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STUDY ON BLOOD GLUCOSE LEVELS PREANALYTICALLY PROCESSED ON DIFFERENT SAMPLE CUPS OVER A PERIOD OF TIME

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ABSTRACT The aim of the study is to compare random blood glucose levels processed in three different types of collection vials(i.e. Clot, EDTA, NaF) with increase in time. Blood samples are collected from 30 non-diabetic healthy individuals and are filled in equal amounts separately to clot vials, EDTA vials and NaF vials. After that serum was separated from each vials before estimation by centrifugation over 10 minutes in 3000 rpm and analyzed for blood glucose levels at hourly interval for next 5 hours by a Vitros 250 autoanalyser. The glucose level of the samples tend to fall with increase in time. It falls more rapidly in clot vials than EDTA vials. The fall is minimum in samples collected in NaF vials.

KEYWORDS : random blood glucose, Clot, EDTA, NaF.

INTRODUCTION:

Clinical biochemical tests have been performed to diagnose, predict and monitor disease for patients including annual health checkups for normal people [1].It is important to recognize that the preanalytical handling of blood samples intended for glucose measurement can influence the laboratory results[2].When left unprocessed, glycolysis occurs in the cellular component of a blood sample and may consume 5% -7 % of the sample's glucose content per hour [3].This is particularly relevant while conducting community or public health efforts, where the blood samples are collected in the field and several hours may elapsed from time of collection to laboratory analysis[4].

Blood coagulates by the transformation of soluble fibrinogen into insoluble fibrin. Anticoagulants are compounds that help prevent the clotting (coagulation) of blood. Glucose estimation requires the use of an anticoagulant. When blood is shed or collected, the cells do 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 of time. Glycolysis can be prevented with an enzyme inhibitor. The commonest inhibitor for this purpose is sodium fluoride which is usually used in conjunction with an anticoagulant potassium oxalate. Fluoride actually inhibits the enzyme enolase which is found in the metabolic pathway of glucose [5].

There are numerous publications detailing a variety of handling methods that can reduce loss of glucose [6]. The American Diabetes Association suggests prompt placement of blood samples in ice slurry or immediate separation of plasma from blood cells can halt glycolysis [7]. 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 NaF to blood tubes. Although NaF has been shown to completely arrest glycolysis by four hours, it has little to no effect on the rate of glycolysis during the first 1-2 hours [8].

This study was focused whether there were any difference in blood glucose level with increase in time .Furthermore, we explored the glucose values obtained by three different methods that are same or different, which will be cost benefit for the patients /health sector in developing countries as well as decreasing level of mistake in multiple blood collection vials.

MATERIALS AND METHOD :

All samples were obtained from venous blood of non-diabetic healthy subjects. About 9mL of the subject's blood were collected and put equally into three sample tubes; clot vials(BD Vacutainer),EDTA vials(AcCuvet K_3 EDTA) and NaF vials(JK fluoride). 0.5 mL blood samples were withdrawn and put in similar vials and then centrifuged at 3000 rpm for 10 minutes at room temperature before estimating glucose levels. The concentration of random blood glucose were determined immediately after collection using Vitros 250 dry chemistry autoanalyser (ortho-clinical diagnostics-Johnson & Johnson) and the procedure was repeated at hourly interval for next 5 hours.

Quality control: Adequate laboratory parameters were followed.
Period of study: July 2017 to December 2017.
Statistical Analysis: Done in Microsoft excel.

RESULTS AND OBSERVATION:

TABLE 1 : Table showing random blood glucose level (Mean ± SD) as per CLOT and EDTA vials and the comparative P value.

	CLOT	EDTA	P value
Immediately	97.3±17.0	98.5±15.8	0.7780
1 hour	94.0±16.6	95.0±16.3	0.8147
2 hour	89.8±16.6	92.0±16.1	0.6043
3 hour	84.6±16.5	89.2±15.5	0.2703
4 hour	80.0±16.2	85.8±15.6	0.1631
5 hour	72.2±16.6	81.5±15.4	0.0283

Observation: Random blood glucose in CLOT vials was found to be significantly lower than in EDTA vials in fifth hour.

TABLE 2 : Table showing random blood glucose level (Mean ± SD) as per EDTA and NaF vials and the comparative P value.

