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The Effect of Delayed Specimen Separation on Phosphorus and Glucose in Clinical Laboratories in Healthy Adults

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Abstract

Background: *Pre-analytical errors are considered the most common causes of inaccurate laboratory results. Delayed separation of blood specimens is a critical factor affecting the stability of various analyses. The* ***objective*** *of this study is to assess the effect of delayed specimen separation on Phosphorus and Glucose in clinical specimens.*

Methodology: *One hundred twenty samples of whole blood were collected from apparently healthy subjects aged 20-40 years, divided into three containers. The first one was centrifuged immediately. The second was delayed for one hour, and the last one was delayed for three hours. Then, glucose and phosphorus were measured.*

Results: *Significant increase between one and 3 hours in the phosphorus level, and decrease between one and 3hours in the glucose level when compared to baseline time.*

Conclusion: *Phosphorus is elevated, while glucose undergoes glycolysis in erythrocytes and leukocytes, causing progressive declines in measurable levels. The review highlights mechanisms, timelines of degradation, and strategies to ease these effects, including prompt centrifugation, use of glycolysis inhibitors, and adherence to standardized protocols.*

Keywords: Effect of Delayed Specimen Separation, Phosphorus, Glucose, Clinical Laboratories

INTRODUCTION:

Accurate biochemical measurements rely heavily on pre-analytical integrity, with specimen handling constituting a major determinant of reliability [1]. Among the

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various pre-analytical variables, the time elapsed between sample collection and separation of plasma or serum from cellular components is especially significant [2]. Phosphorus and glucose, two routinely measured analyses, are particularly vulnerable to delayed processing. Understanding the biochemical and cellular mechanisms underlying these changes is crucial for improving laboratory quality management and patient outcomes [3-5].

Standard guidelines for blood sample handling state that plasma or serum should be separated (20-30 min) from cells as soon as possible after clot formation is complete to avoid clot-induced changes in the concentration of serum analyses [2,6], at the same time this is necessary for particular analyses, it might be assumed that many blood analyses deteriorate within a matter of hours in unseparated samples kept at ambient temperature, for most routine assays in a clinical laboratory, serum is the sample; the laboratory receives the specimen in the form of whole blood, and then separates the serum from the clot by centrifugation for clinically useful and reliable test results [7,8], the interval between blood collection and serum separation must be controlled now there is reliable evidence that up to 70% of laboratory errors are attributable to extra-analytical issues [9]. The development of blood collection tubes that contain gel and form a barrier after centrifugation has markedly improved serum analytic stability in the primary tube and removed the need for liquating the serum [10]. Several studies reported the effect of the delay of centrifugation and exposure of the specimen to variable temperatures and times during transport before being centrifuged [11]. In addition, the turnaround time is also an important consideration in plasma glucose estimation [12]. Prolonged contact of plasma with the cellular part of blood leads to a reduced plasma glucose level due to utilization by red cells, white cells, and platelets [13].

Analyze stability time described by the World Health Organization (WHO), and clinical laboratory specimen intervals according to Clinical and Laboratory Standards Institute (CLSI) guidelines, depending on timing for processing specimens to ensure accurate test results [14,15]. Often difficult to apply in the clinical settings, as the time taken to transport samples from the collection Centre to the laboratory and the time interval between centrifugation and processing are usually laboratory-dependent variables, the present research aimed to assess the effect of time delay in the analysis of plasma glucose concentration and serum phosphorus concentration in healthy adults.

MATERIALS AND METHODS

Study design

Quasi-experimental study.

Study area and duration

White Nile University, Kosti, Sudan, during the period of October to November 2021.

Study population:

A total of 120 blood samples, divided into three groups according to time measurement for glucose and phosphorus, each group included 40 participants.

Inclusion Criteria: Healthy adult volunteers aged between 20 and- 40years, both sexes (males and females) agreed to participate in this study.

Exclusion Criteria: Patients with chronic disease, hemolysis, and lipemic samples, and those who refused to participate in this study were excluded.

Data Collection: Data were collected by direct interview and questionnaire, which included personal and clinical information and measurement of glucose and phosphorus levels according to the standard method described by the manufacturer's procedure.

Ethical Approval: Ethical approval was obtained from the ethical and research committee of the White Nile University, Faculty of Medical Laboratory Sciences, and a verbal consent form was obtained from all adult participants after discussing the objectives of the study according to the Declaration of Helsinki (DOH).

Data Analysis: The Data was analyzed using the statistical package for the social Science version 20. p-value is considered significant if less than 0.05.

