PRE-ANALYTICAL VARIATION IN GLUCOSE CONCENTRATION DUE TO ATMOSPHERIC TEMPERATURE & CLOT IN BLOOD SPECIMENS

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ABSTRACT

Objective: To determine the effect of temperature and contact of clot with serum on laboratory results of glucose concentration in blood.

Study Design: Quasi-experimental study.

Place and Duration of Study: December 2014 to August 2015 at the laboratory of Shoaib Hospital, Fateh Jang, Attock Pakistan.

Material and Methods: Samples were collected for estimation of blood glucose (Random) concentration from patients reporting to the hospital. Blood specimens (n=94) of such volunteers were analyzed for glucose level. Each sample was put up in five tubes. When the blood clotted the serum from tube-1 was analyzed for glucose level within 30 minutes. In tube-2 & tube-3 serum was kept for 24 hours at room temperature & refrigerator temperature respectively before glucose estimation. In tube-4 & tube-5 serum was not separated from clot and kept at room temperature & refrigerator temperature respectively before glucose estimation. The value of tube 1 was taken as reference value for comparison with other parts of the specimen. The equipment used for blood glucose level estimation was semi auto chemistry analyzer (Rayto, China). The kit used for analysis was Glucose – Liquizyme (Germany).

Results: The difference between the mean reference value (tube-1) and refrigerated serum without clot (tube-3) was 4.63 mg/100 ml while that of unrefrigerated portion (tube-2) had a difference of 10.68 mg/100 ml. The mean of unrefrigerated (tube-4) and refrigerated (tube-5) portions of serum kept with the clot had difference of 42.05 mg/100 ml and 25.84 mg/100 ml respectively. The fall in the blood glucose level in all (n=94) the samples in the tube number 3 (serum separated & kept at refrigerated temperature) was 4.63 mg/100 ml ± 3.68 (Mean ± SD) and it ranged from 0 to 20 mg/100 ml whereas fall was maximum in the tube number 4 (serum with clotted blood & kept at room temperature) was 42.04 mg/100 ml ± 10.61 (Mean ± SD) and it ranged from 13 to 82 mg/100 ml. The sample in tube 3 provided the best results as compared to all the other tubes (p=<0.0001). When the serum was kept with clot there was significantly lesser fall when the sample was kept at refrigerated temperature (tube 4) than at room temperature (tube 5) (p=<0.0001). When comparing the fall in blood glucose in sample kept at room temperature but clot separated (tube-2) with sample kept at refrigerated temperature but clot was not separated (tube-5) there was a significantly less fall in glucose in tube-2 (p=<0.0001) indicating that reduction factor of clot is more contributor than the temperature.

Conclusion: There is maximum resistance in fall in glucose level after 24 hours when the blood sample was kept at refrigerated temperature & clot was removed before preservation. If refrigeration facilities are not available, it would be appropriate to remove clot before preservation at room temperature to get the consistent results as far as possible.

Keywords: Atmospheric temperature, Blood glucose estimation, Clot, Pre-analytic variation.

INTRODUCTION

Glucose is one of the least stable analytes in blood[1]. Erythrocytes have membrane associated glycolytic enzyme complexes and utilize glucose by glycolysis[2-4] which continues in the collected blood samples. Glucose concentration in the serum of the blood samples therefore continues to fall if the serum and clot are not separated. Moreover, white blood cells also carry out

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glycolysis and reduce the concentration of glucose in collected blood samples[5,6].

