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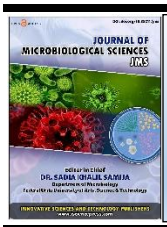

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Utility Assessment of Glyceraldehyde additive for the preservation of blood glucose and its interferences with other clinical chemistry parameters

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Abstract

Sodium fluoride is a commonly used preservative for blood glucose, but it causes interference in the analysis of other chemistry parameters. In order to decrease the economic burden on lab parameters and overcome the problem of interfering with chemical analysis by sodium fluoride an additive that has good glucose preserving ability without affecting other chemistry parameters is used. In this regard, we aimed the cross sectional comparative study to evaluate the utility of glyceraldehyde additive for the preservation of glucose and its interferences with other clinical chemistry parameters. The blood glucose, urea, sodium, and potassium levels in blood samples were taken from 25 volunteers in three different tubes (sodium fluoride, plain, and glyceraldehyde tube). There was no significant difference between glucose, Urea, sodium, and potassium levels of glyceraldehyde tube and Sodium fluoride tube after initial measurement of 30 minutes and later 8 hours, but glucose levels were seen significantly lower in plain tube at 8 hours measurement than initial measurements. It was seen as significant that the use of a single glyceraldehyde tube should be enough and affordable to avoid extra tube costs.

Key words: sodium fluoride, glyceraldehyde, University of Health Sciences, glucose, and urea

Introduction

Blood glucose can be measured from serum or plasma (Frank *et al.*, 2012). Glycolysis occurs in blood samples and glucose level decreases by 5-7% that is 10 mg/dl (0.6 mmol/dl) in one hour (Greene *et al.*, 2010). Therefore either glycolytic inhibitor should be used as glucose preservatives or it should separate from cells within 30 minutes of collection (Stapleton *et al.*, 2017). Different preservatives are used to prevent glycolysis and to maintain glucose levels in blood samples, but sodium fluoride is one of the most commonly used glucose preservatives (Pasqualetti *et al.*, 2017). Sodium fluoride is available commercially in the grey top tubes in combination with potassium oxalate or disodium EDTA (Na_2EDTA). Effective concentration of sodium fluoride is 2.5 mg/ml and potassium oxalate is effective at concentration of 2.0mg/ml[2,]. This combination stops the utilization of glucose by red cells and prevents the clotting of samples (Narayanan, 2000). Fluoride ions also inhibit the action of urease in high concentration and also affect the measurement of electrolytes (sodium and potassium), consequently, the sample may become unsuitable for estimation of electrolytes and urea (Ganapathy *et al.*, 2016). Other antiglycolytic agents are also available commercially which makes it possible to measure other analytes (urea and electrolytes) from the same sample container. One of these antiglycolytic agents is glyceraldehyde (Goto *et al.*, 1994). It makes sorbose-1-phosphate, by combining with dihydroxyacetone phosphate which prevents glycolysis by inhibiting the action of hexokinase. Glyceraldehyde is effective in preserving blood glucose at a low concentration of 0.22 mg/ml of blood (Mangukiya *et al.*, 2013). Moreover, it does not affect electrolytes (sodium and potassium) and urease enzymes thus making it suitable for estimation of other chemistry analytes as well (Landt, 2000). Different studies tested glyceraldehyde for its antiglycolytic effect and its interference with other chemistry

parameters (Banerjee *et al.*, 2018; Michailidou *et al.*, 2022), but its effects on glucose and other parameters are still needed to check. Therefore current study is aimed to access the utility of glyceraldehyde additive for the preservation of glucose level as well as the accuracy of clinical chemistry parameters by comparing the inner tube and intra tube glucose levels of glyceraldehyde and sodium fluoride for initial 30 minutes measurement and later 8 hours measurement.

Material and methods

In this cross-sectional comparative study, samples were collected from 25 volunteers in the chemical pathology department of the University of Health Sciences (UHS) Lahore. The study was approved by the ethical review committee of UHS. The sample size was calculated by Cochran's formula keeping the confidence level 95% and margin of error from 5% to 7%. The calculated size was 5 but to increase the authenticity of the study sample size was increased to 25 for each tube. Commercially prepared sodium fluoride and plain tubes were used, and glyceraldehyde tube was prepared by adding 45 microliters of 5mmol/L glyceraldehyde in a heparinized tube. Heparin was used as an anti-coagulant whereas glyceraldehyde was tested for its antiglycolytic action. Tubes were dried for 48 hours at 37°C. After complete drying, tube were sealed tightly by screw caps. Samples were collected after taking written consent and giving comprehensive information related to the study. Eight (8) ml of intravenous blood was drawn from each volunteer in full hygienic condition. After collection of 8 ml blood, it was divided into 3 separate vacutainer tubes; 2 ml blood in sodium fluoride (NaF) vacutainer, 3 ml blood in plain vacutainer (with clot activator), and remaining 3 ml in D, L-glyceraldehyde vacutainer. All samples were centrifuged and were tested after 1 hour for blood glucose from Sodium fluoride and D, L-glyceraldehyde vacutainer and for urea and Sodium and Potassium from D, L-glyceraldehyde vacutainer. Similarly all the samples were tested after storage of 8 hours at room temperature. Blood glucose and Urea were tested by a semi-automated chemistry analyzer Microlab 300. Sodium and potassium were measured on HumaLyte Plus3 by ISE. Two samples t-test was used for comparative analysis. The p-value less than or equal to 0.05 was considered statistically significant in all statistical tests.

