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The Effect of Storage Time and Different Anticoagulants on Fasting Blood Glucose Concentration

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Abstract: The comparative stabilizing effects of storage time and the anticoagulants; fluoride oxalate, EDTA, sodium citrate, and serum on ice slurry; on fasting blood glucose level were determined using the spectrophotometry method. Fasting blood samples were taken from 75 non-diabetic male people, and the blood glucose levels determined at 30 min intervals for a maximum time of 3 hours. Our results showed that the rate at which plasma glucose changes with time varies with specific anticoagulants. From the results, it was observed that the rate at which the blood glucose decreases with time vary with specific anticoagulants. It was noticed that random blood glucose in sodium citrate, EDTA, fluoride oxalate and serum on ice slurry decreased at a mean value of 28.4mg/dl, 58mg/dl, 15.4mg/dl and 60.2mg/dl dl after 3 hours respectively. With respect to the concentration of glucose before storage, this suggests that storage of blood using fluoride oxalate as an anticoagulant tends to better preserve the glucose level over a long period of time. Transport on ice slurry and rapid separation of serum within 30 min can inhibit glycolysis without the addition any anticoagulants (% reduction 1.3). Thus, from our findings, it is obvious that irrespective of the specimen type, time of collection or type of anticoagulant, the concentration of blood glucose remained unstable during storage. It is therefore suggested that analysis of blood glucose should be carried out immediately after collection of specimen or within the shortest possible time after storage in an anticoagulant to obtain a reliable result.

Keywords: anticoagulants, fasting blood glucose, glycolysis, sample collection.

INTRODUCTION

Most energy for cellular activities is derived from glucose, and more than 70% of the energy used by the human body is provided by the glucose oxidation process, which is important in maintaining the body's normal physiological functions (Zhu et al., 2017)). The blood sugar concentration or blood glucose level is the amount of glucose present in the blood of a human. Normally in mammals, the body maintains the blood glucose level at a reference range between 70 and 100 mg/dl before-meal (Baker et al., 1969). Glucose can be measured in whole blood, serum or plasma (Richard, 2001). Collection of blood specimen for measurement of blood glucose level should be done on the day and time requested. This is because collection times are related to food intake (Ochei and Kolhatkar, 2000). There are two different methods of determining glucose level: The chemical method and the enzymatic method. The chemical method exploits the non-specific reducing property of glucose in reactions with an indicator substance, which concomitantly changes color on its reduction (Louie et al., 2002). The enzymatic method has reached an

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advanced stage where the enzymes could be immobilized in electronic machines or devices for easier and faster analysis (Chernow et al., 1996). Glucose estimation using plasma or whole blood requires the use of an anticoagulant, which are compounds that help prevent the clotting of blood. When blood is shed or collected, the cell does not die immediately. They continue to metabolize and use up glucose as a source of energy via the glycolytic process. Glucose thus disappears from whole blood on standing over a period. Glycolysis can be prevented with an enzyme inhibitor (Lawrence et al., 2008).

The commonest inhibitor for this purpose is sodium fluoride, which usually used in conjunction with an anticoagulant potassium oxalate. Fluoride actually inhibits the enzyme enolase that found in the metabolic pathway of glucose and has a little effect on glucose oxidase and peroxidase enzymes. It also inhibits bacterial growth (Lawrence et al., 2008). Another widely used anticoagulant is Ethylene Diamine Tetra acetate (EDTA). When EDTA added to a blood sample, it chelates the calcium needed for blood clotting and thereby preventing the formation of fibrin. It forms an insoluble calcium salt by chelation. It was the purpose of this study to measure serum and plasma glucose concentration using different anticoagulants at different storage periods of time. Comparison of these anticoagulants helps to choose the suitable anticoagulant and to detect the effect of each anticoagulant at a specific storage time on serum and plasma glucose level. To our knowledge, no studies have been conducted on the investigation of the stability of blood glucose at different laboratory storage times and in different anticoagulant tubes in Libya.

MATERIALS AND METHODS

The study was carried out in University Clinic and biochemistry laboratory at Chemistry Department, Faculty of science, Omar Al-Mukhtar.

Study population and sample size: The study was covering 75 apparently healthy male individuals, randomly selected, and lived in El-Beida City from different age groups (49.7±11.45).