Level of glucose concentration in blood samples falls with passing time due to glycolysis by blood cells which inevitably occurs during transportation and processing. Zhang et al[1] has shown decrease of 50% at 24 hrs in glucose concentration in serum that was left in contact with clot. Vesper et al have found a decline of 50% in glucose concentration in the EDTA whole blood stored at room temperature within 8 hours and 90% after 24 hours[7]. Serum is preferable to whole blood with anticoagulant because the anticoagulant may interfere with some methods of glucose estimation or with the analysis of other analytes[8]. However, if on clotting serum is separated without delay these analytes may be analysed in addition to glucose without such interference[1]. It has been found that glucose concentration continues to fall after collecting the blood in tube with an anticoagulant sodium fluoride[1,9-11]. If however, the pH of the specimen is markedly reduced by acidification the function of the phosphorlyting enzymes may be blocked[5,10]. A loss of 38% in glucose concentration was seen in 8 hours in whole heparinized blood in a study done by Michael Landt[8]. An antiglycolytic agent has to be added to inhibit the glycolytic enzymes. Special tube with reagent to seal off the plasma from the cellular component has also been recommended[11]. Chilling of the blood sample has been recommended as a preservation method to reduce glycolysis[3,8,12]. Various procedures for preservation are being adopted in our set up particularly at small laboratories before estimating the glucose level in the blood samples. Keeping in view the higher atmosphere temperature and poor storage facilities in various small laboratories in our set up it will not be possible to get the reproducible results and will demonstrate the doubtful results particularly in borderline cases. It would be more appropriate to know the correct procedure of storage of blood sample before estimating the blood glucose level. This study was planned to determine the effect of temperature and contact of clot with serum on laboratory results of glucose concentration in blood in peripheral hospital laboratory.

MATERIAL AND METHODS

This quasi experimental study was planned at the laboratory of Shoaib Hospital Fateh Jang, Attock Pakistan from December 2014 to August 2015. The blood samples for glucose estimation (Random) were collected from volunteers who reported the hospital during the period of study for some other tests like blood complete picture and provided the verbal consent for the study. Sample size was calculated by using confidence level as 95%, power of test=90% Pooled standard deviation=1 test value of population mean=7.06, Anticipated Population mean=8.65.

Ninety four blood samples were collected by non probability purposive sampling technique. The samples were excluded if the individual had abnormal blood cell count[2,9]. The individuals were offered free of cost glucose level estimation if they agreed to give the blood sample for this study. During winter average room temperature was 25°C and average refrigerator temperature was 50°C & during summer average room temperature was 32°C. Average refrigerator temperature was 60°C. Samples were collected in plain tubes. Each specimen was divided in five tubes. When the blood clotted the sample was handled as follows:

Tube-1: Serum was separated and analyzed for glucose concentration, immediately on clotting (30 min after collection).

Tube-2: Serum was separated from the clot and kept at room temperature. Test was performed at 24hrs from the time of sample collection.

Tube-3: Serum was separated from the clot and kept in refrigerator. Test was performed at 24 hours from the time of sample collection.

Tube-4: Serum was left in contact with the clot and kept at room temperature. Test was performed at 24 hours from the time of sample collection.

Tube-5: Serum was left in contact with the clot and kept in refrigerator. Test was performed at 24hrs from the time of sample collection.

The value of tube 1 was taken as reference value for comparison with other parts of the specimen. The parts of each specimen in other four tubes were tested after 24 hours.

The time it took from sample collection to separation of serum was 30 minutes (considering clotting time as 20-30 min)[13]. This interval has been accepted as optimum time interval to permit proper clot formation but not too long to cause pre-analytical variation in the test result[1,3,14]. The specimens were collected in the laboratory reception on the same floor. Therefore there was neither delay due to transportation of the specimens nor fluctuation of environmental temperature.

The kit used for glucose estimation was Glucose–Liquizyme (Germany) & the equipments used for cell count and blood glucose level estimation were auto hematology analyzer (Rayto, China) and semi auto chemistry analyzer (Rayto, China) respectively. These equipments have been well established for their reliable function. These equipments are regularly inspected by qualified engineering personnel and their fitness record is documented. The same reagents were used for analysis of all study samples. The operator and the laboratory environment were also the same for all sample analyses.

Refrigerator and room temperatures were recorded three times in 24 hours and documented in accordance with the standard operating procedures (SOPs) at this laboratory.

Table-I: Blood glucose level in all the five test tubes kept at different condition containing same blood sample (n=94).

	Tube-1	Tube-2	Tube-3	Tube-4	Tube-5
Range of Blood Glucose (mg/100 ml)	78 - 283	61-238	22-263	32-201	46-244
Mean blood glucose (mg/100 ml)	100.28	89.60	95.65	58.23	74.44
Standard Deviation	27.37	22.54	25.38	23.97	25.92
Standard Error of Mean	2.82	2.33	2.62	2.47	2.67
95% CI (mean Blood Glucose) mg/100 ml	94.75 – 105.81	85.04 – 94.16	90.52 – 100.78	53.39 – 63.08	69.20 – 79.68

Table-II: Fall in blood glucose level in all the four test tubes (tube 2 to tube 5) kept at different condition containing same blood sample (n=94).

