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EFFECT OF SPECIMEN STORAGE TIME ON SERUM GLUCOSE ESTIMATION

Biochemistry

Dr. Nitin Sharma* Associate Professor, Dept. of Biochemistry, Dr. S.N. Medical College, Jodhpur (342001), Rajasthan. *Corresponding Author

Siddharth Mundotiya Pursuing M.Sc. (Medicine) Biochemistry, Dept. of Biochemistry, Dr S. N. Medical College, Jodhpur (342001), Rajasthan.

Dr. Ranjana Mathur Sr. Professor & Head, Dept. of Biochemistry, Dr. S.N. Medical College, Jodhpur (342001), Rajasthan.

ABSTRACT

In rural areas of Jodhpur, due to inconsistent power supply or delay in transportation of specimen from the local collection centres to the clinical laboratory, early separation of serum from cells is not practically possible. The study aimed to analyze the impact of specimen storage time on serum glucose levels in healthy adults and to evaluate the pattern of change in glucose level from the time of collection in the laboratory up to 4 hours after collection.

A total of 50 samples were analyzed. Glucose concentrations in Group – 1 serum samples (which were separated from clot / blood cells within 15 – 30 minutes of blood collection process) were consistently and significantly higher (129.68±12.45) when compared with the samples of Group – 2 (118.32±18.35) with a difference of 11.36 or 9.16 % (P = 0.0005), Group – 3 (111.62 ±11.77) with a difference of 18.06 or 14.97% (P <0.0001) and Group – 4 (105.88±14.93) with a difference of 23.8 or 20.21% (P <0.0001). We recommend that the health care providers must ensure quick delivery of samples for early separation of serum from cellular components.

We also recommend that if separation of serum within 15-30 minutes is possible, use of NaF fluoride tubes for glucose estimation during blood collection is not necessary. Constant evaluation of skills acquired by phlebotomists or nurses is required to check whether ISO guidelines are being followed.

KEYWORDS

glucose, hyperglycemia, preanalytical error, sample collection, laboratory diagnostics

Diabetes Mellitus (DM) is a group of metabolic diseases that occurs either when the pancreas does not produce enough insulin or when the body cannot effectively use the insulin it produces. The chronic hyperglycemia, due to diabetes results in the impaired function of the eyes, kidneys, blood vessels, heart and nerves [1]. It is a major health problem from both national and worldwide perspectives. According to the WHO in 2014, 8.5% of adults aged 18 years and older had diabetes. In 2015, diabetes was the direct cause of 1.6 million deaths.

The diagnosis and management of diabetes depends on reliable blood glucose analysis and measurement. It is important to recognize that the pre-analytical handling of blood samples intended for glucose measurement can influence the laboratory results [2].

Several researchers in the past have analyzed the methods of sample handling and discussed the effect of specimen storage time on serum glucose levels. Previous studies have reported that glycolysis occurring in the cellular component of a blood sample may consume up to 5%–7% glucose per hour, resulting in declined glucose values that could lead to delayed diagnosis resulting in an increased morbidity and mortality rate [3,4,5]. Therefore it is highly recommended to separate the serum from the cellular components / clot within 15-30 minutes after phlebotomy.

However, such practice may not always be possible when transporting samples from the field to the clinical laboratory. A widely used technique involves the addition of sodium fluoride (NaF) to blood tubes. NaF inhibits enolase, an enzyme in the glycolytic pathway [6]. Although NaF has been shown to completely arrest glycolysis by four hours, it has insignificant effect on the rate of glycolysis during the first 1-2 hours [7].

According to the WHO, earlier development of neonatal hypoglycaemia represents more severe disease, and is more likely to result in permanent central nervous system damage, such as reduced IQ and cerebral palsy [8]. Therefore, accurate neonatal blood glucose measurements are important to prevent and cure neonatal hypoglycaemia [9].

The present study has been carried out in the Jodhpur city which is situated in the north-western Indian state of Rajasthan. In this region the following factors can increase the storage time of blood samples:

- In rural areas, due to inconsistent power supply specimens for analysis are left on the laboratory bench at ambient temperature for several hours before analysis could be performed.
- Several laboratories provide house to house collection service to take blood samples from home. In such cases phlebotomists collect and transport the samples from patient's residence to the laboratory by their own vehicles. Depending on the number of calls, traffic and distance the sample transport time varies from day to day.
- Even in large clinical settings where an average total number of samples could reach 1500 – 2000 per day, delay in transport of samples could occur from the sample collection facility to the laboratory because of continuous inflow of patients and limited laboratory staff.

