www.jmscr.igmpublication.org
Index Copernicus Value: 79.54
ISSN (e)-2347-176x ISSN (p) 2455-0450
crossrefDOI: https://dx.doi.org/10.18535/jmscr/v7i2.31

IGM Publication
Journal Of Medical Science And Clinical Research
An Official Publication Of IGM Publication

Research Article

Comparative Analysis of Random Blood Glucose Levels in Serum, Plasma and Whole Blood Using Glucose Oxidase and Hexokinase Methods under Spectrophotometric and Electrochemical Platforms

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Abstract
Diabetes is, a condition associated with the impairment of the body's ability to produce or utilise the hormone insulin. This leads to abnormal metabolism of carbohydrates and elevated levels of glucose in the blood. This serious non-communicable disease has been on the rise in Kenya partly because many people are not aware that they are diabetic since there are no serious early symptoms associated with the disease. It is estimated that the prevalence of diabetes in Kenya ranges from 2.7% (rural settings) and 10% (urban areas) affecting both the affluent and non-affluent population. It is expected that the actual numbers could be higher since many cases go unreported for lack of regular screening in the general population. In Kenya there is no study that has been conducted to compare the performance of current blood glucose testing methods being used. The suitability of serum or plasma as alternatives to whole blood has also not been well researched. The sample of choice for glucose testing has always been whole blood, either in fluoride or from a finger prick. The aim of the study was to compare the results obtained from these three sample types (whole blood, serum and plasma) using two methods: glucose oxidase and hexokinase methods. The investigation was carried out using 300 study subjects that included 150 diabetic patients attending diabetic clinic either for management, diagnosis or monitoring of blood sugar and 150 healthy individuals in the blood donation centre at Kenyatta National Hospital Nairobi County. The analytical instruments used were glucometers and a spectrophotometer to compare blood glucose levels in serum, plasma and whole blood using electrochemical and spectrophotometric platforms. While comparing the serum and plasma glucose concentration using hexokinase method under electrochemical and spectrophometric platforms, the mean difference for the two protocols was 0.145 which was found to be statistically insignificant (p=0.342) using paired T-test. Similarly while comparing the same using glucose oxidase method under the electrochemical and spectrophotometric platform, the mean difference for the two protocols was 0.012 which was found to be statistically insignificant (p=0.135) using paired T-test. The results revealed that the two methods used in glucose concentration analysis were similar irrespective of the method or sample used. There was no significant difference in their means. Either of the two methods can be used interchangeably in the analysis of glucose and either serum or plasma in fluoride can be used as a specimen of choice.
Keywords: *Glucose oxidase, Hexokinase, electrochemical , spectrophotometric.*

Introduction

Diabetes (commonly called diabetes mellitus) is a metabolic condition (non-communicable disease/ lifestyle disease) in which the body's ability to produce or respond to the hormone insulin is impaired. This results in abnormal metabolism of carbohydrates leading to elevated glucose levels in blood (hyperglycaemia) which marks the diagnosis of the disease. Diabetes affects a large population in the world many of whom are not aware of their condition with diagnosis being done either too late, done through medical outreach camps or on arrival to the hospital with related complications with hyperglycaemia being the hallmark of the disease [1].

It was estimated that 415 million people lived with diabetes worldwide as of 2015 with diabetes Type 2 estimated to have 90% of the cases. This represented 8.3% of the total adult population for both male and female [2]. The number has quadrupled since 1980 thus calling for health systems to be able to diagnose, treat and care for diabetic patients. There is an estimated 40 million living with Type 1 diabetes[3]. The disease doubles ones risk of early death with an approximate 1.5 to 5.0 million deaths in every year from 2012 to 2015 resulting from the disease. Globally in 2014 it was estimated that the economic cost for diabetes was US$612 billion, out of this 80% was found in the developing world [2]. This figure was expected to rise to around 18.6 million by 2030 if no interventions are done to try and curb the disease. The economic burden results from medical costs and this can impose burden not only to the people living with the disease and their families but also on the health-care system and the national economy[4]. In Kenya about 1% of the deaths in 2012 were directly attributed to diabetes (WHO, 2012) though this could be an underestimate since patients with diabetes do not die directly with the disease but from complications [2].

