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ORIGINAL ARTICLE

A comparison of fluoride-oxalate and plain (serum gel) tube on glucose measurement

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A continuing problem in the accurate measurement of glucose is its decline in concentration due to erythrocytic glycolysis after sampling, transport and processing. Eliminating this problem requires the use of an anti-glycolytic agent that can be added to the sampling tubes without altering cellular integrity while measuring blood glucose. This study was therefore conducted to compare fluoride-oxalate and plain tube on glucose measurement at the Komfo Anokye Teaching Hospital. A total of 100 subjects were recruited from an adult population at the Komfo Anokye Teaching Hospital. Six milliliters of venous blood sample was collected from each patient and 1ml each was dispensed into three separate fluoride-oxalate and plain tubes and were centrifuged at different time intervals to obtain plasma and serum respectively. 1%, 4% and 8% of the subjects presented with hypoglycaemia at immediate, after 1 hour and 2 hours fluoride-oxalate while that of plain tubes were 3%, 6% and 14% respectively. 58%, 53% and 49% of the subjects presented with hyperglycaemia at immediate, after 1 hour and 2 hours for fluoride-oxalate while that of plain tubes were 58%, 49% and 47%. Decrease in glucose concentration after 1 hour and 2 hours in fluoride-oxalate tubes were 6.5% and 13% respectively while those in plain tubes were 8.9% and 16.7%. Though, fluoride-oxalate does not completely inhibit erythrocytic glycolysis within two hours, its effect when left on the bench at different time intervals does not show a significant change in test results. Plain tubes however show significant change in test results at different time intervals since they do not contain any antiglycolytic agents needed to preserve blood glucose.
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INTRODUCTION

Blood glucose estimation is one of the biochemical tests that are used to diagnose diabetes mellitus and its associated risk conditions. Its estimation with other biochemical tests such as lipid profiles are needed to assess the risk involved in diseases like cardiovascular diseases, obesity, coronary disease, stroke et cetera (Aronne and Isoldi, 2007). It is one of the most commonly measured components of blood, because of the central role of glucose in metabolism and the high prevalence of diseases of glucose homeostasis (Sidebottom *et al.*, 1982). A continuing problem in the accurate measurement of glucose is the loss of glucose from specimens due to glycolysis by erythrocytes during transport and processing (Sidebottom *et al.*, 1982).

In recent years, this phenomenon has been more evident, as more specimen are transported to distant laboratories for analysis. Several approaches have been proposed to minimize glycolysis, including centrifugation/decantation of plasma immediately after specimen collection (Sidebottom *et al.*, 1982), refrigeration/cooling on ice during transport (Lin *et al.*, 1976), addition of anti-glycolytic agents such as iodoacetate (Chan *et al.*, 1989), fluoride (DenisBeven, 1924) or mannose (Nakashima *et al.*, 1987) to the collection tubes, and the use of glucose analyzers designed for near-patient testing, at

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the bedside (Innanen *et al.*, 1997; Junker *et al.*, 2010).

From a practical standpoint, the best way to achieve this goal is to discover an anti-glycolytic agent that can be added to collection tubes but would not alter cellular integrity or interfere with common analytical methodology. Such an agent should also be effective at low concentration (minimizing volume addition to avoid dilution errors), dissolve rapidly during the collection process, be non-toxic, be stable in the room-temperature storage environment of blood collection devices, and be inexpensive (DenisBeven, 1924). There is paucity of research data in Ghana to ascertain the right tube quality and bench time appropriate for glucose assays hence the need for this study.

MATERIALS AND METHODS

Study design and population

This cross sectional study was conducted among the adult population in Kumasi, Ashanti region, Ghana between February to March, 2009. One hundred subjects were recruited; 75 subjects from the diabetic clinic of the Komfo Anokye Teaching Hospital (KATH) and 25 subjects known to be non-diabetic were also added to act as controls.