Due to the above mentioned reasons immediate sample separation and analysis may not be practicable leading to misdiagnosis of diabetes and consequent health complications.

The purpose of this study was to evaluate the impact of specimen storage time on estimated serum glucose levels in healthy adults and assess the pattern of change in glucose level from the time of collection in the laboratory up to 4 hours after collection.

Methodology:

50 healthy adults (not suffering from any metabolic disease or disorder), 20 to 61 years of age, were included in the study. This study has been conducted in the Department of Biochemistry, Dr. S. N. Medical College and its Associated Group of Hospitals, Jodhpur. Subjects have been selected through random sampling. Participants who volunteered in the study were asked to arrive between 7:30 AM and 8:30 AM after observing a 10–12 hour overnight fast. Blood samples were collected only after taking prior written consent in the prescribed form with prior approval from the institutional Ethics Committee of Dr. S. N. Medical College, Jodhpur. Serum glucose will be estimated using a fully automated clinical chemistry analyzer EM 360 – Transasia Biomedicals Pvt. Ltd., India.

After collection of blood, specimen was divided into four groups as follows:

Group-1: serum separated from clot / blood cells within 15 – 30 minutes of blood collection process and concentration of glucose measured to obtain the baseline standard values to be compared with other concentrations of future time points.

Group-2: serum separated exactly after 1 hour of blood collection process and concentration of glucose measured and compared with baseline standard values.
Group-3: serum separated exactly after 2 hour of blood collection process and concentration of glucose measured and compared with baseline standard values.
Group-4: serum separated exactly after 4 hour of blood collection process and concentration of glucose measured and compared with baseline standard values.

Exclusion criteria:
1. All patients suffering from any metabolic disease or disorder were excluded from this study.
2. Any patient with suspected anemia and having low body weight were not included.
3. Hemolyzed samples were excluded.
4. Subjects who denied filling the written consent forms as per the ethical guidelines.

Sample collection: Phlebotomy was performed; 8 ml blood sample was collected by venipuncture and divided equally into four different plain blood collection vacutainers, containing no additive, pre-labelled as Group-1, Group-2, Group-3 and Group-4. Two different sample identifiers were used along with date and time of specimen collection.

Samples were delivered to the departmental research laboratory. Serum tubes were allowed to stand at room temperature (~25°C) until clot formation and centrifuged according to the scheduled time intervals as discussed above.

Centrifugation: After blood collection, Group-I serum specimen were subjected to centrifugation (within 15 – 30 minutes at RCF between 2000 and 3000 g) for 10 minutes. The remaining 3 specimens were allowed to stand at room temperature (~25°C) for 1 h, 2 h and 4 h and were then sent for centrifugation and analysis as per schedule discussed above.

Measurements of serum Glucose: Immediately after centrifugation, the serum samples from each patient were assayed for glucose levels in duplicate using glucose oxidase / peroxidise method[10] on fully automated clinical chemistry analyzer, EM 360 – Transasia Biomedicals Ltd., India. The instrument was equipped with standard reagents, calibrators and quality control material. Four serum glucose estimations (in duplicate) were performed on each group of specimen, results tabulated, giving rise to total 16 results per patient according to the time of centrifugation and analysis.

Between-group comparisons were performed using the Student's t test. For all statistical evaluations, a two-sided P value of 0.05 or less was considered to be statistically significant.

Results:
The study sample included 50 non-diabetic healthy adults with an average age of 33 years (range was 20 to 61 years).

Glucose concentrations in Group – 1 serum samples (which were separated from clot / blood cells within 15 – 30 minutes of blood collection process) were consistently and significantly higher (129.68±12.45) when compared with the samples of Group – 2 (118.32±18.35) with a difference of 11.36 or 9.16 % (P = 0.0005), Group – 3 (111.62 ±11.77) with a difference of 18.06 or 14.97% (P <0.0001) and Group – 4 (105.88±14.93) with a difference of 23.8 or 20.21% (P<0.0001) (Table – 1).

Almost similar results were observed when statistical comparisons were made separately for females and males (Table – 2 and Table –3).

