

D, L Glyceraldehyde- Will it be an effective Antiglycolytic Agent?

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Abstract

Aim: This study aimed to compare the effectiveness of D, L Glyceraldehyde (GLY) with Plain Sodium Fluoride (NaF) as an antiglycolytic agent and its interferences in the estimation of Serum creatinine by Modified Jaffe's method and Serum Sodium and Potassium by ion selective electrode (ISE) method.

Materials and Methods: Eighty random blood samples were collected from the OP patient after obtaining explicit consent. Serum Glucose, Creatinine, Sodium and Potassium were estimated in all the three tubes and the results were compared across.

Statistical Analysis: ANOVA test was done to compare the analytical values obtained from all the three tubes.

Results: Mean Glucose concentration in Plain Tube with Clot activator (PTC), NaF tube and the GLY tube were 142.25 mg/dl, 124.41mg/dl and 139.23mg/dl respectively. p-value for GLY tube vs NaF was 0.483, GLY tube vs PTC was 0.970 and NaF vs PTC was 0.349 (p value < 0.05 considered significant). There was Positive interference with creatinine measurement by modified Jaffe's method (p<0.0001) and for Serum Potassium estimation by ISE method (p<0.0001). Both sodium and potassium estimation in NaF tube showed significantly increased values when compared to analysis in PTC.

Conclusions: For preventing ex vivo glycolysis, D, L-Glyceraldehyde coated tubes is as good as PTC tube for glucose estimation by glucose oxidase peroxidase (GOD-POD) method. D, L Glyceraldehyde coated tubes can be used for the estimation of serum sodium but not for potassium by ISE method and creatinine by Modified Jaffe's method. PTC tube cannot be replaced by GLY tube for routine chemistry analysis.

Keywords: D, L Glyceraldehyde, Plain tube with clot activator, Sodium Fluoride, Antiglycolytic agent, Glycolysis, Glucose.

Introduction

Glucose is one of the most commonly analysed components in all clinical laboratories as glucose concentration is indispensable in defining Diabetes mellitus (DM). Diabetes mellitus is diagnosed based on the well-established cut-off values originating from the worldwide-accepted guidelines. According to the American Diabetes Association (ADA) and the World Health Organization (WHO), to define DM, it requires >2 independent estimations of fasting plasma glucose concentration of >126 mg/dl, random plasma glucose concentration of >200 mg/dl or 2 h post-oral glucose tolerance test plasma glucose concentration of >200 mg/dl^[1]. Categorization of individuals to the most pertinent diagnostic category is critically reliant on the availability of precise glucose measurements.

Glucose concentration is inversely proportional to serum contact time^[2]. Ex vivo glycolysis by cellular components in blood decreases the blood glucose concentration if there is delay in the transport and/or sample processing or leukocytosis. The WHO has specified that glucose concentrations should be done only on serum with immediately removed cellular components or else glycolysis will result in an erratic underestimation of the true concentration^[7]. Glycolysis is lessened by immediate centrifugation and sample refrigeration or by adding antiglycolytic agents like iodoacetate, D-mannose, sodium fluoride (NaF) but each method has its own major drawbacks.

In the everyday clinical scenario, early separation of serum is found to be practically not feasible. So blood collection tubes containing NaF has become increasingly accepted as a means of curtailing ex vivo glycolysis in clinical practice and is endorsed by WHO. But the disadvantage with NaF is that its antiglycolytic action is delayed for upto 4 hours and during the first 1–2 hours it has only little or no effect, also it interferes with certain enzyme reactions for example, urease^[6]. Various studies have shown that transport of fluoride into the human erythrocytes is even though rapid across the erythrocyte membrane but the antiglycolytic effect is delayed because glycolytic enzymes upstream of enolase remain active and continue to metabolize glucose until substrates are exhausted^[3-5].

Glyceraldehyde in the recent days is considered to be the newer antiglycolytic agent. It exist either as D or L isomers. In the recent past D-Glyceraldehyde was used as invitro stimulus of insulin secretion from pancreatic β cells^[9]. Commercially it is available either as separate isomers or as racemic mixture. Trials have shown that the L isomer was responsible for all or most of the antiglycolytic activity of the racemic mixture^[8]. Glyceraldehyde has its own major advantages than NaF by exerting its action at hexokinase level thus preventing initial one hour glycolysis.

D, L Glyceraldehyde minimizes dilution effect (L-Glyceraldehyde is fully active at concentrations as little as 2.5mmol/L), maintains cell membrane integrity,

stable at room temperature, dissolves rapidly, soluble at higher concentration, inexpensive and nontoxic.

Generally glucose analysis are requested in combination with other tests, thus an ideal antiglycolytic agent added should not interfere with other analyte methodologies. The purpose of my study is to explicate any pre-analytical handling of blood samples intended for glucose measurement can influence the laboratory results and the interferences of the added antiglycolytic agents interferes in the estimation of the serum Sodium, Potassium and Creatinine.

Materials and Methods

All procedures concerning human subjects or patients were permitted by the Institutional Ethical Committee. Explicit written consent was obtained from the patients. D, L-Glyceraldehyde was procured from Sigma Aldrich.