There are various methods used in the laboratory for analysing glucose levels in blood these include glucose oxidase, glucose dehydrogenase and hexokinase methods. In the hexokinase method, nicotinamide adenine dinucleotide (β-NADH) is measured and it is directly proportional to the glucose concentration in the sample. When using glucose oxidase the glucose concentration is measured indirectly by detecting the levels of quinoneimine. According to Freckmann blood glucose levels vary among the GOD oxygen-sensitive system. For proper diagnosis and management of diabetes, there is need to compare screening methods performance and also selection of sample. Different researchers have conducted studies on effect of oxygen on glucose using different methods[5] but none has compared the performance of the methods which is the purpose of our study.

Materials and Methods

This cross-sectional comparative study was carried out at the medical outpatient clinic and the blood donation centre after obtaining an informed consent form duly signed by the subject. All samples collected were divided in to three, prepared and analysed for glucose levels using glucose oxidase and hexokinase methods under electrochemical and spectrophotochemical platforms. In any case analysed total confidentiality was observed and no significant harm was reported during the whole study time.

For relative suitability of serum, plasma and whole blood for blood glucose determination, fluoride plasma, whole blood and serum samples were compared for the same patient. The analysis of these samples was carried out within the first 30 minutes of drawing the blood.8ml of whole blood was drawn from each of the participants through venipunture. This blood was then separated into three different tubes which were labelled with letters and serial numbers specific to each participant as follows: contained 2mls, B,3mls and C, 3mls of the aliquoted blood sample Tubes A and B contained fluoride as an anticoagulant and were for the unprocessed whole blood and serum preparation, respectively. The aliquots in tube C which was a plain tube (no anticoagulant) were for plasma preparation.The tube containing 3ml of sample (un-anticoagulated) was let to stand for 10mins and spun at 2500RPM for 5

minutes; serum separated and put in a sample vial. The tube containing 3ml of sample (anticoagulated) was let to stand for 10mins and spun at 2500RPM for 5 minutes; plasma separated and put in a sample vial. The samples were then analysed by glucose oxidase and hexokinase methods under electrochemical and spectophotochemial platforms for glucose levels.

Results

A total of 300 adult participants both male and female aged 18 years and above were recruited for the study. Out of the 300 recruited one declined to allow their blood sample to be drawn. Therefore blood samples were obtained from 299 participants. From these 299, 149 (49.8%) were diabetic whereas 150 (50.2%) were non-diabetic randomly selected from diabetic clinic and blood donation centre respectively. The total number of participant's distribution according to gender was 49.7% for males and 50.3% for females, with a percentage of 40.7% males and 59.3% females for the diabetic and 59.3% male and 40.7% female for the non-diabetics. The mean serum glucose concentrations for electrochemical technique using glucose oxidase and hexokinase analytical methods were 7.337 and 7.452 respectively. The means difference for the two protocols was 0.115 which was found to be statistically insignificant (p=0.431) using paired T-test. The mean serum glucose concentration for spectrophotometric technique using glucose oxidase and hexokinase analytical methods were 7.325and 7.445 respectively. The means difference for the two protocols was 0.12 which was found to be statistically insignificant (p=0. 0.532) using paired T-test. The mean plasma and serum glucose concentration using glucose oxidase method under spectrophotometric technique were 7.325 and 7.415 with mean difference of 0.09 and statistical t value of 1.097 which was statistically significance at p=0.018.On the other hand, the mean plasma and serum glucose concentration using hexokinase method under spectrophotometric technique were 7.469 and 7.445 with mean difference of 0.024 and statistical t value of 1.041 which was statistically significance at p=0.039. Serum and plasma specimens from two hundred and ninety-nine study subjects were also used to determine whether the two types of specimens have any difference in sensitivity and specificity when run using the two methods under different platforms. The sensitivity and specificity for the GOD method were 100% and 70% respectively with a PPV and NPV was 51% and 100% respectively.

Table 1 Comparison of serum and plasma glucose concentration using hexokinase analytical method under electrochemical and spectrophotometric platforms.