The study was approved by the local Committee on Human Research Publication and Ethics (Ref No. CHRPE / KNUST /KATH / 23-11-08). Permission was also sought from the Head of the Chemical Pathology Department of the Komfo Anokye Teaching Hospital before data collection started. The participation of the respondents was voluntary and informed consent was obtained from each participant.

Sample Collection and Analysis

After an overnight fast of 12-16 hours, 6 ml of venous blood samples was collected from each patient and 1 ml each was dispensed into three separate fluoride-oxalate tubes and three separate plain tubes. The blood samples were centrifuged at 4000 rpm for 4 minutes to obtain plasma from the blood sample in the fluoride-oxalate tubes and serum from clotted samples at different time interval (i.e. immediate, after one hour and after two hours spin) with a turnaround time (TAT) of 30 minutes for samples to be run immediately (that is for the immediate samples, the time between sample collection and sample analysis took 30 minutes which represents the turnaround time). Glucose in the samples (plasma and serum) was measured manually with a spectrophotometer (Micke, USA) using the glucose oxidase test kit and absorbance were read at 500 nm.

Data analysis

The data was analyzed with GraphPad Prism® version 5.0 and Microsoft Excel and expressed as mean ± standard deviation. The 95% confidence interval or the p-value was calculated. In all statistical tests, a value of $p< 0.05$ was considered significant.

RESULTS

From Figure 1A, diabetic participants glucose concentration in both fluoride-oxalate and plain tubes declined with time with the decline in the plain tube greater than that of fluoride-oxalate. The mean glucose concentration for both immediate fluoride-oxalate and immediate plain values were the same. After 1 hour, the rate of decrease for plain tube values was greater than that of the fluoride tube values as well as that for after 2 hours. From Figure 1B, for non-diabetic patients, the mean glucose concentration for immediate fluoride-oxalate and plain tubes were almost the same. After 1 hour, the rate of decrease in fluoride-oxalate and plain tubes values were the same. But after 2 hours, the rate of decrease in plain tubes values was greater than that for fluoride tube values.

The mean difference in glucose concentration between immediate fluoride and plain values is 0.0 (Figure 2). Mean difference in glucose concentration after 1 hour in both fluoride oxalate and plain (serum gel) tubes values was 0.2 (Figure 3). Mean difference in glucose concentration between after 2 hours fluoride oxalate and plain (serum gel) tubes values was 0.3 (Figure 4).

The number of study participants who presented

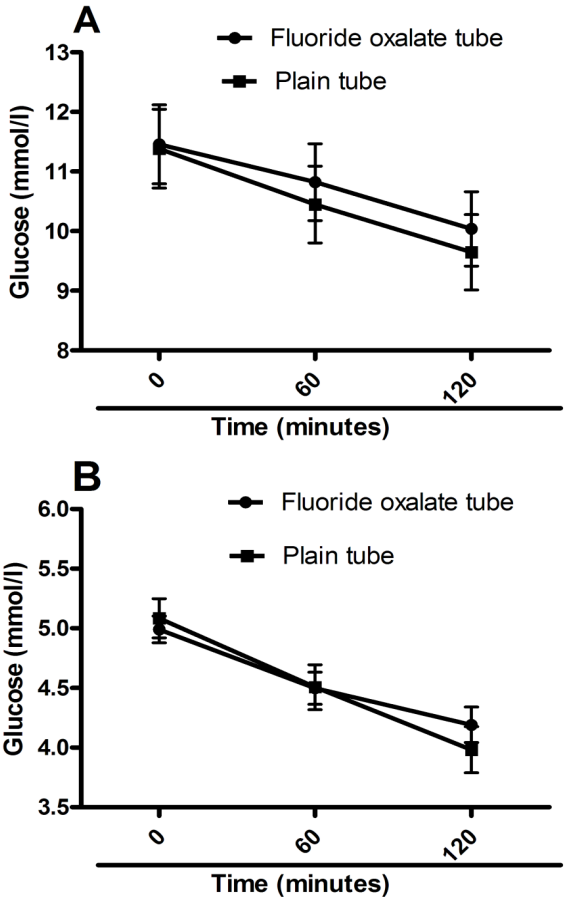

Figure 1: Representative changes in mean glucose concentration with time in blood samples collected into fluoride-oxalate tubes and plain tubes for (A) diabetic and (B) non-diabetic patients.

