Comparative Stabilizing Effects of Some Anticoagulants on Fasting Blood Glucose of Diabetics and Non-diabetics, Determined by Spectrophotometry (Glucose Oxidase)

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Abstract: The comparative stabilizing effects of the anticoagulants; Fluoride oxalate, EDTA and Heparin on fasting blood glucose level were determined, using the spectrophotometry (glucose oxidase) method. Fasting blood samples were taken from ten (10) diabetic patients and ten (10) non-diabetic people, and the blood glucose levels determined at 30 min intervals for a maximum time of 2 h. Our results showed that the rate at which plasma glucose changes with time varies with specific anticoagulants. With Fluoride oxalate and Heparin, it increased by 1.77 and 6.67%, respectively, while with EDTA it decreased by 4.0%, within the first 30 minutes for non-diabetics. For diabetics, within the same period, with Fluoride oxalate and Heparin it increased by 1.9 and 3.7%, respectively, while with EDTA it decreased by 3.6%. Blood glucose levels were however shown to increase significantly (p<0.05) at 60 min (1 h) with the three anticoagulants in diabetics and non-diabetics. But at 120 min (2 h) plasma glucose in Fluoride oxalate, EDTA and Heparin decreased by 16.4, 17.5 and 8.5%, respectively for non-diabetics and by 10.6, 9.8 and 8.6%, respectively for diabetics. These showed that Fluoride oxalate had a better stabilizing effect on plasma glucose within the first 30 minutes while Heparin is a better stabilizer at after 120 min.

Key words: Anticoagulants, EDTA, fluoride oxalate, glucose oxidase, heparin, plasma glucose

INTRODUCTION

Blood glucose refers to the amount of glucose present in a mammal’s blood. Normally, the blood glucose level is maintained at a reference range between 4 to 6mM. Glucose can be measured in whole blood, serum or plasma. An anticoagulant is a substance that prevents the clotting of blood. Anticoagulants can be used endogenously or/and exogenously. The endogenous anticoagulants help prevent formation of hard clots in the blood by decreasing the ability of the blood to clump together. They are also called “blood thinners”. They include medications that slow-down blood clotting time. The exogenous anticoagulants are used during analysis of blood samples. As medication, anticoagulants are administered to patients to stop thrombosis (blood clotting inappropriately in the blood vessels). This is useful in the primary and secondary prevention of deep vein thrombosis, pulmonary embolism, myocardial infarction and strokes in those who are predisposed (Schrezenmeier, 1995).

The exogenous anticoagulants are compounds that have been developed using several mechanisms of action. They include heparin, Ethylene Diamine Tetra-acetate (EDTA), fluoride oxalate etc. Heparin acts by forming a complex with anti-thrombin; this complex inhibits factor X (stuart prower) and thrombin which is required in the formation of fibrin. EDTA acts as a chelator and binds to calcium which is required for coagulation thereby making calcium unavailable and inhibiting clotting. Fluoride oxalate acts as an inhibitor of glycolysis.

Heparin is a naturally occurring anticoagulant produced by basophils and mast cells (Guyton and Hall, 2006). Heparin, a highly sulfated glycosaminoglycans, is widely used as an inject-able anticoagulant and has the highest negative charge density of any known biological molecule (Cox and Nelson, 2004). Native heparin is a polymer with a molecular weight ranging from 3 kDa to 50 kDa, although the average molecular weight of most commercial heparin preparations is in the range of 12 kDa to 15 kDa (Bentolila et al., 2008). Heparin is a linear poly-disperse polysaccharide consisting of repeating units of alpha 1→4 linked pyranosyluronic acid and 2-amino-2-deoxyglycopyranose (glucosamine) residues (Comper, 1981). EDTA is a colorless, water-soluble solid produced on a large scale for many applications. Its prominence as a chelating agent arises from its ability to “sequester” di-
and tricationic metal ions such as Ca\(^{2+}\) and Fe\(^{3+}\). After being bound by EDTA, metal ions remain in solution but exhibit diminished reactivity. The compound was first described in 1935 by Ferdinand Munz, who prepared it from ethylenediamine and chloroacetic acid (Munz and Robert, 1987). Today, EDTA is mainly synthesized from ethylenediamine (1,2-diaminoethane), formaldehyde (methanal), and sodium cyanide. EDTA is used to detoxify metal ions in chelation therapy for e.g. mercury and lead poisoning (Debusk et al., 2002). Similarly, it is used to remove excess iron from the body. EDTA exhibits low toxicity with LD\(_{50}\) of 2.0-2.2 g/kg. It has been found used to remove excess iron from the body. EDTA exhibits diminished reactivity. The compound was first described in 1935 by Ferdinand Munz, who prepared it from ethylenediamine and chloroacetic acid (Munz and Robert, 1987). Today, EDTA is mainly synthesized from ethylenediamine (1,2-diaminoethane), formaldehyde (methanal), and sodium cyanide. EDTA is used to detoxify metal ions in chelation therapy for e.g. mercury and lead poisoning (Debusk et al., 2002). Similarly, it is used to remove excess iron from the body. EDTA exhibits low toxicity with LD\(_{50}\) of 2.0-2.2 g/kg. It has been found to be cytotoxic and weakly genotoxic in laboratory animals. Oral exposures have been noted to cause reproductive and developmental effects (Lanigan and Yamarik, 2002).

