Lag Period of Fluoride Oxalate in Plasma Glucose Stabilization

Adamu, A. I[1]., Ahmad, M.B[1]. and Ekanem, M.E[2].

[1]Department of Medical Laboratory Science, Faculty of Allied Health Science, College of Health Science, Bayero University, Kano-Nigeria.

[2]Department of Chemical Pathology, Ahmadu Bello University Teaching Hospital, Zaria-Nigeria.

Abstract

Introduction: A major problem in the accurate measurement of glucose is its decrease in concentration due to erythrocytic glycolysis after sampling. Eliminating this problem requires the use of an anti-glycolytic agent that can be added to the sampling tubes to stabilize the glucose level before analysis.

Aim: This research was aimed at determining the lag period of fluoride oxalate in plasma glucose stabilization at Ahmadu Bello University Teaching Hospital Shika –Zaria.

Methods: Five milliliters of venous blood sample was collected from 40 subjects (20 diabetic and 20 apparently healthy) each patient and 1.2ml each was dispensed into four separate fluoride-oxalate (20mg/ml) tubes and were centrifuged at 0, 30, 60, and 120 minutes time to obtain plasma respectively. The glucose concentrations of the separated samples was analysed by glucose oxidase method.

Results: The mean glucose concentrations at 0, 30, 60 and 120 minutes were 9.92±0.84, 9.64±0.83, 9.48±0.83, 9.47±0.82 and 5.38±0.33, 5.12±0.34, 4.95±0.32 and 4.95±0.32 for diabetic and normal samples respectively. No significant decrease ($p>0.05$) were observed in the mean plasma glucose concentrations between samples separated at 60 minutes compared to 120 minutes in both group of subjects.

Conclusion: This shows that the fluoride-oxalate solution almost completely inhibit the loss of plasma glucose 60 minutes after mixing it with the blood. This was observed in both the normal and diabetic samples. This showed that a lag period of about 1 hour (from 0 to 60 minutes period) is required by fluoride-oxalate solution to completely stabilize plasma glucose. When fluoride-oxalate solution is used for plasma glucose assay, the blood sample should be separated immediately to prevent a significant loss of the plasma glucose due to its long lag period.

Keywords: Fluoride Oxalate, Glucose Estimation, Lag Period, Glucose Stabilization.

Introduction

Glucose a very important carbohydrate in biology is a monosaccharide or simple sugar also known as grape sugar, blood sugar or corn sugar. Its molecular formula is $C_6H_{12}O_6$. It is most often produced by the hydrolysis of natural glycosides. Glucose is a normal constituent of the blood of animals and humans (Redmond, 2007). Most of the glucose molecules found in the human body are of the D-configuration in which their hydroxyl groups are writtenin the right side of the structures (Nelson and Cox, 2005).

Glucose enters the bloodstream after absorption and is carried to the brain and muscles for energy (ATP) generation. Excess glucose is stored in the liver (as glycogen) and fat cells. Starch and glycogen are storage polymers of glucose in humans. They are also the major sources of energy in the diet. Glucose is formed by the hydrolysis of many carbohydrates (Nelson and Cox, 2005).

Citation: Adamu, A. I., Ahmad, M.B. and Ekanem, M.E. (2016): Lag Period of Fluoride Oxalate in Plasma Glucose Stabilization. *BJMLS. 1(1): xxx*

Glucose is rapidly metabolized to produce ATP (Adenosine triphosphate), a high energy end product. Glucose is oxidized through a large series of reactions that extract the greatest amount of possible energy of from it. If glucose metabolism occurs in the presence of oxygen(aerobically), the net production are 36 molecules of ATP from one molecule of glucose and 2 molecules of ATP, if glucose metabolism occurs in the absence of oxygen (anaerobic) (Szablewski, 2011).

