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Original Research Article

Study of various glycolytic inhibitors for preservation of blood glucose and clinical biochemical parameters

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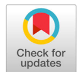

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ABSTRACT

Introduction: Sample for plasma glucose estimation is taken in fluoride Vacutainer and that is to prevent glycolysis and so that accurate glucose estimation can be done.
Objectives: The objective of the study is to study various glycolytic inhibitors for preservation of blood glucose and other common clinical biochemical parameters.
Materials and Methods: This prospective study was conducted at new civil hospital Surat. Study includes total 100 participants. We have prepared 5 mmol/L DL Glyceraldehydes containing Vacutainer. Blood sample from all participants were taken in 3 Vacutainer like plain Vacutainer, fluoride Vacutainer and DL Glyceraldehydes containing Vacutainer. Various biochemical investigations were performed from all above collected blood samples to see the difference of significant among them by calculating p value. P value less than 0.05 was considered as a difference of significant.
Results: There is no significant changes in results of glucose between fluoride and glycrealdehyde containing Vacutainer. Biochemical parameter like SGPT, Creatinine, Total Bilirubin, Albumin, cholesterol, Total protein, Uric acid, electrolytes also does not show any significant difference between DL-Glyceraldehyde and plain Vacutainer. The Result of Serum Creatinine was found to be high from Glyceraldehyde containing Vacutainer as compared to plain Vacutainer and difference among them is highly significant.($p<0.001$)
Conclusion: From our study we would like to conclude that. DL -Glyceraldehyde containing vial for many biochemistry related parameter analysis is better option as it is save additional use of vaccutte, except for serum creatinine (by alkaline picrate method). So DL Glycrealdehyde cannot be solely used as alternative for plain and fluoride containing Vacutainer.

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1. Introduction

Estimation of blood glucose is routinely required now a days because there are many metabolic disorders that has direct connection with blood glucose level like diabetes mellitus, lipid storage disorder, hypertriglyceridemia etc.[1,2]

A continuing problem in the accurate measurement of glucose is the loss of glucose from specimens because of glycolysis by erythrocytes during transport and processing. In recent years this phenomenon has been more evident as laboratory services have consolidated and many more specimens are transported to distant laboratories for analysis. Several approaches have been proposed to minimize glucose loss, including centrifugation /decantation of plasma immediately after specimen collection, refrigeration /cooling on ice during transport, addition of antiglycolytic agents such as iodoacetate, fluoride or mannose to the collection tubes; and the use of glucose analyzers designed for near -patient testing, at the bedside.[3,4]

All of these approaches are in current use, and the use of fluoride in blood collection tubes is prevalent in circumstances where substantial delay between collection and analysis is anticipated; however, all have substantial limitations. To various degrees, these approaches are

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limited in efficacy by incomplete inhibition of glycolysis, interference in testing for co -analytes (e .g., electrolytes, creatinine , and urea), disturbance of cellular integrity (e .g., haemolysis), or promotion of leakage of intracellular potassium.[5–7]

Considerable effort has been expended in the past to find a highly effective preservative of glucose for blood collection that does not interfere in other common clinical chemical tests, does not cause haemolysis or other loss of cellular integrity, is nontoxic, is stable for storage at room temperature, and is inexpensive. While fluoride is the single most common preservative used for blood glucose measurement, many other strategies are in use.

Among them most commonly used glycolytic inhibitor is sodium fluoride.[5] Fluoride forms a complex with magnesium and phosphate ions. This complex binds to the active site of enolase that is involved in glycolysis resulting in competitive inhibition of the enzyme so that glycolysis can be prevented and exact estimation of blood sugar level can be done. But the main problem is that we cannot perform other clinical biochemistry tests like Liver function tests, renal function test, lipid profile etc.[8,9] and for that we have to take blood sample in plain Vacutainer so that cost per test is increased and also more amount of patients blood is required and it also leads to mistake in laboratory barcode system.

To solve all this problems we have tried DL Glycealdehyde as an alternative of fluoride so that all tests can be performed in single Glyceraldehydes containing Vacutainer.

2. Materials and Methods

This Study was conducted at N ew civil hospital, Surat, Gujarat by Department of B iochemistry from 210-2013 after obtaining permission of institutional ethical committee.

