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Decrease In Glucose Levels Over A Period Of Time In Samples Collected In Sodium Fluoride, Edta And Clot Activator Tubes(A Comparative Study)

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Abstract: BACKGROUND: Glucose is one of the least stable analytes in blood sample. Estimation of true glucose level is important for diagnosis of diabetes at the earliest, monitoring control of blood sugar and also to identify high risk patients. Erythrocytes in blood utilize glucose by glycolysis by the glycolytic enzyme complexes present in their membrane. There is also reduction in blood sugar level in collected and stored blood due to glycolysis occurring in white blood cells. To avoid this reduction in the glucose level, varieties of methods are employed. Placing the blood samples in ice slurry and immediate separation of plasma from blood cells may prevent the reduction of glucose level. But this practice may not always be possible when transporting samples from the field to the clinical laboratory. To decrease glycolysis during storage, conventionally, sodium fluoride is added to blood. Other technique like use of serum gel separator is also useful in slowing down the glycolysis. It is further demonstrated by that acidification of blood samples using a combination of citrate buffer, NaF and EDTA was more effective in arresting glycolysis during storage **METHOD** : The samples was collected from the healthy volunteers with in the age group of 18 to 27 blood samples from each volunteer was analysed in duplicate with serum and plasma immediately after separation from clot and anticoagulants, to obtain the baseline result and retested after 2 hours and 4 hours of storage at room temperature. The result was compared and any significant decrease in glucose value was observed and subjected to statistical analysis. **RESULT**: In this study blood samples were collected from 50 individuals and tested glucose content in three different conditions of storage.
Samples were divided in to three, serum from the blood clot, plasma from EDTA treated blood and plasma from NaF treated blood. After 30 min, 2 h and 4 h of storage, glucose content of the serum was analyzed. There was no significant difference of glucose level in clot, EDTA and NaF, if performed analysis within 30 minutes. However after 2 hr, glucose content decreased in the clot and EDTA treated blood, but after 4 hr glucose concentration decrease in NaF treated samples. 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

Index Terms - Glucose, Sodium Fluoride (NaF), EDTA and Clot Activator Tubes

I. INTRODUCTION

Blood glucose determination is one of the most common clinical diagnostic tests. Accurate and precise measurement of blood glucose is of great importance in the diagnosis and management of diabetes. Serum or plasma samples not promptly separated from RBC may contain low glucose and high lactate concentrations due to the continue uptake and metabolism of glucose by RBC *in vitro*. Once the blood is drawn, the concentration of glucose will continue to decrease because of glycolysis, which will occur in erythrocytes, white blood cells and platelets. 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, Ethylene Diamine Tetra Acetic Acid, Oxalates, and Sodium Fluoride. Separation of serum from RBC within 15 to 30 minutes is considered necessary to prevent significant alterations in blood glucose concentration. Large variable changes in lactate concentration can occur immediately after specimen collection, depending on temperature, pH, and glycolytic rate. 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. Sodium fluoride (NaF) inhibits several glycolytic enzymes by complexion with their cofactor, magnesium ion. Tubes containing NaF and potassium oxalate, the latter and anticoagulant, are recommended for the collection of human and animal blood samples to be analyzed for glucose and particularly lactate, to avoid artifactually changes resulting from glycolysis. Sodium fluoride, potassium oxalate, or both, depending on their concentrations, may cause shrinkage and to a lesser extent, lysis of RBC, The dilution effect of NaF/Ox-induced RBC lysis would be expected to be greater in species whose RBC contained lower intracellular concentrations of glucose (or lactate) compared to plasma. In this study to compare the results of glucose analysis routinely processed serum and plasma samples from healthy voluntaries. Results of these analyses were investigated further by evaluation of storage time and temperature on glucose concentrations in vitro and the effect of Clot, EDTA, and NaF on RBC volume.

II. MATERIALS AND METHODS

A total of 50 healthy students volunteer the age range of 18 to 27 were selected for sample collection. In this study, the healthy criteria for selection of them for sampling were based on their clean habits. Before sampling, the first step to the collection of identify of the volunteers by two forms. After the blood collection draw with the blood from tube followed the order of draw, samples were collected in three tubes. One plain tube for clotted blood second one tube contains EDTA and the other tubes containing NaF. Then wait for 3o minutes all the samples was centrifuged at 2500 rpm for 10 min and the clear plasma and serum was used in the assay. Samples were maintained at 22 ± 2 °C for room temperature. Serum, EDTA and NaF samples were centrifuged after 30 min, 2 and 4 hours for spectrophotometric analysis of glucose.

2.1 Statistical analysis

Analysis of variance (ANOVA) was used to evaluate change in glucose concentrations over time for *in vitro* experiments. Results were considered significant when $p < 0.5$.