Blood glucose concentration or level is tightly regulated in the human body and normally this blood glucose level is maintained at a specific reference range. The normal amount of glucose circulating in the blood is about 3.3 to 7.0g (assuming an ordinary adult blood volume of 5-6 liters, plausible for an average adult make and 4-5 liters for an average female). Glucose levels rise after meals for an hour or two and are usually lowest in the morning, before the first meal of the day. For this reason, patients going in for fasting blood glucose tests are advised to go before breakfast (fasting) (Nordlie *et al*., 1999).

The value of the blood glucose level has being kept in a remarkably narrow range due to the homeostatic effect of many factors, of which hormone regulation is the most important. The concentration of glucose is hormonally regulated. Fluctuations in blood glucose levels due to dietary intake or vigorous exercise are counterbalanced by a variety of hormonally triggered changes in the metabolism of several organs (Nelson and Cox, 2005).

Blood coagulates by the transformation of soluble fibrinogen into insoluble fibrin. Anticoagulants are compounds that help prevent the clothing (coagulation) of blood. Glucose estimation using plasma or whole blood 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. Normally, glycolysis decreases serum glucose by approximately 5 to 7% per hour (5 to 10mg/gl)in normal uncentrifuged coagulated blood at room temperature. This causes an inaccuracy in plasma glucose measurement in a blood sample collected in to a coagulated or an anticoagulated bottle that does not contain an inhibitor of glycolysis (Sacks, 2001).

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 ranges from severe toxins to life saving pharmaceuticals such as efivirenz and refractory materials such as calcium fluoride to highly reactive sulphur tetrafluoriden (Aigueperse *et al*., 2005). The range of fluoride is considerably large as fluorine forms compounds with all elements except helium and neon. Compounds containing fluoride anions and in many cases those containing covalent bonds to fluorine are called fluorides. The silicon–fluoride (Si–F) linkage is one of the strongest single bonds. Many fluoride minerals are known but paramount in commercial importance is fluorite and fluoropatite (Greenwood *et al*., 1997).

Fluoride actually inhibits the enzyme enolase which is found in the metabolic pathway of glucose and has little effect on glucose oxidase and peroxidase enzymes (Lawrence *et. al.,* 2008).

This research intends to determine the lag period of fluoride oxalate in plasma glucose stabilization.

Materials and Methods

Standard laboratory materials, equipments and reagents were used in this research.

The subjects were recruited from an adult population at the Ahmadu Bello University Teaching Hospital Shika -Zaria. A average of three samples were collected and analysed in a day and this was due to the tediousness of the practical aspect of the research work.

The method used to assay the plasma glucose concentration was kit-based glucose oxidase method modified by Trinder (1969).

Blood Sampling

A blood sample was collected from each of the fourty indivual volunteer aged between 18- 45 years; 20 of which were diabetic (group A) while the other 20 were apparently healthy (group B) individuals. From each individuals, 5ml of venous blood was collected from each individual participant.

Experimental design

Each blood sample collected was divided into four separate plain blood bottles labelled 1,2,3 and 4 and they were treated as follows:

Bottle labelled 1: The blood (1.2ml) in this bottle was centrifuged (at 4000 rpm for 10 minutes) immediately after mixing and the plasma sample was separated into a correspondingly labelled dry plain plasma bottle and it was recorded as being separated at approximately 0 minutes (0 minute time).

Bottle labelled 2: The blood(1.2ml) sample in this bottle was centrifuged 30 minutes after collection, separated and recorded as being separated at approximately 30 minutes (30 minute time)

Bottle labelled 3 and 4: The blood(1.2ml each) samples in these bottles were separated at 60 and 120 minutes respectively after collection, and they were labelled as such.

These gave a total of 160 samples and they were analysed for plasma glucose level.

Analysis of Result

Results were expressed as Mean ± SD. The means and standard deviations were calculated and paired *t*-test was used to calculate the *P* values. P-values of less than 0.05 were considered significant.

Result

The mean glucose concentrations in the plasma samples separated at approximately 0 minutes after mixing with fluoride oxalate solution in both diabetic (group A) and normal (group B) subjects were found to be 9.92 ± 0.84 mmol/L and 5.38 ± 0.33 mmol/L respectively (Table 1).