	Fall in Blood glucose in mg/100 ml after 24 hours			
	Tube-2	Tube-3	Tube-4	Tube-5
Range of Fall (Blood Glucose)	1.0-46.0	00-20.0	13.0-82.0	4.0-56.0
Mean of fall (Blood glucose)	10.68	4.63	42.04	25.84
Standard Deviation	7.82	3.68	10.61	9.09
Standard Error of Mean of Fall in Glucose	0.81	0.38	1.09	0.94
95% CI of Fall in Glucose	9.10–11.48	3.81–5.05	40.96–45.46	24.66–28.33

Quality assurance is also regularly carried out at this laboratory through quality control and standard reagents. Moreover, this laboratory is also registered with National External Quality assurance programme, Pakistan (NEQAAP).

Data analysis were done by using Statistical Package for Social Sciences version 21 for Windows software. Descriptive statistics was used for calculation of means, standard deviation & percentages for quantitative variables & paired samples t-test was applied to compare the means of fall of glucose level after 24 hours when

samples are kept at different temperature & with & without clot. The difference was considered to be significant when *p*-value was <0.05.

RESULTS

The glucose concentration determined in serum 30mins after collection of sample was taken as reference value (tube-1). The mean blood glucose level in 94 samples was 100.28 ± 27.37 mg/100 ml (Mean ± SD). The mean blood glucose levels in all the tubes (1 to 5) are depicted in the table-I. The difference between the mean reference value (tube-1) and refrigerated serum without clot (tube-3) was 4.63 mg/100 ml while that of unrefrigerated portion (tube-2) had a difference of 10.68 mg/100 ml. The mean of unrefrigerated (tube-4) and refrigerated (tube-5) portions of serum kept with the clot had difference of 42.05 mg/100 ml and 25.84 mg/100 ml respectively (table-I). The mean of fall in the blood glucose level in all (n=94) the samples in the tube number 3 (serum separated & kept at refrigerated temperature) was 4.43 ± 3.68 (Mean ± SD) and it ranged from 0 to 20 mg/100 ml whereas mean of fall was maximum in the tube number 4 (serum with clotted blood & kept at room temperature) was 42.04 ± 10.61 (mean ± SD) and it ranged from 13 to 82 mg/100 ml (table-II). The sample in tube 3 provided the best results as compared to all the other tubes (*p*=<0.0001). When the serum was kept with clot then there was significantly lesser fall when the sample was kept at refrigerated temperature (tube-5) than at room temperature (tube 4) (*p*=<0.0001). When comparing the fall in blood glucose in samples kept at room temperature but clot separated (tube-2) with samples kept at refrigerated temperature but clot was not separated (tube-5) there was a significant less fall in glucose in tube-2. (*p*=<0.0001) indicating that reduction factor of clot is more contributor than that of temperature.

DISCUSSION

The study done by Zhang et al has demonstrated that if serum is left with clot for 24 hours, there was decrease of 50% in glucose concentration[1]. Our study has also shown similar results. Similar decrease was noted by Vesper et al if EDTA blood is stored at room temperature for 8 hours and even 90% reduction if kept for 24 hours[7]. Serum is preferable to whole blood with anticoagulant because the anticoagulant may interfere with some methods of glucose estimation or with the analysis of other analytes[8]. However, if on clotting serum is separated without delay these analytes may be analysed in addition to glucose without such interference[1].

Sodium fluoride inhibit enolase which acts late in glycolytic pathway and it has been found that glucose concentration continues to fall after collecting the blood in tube with an anticoagulant sodium fluoride[1,3,10,11]. Glycolysis may however, be inhibited in early phase if pH of the specimen is markedly reduced by acidification which blocks the function of the hexokinase & phosphofructokinase[5,10]. A loss of 38% in glucose concentration was seen in 8hrs in whole heparinized blood in a study done by Michael Landt[8]. An antiglycolytic agent has to be added to inhibit the glycolytic enzymes. Chilling of the blood sample has been recommended as a preservation method to reduce glycolysis[2,3,6]. These studies showed that despite all recommended precautions a fall in blood glucose concentration does occur by 30 minutes after drawing the blood sample. During routine work in hospital laboratory it may be manageable to perform the analysis immediately for some specimens it is generally not possible to immediately analyse every specimen after collection and concentration of glucose will fall from its actual level in specimens as time passes. In the whole blood concentration of other components to be simultaneously analysed will also be affected by the duration of preservation and temperature[1].