Preparation of GLY tube: Concentration of GLY used for antiglycolytic effect in this study was 5 millimoles/Litre. Molecular weight of GLY is 90.07794 grams/mole

Stock solution of GLY: Stock= 3 Grams %

GLY tubes were prepared by adding 30 micro liter of D, L-glyceraldehyde with the concentration of 5mmol/L. Coated tubes were allowed to dry for a period of 48 hours at 37°C. After complete drying, tube cap was applied. Final concentration of D, L-glyceraldehyde was 5mmol/l.

Eighty random blood samples were collected from the OP patient, explicit written consent was obtained

from the patients. Six ml of venous blood was extracted from each patient and it was equally distributed - 2ml in PTC tube (5 inversions), 2ml in NaF tube (8 inversions) and 2ml in the tubes coated with 5mmol/L of GLY (8 inversions). Serum from the PTC were separated after 30 minutes and refrigerated at 4°C until analyzed. Sera from the NaF and GLY tube were put at room temperature at 25°C for a period of 8hrs and were centrifuged for a period of 10 minutes at 3000 RPM in R-8C BL Bench top Remi centrifuge. Three aliquot were made from serum separated from above samples.

Quality control: Serum Glucose, Creatinine, Sodium and Potassium analyses were done using RANDOX calibrator (lot no 2351-562UE) and 2 level of Controls (lot no 768UN and lot no 501UE) used for checking internal quality. One aliquot was used for the estimation of Serum glucose by GOD-POD method and the other two for the estimation of serum electrolytes by ISE (Roche 9180 Electrolyte Analyzer) and Creatinine by Modified Jaffe's method in Merkmicrolab 300 semi autoanalyzer and the analyte values were compared across the three tubes.

Results

In this study serum acquired from PTC tube in which serum was separated after 30 minutes of blood collection and refrigerated at 4°C. Serum Glucose, Creatinine, Sodium and Potassium were estimated in all the tubes and the values were compared across the three test tubes. ANOVA test was done to compare the analytical values.

Table 1: Serum glucose values across PTC, NaF and GLY coated tubes

Analytes Estimated	PTC Mean \pm SD	NaF Tube Mean \pm SD	Gly Coated Tube Mean \pm SD
Glucose (mg/dl)	142.25 \pm 80.832	124.41 \pm 80.466	139.23 \pm 82.628

Table 2: Multiple comparison of glucose values across PTC, NaF & GLY coated tubes

Analyte Compared	Total number of samples	Preservatives Compared		p Value (Level of significance)
		PTC	NaF	
Serum Glucose (mg/dl)	80 Random Samples	PTC	NaF	0.349
		PTC	GLY	0.970
		NaF	PTC	0.349
		NaF	GLY	0.483
		GLY	PTC	0.970
		GLY	NaF	0.483

p value < 0.05 considered significant

Glucose values were compared across three tubes. Sera from GLY coated tube (5mmol/L), NaF tube and from PTC were estimated for glucose by Glucose oxidase peroxidase(GOD-POD) method. Mean glucose concentration in PTC, NaF tube and the GLY tube (5 mmol/L) was 142.25 mg/dl, 124.41mg/dl and 139.23mg/dl respectively. Multiple comparison showed p-value for GLY tube vs. NaF was 0.483, p-value for GLY tube vs. PTC was 0.970 and p-value for NaF vs. PTC was 0.349(p value < 0.05 considered significant).

Table 3: Serum Creatinine, Sodium, Potassium values across PTC, NaF & Gly coated tubes

Analytes Estimated	PTC Mean \pm SD	NaF Tube Mean \pm SD	Gly coated tube Mean \pm SD
Serum Creatinine (mg/dl)	0.92 \pm 0.51	0.89 \pm 0.47	1.70 \pm 0.55
Serum Sodium (mg/dl)	140 \pm 3.11	144 \pm 3.69	140 \pm 3.04
Serum Potassium (mg/dl)	4.2 \pm 0.61	Very High	5.1 \pm 0.6

Table 4: Multiple comparison of Creatinine, Sodium & Potassium values across PTC, NaF & Gly coated tubes

Analytes Compared	Total number of samples	Preservatives Compared		p Value
Serum Creatinine	80	PTC	NaF	0.955
		GLY	PTC	<0.0001
		NaF	GLY	<0.0001
Serum Sodium	80	PTC	NaF	<0.0001
		GLY	PTC	0.694
		NaF	GLY	<0.0001
Serum Potassium	80	PTC	GLY	<0.0001

p value < 0.05 considered significant

Sera from GLY coated tube (5mmol/L), NaF tube and from PTC were estimated for creatinine by modified Jaffe's method and Sodium and Potassium by ISE method. There was no significant difference in values of serum Creatinine between the plain tube and NaF tube but significant difference was noted between GLY tube and both plain tube and NaF ($p < 0.0001$). Serum sodium showed no statistical difference between GLY tube and plain tube. For Serum Potassium estimation significant difference exist between GLY tube and plain tube ($p < 0.0001$). Both sodium and potassium estimation in NaF tube showed significantly increased values when compared to PTC.