Results

Inter tube comparison for glucose levels (1 hour and 8 hours after collection) between Sodium Fluoride Tube and Glyceraldehyde Tube was performed by two-sample t-test. The mean results of both tubes were not significantly different with a p-value of 0.66 for 1-hour analysis and 0.78 for 8 hours after collection analysis. Intra tube comparison between 1-hour glucose levels and 8-hour glucose levels of Sodium Fluoride tube and Glyceraldehyde Tube were carried out and here we found a significant difference for both tubes with a p-value less than or equal to 0.05 (Table 1).

Table 1: Comparison of Glucose results between Sodium Fluoride tube and Glyceraldehyde at 1 hour and 8 hours after collection.

Test	Sodium Fluoride Tube (n= 25)	Glyceraldehyde Tube (n= 25)	P-Value
Glucose (1 hour)	110.5±12.1	109 ± 12.2	0.66
Glucose (8 hours)	103 ± 12.6	102 ± 13.4	0.78
P-value	0.05	0.05	
Key: Values p<0.05 are found statistically significant			

Inter tube levels of Urea, Sodium, and Potassium were compared between Plain Tube and Glyceraldehyde Tube, for a 1-hour assessment after collection and 8 hours after collection. The results of both tubes at both intervals were comparable with each other with a p-value >0.05.

Intra tube comparison of Urea, Sodium, and Potassium results at 1 hour and 8 hours assessment after collection for Plan tube and Glyceraldehyde tube was carried and intra tube results of both tubes were not significantly different for 1-hour assessment and 8-hours assessment (Table 2)

Table 2: Comparison of Urea, Sodium and Potassium results between Sodium Fluoride tube and Glyceraldehyde at 1 hour and 8 hours after collection.

Test	Plan Tube (n= 25)	Glyceraldehyde Tube (n= 25)	P-Value
Urea (1 hour)	24 ± 5.3	24 ± 5.3	1.00
Urea (8 hour)	24 ± 4.5	24 ± 5.6	1.00
P-Value	1.00	1.00	
Sodium (1hour)	143 ± 4.4	141 ± 4.9	0.13
Sodium (8hour)	142 ± 3.1	141 ± 3.9	0.32
P-Value	0.35	1.00	

Potassium (1hour)	4.3 ± 1.0	4.4 ± 1.1	0.73
Potassium (1hour)	4.1 ± 1.8	4.5 ± 0.5	0.28
P-Value	0.62	0.79	

Discussion

In our study, we found that the results of glucose between sodium fluoride tubes and glyceraldehyde tubes at 30 minutes after collection and at 8 hours after collection were almost similar, and the p-value was greater than 0.05, but intra tubes results of both tubes at 8 hours after collection were significantly lower than initial results. This result was in accordance with the study of Genapathy *et al*, who reported no significant difference in mean glucose concentrations of glyceraldehyde tube and sodium fluoride tube (p value= 0.48) after 8-hours storage at room temperature[3] but this study of Genapathy *et al* did not compare 8-hours results with 30 minutes results of these samples. It has been described previously that during the first three hours, the concentration of blood glucose decreases slowly in the sodium fluoride tube, and then it becomes stable for 3 to 8 days (Frank *et al.*, 2012). To overcome this problem, it is recommended that sodium fluoride preserved blood samples should be analyzed within 30 minutes of collection, if the delay is suspected, then plasma should be separated within 30 minutes to get reliable results (Greene *et al.*, 2010). A decrease in glucose levels during the first three hours has also been advocated by del Pino *et al*., who compared the glucose levels in sodium fluoride tubes at different intervals and found significant differences till 180 minutes after collection (Stapleton *et al.*, 2017). However, Sodium fluoride is still a commonly used tube for glucose analysis and the results of the glyceraldehyde tube are comparable with sodium fluoride tube with the advantage of less or no interferences with other chemistry parameters like urea, sodium, and potassium in the samples preserved in glyceraldehyde (Pasqualetti *et al.*, 2017). Another study reported addition of citrate buffer to routinely used glucose preservatives to get immediate preservation (Narayanan, 2000). Transportation of samples in ice slurry is also reported in previous studies (Ganapathy *et al.*, 2016). According to a current study, glucose results are reliable till 4 hours at room temperature (Goto *et al.*, 1994). Hence, current study recommends the use of glyceraldehyde as a preservative agent because of its efficacy as compare to other tested preservative. Preservation of glucose by D, L-Glyceraldehyde coated tubes and reliability of results have been reported in previous studies (Landt, 2000; Mangukiya *et al.*, 2013). We also compared the results of sodium and potassium on the ISE (Ion Selective Electrodes) method between glyceraldehyde and plain tubes and urea on the spectrophotometric method at 30 minutes after collection and 8 hours after collection. Here we found excellent comparable results between both tubes at both intervals. During intra-tube results comparison at both intervals, we also found no significant variations and the p values were greater than 0.05 for both comparative analyses (Table 2). Findings of current study are in line with previous studies for the assessment of glyceraldehyde interferences with clinical chemistry parameters (Landt, 2000; Mangukiya *et al.*, 2013). Critical reviews by other scientists also indicated that glyceraldehyde has good preservative characteristics for glucose and acceptable additive having no interferences with other chemistry parameters like electrolytes, hormones, and Enzyme measurements (Banerjee *et al.*, 2018; Pasqualetti *et al.*, 2017).

Conclusion and recommendations

It is concluded from this observation that Glyceraldehyde tube results are comparable for glucose with a sodium fluoride tube as well as for urea, sodium and potassium with a plain tube so we may use a single Glyceraldehyde tube for glucose as well as chemistry parameters.

- Large scale study should be done to further strengthen and verify the results of the current study.
- A study should be designed in such a way that glucose levels should be monitored every hour to see the rate of decrease in glucose values to check how long the NaF tube and Glyceraldehyde tube remain effective for the preservation of blood glucose.

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