Similarly when serum glucose concentrations of Group – 2 samples (118.32±18.35) (serum separated exactly after 1 hour of blood collection process) were compared with Group – 3 samples (111.62 ±11.77) (serum separated exactly after 2 hour of blood collection process), the difference calculated was 6.62 or 6.22% (P = 0.0322) which was significantly higher (Table – 1).

And when serum glucose concentrations of Group – 3 samples (111.62 ±11.77) (serum separated exactly after 2 hour of blood collection process) were compared with Group – 4 samples (105.88±14.93) (serum separated exactly after 4 hour of blood collection process), the difference calculated was 5.74 or 5.28% (P = 0.0353) which was significantly higher (Table – 1).

Conclusion:
The aim of the study was to analyze the effect of specimen storage time on the determination of glucose concentrations in the human serum samples. The fact that in the rural areas of Rajasthan where power supply remains inconsistent or in cases when blood collection process occurs in the field or in places distant to the actual clinical laboratory, there would be a delay in the processing of specimen leading to false low blood glucose levels. It has been observed previously by other researchers and in one such study it has been concluded that in blood samples from human beings, glucose concentration decreases in vitro at a rate of 0.36-0.56 mmol/L (6-10 mg/dL) per hour at 25°C due to continued consumption of glucose by cellular components via glycolysis[11].

In this study, the concentration of serum glucose was highest in case when the serum was separated within 15 – 30 minutes after phlebotomy. During the initial first hour, the decrease was maximum, 9.16% which continued with time but at a slower rate (Fig.1). This difference was of similar magnitude when compared separately for females and males (Fig.2. and Fig.3.).

Based on our research, we recommend that the health care providers or technicians involved in phlebotomy must ensure quick delivery of samples and separation of serum from RBC and other cellular components should take place within 15 to 30 minutes in order to prevent significant alterations in blood glucose concentration. These observations were supported by other researchers in the past [12,13,14]. If the delivery of samples to the clinical laboratory is not possible, in that case all collected blood samples must first be taken to a facility where serum separation could be done and the separated serum samples should be kept on ice slurry up till delivery to the concerned laboratory to ensure accuracy of the results.

Previously some researchers have evaluated the effectiveness of sodium fluoride (NaF) and other anti-glycolytic agents in preserving blood glucose. They found a continued decline in glucose concentrations in the initial 3-4 hours after which glucose values remain stable for 3 days 15. It has been observed that NaF has no effect in preserving blood glucose in the initial first hour but inhibits glycolysis considerably in the second hour and strongly or completely inhibiting it by the third and fourth hour [7]. In this present study we recommend that in a modern clinical setting where the diagnostic laboratories are following the highest standards of quality and are able to separate the serum from the cellular components within 15-30 minutes, use of NaF fluoride tubes for glucose estimation during blood collection is not necessary.

Diabetes is a progressive disease that if left undiagnosed and untreated can result in serious illness, disability and death from complications. Therefore timely detection of this disease is essential in the management and to avoid complications. Individuals who do not meet the criteria for diabetes due to wrong blood processing practices and falsely decreased glucose levels due to the reasons mentioned above, may be subjected to compromised quality of life and undue increase in the healthcare costs. In this regard, we recommend that the phlebotomists/technicians/staff nurses involved in the blood collection process along with laboratory personnel responsible for transportation of the specimen must be trained and evaluated in the pre-examination processes as per the highest quality guidelines prescribed by Clinical and Laboratory Standards Institute (CLSI) and the International Organization for Standardization (ISO -15189).

Table 1: Comparison of serum Glucose concentrations between different Groups. Data are mean + SD

Difference in Groups	SERUM BLOOD GLUCOSE LEVEL (mg/dl) (Mean + SD) n=50		Difference b/w Groups	Range of Differences	% Difference	p value
Group 1 v/s Group 2	Group-1= 129.68±12.45	Group 2 = 118.32± 18.35	11.36±3.14	2-21	9.16%	0.0005

Group 1 v/s Group 3	Group-1 =129.68± 12.45	Group 3 =111.62 ±11.77	18.06±2.42	7-31	14.97%	<0.0001
Group 1 v/s Group 4	Group-1 =129.68± 12.45	Group 4 = 105.88± 14.93	23.8±2.75	12-47	20.21%	<0.0001
Group 2 v/s Group 3	Group 2 = 118.32±18.35	Group 3 =111.62 ±11.77	6.7±3.08	1-18	5.83%	0.0322
Group 3 v/s Group 4	Group 3 =111.62 ±11.77	Group 4 = 105.88± 14.93	5.74 ± 2.69	1-16	5.28%	0.0353