Sample type	platform	N	Mean	SD	SEM	95% CI		MD	t-value	Sig. (2tailed)
						Lower	Upper			
Serum	electrochemical	299	7.452	4.311	0.249	0.085	0.281	0.017	1.980	0.193
	spectrophotometric	299	7.469	4.194	0.243					
Plasma	electrochemical	299	7.590	4.303	0.249	0.024	0.344	0.145	1.685	0.342
	spectrophotometric	299	7.445	4.216	0.244					

Key: N=number, SD=standard deviation, SEM =standard mean error, CI=confidence interval, MD=means difference

Table 2 Comparison of serum and plasma glucose concentration using glucose oxidase analytical method under electrochemical and spectrophotometric platform

Sample type	technique	N	Mean	SD	SEM	95% CI of the difference		MD	t-value	Sig. (2tailed)
						Lower	Upper			
Serum	electrochemical	299	7.561	4.244	0.361	3.6889	4.3442	0.084	1.126	0.185
	spectrophotometric	299	7.445	4.216	0.244					
Plasma	electrochemical	299	7.337	4.057	0.350	3.5724	4.2098	0.012	1.028	0.135
	spectrophotometric	299	7.325	4.116	0.244					

Key: N=number, SD=standard deviation, SEM =standard mean error, CI=confidence interval, MD=means difference

Table 3 Comparison of Hexokinase and Glucose Oxidase analytical methods

Sample type	platform	Method	N	Mean	SD	SEM	95% CI of the difference		MD	t-value	Sig. (2tailed)
							Lower	Upper			
serum	electrochemical	GOD	299	7.337	4.244	0.361	3.256	4.111	0.115	1.282	0.431
		HK	299	7.452	4.311	0.249					
	spectrophotometric	GOD	299	7.325	4.216	0.244	3.421	4.251	0.12	1.244	0.532
		HK	299	7.445	4.194	0.243					
plasma	electrochemical	GOD	299	7.561	4.057	0.350	3.419	4.418	0.029	1.372	0.751
		HK	299	7.590	4.303	0.249					
	spectrophotometric	GOD	299	7.415	4.116	0.244	3.221	4.331	0.054	1.333	0.673
		HK	299	7.469	4.216	0.244					

Key: N=number, SD=standard deviation, SEM =standard mean error, CI=confidence interval, MD=means difference, HK= hexokinase, GOD= glucose oxidase

Table 4 Difference between glucose concentration in plasma and serum specimens used for glucose analysis

Specimen	Method	platform	N	mean	SD	SEM	95% CI of the difference		MD	t	sig
							L	U			
P	GOD	electrochemical	299	7.561	4.244	0.361	3.111	4.212	0.224	1.324	0.013
S			299	7.337	4.057	0.350					
P	HK	electrochemical	299	7.59	4.311	0.249	3.108	4.123	0.138	1.124	0.044
S			299	7.452	4.303	0.249					
P	GOD	spectrophotometric	299	7.415	4.216	0.244	3.042	4.202	0.09	1.097	0.018
S			299	7.325	4.116	0.149					
P	HK	spectrophotometric	299	7.469	4.194	0.243	3.117	4.531	0.024	1.041	0.039
S			299	7.445	4.358	0.341					

KEY:S=serum, P=plasma, GOD=glucose oxidase, HK=hexokinase, N=number,SD=standard deviation, SEM=standard mean error, CI=confidence interval, L=lower limit, U= upper limit, MD= mean difference, t=t value, sig= significance difference.

Table 5 Evaluating the performance characteristics of the glucose oxidase method Vs hexokinase method under electrochemical and spectrophotometric platforms.