2.1. Patient selection

Samples were taken from consenting indoor patients of New Civil Hospital.(n=100)

2.2. Stock solution of D, L-Glyceraldehyde

3% stock solution was prepared by adding 3 gm of D, L-glyceraldehyde in 100 ml of water.

2.3. Preparation of D,L-Glyceraldehyde Tubes

D,L-Glyceraldehyde Tubes was prepared by adding 60 micro liter of D, L-glyceraldehyde of 5 mmol/l after removing vacuum. Tube was allowed to dry for a period of 48 hours at 37 ´C. after complete drying vacuum tube its cap was reapplied. Final concentration of D,L-glyceraldehyde was 5 mmol/l.

2.4. Sample collection

10 ml of venous blood was collected from consenting indoor patients of New Civil Hospital admitted at various ward of New civil hospital, Surat. Sample was collected in supine position under full aseptic precaution after taking written consent and giving complete information regarding to study. Blood was collected from Median cubital vein. After collection of 10 ml blood, it was distributed in a 3 separate vacutainer Tube; 2 ml blood in fluoride vacutainer tube, 4 ml blood in plain vacutainer (with clot activator) and remaining 4 ml is in D, L-glyceraldehyde Vaccutainer Tube. Dummy identity number was given to each participant involve in study. Same identity number also given on vacutainer tube.

All above samples were put at room temperature at 25´C for a period of 8 hr. in clinical biochemistry laboratory of New Civil Hospital. After 8 hr, all above sample was centrifuged at 3000 RPM in R-8C BL Bench top Remi centrifuge for a period of 10 minutes. Aliquot ware prepared from serum/plasma separated from above samples.

Samples were analyzed at biochemistry laboratory of New civil Hospital, Surat in fully auto analyzer ERBA XL 640. Along with quality control sera of normal and abnormal range. Following parameters were analyzed

2.5. Statistical analysis

Results of all examinations were exported from the analyzer in to Microsoft office spreadsheet. Mean and Standard deviation (SD) was calculated for all parameters. P value was calculated by using online student t test calculator and Graph pad Prism software.

3. Results and Discussion

After analysis of various biochemical parameters, P-value is calculated by using online student t-test calculator. In case of glucose, comparison was done between Glyceraldehyde vs. Fluoride, Glyceraldehyde vs. plain and Fluoride vs. plain (Table 2). If P-value is <0.05, then the difference between them is consider significant and if It is >0.05, then it is consider as a non-significant. While rest of other common clinical parameters, comparison was done between plain tube and Glyceraldehyde containing tube. (Table 3)

Graph 1 showing that Average Glucose concentration in 5 mmol/L DL-Glyceraldehyde, Fluoride and Plain tube each was 124 mg/dl, 120 mg/dl and 90 mg/dl respectively after 8 hours.

P-value for glyceraldehyde tube vs. Fluoride tube was 0.5432, P-value for glyceraldehyde tube vs. Plain tube was 0.0085 and P-value for Fluoride tube vs. Plain tube was 0.0078.(Table 2)

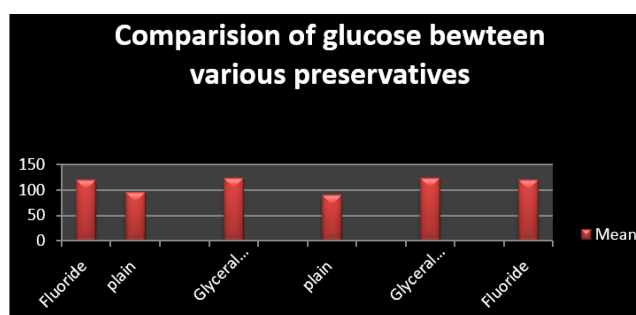
The P-value of Glucose between Fluoride and Glyceraldehyde containing tube is 0.54, that is non significant. It indicates that Glyceraldehyde prevents glycolysis in blood for at least up to 8 hours at room temperature

