ORIGINAL ARTICLE

Glucose level variation in blood with Sodium Fluoride and in Serum

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ABSTRACT

Background: Blood samples for glucose estimation are being taken in containers with or without some additives to get correct level. There is need to determine whether some additive is required to reduce the cost of containers.
Design: Quasi-experimental
Place & Duration: January 2017 to December 2017 at the laboratory of Shoaib Hospital, Fateh Jang, Attock.
Aim: To determine the difference in decrease in glucose level in serum and plasma(Sodium Fluoride (NaF) and EDTA)after 24 hours.
Methods: Blood Specimens (n=134) were collected from each individual in two tubes. In tube -1, the serum was separated immediately after clotting and glucose level was estimated within 30 minutes. In second tube containing NaF and EDTA, plasma was separated for glucose level estimation. Then these tubes were kept for 24 hours at room temperature before glucose estimation second time. Glucose –Liquizyme (Germany) kit was used for glucose estimation using Semi auto chemistry Analyzer (Rayto, China).
Results: There were a total of 134 patients from whom blood samples were taken. The age range was from 19 years to 65 years. Among them 78 were males and 56 were females. The mean glucose level in all the plasma samples was 100.73±30.21 mg/100ml (Mean+SD) whereas mean glucose level in serum was also 100.70±30.51 mg/100ml (p=0.833). However, the mean glucose level in plasma after 24 hours was 92.33±28.87 mg/100ml, whereas it was 96.48±29.05 mg/100ml in serum (p=<0.0001).
Conclusion: There was no significant difference of glucose level in plasma (with NaF and EDTA) and serum performed within 30 minutes. However, after 24 hours the fall in plasma glucose level was more than that of serum. Use of serum will help in reduction of extra expenses incurred with use of collection tubes with NaF and EDTA.
Keywords: Plasmaglucose level, EDTA, Sodium fluoride, Serum glucose level

INTRODUCTION

Glucose is one of the least stable analytes in blood[1]. Erythrocytes utilize glucose by glycolysis because of the presence of glycolytic enzyme complexes at their membrane[2,3]. The reduction in glucose in collected blood specimens is also caused by glycolysis due to white blood cell glycolysis[4].

Chemical agents that prevent coagulation of blood drawn from body are used when whole blood or plasma is required for analysis of its contents. Some of these agents, the anticoagulant, are heparin, salt of Ethylene Diamine Tetra Acetic Acid (EDTA), oxalates, and sodium fluoride (NaF). There is a decrease of 50% to 90% in glucose concentration in clotted and EDTA blood in 24 hours[5]. It is suggested that certain methods of glucose estimation may be affected by anticoagulants[6]. Glucose concentration keeps on decreasing if the blood is collected with NaF[7,8]. However, acidification also affects the working of the phosphorylating enzymes[4,8]. Landt demonstrated a significant decrease in glucose leve l(38%) within 8hrs in anti-coagulated blood (Heparinized)[6]. The separation of plasma from cell and chilling of the blood sample can reduce glycolysis[2, 6,9]. There are several methods available for preservation of blood specimens before determination of glucose level particularly in peripheral laboratories. There are other factors like higher temperature and compromised facilities for storage in small peripheral laboratories which cause failure in getting reproducible results. The results in such laboratories would be doubtful specifically in borderline cases. This study was planned to compare the results of glucose level in blood specimens with NaF and the use of serum in a laboratory with limited facilities.

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MATERIALS & METHODS

Specimens were collected from patients for determination of random Blood Glucose level irrespective of age and sex. These patients attended the hospital for some laboratory tests like Blood Complete Picture. After getting the consent for this study these patients delivered blood specimen for Glucose Level (Free of cost). If blood complete picture in these individuals revealed no abnormality they were included in the study[7]. Blood samples (n=134)from these subjects were used for study testing from January 2017 to December 2017. The study was conducted at Shoaib Hospital, Fateh Jang, Attock. Samples were collected in two tubes: one plain tube for clotted blood and the other tube containing NaF and EDTA.