In testing for blood glucose level, sodium fluoride is used as a glycolytic inhibitor to preserve the blood glucose level combined with anticoagulant, potassium oxalate. It has long been recognized that blood sample is subjected to in vitro glycolysis, which is enhanced by the presence of leucocytes and diminished by sample refrigeration (Totsoi, 2004). Most researchers have concluded that addition of sodium fluoride to blood sample is capable, in at least some parts of decreasing ex-vivo glycolysis which results to decline in glucose concentration of the blood sample (Chan et al., 1989). Fluoride oxalate is an anticoagulant which binds to ionized calcium, thereby preventing blood from clotting. Fluoride oxalate’s ability to prevent clotting by binding to Ca\(^{2+}\) is not as strong as that of EDTA.

In many laboratories in this part of the world, blood samples are collected and kept with the intention to test for glucose. These blood samples may not be properly stored, and when tested for glucose, a wrong result may be gotten. On another note, anticoagulants may be used indiscriminately giving room for errors in results. To this effect, it became necessary to investigate the changes in blood glucose concentrations with time, in blood samples stored or collected in different anticoagulants, using the spectrophotometry method (Glucose-oxidase kit).

**MATERIALS AND METHODS**

**Specimen type:** Fasting blood samples were gotten from ten (10) diabetic and ten (10) non-diabetic patients at Igbinedion University Teaching Hospital (IUTH).

**Anticoagulants:** The three (3) anticoagulants used are Fluoride oxalate, EDTA and Heparin.

**Specimen collection:** About 6 mL of the subjects’ blood were collected via venipuncture and put into three sample bottles (2 mL in each) containing the three anticoagulants; Fluoride oxalate, EDTA and Heparin. The blood samples were then centrifuged and the plasma separated from the blood cells.

**Glucose determination:** The concentrations of glucose in the plasma were determined immediately after collection spectrophotometrically, using Glucose-oxidase test kit (from Randox Laboratory Limited, U. K.). The procedure was repeated at every 30 min interval for 2 h.

**RESULTS AND DISCUSSION**

The assay of glucose in blood samples stored in bottles containing anticoagulants is a common practice in this part of the world. When blood samples are collected, they are stored in their native state by preserving them in anticoagulant bottles (Oduola et al., 2006). Our results showed that though their native state was preserved, the blood glucose level when assayed in different anticoagulant bottles at different time varies. Trinder (1969) reported that plasma glucose levels remain quite stable for 6 h at room temperatures (25-35°C). This however is not in agreement with our results as it seems that plasma glucose levels are not exactly stable within the stated time. It was observed that the rate at which plasma glucose changes with time varies with specific anticoagulant. With fluoride oxalate and heparin, it increased by 1.77 and 6.67%, respectively, while with EDTA it decreased by 4.0% within the first thirty (30) min for non-diabetics (Table 1). Within the same period, for the diabetics (Table 2), with fluoride oxalate and heparin it increased by 1.9 and 3.7%, respectively while with EDTA it decreased by 3.6%. This suggests that fluoride oxalate has stronger stabilizing ability for blood glucose within the first 30 min. This may be due to the ability of fluoride ion to inhibit the activities of the glycolytic enzyme enolase, thereby stopping further breakdown of glucose.

Our results also showed that at 120 min, plasma glucose in fluoride oxalate, EDTA and heparin decreased by 16.4, 17.5 and 8.5%, respectively for non-diabetics (Table 1) and by 10.6, 9.8 and 8.6%, respectively for diabetics (Table 2). This shows that although fluoride oxalate was most stable in the first 30 min, heparin had better stabilizing ability after 120 min. This suggests that the anticoagulant action of heparin in binding to antithrombin thereby forming a complex that inhibits the procoagulant proteinases, stuart prower factor and thrombin (Linhardt and Gunay, 1999); is also effective in maintaining the glucose level of blood samples to an extent.

Blood glucose levels increased significantly at 60 min with all three anticoagulants in diabetics and non-diabetics likewise. The reductions were less in diabetic blood samples which showed 10.6, 9.8 and 8.6%, decreases for fluoride oxalate, EDTA and heparin.
respectively after 120 min when compared to the non-diabetics. This could be due to the presence of compounds or macromolecules in the blood of these patients, which may include their medications.

Our findings suggest that blood glucose analysis results would be most reliable when blood samples are stored in fluoride oxalate bottles for the first 30 min. However, if the blood samples must be stored for up to 120 min, then heparin is a better choice.

**CONCLUSION**

Our results showed that although Fluoride oxalate had stronger stabilizing effect on plasma glucose than EDTA and Heparin in the first 30 min, Heparin had better stabilizing effect after 120 min. This suggests that Lithium Heparin is the best choice of anticoagulant for analysis of sample which will be stored for 2 h.

**ACKNOWLEDGMENT**

The Authors are grateful to the authorities of Department of Biochemistry, Igbinedion University, Okada (Edo State, Nigeria) and the authorities of the Department of Chemical Pathology Laboratory, Igbinedion University Teaching Hospital, Okada, for providing the necessary laboratory facilities.

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