III. RESULT

Blood sample was collected from a total of 50 patients (150 samples). The age range was from 18 years to 30 years, including males and females there was significant different in glucose concentration after 30 min, 2 hr and 4 hr in clotted samples ($p < 0.00001$). In the EDTA treated sample, the mean glucose level varied after 30 min, 2 hr and 4 hr and was 83.7 ± 13.19 mg/dl, 79.88± 13.75 mg/dl, and 72.8 ±15.3, respectively. There was a significant difference in glucose concentration in EDTA samples after 30 min, 2 hr, and 4 hr ($p < 0.000672$). After 30 min, 2 hr and 4 hr the mean glucose level of NaF treated sample was 87.2± 13.09 mg/dl, 87.04 ± 12.82 mg/dl, and 87.02± 12.59 mg/dl, respectively. There was no significant difference among mean blood glucose level in NaF treated sample after 30 min, 2 hr and 4 hr ($p< 0.05$). The present study revealed no significant variation in the glucose level of plasma obtained after NaF treatment and serum ($p>0.05$). After 24 hr, there was significant fall in plasma glucose level in NaF than that of serum ($p <0.0001$). The efficacy of collection methods and an average glucose level after 30 min, 2 hr and 4 hr was described in Fig. 1. Use of serum will help in the reduction of extra expenses incurred with the use of collection tube with NaF.

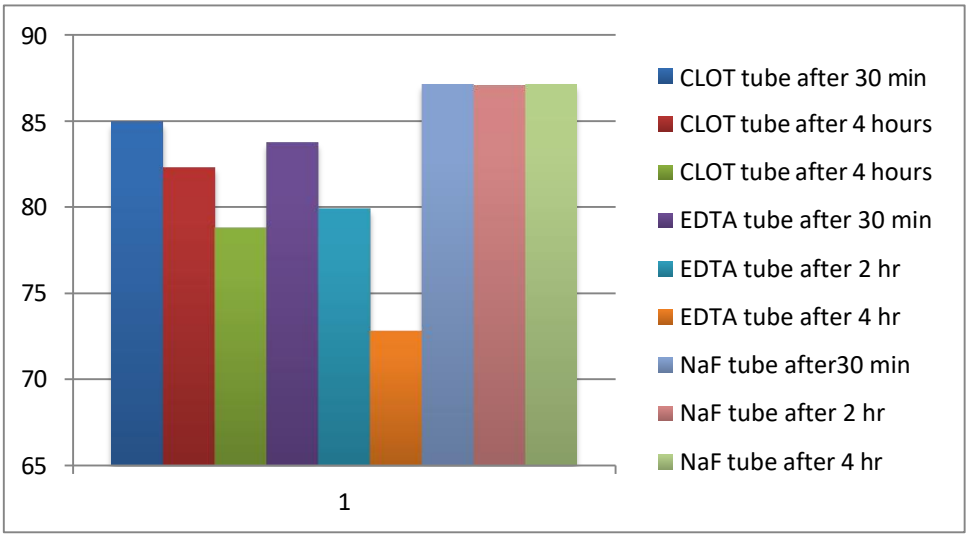

Fig. 1. Efficacy of Sodium Fluoride, EDTA and Clot Activator within 30 min, 2 hr and 4 hr.

IV. 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. 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. Its early diagnosis successful treatment and assessment of risk of developing diabetes depend on measurement of glucose concentration accurately.

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. 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. 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. In this study an attempt has been made to analyze the glucose content with various anticoagulants at various storage times. Also, 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. With respect to the concentration of glucose before storage, previous studies suggested that storage of blood using fluoride oxalate as an anticoagulant tends to better preserve the glucose level over a long period of time. For estimation of blood glucose concentration is measured in blood with serum from the clot, plasma from EDTA and NaF treated blood, there is a linear decrease in glucose after 30 min, 2 h and 4 h. A total of 50 specimens have been used for these analyses. The average mean value, standard deviation and percentages for quantitative variable were determined using descriptive statistics. ANOVA was used to compare the mean difference of glucose concentration in serum from the clot and EDTA and NaF treated serum after 30 min 2 hr and 4 hr ($p<0.000672$). There was significantly less mean change of glucose in serum after 2 hr, NaF ($p=<05$) no significant difference among mean blood glucose level in after 4 hr. The amount of blood glucose level varied based on glycolysis.

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 2 mg/ml blood. NaF inhibited enolase, thus inhibiting glycolysis. NaF and potassium oxalate are mixed in the ratio 1 : 3. α-D glucose is converted to 2-phosphoglycerate through hexokinase, phosphohexaseisomerase, phosphofructokinase, glyceraldehye 3-phosphate, dehydrogen phosphoglycerate kinase, and mutase. None of these enzymes is inhibited by fluoride. Therefore continues

to fall due to glycolytic activity. Conversion 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.

It is preferable to use serum for such estimation. However serum separated immediately after clotting such interference can be avoided during analysis of glucose and these analytes. It decreased steadily as compared to the value before storage. This actually shows that anticoagulants cannot stop, in totality, the breakdown of glucose (glycolysis). Thus, over a long period of time, the concentration of glucose may reduce continuously 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. 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. 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. There is also a suggestion that clotting consumes glucose. The amount of the differences will vary with the glycolysis rate in the individual specimen and the time elapsed between collection and centrifugation. 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. In this study, 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

V. CONCLUSION

In this study blood samples were collected from 50 individuals and tested glucose content in three different conditions of storage. Samples were divided in to three, serum from the blood clot, plasma from EDTA treated blood and plasma from NaF treated blood. After 30 min, 2 h and 4 h of storage, glucose content of the serum was analyzed. There was no significant difference of glucose level in clot, EDTA and NaF, if performed analysis within 30 minutes. However after 2 hr, glucose content decreased in the clot and EDTA treated blood, but after 4 hr glucose concentration Decreased in NaF treated samples. 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

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