Table 1 Plasma Glucose Levels at Time Intervals from 0 to 120 Minutes

TIME/SUBJECTS	GROUP A	GROUP B
0 Minute	$9.92 \pm 0.84^{a,b,d}$	$5.38 \pm 0.33^{e,f,h}$
30 Minutes	$9.64 \pm 0.83^{a,c}$	$5.12 \pm 0.34^{e,g}$
60 Minutes	$9.48 \pm 0.83^{b,c}$	$4.95 \pm 0.32^{f,g}$
120 Minutes	4.97 ± 0.82^{d}	4.95 ± 0.32^{h}

Legend: Results are expressed as mean ± SD. Values with the same alphabetical superscripts for the same group in a column are statistically significant with respect to each other (P<0.05).

The values were significantly higher (p<0.05) than the mean plasma glucose concentration of both group A and B subjects separated at 30 minutes (9.64 ± 0.83mmol/L and 5.12 ± 0.34 mmol/L respectively; p<0.05). The decrease in mean plasma glucose concentration between 0 and 30 minutes in both group A and B were 0.28 mmol/L (28%) and 0.26 mmol/L (26%) respectively, table 2 and 3 respectively.

Table 2 Differences and Percentage Losses of Plasma Glucose of Group A Subjects from 0 to 120 minutes

Time Intervals (minutes)	Differences (mmol/L)	Percentage loss (%)
0	0	0
0 to 30	0.28	2.8
30 to 60	0.16	1.7
60 to 120	0.01	0.1
30 to 120	0.17	1.8
0 to 60	0.44	4.4
0 to 120	0.45	4.5

The mean plasma glucose (mean±SD) of group A and group B at 60 minutes were significantly lower ($p<0.05$) than their mean plasma glucose values obtained at 0 minutes (9.48 ± 0.83) and 30 minutes (4.95 ± 0.32) as shown in table 1. The decrease observed in the mean plasma glucose concentration between 30 minutes and 60 minutes were 0.16 mmol/L (1.7%) and 0.17 mmol/L (1.8%) for group A and B respectively, Tables 2 and 3 respectively .

Statistically significant difference ($p<0.05$) were observed between the mean results obtained from both group A (9.92 ± 0.84 and 9.48 ± 0.83) and B (5.38 ± 0.33 and 4.95 ± 0.32) at approximately 0 minutes and 60 minute. These difference were 0.44mmol/L (4.4%) and 0.43 mmol/L (4.3%), representing the degree of loses in plasma glucose for group A and B respectively between 0 and 60 minutes (Tables 2 and 3 respectively).

Table 3 Differences and Percentage Losses of Plasma Glucose of Group B Subjects From 0 to 120 minutes.

Time Intervals (minutes)	Differences (mmol/L)	Percentage loss (%)
0	0	0
0 to30	0.26	2.6
30 to 60	0.17	1.8
60 to 120	0.00	0.0
30 to 120	0.17	1.8
0 to 60	0.43	4.3
0 to 120	0.43	4.3

There were no statistically significant difference ($p>0.05$) between the mean plasma glucose concentrations of group A (9.48 ± 0.83) and group B (4.95 ± 0.32) at 60 minutes and their corresponding values (9.47 ± 0.82 and 4.95 ± 0.32 respectively) at 120 minutes. Table 1

There were statistically significant decrease ($p<0.05$) between the mean values of plasma glucose concentration of samples separated at 0 minute time (9.92 ± 0.84 and 5.38 ± 0.33) and 120 minutes (9.47 ± 0.82 and 4.95 ± 0.32) for group A and B respectively, after mixing with fluoride oxalate solution with the samples. The actual loses of plasma glucose in groups A and B from 0 time to 120 minutes were 0.45mmol/L (4.5%) and 0.43mmol/L (4.3%) for group A and B respectively as shown in table 2 and 3 respectively.