All methods of presentation of blood specimen for measuring glucose concentration mentioned have disadvantages of either incomplete inhibition of glycolysis, interference in determination of co-analytes, damage to cellular integrity or extracellular movement of potassium[8]. Permitting the blood to clot and

separate the sera of the blood samples therefore has advantage over using the whole blood with addition of anticoagulants, antiglycolytic agents, chilling or acidification as there is only insignificant fall in glucose concentration from the level obtained immediately on collection of blood specimen.

CONCLUSION

There is maximum resistance in fall in glucose level after 24 hours when the blood sample was kept at refrigerated temperature & clot was removed before preservation. If refrigeration facilities are not available, it would be appropriate to remove clot before preservation at room temperature to get the consistent results as far as possible.

CONFLICT OF INTEREST

This study has no conflict of interest to declare by any author.

REFERENCES

1. Zhang DJ, Elswick RK, Miller WG, Bailey JL. Effect of serum-clot contact time on clinical chemistry laboratory results. Clinical chemistry 1998; 44 (6): 1325-1333.
2. Burns DE, Knowler WC. Stabilization of glucose in blood Samples: Why it Matters. Clinical Chemistry 2009; 55(5): 850-852.
3. Sacks DB, Horvath AR. It's time for a better blood collection tube to improve the reliability of glucose results. Diabetes care 2013; 36: e2.
4. Campanella EP, Chu H, Anstee DJ, Galan JA, Tao WA, Low PS. Identification of the Components of a glycolytic enzyme metabolon on the Human Red Blood Cell Membrane. The Journal of Biological Chemistry. 2013; 288(2): 848-858.
5. Gambino R, Piscitelli J, Ackattupathil TA, Theriault JL, Andrin RD, Sanfilippo ML, et al. Acidification of blood is superior to sodium fluoride alone as an inhibitor of glycolysis. Clinical chemistry 2009; 55 (5): 1019-1021.
6. Lin YL, Smith CH, Dietzier DN. Stabilization of blood glucose by cooling with ice: an effective procedure for preservation of samples from adults and newborns. Clinical Chemistry 1976; 22 (12): 2031-2033.
7. Vesper HW, Archibold E, Porter KH, Myers GL. Assessment of a reference procedure to collect and analyze glucose in capillary whole blood. Clinical chemistry 2005; 51 (5): 901-903.
8. Landt M. Glyceraldehyde preserves glucose concentrations in whole blood specimens. Clinical Chemistry 2000; 46 (8): 1144-1149.
9. Sacks DB. A1C versus glucose testing: A comparison. Diabetes Care 2011; 34: 518-523.
10. Gambino R. Sodium fluoride: An ineffective inhibitor of glycolysis. Annals of Clinical Biochemistry 2013; 50: 3-5.
11. Chan AYW, Swaminathan R, Cockram CS. Effectiveness of sodium fluoride as a preservative of glucose in blood. Clinical Chemistry 1989; 35 (2): 315-317.
12. Messana I, Ferroni L, Misiti F, Girelli G, Pupella S, Castagnola M, et al. Blood bank conditions and RBCs: the progressive loss of metabolic modulation transfusion 2000; 40: 353-360.
13. Haverstick DM, Groszbach AR. Specimen collection & Processing. In: Burtis CA, Ashwood ER, Burns DE, eds., Teitz Textbook of clinical chemistry and molecular diagnostics, 5th edition. WB Saunders: New Delhi, 2012; pp 145-162.
14. Christopher MM, O' Neill S. Effect of specimen collection and Storage on Blood Glucose and Lactate concentrations in healthy, hyperthyroid and diabetic cats. Veterinary Clinical Pathology 2000; 29 (1): 22-28.