with hypoglycaemia for immediate fluoride-oxalate results was 1, but increased to 4 and 8 after 1 hour and after 2 hours fluoride oxalate values respectively. The number of study participants that presented with normoglycaemia for immediate fluoride oxalate results was 41, but increased to 43 after 1 hour and after 2 hours fluoride-oxalate values respectively. The number of study participants that presented with hyperglycaemia for immediate fluoride-oxalate results was 58, but decreased to 53 and 49 after 1 hour and after 2 hours fluoride-oxalate values respectively (Table 1).

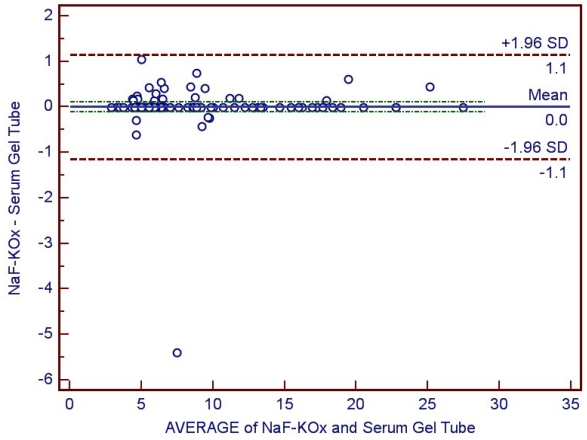

Figure 2: Bland-Altman graph of difference between glucose concentrations in immediate fluoride– oxalate and immediate serum-gel tube values (mmol/l) in 100 subjects

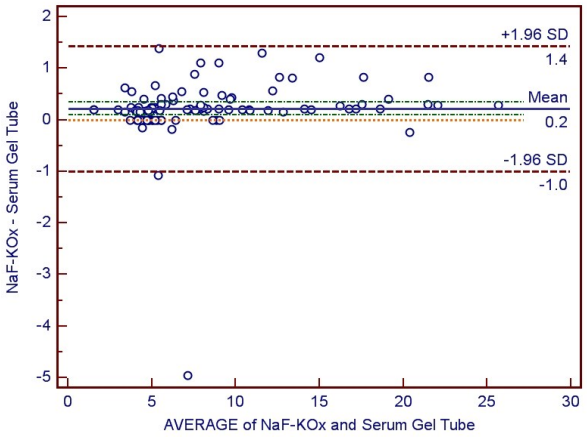

Figure 3: Bland-Altman graph of difference between glucose concentrations in after 1hour fluoride– oxalate and after 1hour serum-gel tube values (mmol/l)

The number of study participants that presented with hypoglycaemia for immediate plain tubes results was 3, but increased to 6 and 14 study participants after 1 hour and after 2 hours plain tubes values respectively. The number of study participants that presented with normoglycaemia for immediate plain tubes results was 39, but increased to 45 and decreased to 39 after 1 hour and after 2

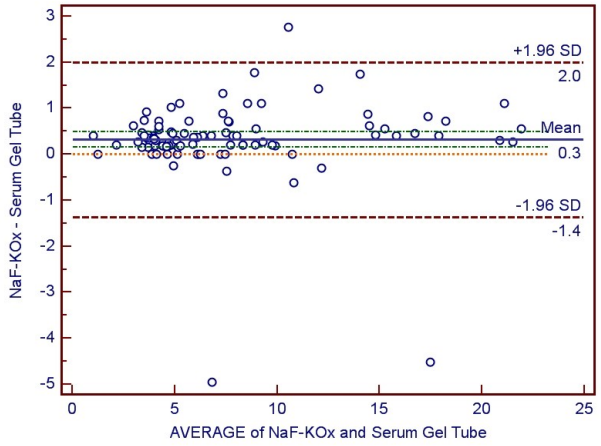

Figure 4: Bland-Altman graph of difference between glucose concentrations in after 2 hour fluoride– oxalate and after 2 hour serum-gel tube values (mmol/l)

hours plain tubes values respectively. The number of study participants that presented with hyperglycaemia in immediate plain tubes results was 58, but decreased to 49 and 47 after 1 hour and after 2 hours plain tubes values respectively (Table 1).