Discussion

The Loss of glucose due to storage is a serious problem which arises due to glycolysis. Such decreases in glucose concentration will lead to wrong diagnoses in the large proportion of the population who have glucose concentrations near the cut-points for diagnosis of diabetes. Several approaches have been proposed to minimize erythrocytic glycolysis during glucose measurement. In Nigeria however, Fluoride-oxalate is the main anticoagulant used in preserving glucose in most laboratories.

In the current study the mean plasma glucose concentrations of 0 minute compared to 30 minutes, and 30 minutes compared to 60 minutes (Table 1) showed that there were statistically significant loss of plasma glucose ($p<0.05$) in both group A and B samples. This indicates that the fluoride does not significantly inhibit the activity of the enzyme (enolase) in the period between 0 and 60 minutes. These results are in agreement with the one reported by Chan *et al.* (1989), were he reported that the fluoride does not prevent loss of plasma glucose during the 30, 60 minutes (or longer). Lean and David (2008) also made similar observation. Fluoride inhibits enolase, which is far downstream in the glycolytic pathway(Mikesh and Bruns, 2008). Enzymes upstream of enolase remain active and continue to metabolize glucose until substrates are exhausted. Thus the anti glycolytic action of fluoride is delayed for up to four hours (Chan *et al*., 1989).

The result also demonstrated about 4.5% and 4.3% loss of plasma glucose in group A and B respectively which was in accordance with the findings of Gambino *et al.*(2009), who reported about 4.6% plasma glucose loss per two hours. This is in contrast to the findings of Amegashie *et al*., 2015 who reported a percentage decrease in glucose concentration between immediate and after 1 hour in fluoride oxalate tubes to be 6.5%, while that between immediate and after 2 hours 13% and that between after 1 hour and after 2 hours was 7.1%. They concluded that their result indicates that when blood samples stored in fluoride-oxalate tubes are left for more than 2 hours on the bench, glucose concentration decreases by 13%.

The possible reasons for the significant decrease in the mean plasma glucose concentration between 0 and 60 minute is that, after entry of the fluoride in to the erythrocyte, although it rapidly blocks enolase (Lean and David, 2008; Feig *et al*., 1971), other enzymes such as phosphoglycerate kinase, glyceraldehyde 3-phosphate dehydrogenase, fructose1,6-bisphosphate aldolase and hexokinase which are upstream of enolase on the glycolytic pathway remain active (Bryan, 2008). Thus, glucose continues to be metabolized to glucose-6-phosphate which is further metabolized to other phosphorylated metabolites of glucose, all of which accumulates in the cells. So glucose continues to decrease in the plasma (Lean and David, 2008) though it is not converted to pyruvate or lactate.

With glycolytic pathway blocked, other pathways may also metabolize phosphorylated sugars, an example of such pathway is pentose phosphate pathway. Such metabolism will continue until equilibrium states are reached for the several reactions involved. In particular, the rate of phosphorylation of glucose to glucose-6-phosphate will decrease because this rate depends on the supply of ATP. The concentration of which decrease in erytrocyte by almost 90% sixty (60) minutes after addition of fluoride (Feig *et al*., 1971) and this ATP depletion is enough to significantly inhibit the utilization of glucose by other pathways thus stabilizing blood glucose just about 60 minutes after adding the fluoride oxalate solution. Chapman and Kuchel, (1990) also made similar observation but disagreed with the postulated existence of "barrier to the movement of glucose into the erythrocyte and leucocytes" since ^{19}F NMR spectra of sodium fluoride in suspensions of human erythrocytes were seen to yield separate resonances for the F^- populations inside and outside the cells.

