 h) had 5.8% lower glucose results compared to those obtained from citrate buffer tube. Glucose levels decreased significantly in both citrate and sodium fluoride tubes [Table 2].
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Table 1: Mean of glucose values at different precentrifugation time

| Sample at different centrifugation times | Mean±SD glucose (mg/dl) |
|------------------------------------------|-------------------------|
| Citrate 0                                | 105.8±19.5              |
| NaF 0                                    | 99.6±18.3               |
| Citrate 1                                | 103.6±20.8              |
| NaF 1                                    | 96.7±18.3               |
| Citrate 2                                | 100±21.3                |
| NaF 2                                    | 92.5±19.7               |

Subscript 0, 1, 2 indicate centrifugation time at 0 h, 1 h and 2 h respectively. NaF: Sodium fluoride tube; Citrate: Citrate buffer tube; SD: Standard deviation

Table 2: Difference in glucose levels between citrate buffer and sodium fluoride tubes at different precentrifugation times

| Pairs (n=44) | Means of differences (mg/dl) | 95% CI     | P       |
|--------------|-----------------------------|------------|---------|
| Citrate 2-Citrate 1 | 2.18                         | 0.64-3.72  | 0.0067  |
| Citrate 2-Citrate 2 | 5.75                         | 3.65-7.85  | <0.0001 |
| Citrate 2-Citrate 3 | 3.57                         | 1.83-5.31  | 0.0002  |
| Citrate 2-NaF 1 | 6.11                         | 3.53-8.69  | <0.0001 |
| Citrate 2-NaF 2 | 6.9                          | 3.96-9.88  | <0.0001 |
| Citrate 2-NaF 3 | 7.4                          | 4.99-9.96  | 0.0001  |
| NaF 2-NaF 1 | 2.99                         | 1.79-4.19  | <0.0001 |
| NaF 2-NaF 2 | 7.11                         | 4.34-9.83  | <0.0001 |
| NaF 2-NaF 3 | 4.13                         | 1.77-6.45  | <0.001  |

Subscript 0, 1, 2 indicate centrifugation time at 0 h, 1 h and 2 h respectively. NaF: Sodium fluoride tube; Citrate: Citrate buffer tube; CI: Confidence interval

Discussion

Estimation of true plasma glucose level is important not only for diagnosis of diabetes at the earliest, but also to identify high risk patients correctly. Many studies have reported lower results for plasma glucose obtained from sodium fluoride tubes compared with immediately centrifuged serum gel separator tubes.[8-10]

This indicates delayed and inefficient inhibition of glycolysis by sodium fluoride leading to false low results. It is well-documented that small increments in fasting glucose levels may increase the risk of developing diabetes significantly. A person with fasting plasma glucose between 87 and 90 mg/dl has an age-adjusted risk of developing diabetes that is 1.81 times that of a person with fasting plasma glucose 82 mg/dl.[11] This apparently small difference of 5 mg/dl nearly doubles the risk which may be missed due to ineffective inhibition of glycolysis by sodium fluoride.

Replacement of sodium fluoride with a better and more effective glycolysis inhibitor is certainly overdue and the change in guidelines for laboratory estimation of plasma glucose is a welcome step forward. There is also some suggestion on the need to redefine the diagnostic cut-offs for diabetes using new data from samples that follow the new guidelines since all the currently defined concentration cut-offs used to classify diabetes mellitus patients were derived from plasma glucose data using sodium fluoride tubes.[7] A well-planned elaborate study done by del Pino et al.[4] has produced the results contrary to this view. In their study done in three phases, the authors first validated the use of citrate buffer tubes for glucose estimation, and then compared the results at different precentrifugation times in both citrate and sodium fluoride tube. They have found statistically significant difference in glucose results in these two types of collection tubes at 0 h, 1 h and 2 h delay in centrifugation. The mean difference in results ranged from 2.9 mg/dl at 0 h to 8.1 mg/dl at 2 h in citrate buffer and sodium fluoride tube. We have found this difference to range from 6.1 mg/dl to 7.4 mg/dl though we cannot truly compare our results with their study as the composition of commercially available citrate buffer tubes used by them may be different from the tubes prepared by us.

del Pino et al. then used the citrate tube for glucose estimation in patient samples and assessed the statistical significance of changes on diagnostic test results with reference to the cut-offs used. They have found no statistically significant increase in diagnosis of diabetes mellitus cases though they were able to obtain more reliable glucose results that were less affected by glycolysis.[4]

Our study also highlights the fact that there is statistically significant fall in glucose levels if sodium fluoride tubes are not cooled immediately and there is delay of even 3 min in separation of plasma. This decrease in glucose levels continues further lowering the results till centrifugation is done, which may be delayed for varied time intervals depending on the efficiency of sample transport system and laboratory turnaround time. A major limitation of our study is that there was significant fall in glucose results in citrate buffer tubes also though they were still statistically significantly higher than those obtained from sodium fluoride tubes. This issue can be solved by doing different experiments to improve the composition of citrate buffer tube till their commercial supply is available in India.

Conclusions

There is need to change the preanalytical conditions to prevent any loss of glucose by glycolysis in samples for glucose estimation. This only will enable estimation of true glucose levels and thus accurate diagnosis and management of diabetes mellitus.

Acknowledgments

We would like to thank all the volunteers for participating in the study. We acknowledge the support from the Biochemistry...
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laboratory personnel in the time bound centrifugation and processing of the samples.

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How to cite this article: Gupta S, Gupta AK, Verma M, Singh K, Kaur A, Chopra B, et al. A study to compare the plasma glucose levels obtained in sodium fluoride and citrate buffer tubes at a tertiary care hospital in Punjab. Int J App Basic Med Res 2016;6:50-3.

Source of Support: Nil. Conflict of Interest: None declared.