Inhibition of Glycolysis for Glucose Estimation in Plasma: Recent Guidelines and their Implications

Shalini Gupta · Harpreet Kaur

Received: 6 August 2013 / Accepted: 11 November 2013 / Published online: 22 November 2013
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Dear Sir

The most recent guidelines 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 recommendation reflect upon the inability of sodium fluoride to inhibit glycolysis effectively.

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 [2]. Transport on ice slurry and rapid separation of serum within 30 min can inhibit glycolysis without the addition of sodium fluoride and in fact works better as shown by few studies [3, 4]. But this would be impractical to expect that every sample be cooled and separated soon after collection. An effective inhibitor of glycolysis is

required to enable to laboratories to estimate true glucose levels in blood.

American Association for Clinical Chemistry (AACC) and American Diabetes Association (ADA) recommend the use of citrate buffer which is considered a rapid inhibitor of glycolysis. Effectiveness of citrate buffer in preventing glycolysis is based on scientific data which dates back to 1988 when Uchida et al. [5] first described this method of acidification of serum. 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 a citrate buffer. The inhibitory effect of acidification is sustainable for approximately 10 h at 25 °C. The method has got patent in US and Terumo Medical Corporation manufactures a blood collection tube that contains the ingredients identified in patent. These ingredients are a granular mixture of a citrate buffer, sodium fluoride and disodium EDTA with a combined mass of 7.5 g/L of blood. The tube is not available worldwide and currently being used in Europe [6].

Gambino et al. published a study in 2009 to show the effectiveness of citrate buffer tubes compared to the sodium fluoride tube which contained sodium fluoride as antiglycolytic agent and sodium oxalate as anti-coagulant. 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. The mean glucose concentration decreased by 0.3 % at 2 h and by 1.2 % at 24 h when blood was drawn into citrate buffer tubes. In contrast, the mean glucose concentration decreased by 4.6 % at 2 h and by 7.0 % at 24 h when blood was drawn into sodium fluoride tube [6]. In another recent study reported from Spain, 70 paired samples were processed for glucose in three different sets

S. Gupta (✉) · H. Kaur
Department of Biochemistry, Gian Sagar Medical College & Hospital, District Patiala, Ramnagar 140601, Punjab, India
e-mail: shalinidr2000@yahoo.com

H. Kaur
e-mail: jeenu2k18@rediffmail.com

of pre analytical conditions. Three pairs of samples were taken in citrate buffer tube and sodium fluoride-potassium oxalate tubes which were centrifuged after keeping for 0, 120 and 180 min respectively at room temperature. The authors observed that the means of differences between results obtained using citrate tubes were not significant when comparing 0, 120 and 180 min of delay. However, on comparing citrate with sodium fluoride tubes, the differences were significant and ranged from 2.9 mg/dL at 0 min to 8.1 mg/dL at 180 min. Also the differences between sodium fluoride tubes results at 0, 120 and 180 min were significant. After getting these results, their laboratory started using citrate buffer tubes for glucose samples and assessed the statistical significance of changes on diagnostic test results with reference to the cut-offs used. They did not find any 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 [7].

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. Tirosh et al. [8] have well documented that how small increments in fasting glucose levels increase the risk of developing diabetes. 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. Thus, a difference as small as 5 mg/dL nearly doubles the risk. The failure of sodium fluoride to properly control glycolysis falsely increases the within-person biological variability of plasma glucose and hinders our ability to accurately quantify the risk of developing diabetes for an individual patient [4]. The non-uniformity of preanalytical conditions for glucose estimation across clinical laboratories further adds to the problem.

The recommendations of ADA published in 2002 and WHO guidelines of 2006 clearly indicate that venous plasma is the preferred sample for glucose estimation [9, 10]. However, in most laboratory panels, serum is the most suitable sample for all other chemistries performed, and so “panel” glucose is usually serum glucose. The requirement that serum samples must be allowed to clot before serum glucose is tested significantly increases turnaround time for glucose results compared with plasma results [11]. There is also some suggestion that clotting consumes glucose [6]. Therefore, serum glucose concentrations will always be lower than plasma glucose if glycolysis in a plasma sample is inhibited immediately. The amount of the difference will vary with the glycolysis rate in the individual specimen and the time elapsed between collection and centrifugation [4]. The comparison of paired blood samples for serum & heparinized plasma collected and stored at same ambient temperature and centrifuged at same time produced higher

glucose values in plasma [6]. On the other hand studies comparing the results of serum gel separator tubes with those of fluoride tubes have reported higher glucose values in serum samples [3, 4, 12]. This is because when serum is collected in tubes with a clot activator and serum gel separator, and promptly centrifuged, glycolysis is stopped quickly due to separation of serum from the cellular components [12].

The use of serum for glucose estimation is not uncommon in India. But collection of sample in gel separator tubes and prompt centrifugation is not a routine in most of such laboratories. This means that the practice of using serum sample for glucose estimation could be leading to many wrong reports, responsible for false variation in results of an individual obtained from different laboratories as well as misclassification of at risk patients.

In light of the recent guidelines and scientific evidence, we need to reconsider the way glucose samples are handled before their analysis. Sodium fluoride is only partially effective in arresting glycolysis and should be replaced by citrate buffer which exhibit immediate action. WHO and ADA have stressed on the need of putting the plasma glucose samples immediately on ice slurry and centrifugation within 30 min [1, 10]. This may be difficult but not impossible to achieve as a short term measure for accurate reporting of glucose levels. Mechanisms can be developed to either centrifuge the glucose samples immediately in the sample collection area and transport to central lab on ice packs or they can be directly sent to laboratory on priority basis immersed in ice slurry, where immediate processing could take place. Moreover heparin tubes are available in which, glucose levels measured after immediate centrifugation has been used as the reference concentration to compare the results in various studies [6, 7]. Meanwhile efforts should be made to make citrate buffer tubes available worldwide or develop collection tubes with similar anti glycolytic action. The issue needs to be addressed in an integrated manner so that the pre analytical conditions for glucose estimation could be standardized in a suitable, efficient and cost effective manner.

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