Table 1: General characteristics on the number of subjects presenting with hypoglycaemia, normoglycaemia and hyperglycaemia in blood samples stored in fluoride-oxalate and plain tubes at different time interval

Variables	Hypoglycaemia	Normoglycaemia	Hyperglycaemia
Fluoride-oxalate	< 3.6	3.6-6.0	> 6.0
0	1%	41%	58%
60	4%	43%	53%
120	8%	43%	49%
Plain tube	< 3.6	3.6-6.0	> 6.0
0	3%	39%	58%
60	6%	45%	49%
120	14%	39%	47%

The percentage decreases in glucose concentration between immediate and after 1 hour fluoride-oxalate values is 6.5%. The percentage decreases in glucose concentration between immediate and after 2 hours fluoride-oxalate values was 13%. The percentage decrease in glucose concentration between after 1 hour and after 2 hours fluoride-oxalate values was 7.1% (Table 2).

The percentage decreases in glucose concentration between immediate and after 1 hour plain tubes values was 8.9%. The percentage decreases in glucose concentration between immediate and after 2 hours plain tubes values was 16.7%. The percentage decrease in glucose concentration between after 1 hour and after 2 hours plain tubes values was 8.6% (Table 2).

Table 2: Percentage decrease in glucose concentration within fluoride-oxalate and plain tubes at different time interval

Variables	% decrease
Fluoride 0 vrs fluoride 60	6.5%
Fluoride 0 vrs fluoride 120	13%
Fluoride 60 vrs fluoride 120	7.1%
Plain 0 vrs Plain 60	8.9%
Plain 0 vrs Plain 120	16.7%
Plain 60 vrs Plain 120	8.6%

DISCUSSION

Several approaches have been proposed to minimize erythrocytic glycolysis during glucose measurement. In Ghana however, Fluoride-oxalate is the main anticoagulant used in preserving glucose in most laboratories. Out of the 75 participants recruited from the Diabetic Clinic at Komfo Anokye Teaching Hospital for this study, only 58 participants presented with hyperglycaemia after immediate analysis and 17 were found to have normoglycaemic. The 17 participants who present-

ed with normoglycaemia may have responded to the management of the condition at the Diabetic clinic. In this study, blood glucose concentration in both fluoride-oxalate and plain tubes decreased with time but the rate of decrease in plain tubes was higher than that of fluoride-oxalate. The magnitude of decrease of the blood glucose concentration in the plain tubes was however, statistically significant ($p<0.05$) after 1 hour and 2 hours.

In the immediate fluoride and plain tubes values, erythrocytic glycolysis had less effect on glucose concentration due to the immediate centrifugation and separation of plasma and serum respectively from red blood cells thus it was not able to lower the blood glucose concentration as compared to the concentrations after 1 hour and 2 hours values for both fluoride-oxalate and plain tubes. The reduced glucose concentration observed after 2 hours for blood stored in both fluoride and plain tubes may be attributed to the delay in the separation of plasma and serum from red blood cells respectively which led to erythrocytic glycolysis and also the partial effect of fluoride on blood glucose (Chan *et al.*, 1989).

The bias between immediate fluoride-oxalate and plain tubes was 0.0 indicating that the glucose concentration values in both tubes do not show a significant change in results therefore blood samples for glucose measurement can be stored in both fluoride and plain tubes when samples are scheduled to be analyzed immediately. One percent (1%) of the participants presented with hypoglycaemia when glucose concentration was measured immediately after sample collection and this increased to 4% and 8% of the total participants when measured after 1 hour and 2 hours respectively in blood samples stored in fluoride-oxalate tubes. This indicates that a number of normoglycaemic patients could be termed as being hypoglycaemic if their blood samples are stored in fluoride-oxalate tubes and left on the bench for an hour or more.