Glucose analysis Method	**Performance characteristics**			
	Sensitivity (%)	**Specificity (%)**	**PPV (%)**	**NPV (%)**
Hexokinase	100	100	100	100
Glucose oxidase	100	70	51	100

Discussion

Results generated from the clinical laboratory are mostly utilized either to make a diagnosis or determine the prognosis of any pathological disorder. It is of paramount important therefore that these results are reliable and goes a long way in ensuring that the clients get the benefit of the services offered in the laboratory. The current study incorporated internal quality control procedure in every step of the undertaking in the generation of study results. The internal quality control results produced in this study were within the expected range according to the assigned glucose reagent kits manufacturer values. The internal quality control was found to be accurate and precise for both dry and wet chemistry glucose analytical procedures undertaken in the current study. Other qualitative and quantitative studies undertaken here in Kenya are in agreement with this good laboratory practice.[6] in a study on quantitative reference ranges for fasting profiles and oral glucose tolerance test for healthy adults from metropolitan region of Kenya emphasized the need to carry out internal quality control procedure prior to analysing specimens from clients as a measure of ensuring that the results generated are accurate and precise.

The current study was able to make use of whole blood, plasma and serum as the specimens of choice since these are the type of specimens used for analysis of glucose concentration in a routine clinical laboratory. Other studies carried out elsewhere have widely used the three specimens in

undertaking similar studies on glucose concentration[7]. The choice of the two categories of study population that is the diabetic and non-diabetic study subjects was made to ensure that the analysis is not baised. Normal study subjects enabled the study to use glucose concentration that is within the normal reference ranges using the studied techniques that is wet and dry chemistry. The anticipation of the current study was to reveal whether the glucose concentration in the three types of specimen is affected by the type of technique applied in a clinical laboratory. Studies carried out in other parts of the world are in agreement with the current study as far as the use of normal subjects referred in this study as non-diabetic study subjects. On the other hand, the current study had to use diabetic patients as study subjects, so as to determine whether the application of dry or wet chemistry analytical techniques in the analysis of high concentration of glucose is affected by the type of specimen used from a single subject. The utilization of diabetic patients as study subjects in an effort to establish the effect on the techniques applied on the three specimens under study is in agreement with a study carried out on a similar subject by[8].

It is very clear from the current study findings that the two methods applied in glucose analysis, are able to pick high glucose concentration in diabetic study subjects as well as picking normal glucose concentration in non- diabetic study subjects. This picture of glucose concentration is irrespective of the type of glucose analytical method applied. The current study has established that the glucose concentration in whole blood is lower than the glucose concentration in both serum and plasma of the study subjects. This trend is expressed in the two methods used that is glucose oxidase and hexokinase methods. The reason attributed to this low glucose concentration in whole blood is that glycolysis taking place in the blood cells utilizes glucose therefore lower the glucose concentration in the whole blood specimen. The current study findings are similar to other studies carried out elsewhere in the world.[9] researching on the impact of blood sample collection and processing methods on glucose levels in community outreach studies established similar findings of low glucose levels due to glycolysis taking place in whole blood specimen. Serum and plasma specimens when analysed for glucose concentration produce similar results irrespective of the type of method used for analysis. This similarity in glucose concentration in both serum and plasma is expressed in glucose concentration for both diabetics and non-diabetics study subjects. The current study findings on the similarities of glucose concentration in serum and plasma concur with a similar study by Hye Soon Kim2016 which aimed at establishing whether serum and plasma from the same individual using same or different glucose conventional methods generates similar or different results.

The study compared the means of the two methods and we found that there was no significant difference between the means in GOD in relation to HK method. This finding was similar to other studies conducted by Ayyaanar et al, 2018 in their study that showed that there was good correlation between GOD and HK methods.

Due to liberation of reagent market in our country today, clinical laboratories are at liberty to choice which reagent kit to use for analysis of glucose. The current study considered that these reagents kits are representation of the conventional methods widely used in our clinical chemistry laboratories today. The current study also considered the possibilities of health workers making request for glucose analysis using either plasma or serum as the specimen of choice. It is evident from the current study that when plasma specimen is used for the analysis of glucose concentration using either the hexokinase or glucose oxidase analytical methods, the results generated are similar. Likewise, serum specimen generates similar results when the two analytical methods are used for analysis of glucose concentration. The current study finding is in agreement with a study carried out by Jia and Zhang, 2010, which evaluated serum and plasma glucose concentration results generated by routine glucose analytical methods.