First Tube: Serum was collected after clotting within 30 minutes and glucose level was determined. Serum was kept overnight at 22-28°C (room temperature). Then Glucose level was determined again after 24hrs.

Second Tube: Plasma was taken after centrifugation and analyzed for glucose estimation immediately (within 30

minutes). Plasma was kept overnight at 22-28°C (room temperature). Then Glucose level was determined again after 24hrs.

The serum was collected after clotting of the specimen within 30 minutes (clotting time within 20 to 30 minutes)[10]. It has been estimated that 30 minutes is the time interval in which there is not only complete clot formation occurs but also no pre-analytic variations appear in the test result[2, 11]. The samples were collected in the side room of the laboratory working area. Therefore, there was no delay in transportation and no effect of environmental temperature.

Glucose level was determined using Glucose – Liquizyme (Germany) kit. Auto hematology Analyzer (Rayto, China) was used for blood complete picture and Semi auto chemistry Analyzer (Rayto, China) was used determination of blood glucose estimation. The quality control of the equipment is being conducted by qualified technical personnel and properly documented. The same laboratory technician performed the procedure on all the study specimens.

We maintain standard operating procedures (SOPs) in the laboratory to check the Room temperatures were variation.

The hospital laboratory is enlisted with National External Quality assurance program, Pakistan (NEQAAP). Moreover, quality control and standard reagents are being regularly used during testing.

Statistical Package for Social Sciences version 21 was used for data analysis. Calculation of means, standard deviation and percentages for quantitative variableswere determined using Descriptive statistics, while paired samples t-test was applied to compare the means of glucose level in plasma and serum after 30 minutes and 24 hours, and the fall of glucose level in plasma and serum after 24 hours. When p-value was <0.05 the difference was significant.

RESULTS

There were a total of 134 patients from whom blood samples were taken. The age range was from 19 years to 65 years. Among them 78 were males and 56 were females. The mean glucose level in all the plasma samples was 100.73±30.21 mg/100ml (Mean±SD), and the mean glucose level in serum was also 100.70±30.51 mg/100ml. There was no significant difference among mean blood glucose level in plasma and serum (p=0.833). However, the mean glucose level in plasma after 24 hours was 92.33±28.87mg/100ml, whereas it was 96.48±29.05 mg/100ml in serum (p=<0.0001) [Table 1]. Similarly, there was significantly less mean change of glucose in serum after 24 hours (4.19±2.53 mg/100ml) than that of plasma glucose after 24 hours (8.46±3.93 mg/100ml)(p=<0.0001) [Table 1]

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Table 1: Blood Glucose level in plasma and serum after 30 minutes and 24 hours (n=134)

	Plasma		Serum		Glucose Fall in Plasma (After 24 hours)	Glucose fall in Serum(After 24 hours)
	After 30 min	After 24 hours	After 30 min	After 24 hours		
Range of Blood Glucose (mg/100ml)	64 - 316	59-297	65-315	60-306	1-25	1-22
Mean blood glucose (mg/100ml)	100.73	92.33	100.70	96.48	8.54	4.19
Standard Deviation	30.21	28.87	30.51	29.05	3.94	2.54
Median	98	89.5	98	94.5	9	4
SEM	2.61	2.50	2.64	2.51	0.34	0.22
95% CI (mean Blood Glucose) mg/100ml	95.62 – 105.85	95.54 – 105.87	87.44 – 97.22	91.56 – 101.40	7.87 – 9.20	3.77 – 4.62

SEM: Standard error of Mean CI= *Confidence Interval*

DISCUSSION

Glucose is among the blood components that are most frequently estimated in medical laboratories because there is a high prevalence of medical conditions that derange glucose homeostasis[6]. Among these condition diabetes mellitus does not only cripple insidiously but may be lethal.

Measurement of glucose in plasma is widely accepted as a diagnostic criterion for diabetes[7]. Its early diagnosis, successful treatment, and assessment of risk of developing diabetes depend on measurement of glucose concentration accurately[8]. Blood glucose concentration levels are fixed as cut points to classify and manage patients. It is hence very important that the concentration of glucose determined at the laboratory is as near as possible, if not exactly the same, to the actual level in the blood.