Conclusion

The facts from this research work have shown that stabilization of plasma glucose by fluoride oxalate solution is not immediate since it does not prevent significant loss of plasma glucose form 0 times to 60 minutes. The lag period in plasma glucose stabilization with fluoride oxalate solution occurs from 0 to 60 minutes, since the result showed no significant decrease in plasma glucose concentration from 60 minutes to 120 minutes. Therefore the controversy of the existence of lag or no lag period might be resolved based on this work. The percentage glucose loss during the lag period is 4.4%. This also provides a baseline subject for further research on more effective antiglycolytic agents.

Recommendations

From the findings of the present study, it is recommended that, to get a reliable results, glucose determination should be carried out immediately after collection of sample or within the shortest possible time. This is because the concentration of glucose is never stable within the first hour of sample collection.

References

Aigueperse, Jean, P. and Mollard, (2005). Fluoride compounds, *Uilmann's Encyclopedia of Industrial Chemistry*, Wiley-VCH, Weinheim, 307

Amegashie, E. A., Amidu, N. and Owiredu, W. K. (2015). A comparison of fluoride-oxalate and plain (serum gel) tube on glucose measurement. *Jour. of Medic. and Biomedic. Sci.* UDS Publishers Limited 4(1): 34-40.

Bryan, K. (2008). "Glycolysis, Gluconeogenesis, and the Pentose Phosphate Pathway." Principles of Biochemistry.

Chan, A., Swaminathan, R., and Cockram, C. (1989). Effectiveness of sodium fluoride as a preservative of glucose in blood. *Cli. Chem.*, 35(2), 315-317.

Chapman, B. E. and Kuchel, P. W. (1990). Fluoride transmembrane exchange in human erythrocytes measured with ^{19}F NMR Magnetization transfer. *Eur. Biophys. Jour.*19: 41-45

Feig, S. A., Schotiet, S. B. and Nathan D. G. (1971). Energy Metabolism in Human Erythrocyte. Effect of Sodium Fluoride. *Jour .Chin.Invest.* **50**: 1731-1737.

Gambino, R., Piscitelli, J., Ackattupathil, T. A., Theriault, J. L., Andrin, R. D., Sanfilippo, M. C. and Etiennem, M. (2009). Acidification of blood superior to sodium fluoride alone as an inhibitor of glycolysis. *Clin. Chem.* 55(5):1019-21

Greenwood, Norman, N. and Earnshaw, A. (1997). Chemistry of the elements, (2nd edition), Oxford Butterworth – Heinemann, 804

Lawrence, J. M., Contreras, R., Chen, W. and Sacks, D. A. (2008)**.** Trends in the prevalence of pre-existing diabetes and gestational diabetes mellitus among a racially/ethnically diverse population of pregnant women. *Diab. Care* **31** (5): 899-904.

Lean, M. M. and David, E. B. (2008). Stabilization of glucose in blood specimen: *Mechanism of delay in fluoride inhibition of glycolysis*, an article cited in www.pubmed.com

Mikesh, L.M. and Bruns, D.E. (2008). Stabilization of glucose in blood specimens: mechanism of delay in fluoride inhibition of glycolysis. *Jour. Clin Chem.* 54(5):930-932.

Nelson, D.L., Cox, M. M.(2005). Glycolysis and catabolism of hexose. Lehninger Principles of Biochemistry, 4th Edition, *Worth Publishers*, New York **15** p527

Nordlie, R. C., Foster, J. D.and Lange, A. J. (1999). Regulation of glucose production by the liver. *Ann. Rev. of Nutr., 19*, 379-406.

Redmond, W. A. (2007). Microsoft Encarter. *Jour on Glu.*,

Sacks, D. B. (2001). Carbohydrate sited in *Tietz fundermentals of clinical chemistry* 5th ed. By Burtis C. A. and Ashwood E. R. W. B. Saunders company, United State of America. **23**: Pp 427-459

Szablewski, L. (2011). Glucose homeostasis and insulin resistance, *Bentham e-books*, Eisbn 9781-60805-189-2.

Trinder, P. (1969). Determination of glucose in blood using glucose oxidase with on alternative oxygen receptor. *Ann Clin. Biochem.* 6: 24-27.