Exclusion criteria: Hemolytic samples and diabetic samples must be rejected.

Ethical consideration: All participants on this study were informed about the nature of study; blood samples were collected after their agreement.

Sample collection and processing: Vein side was cleaned with 70% alcohol; tourniquet was tied in space before collection. The needle was inserted and 6 ml of blood sample was collected. 2.0 ml from sample were applied to; fluoride oxalate anticoagulant container (Orange color), EDTA anticoagulant container (Green color), and sodium citrate (Pink Color) anticoagulant container and then were immediately separated at 3.000 rpm for 5 minutes at 20°C by using the centrifuge instrument. The serum was then separated from the blood cells in a plane container (Red color) and kept on an ice slurry. Blood samples were examined initially (at zero time), after 30, 60, 90, 120, and 180 minutes from sample collection.

Fasting blood glucose determination: The concentrations of fasting blood glucose in the plasma and serum were determined spectrophotometrically immediately after collection using Glucose-Oxidase test kit (Vitro Scient, Germany) described by (Werner et al., 1970). The procedure was repeated every 30 min interval for 3 h. Results were expressed as mg/dl.

Reference range of random blood glucose: Serum or plasma = 70-120 mg/dl (Trinder, 1969).

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Statistical analysis: The means and standard deviations (SD) were calculated and paired t-test was used to calculate P values.

RESULTS AND DISCUSSION

The assay of fasting blood glucose in samples stored in anticoagulants is a regular practice in this part of the world. When blood samples are collected, they are stored in their native state by preserving them in different anticoagulants. Though their native state is preserved, the blood glucose when assayed in different anticoagulants, at different times, varies. In this study, an attempt was made to compare the changes in blood glucose level over three hours at intervals of thirty minutes. From the results (Table 1), it was observed that the rate at which the blood glucose decreases with time vary with the specific anticoagulant.

Table (1). Effects of some anticoagulants on random blood glucose of some apparently healthy individuals.

Anticoagulant	Blood glucose concentration (mg/dl) during time (minutes)					
	Zero time	30	60	90	120	180
Sodium citrate	100.3±15.1	98.5±17. 7	92.6±21.7	87.1±23.1	78.9±25.2	71.9±29.0
EDTA	102.1±19.8	88.0±23.9	75.2±22.6	61.6±27.8	55.2±30.6	44.1±25.1
Fluoride oxalate	101.7± 23.6	100.9±12.6	100.1±16.1	99.4±12.9	98.1± 25.8	86.3± 29.6
Serum on ice slurry	100.9±22.4	99.5±22.8	79.9±27.2	65.6±29.1	50.1±33.2	40.7±35.4

Data are expressed as mean ± SD; n = 75; Ethylene Diamine Tetra Acetate (EDTA)

It was noticed that random blood glucose in sodium citrate, EDTA, fluoride oxalate and serum on ice slurry decreased at mean values of 28.4 mg/dl, 58mg/dl, 15.4 mg/dl and 60.2 mg/dl after 3 hours respectively. With respect to the concentration of glucose before storage, this suggests that storage of blood using fluoride oxalate as an anticoagulant tends to better preserve the glucose level over a long period of time. This may be due to the ability of fluoride ion to inhibit the activity of enolase, an enzyme in the glycolytic pathway, thereby slowing down the breakdown of glucose (Gupta & Kaur, 2014).

It can also be observed that irrespective of the anticoagulant used, the random blood glucose significantly ($P<0.05$) decreased steadily as compared to the value before storage. This actually shows that anticoagulants can not stop, in totality, the breakdown of glucose (glycolysis). Thus, over a long period of time, the concentration of glucose may reduce to zero level (Tables 2, 3, 4 and 5).

Table (2). Comparison between plasma glucose concentrations at zero with 30, 60, 90,120 and 180 minutes in sodium citrate anticoagulant.

Glucose concentration mg/dl ± SD at different times		
A	B	Mean differences(A-B)
Zero(100.3±15.1)	30 (98.5±17. 7)	1.8
Zero(100.3±15.1)	60 (92.6±21.7)	7.7
Zero(100.3±15.1)	90 (87.1±23.1)	13.2*
Zero(100.3±15.1)	120(78.9±25.2)	21.4*
Zero(100.3±15.1)	180(71.9±29.0)	28.4*

Data are expressed as mean ± SD; n = 75; *represents significant at *P<0.05* between different times.