Table 2: Comparison of serum Glucose concentrations between different groups in females. Data are mean + SD

Difference in Groups	Serum Blood Glucose Level (mg/dl) (mean + Sd) n=26		Difference b/w Groups	Range of Differences	% Difference	p value
Group 1 v/s Group 2	Group-1= 137.92±17.03	Group 2 = 126.23±16.57	11.69 ±4.66	5-19	8.85 %	0.0154
Group 1 v/s Group 3	Group-1 =137.92±17.03	Group 3 = 119.46±15.88	18.46 ±4.57	7-30	14.34 %	0.0002
Group 1 v/s Group 4	Group-1 =137.92±17.03	Group 4 =113.73 ±15.86	24.19± 4.56	12-37	19.23 %	<0.0001
Group 2 v/s Group 3	Group 2 = 126.23±16.57	Group 3 = 119.46±15.88	6.77±4.50	1-18	5.51 %	0.0396
Group 3 v/s Group 4	Group 3 = 119.46±15.88	Group 4 =113.73 ±15.86	5.73±4.40	1-16	4.91 %	0.0741

Table 3: Comparison of serum Glucose concentrations between different groups in males. Data are mean + SD

Difference in Groups	SERUM BLOOD GLUCOSE LEVEL (mg/dl) (Mean + SD) n=24	Difference b/w Groups		Range of Differences	% Difference	p value
Group 1 v/s Group 2	Group-1= 120.75±16.11	Group 2 =109.75 ±11.80	11 ± 4.08	2-21	9.54 %	0.0097
Group 1 v/s Group 3	Group-1 =120.75±16.11	Group 3 = 103.13±13.39	17.62 ± 4.28	11-31	15.75 %	0.0002
Group 1 v/s Group 4	Group-1 =120.75±16.11	Group 4 = 97.37± 12.84	23.38 ± 4.21	14-47	21.44 %	<0.0001
Group 2 v/s Group 3	Group 2 =109.75 ±11.80	Group 3 = 103.13±13.39	6.62 ± 3.64	2-12	6.22 %	0.0101
Group 3 v/s Group 4	Group 3 = 103.13±13.39	Group 4 = 97.37± 12.84	5.76 ± 3.79	2-16	5.75 %	0.0305

Fig.1. In vitro changes in serum glucose concentrations (mean±SD) in 50 healthy adults collected in serum tubes and incubated at 250 Celcius for upto 4 hours.

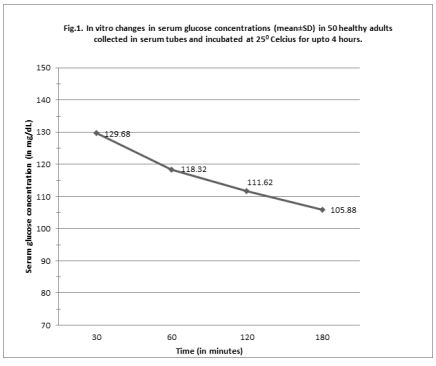


Fig.2. In vitro changes in serum glucose concentrations (mean±SD) in 50 healthy adults females collected in serum tubes and incubated at 250 Celcius for upto 4 hours.

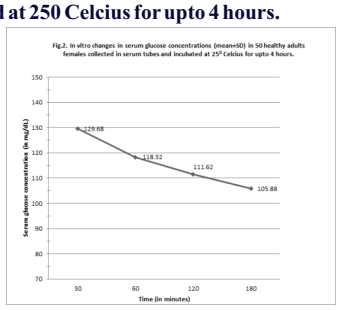
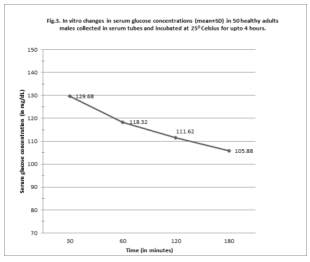


Fig.3. In vitro changes in serum glucose concentrations (mean±SD) in 50 healthy adults males collected in serum tubes and incubated at 250 Celcius for upto 4 hours.



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