Table 1: Name of various biochemical parameters analyzed

Vacutainer tube	Parameters
Fluoride	Glucose
Plain	Glucose, SGPT, Creatinine, Total Bilirubin, Albumin, cholesterol, Total protein, Uric acid, electrolytes
DL -Glyceraldehyde	Glucose, SGPT, Creatinine, Total Bilirubin, Albumin, cholesterol, Total protein, Uric acid, electrolytes

Table 2: Comparision of glucose from various preservative

Parameter	Preservative pair compared	Preservative	Mean ± SD	P value
Glucose (mg/dl)	Fluoride Vs plain	Fluoride	120 ±5	0.0078(S)
		Plain	90 ±6	
	Glyceraldehyde Vs plain	Glyceraldehyde	124 ±7	0.0085(S)
		Plain	90 ±6	
	Glyceraldehyde Vs Fluoride	Glyceraldehyde	124 ±7	0.5432(NS)
		Fluoride	120 ±5	



Graph 1: Showing graphical presentation of comparisons of glucose from various preservative

as efficiently as Fluoride. The P- value of Glucose between Fluoride containing tube and plain tube as well as between Glyceraldehyde containing tube and plain tube is significant because glycolysis remain continuous in plain tube due to absence of glycolytic inhibitor like Fluoride or Glyceraldehyde. Unless, turnaround time for measurement of glucose (from sample collection to analysis of sample is as low as 1 hour, laboratory may not use plain tube for measurement of glucose.

P-value for SGPT, Creatinine, Total Bilirubin, Albumin, cholesterol, Total protein, Uric acid, electrolytes were unaffected by DL-Glyceraldehyde, while there was negative interference with Creatinine measurement by Alkaline Picrate method.

The p-value of serum creatinine by alkaline picrate method for Glyceraldehyde vs. Plain tube is <0.05, so the difference between creatinine of Glyceraldehyde vs Plain tube is considered significant. The bias caused by Glyceraldehyde is positive. It is likely that Glyceraldehyde, like acetoacetate, pyruvate, protein etc. reacts with alkaline picrate causing positive interference in creatinine measurement. Further study is required to find time-window during which glyceraldehyde react with alkaline picrate, weather any change in reagent, incubation period and measurement period can decrease Glyceraldehyde interference to creatinine measurement. This difference can be avoided by estimatings. creatinine by enzymatic method. [10–12]

The ideal approach to eliminating glucose loss would provide reasonably stable glucose concentrations for the period needed for transport to a centralized laboratory, avoid costly near-patient analysis, and yield a specimen that was suitable for analysis of many other common analytes so that separate collection of specimen for those analytes was not necessary. [13,14] From a practical standpoint, the best way to achieve this goal is discovery of an antiglycolytic agent that could be added to collection tubes but did not alter cellular integrity or interfere in common analytical methodologies. Such an agent should also be effective at low concentrations (minimizing volume addition to avoid dilution errors), dissolve rapidly during the collection process, be nontoxic, be stable in the room-temperature storage environment of blood collection devices.

The study done by Michael Landt using DL-Glyceraldehyde by using enzymatic kit for Measurement of serum creatinine in (Vitros 250 and Hitachi 747)fully automated biochemical analyzer does not show significant difference between serum creatinine value of plain tube (without additive) and DL-Glyceraldehyde tube. Measurement of serum creatinine in (Dade Behring RXL) fully automated biochemical analyzer by using Alkaline Picrate method shows positive interference by any form of Glyceraldehyde. [15,16]

4. Conclusion

From our study we would like to conclude that. DL -Glyceraldehyde containing vial for many biochemistry related parameter analysis is better option as it is save

Table 3: Comparison of various biochemical parameters from plain and glyceraldehyde tube