For any particular test reliability and validity must be tested. Validly is testing how accurate the results from a particular test are compared to another method. The current study tested the validity of GOD comparison to HK method in the determination of glucose levels. Measure of validity was done by testing the tests sensitivity, specificity, positive predictive value and negative predictive values in relation to the gold standard when using serum and plasma samples. The sensitivity and specificity of the GOD method was 100% and 70% respectively while for hexokinase the sensitivity and specificity was 100% and 100% respectively.

These were found to be in linewith other studies conducted by[10], in their study that showed that there was good correlation of both GOD and HK methods. This also correlates with the WHO 2003 guidelines on diabetes type 2 screening methods for venous blood sugar.

In this study no significant difference was found between GOD method vis a vie Hexokinase while using plasma sample. But when using serum sample there was a significant difference (p=0.093) when using serum sample and this could be attributed to the fact that glycolysis could have taken place prior to analysis. This is in agreement with other studies carried out carried out by Eldin Abdelsalam, Dirar, & Abdallah, 2010)

It is desirable to have tests that are both highly sensitive and highly specific to the analyte being analysed, though at times this is not usually possible. When choosing the cut-off point there is need to trade-off between sensitivity and specificity since when one is increased the other reduces. When sensitivity increases there is little decrease in specificity up until when high levels of sensitivity is reached. Screening methods should be valid, reliable and reproducible in the population where screening is taking place (WHO, 2003). To ensure this, uniform procedures and methods, standardized techniques, proper functioning equipment and quality assurance were used in this study.

Predictive values of a test are determined by sensitivity, specificity of the test and the prevalence of the disorder in the population being screened. In Kenya the prevalence of diabetes was estimated to be 3.3% (*WHO 2016)* and was projected to increase to 4.5% by 2025 though most of the cases undiagnosed. It is evident from our current study that the two methods are highly sensitive and specific on glucose. This evident on the fact that they were able to pick both high and low glucose levels on the study subjects used during the study. This was in comparison with hexokinase which is considered as the gold standard

The level of agreement between GOD and hexokinase was determined using kappa statistics. A 2X2 contingency table was used to tabulate the results. The Kappa value was 0.52 and this therefore depicts that the level of agreement between the two techniques is substantial. The two methods are able to pick hyper and hypoglycemic status of individuals since they have shown to be having high specificity and sensitivity. Therefore either of the two methods can be used interchangeably for diagnosis, screening and management of diabetes Hexokinase which was used as the gold standard in glucose screening diagnosis and management reported 68 true positive and 231 true negatives 0 false negatives and 1 false positive.

Conclusion

Glucose analysis in our clinical chemistry laboratories can be achieved by the use electrochemical and spectrophotometric analytical techniques with equal chance of generating similar results. This is an assurance that there is no bias in the results generated. Neither of the two techniques has an advantage over the other in relation to the techniques application. Clinical chemistry laboratories can interchangeably use the two techniques in glucose analysis.

Sodium fluoride plasma and serum can be used as specimens of choice for the analysis of glucose concentration for the purpose of diagnosing and monitoring glucose concentration of an individual. The current study has established that these two specimens generates different results. If plasma is to be used as the specimen of choice, then it has to be harvested immediately after collection of whole

blood so as to avoid the reduction of glucose contents due to glycolysis that take place in blood cells with ultimate utilization of glucose. Serum has been found to have lower glucose concentration than plasma.

The two widely used analytical methods that are hexokinase and glucose oxidase can interchangeably be used in the analysis of glucose concentration. The two methods have been found to have the same diagnostic value in terms of result generation using one type of specimen (serum/ plasma). Clinical laboratories can make use of reagent kits formulated with hexokinase and glucose oxidase analytical methods.

Recommendations

1) Similar study to be carried out using the same analytical techniques and methods on other specimens such as cerebral spinal fluids.
2) Similar studies to be carried out using whole blood and heparinized plasma to determine whether there will be any similarities or differences with the current study findings
3) The current study recommends that results generated using plasma as specimen of choice should be interpreted using glucose reference ranges established using plasma of healthy individuals. On the other hand, results generated using serum as specimen of choice should be interpreted using glucose reference ranges established using serum of healthy individuals

Sources of fund: Africa-ai-Japan
Conflict of interest: None declared

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