For estimation of blood glucose concentration, anticoagulants are required if whole blood or plasma is required for analysis. If glucose concentration is measured in blood with heparin, there is a linear decrease in glucose concentration due to glycolysis[6]. Various methods are, therefore, adopted to control glucose loss in blood specimens, including the use of fluoride. NaF is a weak anticoagulant but is often added as a preservative along with oxalate or EDTA. It is effective at a concentration of 2mg/ml blood. NaF inhibits enolase, thus inhibiting glycolysis. NaF and potassium oxalate are mixed in the ratio 1:3. α D glucose is converted to 2 phosphoglycerate through steps catalyzed by hexokinase, phosphohexaseisomerase, phosphofructokinase, glyceraldehyde 3 phosphate, dehydrogen phosphoglycerate kinase, and mutase. None of these enzymes is inhibited by fluoride. Glycolysis, therefore, continues uninterrupted from α D glucose to 2 phosphoglycerate, despite the addition of fluoride to the blood sample taken for estimation of glucose concentration. The concentration of glucose in the sample, therefore, continues to fall due to glycolytic activity. Conversion of 2 phospho-glycerate to phosphor-enol-pyruvate is, however,

stopped in the presence of fluoride as this step is catalyzed by enolase, the enzyme that is inhibited by fluoride. This inhibition of glycolysis, however, takes 2 – 4 hours[7,8].

In some studies, there is decrease in the mean glucose level (4.6%) after two hours and by 7.0% after twenty four hours in specimens collected in containerswith Sodium oxalate as anticoagulant and NaF as antiglycolytic agent[2, 8]. Samples with increased RBC, WBC, or platelet counts will have a greater glucose loss, and if the environmental temperature is high this loss is even greater[12]. All approaches to preserve glucose, including the use of NaF, chilling, acidification, or separating plasma from cells have disadvantages of either incomplete inhibition of glycolysis and interference by additives in the simultaneous estimation of other analytes, or being impractical[6, 7].

In view of the wide range of interpersonal and inter-sample variability, consideration of the handling of blood samples prior to glucose measurement is important. The belief that NaF has solved the pre-analytical problem is wrong. Besides being ineffective in the first two hours, as stated above, NaF may cause shrinkage and lysis of erythrocytes[6,11]. This may result in dilution of plasma by the cytosol of the erythrocytes, leading to an artificial decrease in blood glucose concentration. The hemolysis makes the specimen unsuitable for estimation of other analytes which are frequently requested along with glucose – like potassium[6]. In one study the glucose concentration in NaF/ oxalate plasma was significantly lower than the mean serum glucose concentration[11].

Various studies have revealed that a fall in blood glucose level often occur by 30 minutes after collection of blood specimen despite all recommended precautions. It is possible that in working hospital laboratory immediate analysis for some of tests but it is generally not possible to perform tests on all the tests immediately and if the test is performed later on the level of glucose keeps on decreasing with time. In the blood with anticoagulant ,level of other components to be simultaneously analyzed will also be affected by the time duration and temperature[1].

NaFkeeps on decreasing blood glucose level as it is known that NaF inhibitsenolase.This enzyme acts in glycolytic pathway[1,2,8].However, acidification blocks the function of the hexokinase and phosphofructokinase resulting in prevention of glycolysis[4,8]. As anticoagulants interferes some methods of glucose and other analytes, it is preferable to use serum for such estimation[6]. However, if serum is separated immediately after clotting, such interference can be avoided during analysis of glucose and these analytes.

CONCLUSION

There was no significant difference of glucose level in plasma (with NaF and EDTA) and serum if performed immediately within 30 minutes. However, after 24 hours, there was significantly more fall in plasma glucose level than that of serum. Use of serum will help in reduction of extra expenses incurred with the use of collection tube with NaF and EDTA.

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