Discussion

The difference in mean of glucose values for GLY tubes and PTC were smaller (139.23mg/dl and 142.25 mg/dl respectively) and not statistically significant. Even though no statistically significant difference exist on multiple comparison of three test tubes, the difference in mean of glucose values between PTC tubes and NaF were greater (142.25 and 124.41mg/dl respectively) compared to difference between PTC and GLY tube possibly due to delay in the inhibition of glycolysis and could also be due to dilution effect^[3-5,7]. With the immediate centrifugation and refrigeration of sample from the PTC is considered as standard, glucose values obtained from GLY tube was as comparable to it. This confirms that GLY can be used as an antiglycolytic agent.

Possibly GLY (free or its phosphorylated forms) mediates its antiglycolytic action by the formation of sorbose-1-phosphate by condensation with dihydroxyacetone phosphate. Sorbose 1 phosphate is an inhibitor of hexokinase^[10].

In recent past effort has been expended to identify an effective antiglycolytic agent that should not interfere with other clinical analyte methodologies. Analyzing stability and solubility of GLY, it appears to be an ideal additive since it is highly soluble (30 g/L), stable at room temperature in the crystalline form and is effective at concentrations as little as 2.5 mmol/L(L isomer)^[8]. This eliminates the volume dilution of the specimen by the additive and become practically more important when collection tubes are partially filled^[8].

In this study it is considered that GLY is causing positive interference in the estimation of creatinine by modified Jaffe's method. Possibly GLY like acetoacetate, pyruvate, proteins etc. reacts with alkaline picrate causing positive interference in creatinine measurement. This findings was in accordance with study conducted by Ketan K. Mangukiya^[5].

Serum potassium estimation by ISE method also shows positive interference by GLY. Possibly this is due to glycolytic inhibition causing absence of ATP production this in turn favours efflux of potassium out of the erythrocytes. These findings were in agreement with the study conducted by Manukiya et al but disagreed with the findings of Landt^[5,8].

Conclusions

D, L-Glyceraldehyde at 5millmoles/L preserves glucose for 8 hours as good as PTC tube in which serum was separated after 30 minutes of blood collection and refrigerated at 4°C until analysed for glucose estimation by GOD-POD method. The NaF value for mean glucose is clearly much lower than that obtained using PTC or GLY as a preservative. GLY as an additive has a positive interference in the Jaffe reaction for creatinine estimation and in the estimation of potassium by ISE

methods. NaF as an additive has a positive interference on the estimation of sodium and potassium.

Future scope of the study

GLY interference with the common clinical analyte methodologies has to be established. With the significant difference in place for the estimation of serum creatinine by modified Jaffe's method in GLY coated tubes, an alternative enzymatic method can be tried.

Conflict of interest

Authors declare that they have no conflict of interest.

Ethical approval

All procedures performed in this studies involving human participants were in accordance with the ethical standards of Institution.

References

1. American Diabetes Association .Standards of medical care in diabetes; 2014. *Diabetes Care*. 2014;37:S14-S80.
2. Dongbo J. Zhang, R K Elswick, W. Greg Miller and Jimmy L. Bailey. Effect of serum clot time on clinical chemistry laboratory results. *American Association for Clinical Chemistry* 1998;44:61325-1333.
3. Y. W. Chan, R. Swaminathan, and C. S. Cockram; Effectiveness of sodium fluoride as a preservative of glucose in blood. *Clinical Chemistry* 1989; vol. 35, no. 2, pp. 315-317.
4. Leann M. Mikesch and David E. Bruns. Stabilization of Glucose in Blood Specimens: Mechanism of delay in fluoride inhibition of glycolysis. *Clinical Chemistry* May 2008; vol. 54 no. 5. 930-932.
5. Ketan K. Mangukiya, Punit Saxena, Nikunj R Patel et. al., Comparison of alternate glycolysis inhibitors with fluoride for preservation of blood for glucose and other common clinical chemistry examinations. *NJIRM*. 2013; Vol. 4(3),97-102.
6. Raymond Gambino, Janet Piscitelli, Tomy A. Ackattupathil, Judy L. Theriault, Reynaldo D. Andrin et. al, Acidification of blood is superior to sodium fluoride alone as an inhibitor of glycolysis. *Clinical Chemistry* 2009;55:5:1019-1021 .
7. W. S. Waring, L. E. Evans, and C. T. Kirkpatrick. Glycolysis inhibitors negatively bias blood glucose measurements: potential impact on the reported prevalence of diabetes mellitus. *Journal of Clinical Pathology* 2007;60:820-823.
8. Michael Landt. Glyceraldehyde Preserves Glucose Concentrations in Whole Blood Specimens. *Clin Chem* 2000;46(8):1144 -1149.
9. J. Davies, S. Tomlinson, A. C. Elliott, and L. Best. A possible role for glyceraldehyde transport in the stimulation of HIT-T15 insulinomacells. *Biochem J*. Nov 15, 1994;304(Pt 1):295-299.
10. H. A. Krebs and Patricia Lund. Formation of glucose from hexoses, pentoses, polyols and related substances in kidney cortex *Biochem J*. Jan 1966;98(1):210-214.