Table (3). Comparison between plasma glucose concentrations at zero with 30, 60, 90,120 and 180 minutes in EDTA anticoagulant.

Glucose concentration mg/dl ± SD at different times		
A	B	Mean differences (A-B)
Zero(102.1±19.8)	30 (88.0±23.9)	14.2*
Zero(102.1±19.8)	60 (75.2±22.6)	26.9*
Zero(102.1±19.8)	90 (61.6±27.8)	40.5*
Zero(102.1±19.8)	120(55.2±30.6)	46.9*
Zero(102.1±19.8)	180(44.1±25.1)	58*

Data are expressed as mean ± SD; n = 75; *represents significant at *P<0.05* between different times.

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Table (4). Comparison between plasma glucose concentrations at zero with 30, 60, 90,120 and 180 minutes in fluoride oxalate anticoagulant.

Glucose concentration mg/dl ± SD at different times		
A	B	Mean differences (A-B)
Zero(101.7±23.6)	30(100.9±12.6)	0.8
Zero(101.7±23.6)	60(100.1±16.1)	1.6
Zero(101.7±23.6)	90(99.4± 12.9)	2.3
Zero(101.7±23.6)	120(98.1±25.8)	3.6
Zero(101.7±23.6)	180(86.3±29.6)	15.4*

Data are expressed as mean ± SD; n = 75; *represents significant at *P<0.05* between different times.

It was noticed that fasting blood glucose in sodium citrate, EDTA, fluoride oxalate and serum on ice slurry decreased at mean percentage values of 28.4%, 56.8%, 13.7% and 59.6% after 3 h (Table 6).

Table (5). Comparison between serum glucose concentrations on ice slurry at zero with 30, 60, 90,120 and 180 minutes.

Glucose concentration mg/dl ± SD at different times		
A	B	Mean differences (A-B)
Zero(100.9±22.4)	30 (99.5±22.8)	1.4
Zero(100.9±22.4)	60 (79.9±27.2)	21*
Zero(100.9±22.4)	90 (65.6±29.1)	35.3*
Zero(100.9±22.4)	120(50.1±33.2)	50.8*
Zero(100.9±22.4)	180(40.7±35.4)	60.2*

Data are expressed as mean ± SD; n = 75; *represents significant at *P<0.05* between different times.

Table (6). Comparison between reduction percentage of serum and plasma glucose levels in different anticoagulants.

Anticoagulant	Mean of % reduction from zero to 30 min	Mean of % reduction from zero to 60 min	Mean of % reduction from zero to 90 min	Mean of % reduction from zero to 120 min	Mean of % reduction from zero to 180 min
Sodium citrate	1.7	7.6	13.1	21.1	28.1
EDTA	13.8	26.3	39.6	45.9	56.8
Fluoride oxalate	0.78	1.57	2.2	3.5	13.7
Serum on ice slurry	1.3	20.8	34.9	50.3	59.6

These also support the possibility for fluoride oxalate to be a better anticoagulant for long-term storage of blood samples for glucose determinations, since the glucose concentration in the blood samples stored in it tends to be comparatively more stable (% reduction 13.7 after 3 h).(Chan et al., 1989)) reported that 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. Transport on ice slurry and rapid separation of serum within 30 min can inhibit glycolysis without the addition of any anticoagulants (Table 6; % reduction 1.3), and in fact works better as shown by few studies (Gambino, 2013; Waring et al., 2007).

The recommendations of American Diabetes Association (ADA) published in 2002 and WHO guidelines of 2006 clearly indicated that venous plasma is the preferred sample for glucose estimation (Organization, 2006; Sacks et al., 2002). 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 and significantly increases turnaround time for glucose results compared with plasma results(Schrot et al., 2007). There is also a suggestion that clotting consumes glucose (Gambino et al., 2009). Therefore, serum glucose concentrations will always be lower than plasma glucose if glycolysis in a plasma sample is inhibited immediately. The amount of the differences will vary with the glycolysis rate in the individual specimen and the time elapsed between collection and centrifugation(Gambino, 2013). The comparison of paired blood samples for serum and heparinized plasma collected and