In blood samples stored in plain tubes, 3% of the participants presented with hypoglycaemia and this increased to 6% and 14% when the glucose concentration was estimated after an hour or 2 respectively. The number of participants who presented with hypoglycaemia was higher in blood samples stored in plain tubes than in fluoride tubes. This indicates that fluoride-oxalate is a better preservative of blood glucose concentration than plain tube. This also suggests that a number of normoglycaemic patients would be termed as being hypoglycaemic if their blood samples are stored in plain tubes and left on the bench for an hour or more. This observation was also made by (Chan *et al.*, 1989) who indicated that artefactual hypoglycaemia could occur in hospital patients whose blood samples were collected into non-anticoagulated tubes.

Fifty eight percent (58%) of the participants presented with hyperglycaemia when glucose concentration was measured immediately after sample collection, and they decreased to 53% and 49% of the participants when their glucose concentration was measured after 1 hour and 2 hours respectively in blood samples stored in fluoride oxalate tube. This suggests that 5% to 9% of the total subjects may have moved from a hyperglycaemic state to a normoglycaemic state when blood samples were left on the bench for more than an hour. Also 58% of the participants presented with hyperglycaemia when glucose concentration was measured immediately after sample collection, and this decreased to 49% and 47% of the participants when glucose concentration was measured after 1 hour and 2 hours respectively in blood samples preserved in plain tubes. This implies that 9% to 11% of the total participants may have moved from a hyperglycaemic state to a normoglycaemic state when blood samples were left on the bench for more than an hour. Thus, some subjects presenting with impaired glucose tolerance and diabetes mellitus may be missed if their blood is left on the bench for more than 1 hour after sample collection thus, resulting in wrong diagnosing of patients. If this error of misdiagnoses continues, a patient may develop the complications associated with diabetes mellitus before the patient is appropriately diagnosed. Chan *et al.,* 1989 have indicated that it is possible some patients with hyperglycaemia may present with artefactual normoglycaemia, thereby missing a diagnosis of diabetes mellitus. The number of study par-

ticipants who presented with hyperglycaemia decreased more in blood samples stored in plain tubes than that stored in fluoride-oxalate tubes.

The percentage decrease in glucose concentration between immediate and after 1 hour in fluoride oxalate tubes was 6.5%, while that between immediate and after 2 hours was 13% and that between after 1 hour and after 2 hours was 7.1%. This 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% which falls in line with the report by (Chan *et al.*, 1992; Nakashima *et al.*, 1987) which states that even in the presence of fluoride-oxalate, glucose concentration decreases by 5-15% within the first 2-4 hours after sample collection. This study was however, conducted over a two hour period.

For plain tubes, the percentage decrease in glucose concentration between immediate and after I hour was 8.9%, while that between immediate and after 2 hours was 16.7% and that between 1 hour and after 2 hours was 8.6%. This shows that when blood samples stored in plain tubes are left on bench for more than 2 hours, glucose concentration decreases by about 16.7%. The percentage decrease in glucose concentration of blood samples stored in plain tubes, left on the bench for more than 2 hours was higher than that stored in fluoride-oxalate tubes. This shows that fluoride has anti-glycolytic properties which preserves blood glucose (Nakashima *et al.*, 1987).

CONCLUSION

This study has shown that though fluoride-oxalate cannot completely inhibit erythrocytic glycolysis, its effect when left on bench at different time interval does not show a significant change in test results. With plain tubes, there was a significant change in test result at different time intervals since plain tubes do not contain any anti-glycolytic agents needed to preserve blood glucose. However, blood samples preserved in plain tubes for glucose assays can be measured immediately after sample. Even though fluoride-oxalate inhibits erythrocytic glycolysis, its effects are manifested after several hours. Therefore, laboratory workers should ensure that the blood samples for glucose estimation are not left on the bench for more than an hour.

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COMPETING INTERESTS

The authors declare that they have no competing interests.

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