	EDTA	NaF	P value
Immediately	98.5±15.8	97.8±16.6	0.8677
1 hour	95.0±16.3	95.0±17.2	1.0000
2 hour	92.0±16.1	93.8±17.2	0.6771
3 hour	89.2±15.5	92.7±17.6	0.4170
4 hour	85.8±15.6	91.0±16.9	0.2206
5 hour	81.5±15.4	90.2±16.9	0.0416

Observation: Random blood glucose in EDTA vials was found to be significantly lower than in NaF vials in fifth hour.

TABLE 3: Table showing random blood glucose level (Mean ± SD) as per CLOT and NaF vials and the comparative P value.

	CLOT	NaF	P value
Immediately	97.3±17.0	97.8±16.6	0.9080
1 hour	94.0±16.6	95.0±17.2	0.8196
2 hour	89.8±16.6	93.8±17.2	0.3632
3 hour	84.6±16.5	92.7±17.6	0.0710
4 hour	80.0±16.2	91.0±16.9	0.0126
5 hour	72.2±16.6	90.2±16.9	0.0001

Observation: Random blood glucose in CLOT vials was found to be significantly lower in the fourth hour and highly significantly lower in fifth hour than in NaF vials.

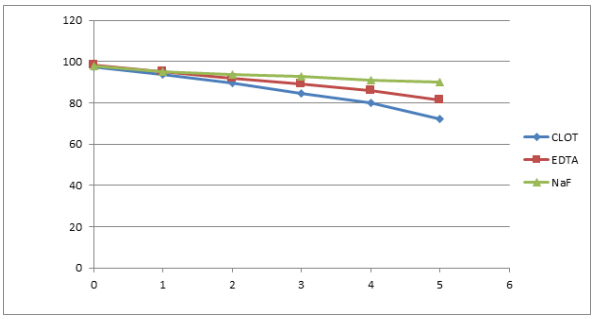

Fig: Representative changes in mean random blood glucose (mg/dL) with time (Hour) samples kept in CLOT, EDTA and NaF vials.

DISCUSSION:

In our study, random blood glucose in clot vials were significantly lower in fifth hour (P=0.0283) than in EDTA vials and similarly glucose level in EDTA vials were significantly lower in fifth hour (P=0.0416) than in NaF vials. But random blood glucose in clot vials were significantly lower in the fourth hour (P=0.0126) and highly significantly lower in the fifth hour (P=0.0001) than in NaF vials.

Sodium fluoride inhibits enolase, an enzyme which is far downstream in the glycolytic pathway. Enzyme upstream of enolase remains active and continues to metabolize glucose until substrates are exhausted. So, for the first two to three hours after blood is mixed with NaF, the rate of glycolysis is identical to that occuring in a paired blood sample collected without the addition of NaF. Thus antiglycolytic action of fluoride is delayed for upto 4 hour.

Nwangwu C.O.Spencer et al states that irrespective of anticoagulant used, the random blood glucose significantly decreased steadily as compared to the value before storage. This actually shows that anticoagulants cannot stop, in totally, the breakdown of glucose (glycolysis). Thus over a longer period of time, the concentration of glucose may reduce to zero level. With respect to the concentration of glucose before storage, it suggests that storage of blood using NaF vials as an anticoagulant, tends to better preserve the glucose level over a longer period of time [5]. This finding is comparable to the present study with decrease in concentration of random blood glucose level irrespective of the vials (Clot,EDTA,NaF) used, but for storage of blood for longer time, NaF vials is a better choice.

A.Y.W.Chan et al states that NaF is slow but effective in preserving blood glucose, having no effect in the first hour but slowing glycolysis considerably by the second hour and more or less completely inhibiting it by the fourth hour. However, for specimens assayed within 1 hour after collection, NaF is not necessary[8]. This finding is comparable to the present study, that the rate of decrease in concentration of random blood glucose level is obvious during first hour in all the vials including NaF vials. Later, in NaF vials, it becomes slow by fourth hour in comparison to the Clot and EDTA vials.

Thus it is obvious that irrespective of the time of collection or type of anticoagulant, the concentration of blood glucose was unstable during storage. It is therefore suggested that analysis for blood glucose concentrations 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 [5].

CONCLUSION:

From the present study it is observed that irrespective of the vials, the concentration of random blood glucose level tend to decrease with time. Thus to get a reliable result, sample should be estimated immediately after collection.

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