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stored at same ambient temperature and centrifuged at same time produced higher glucose values in plasma (Gambino et al., 2009). 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 (Turchiano et al., 2013). This is because when serum is collected in tubes with a clot activator, serum gel separator, and promptly centrifuged, glycolysis is stopped quickly due to the separation of serum from the cellular components (Turchiano et al., 2013). The use of serum for glucose estimation is not uncommon in the world. This means that the practice of using serum sample for glucose estimation could be leading to many wrong reports and responsible for false variation in results of an individual obtained from different laboratories as well as misclassification of at risk patients (Gupta & Kaur, 2014). But WHO and ADA have emphasized on the need of putting the plasma glucose samples immediately on ice slurry and centrifugation within 30 min (Sacks et al., 2011).

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 or transport to central lab on ice packs or they can be directly sent to laboratory on priority as is immersed in ice slurry, where immediate processing could take place (Gupta & Kaur, 2014).

As observed in Tables 2, 3 and 4, they also showed significantly decrease ($P<0.05$) in fasting blood glucose levels in the blood samples stored in all the anticoagulants under study. Thus, from our findings, it is obvious that irrespective of the specimen type, time of collection or type of anticoagulant, the concentration of blood glucose remained unstable during storage. It is therefore suggested that analysis of blood glucose concentrations should be carried out immediately after collection of the specimen or within the shortest possible time after storage in an anticoagulant, so as to obtain a reliable result.

CONCLUSION

From our findings, it is obvious that irrespective of the anticoagulant used, time of collection of specimen or type of specimen, the concentration of glucose is never stable. Thus, to get reliable results, glucose determination should be carried out immediately after collection of samples or within the shortest possible time. Fluoride oxalate is more stable anticoagulant than the other anticoagulants. Reduction percentage was more pronounced between 0-3 hours. For this reason, it is better to measure the concentration of fasting glucose within the shortest time after sample collection.

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تأثير وقت التخزين ومضادات التخثر المختلفة على مستوى السكر في الدم

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المستخلص : دراسة تأثير زمن التخزين على مستوى الجلوكوز في عينة دم مخزنة في مضادات تجلط مختلفة من أوكسالات الفلوريد، إديتا (EDTA)، سيترات الصوديوم وعينة سيرم في ثلج مجروش وذلك باستخدام طريقة الطيف الضوئي. أخذت عينات الدم من 75 ذكراً غير مصابين بمرض السكري، وحددت مستويات السكر في الدم على فترات 30 دقيقة لمدة أقصاها ساعتان، وأظهرت النتائج أن المعدل الذي يتغير فيه جلوكوز البلازما مع مرور الوقت يختلف مع اختلاف مضادات التخثر. لوحظ أن المعدل الذي ينخفض فيه الجلوكوز في الدم مع مرور الوقت يختلف مع اختلاف مضادات التخثر، كما أن الجلوكوز في الدم العشوائي في سيترات الصوديوم وإديتا (EDTA) وأوكسالات الفلوريد والمصل في الثلج المجروش قد انخفض بمعدل متوسط قدره 28.4مليجرام/ دل، 15.4 مليجرام/ دل و60.2 مليجرام/ دل بعد مرور 3 ساعات على التوالي. فيما يتعلق بتركيز الجلوكوز قبل التخزين، يشير هذا إلى أن تخزين الدم باستخدام أوكسالات الفلوريد كمضاد للتخثر، يميل إلى الحفاظ على مستوى الجلوكوز بشكل أفضل على مدى فترة طويلة من الزمن. التخزين على الثلج المجروش والفصل السريع من المصل في غضون 30 دقيقة يمكن أن تمنع التحلل دون إضافة أي مضادات للتخثر (٪ تخفيض 1.3). يمكننا أن نستنتج بغض النظر عن نوع العينة، ووقت جمع أو نوع مضادات التخثر، فإن تركيز الجلوكوز في الدم سيبقى غير مستقر أثناء فترة التخزين الطويلة، ولذلك يقترح إجراء تحليل لجلوكوز الدم مباشرة بعد جمع العينات أو في أقصر وقت ممكن بعد التخزين في مضاد للتخثر، وذلك للحصول على نتيجة يمكن الاعتماد عليها.

الكلمات المفتاحية : مضادات التخثر، مستوى الجلوكوز في الدم، تحلل الجلوكوز، جمع العينات.

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