Parameter	Preservative pair compared	Preservative	Average	P value
Total cholesterol (mg/dl)	Glyceraldehyde Vs plain	Glyceraldehyde	148 ± 9	0.7050
		plain	151 ± 8	
Albumin (gm/dl)	Glyceraldehyde Vs plain	Glyceraldehyde	4.2 ± 1	0.4050
		plain	3.9 ± 0.9	
ALT (U/L)	Glyceraldehyde Vs plain	Glyceraldehyde	35 ± 5	0.8502
		plain	38 ± 6	
Creatinine (mg/dl	Glyceraldehyde Vs plain	Glyceraldehyde	1.5 ± 0.3	**<0.050**
		plain	0.8 ± 0.4	
Sodium (mmol/l)	Glyceraldehyde Vs plain	Glyceraldehyde	139 ± 5	0.5063
		plain	136 ± 4	
Potassium (mmol/l)	Glyceraldehyde Vs plain	Glyceraldehyde	3.90 ± 0.8	0.6050
		plain	4.3 ± 1.0	
Total protein (gm/dl)	Glyceraldehyde Vs plain	Glyceraldehyde	7.5 ± 1	0.5602
		plain	7.7 ± 1.2	
Uric acid (mg/dl)	Glyceraldehyde Vs plain	Glyceraldehyde	4.5 ± 1.0	0.5006
		plain	4.2 ± 0.9	
Total bilirubin (mg/dl)	Glyceraldehyde Vs plain	Glyceraldehyde	1.0 ± 0.3	0.7560

additional use of vaccutte , except for serum creatinine (by alkaline picrate method). So DL Glycrealdehyde cannot be solely used as alternative for plain and fluoride containing Vacutainer.

5. Authorship

All Authors have done Equal contribution for research work.

6. Acknowledgement

We would like to acknowledge the whole Biochemistry department staff as well as lab technicians.

7. Conflict of interest

Nil

References

1. Chan AYW, Swaminathan R, Cockram CS. Effectiveness of sodium fluoride as a preservative of glucose in blood. *Clin Chem*. 1989;35:315–317.
2. Krebs HA, Lund P. Formation of glucose from hexoses, pentoses, polyols and related substances in kidney cortex. *Biochem J*. 1966;98:210–214.
3. Astles R, Williams CP, Sedor F. Stability of plasma lactate in vitro in the presence of antiglycolytic agents. *Clin Chem*. 1994;40:1327–1330.
4. Orang AV, Petersen J, Mckinnon RA, Michael MZ. Micromanaging aerobic respiration and glycolysis in cancer cells. *Mol Metab*. 2019;23:98–126.
5. Feig SA, Shohet SB, Nathan DG. Energy metabolism in human erythrocytes, I: effects of sodium fluoride. *J Clin Invest*. 1971;50:1731–1737.
6. Liu Y, Zhang Z, Wang J, Chen C, Tang X, et al. Metabolic reprogramming results in abnormal glycolysis in gastric cancer: a review. *Onco Targets Ther*. 2019;12:1195–1204.
7. Hall PM, Cook JGH. Fluoride and iodoacetate interfere with glucose determination with Yellow Springs glucose analyzer. *Clin Chem*. 1982;28:387–388.
8. Astles R, Williams CP, Sedor F. Stability of plasma lactate in vitro in the presence of antiglycolytic agents. *Clin Chem*. 1994;40:1327–1330.
9. Weil R, Altszuler N, Kessler J, Amer J. *J Physiol*. 1997;201:251–256.
10. Vaidyarrathan VV, Shastry PS, Ramasarma T. Regulation of the activity of glyceraldehyde-3-phosphate dehydrngenase by glutathione and HzOz. *Mol Cell Biochem*. 1993;129:57–65.
11. Mcdonald LJ, Moss J. Stimulation by nitric oxide of m NAD linkage to glyceraldehyde-3-phosphate dehydrogenase. *Proc Nat Acad USA*. 1993;90:6238–6241.
12. Villee CA, Hastings AB. -. *J Biol Chem*. 1999;181:131–133.
13. Bergmeyer HU. Methods in Enzymatic Analysis, 2nd ed. In: HU. Methods in Enzymatic Analysis. Weinheim: Verlag Chemie ; 1974,. .
14. Goto I, Inaba M, Shimizu T, Maede Y. Mechanism of hemolysis of canine erythrocytes induced by l-sorbose. *Am J Vet Res*. 1994;55:291–294.
15. Landt M. Glyceraldehyde Preserves Glucose Concentrations in Whole Blood Specimens. *Clin Chem*. 2000;46(8):1144–1149.
16. Astles R, Williams CP, Sedor F. Stability of plasma lactate in vitro in the presence of antiglycolytic agents. *Clin Chem*. 1994;40:1327–1330.

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