Results:

The objective of this study is to assess the effect of delayed separation of specimens on glucose and phosphorus, which were measured at three different times: at baseline (immediately), one hour, and three hours. One hundred twenty samples of whole blood from apparently healthy adult participants aged between 20-40 years were enrolled in this study

Glucose:

Table 1 shows a significant difference in the mean concentration level of glucose between immediately (0 hr) and 1 hour, with a P-value of 0.0001.

Table 1: Comparison of Measurement of glucose at Baseline(0hr) and after one hour(1hr)

Model		Unstandardized Coefficients		Standardized Coefficients	T	Sig.	99.0% Confidence Interval for B	
		B	Std. Error	Beta			Lower Bound	Upper Bound
1	(Constant)	-.366	5.039		-.073	.942	-14.030	13.297
	G0h	.851	.051	.938	16.625	0.0001	.712	.990

Coefficients [a]

a. Dependent Variable: G1h

b. Predictors: (Constant), G0h

Table 2: shows a significant difference in the mean concentration level of glucose between 0 hr and 3hr

Model		Unstandardized Coefficients		Standardized Coefficients	T	Sig.	99.0% Confidence Interval for B	
		B	Std. Error	Beta			Lower Bound	Upper Bound
1	(Constant)	.414	2.487		.166	.869	-6.330	7.158
	G1h	.899	.030	.980	30.206	0.0001	.818	.980

Coefficients [a]

a. Dependent Variable: G3h

b. Predictors: (Constant), G1h

Table 3: shows a significant difference in the mean concentration level of glucose between 1hr and 3 hours with a P-value of 0.0001.

Model		Unstandardized Coefficients		Standardized Coefficients	T	Sig.	99.0% Confidence Interval for B	
		B	Std. Error	Beta			Lower Bound	Upper Bound
1	(Constant)	1.569	5.788		.271	.788	-14.124	17.262
	G0h	.750	.059	.900	12.754	0.0001	.590	.909

Coefficients a

a. Dependent Variable: G3h

b. Predictors: (Constant), G 0h

Phosphorus:

Table 4: shows significant difference in mean concentration level of phosphorus(Ph) between 0hr and 1hr with P-value 0. 0001.

Model		Unstandardized Coefficients		Standardized Coefficients	T	Sig.	99.0% Confidence Interval for B	
		B	Std. Error	Beta			Lower Bound	Upper Bound
1	(Constant)	.980	.567		1.729	.092	-.557	2.516
	Ph0	.968	.138	.751	7.018	0.0001	.594	1.342

Coefficients [a]

a. Dependent Variable: Ph1

b. Predictors: (Constant), Ph0

Table 5: shows significant difference in mean concentration level of phosphorus between 0hr and 3hr with P-value 0.0001.

Model		Unstandardized Coefficients		Standardized Coefficients	T	Sig.	99.0% Confidence Interval for B	
		B	Std. Error	Beta			Lower Bound	Upper Bound
1	(Constant)	2.196	.389		5.651	0.0001	1.142	3.249
	Ph0	.744	.095	.787	7.869	0.0001	.488	1.001

Coefficients [a]

a. Dependent Variable: Ph3

b. Predictors: (Constant), Ph0

Table 6: shows a significant difference in mean concentration level of phosphorus between 1hr and 3hr with a P-value of 0.0001.

Model		Unstandardized Coefficients		Standardized Coefficients	T	Sig.	99.0% Confidence Interval for B	
		B	Std. Error	Beta			Lower Bound	Upper Bound
1	(Constant)	2.588	.405		6.391	0.0001	1.490	3.685
	Ph1	.536	.081	.730	6.583	0.0001	.315	.756

Coefficients [a]

a. Dependent Variable: Ph3

b. Predictors: (Constant), Ph1

Table 7 shows there is are decrease in the mean concentration levels of glucose between 0hr, 1hr, and 3hr (97.20, 82.35, 74.45) receptively. Also, the table shows an increase in the mean concentration levels of phosphorus between 0hr,1hr &3hr (4.060,4.910, 5.218) respectively.

Table 7: Statistics the mean concentration levels of glucose and phosphorus at 0hr,1hr and 3hr .

		G0h	G1h	G3h	Ph0	Ph1	Ph3
N	Valid	40	40	40	40	40	40
	Missing	0	0	0	0	0	0
Mean		97.20	82.35	74.45	4.060	4.910	5.218
Median		100.00	80.00	73.00	4.100	5.000	5.200
Mode		93	73[a]	66	4.4	5.0	5.2[a]
Sum		3888	3294	2978	162.4	196.4	208.7

DISCUSSION:

The current study shows a significant increase in mean concentration levels of phosphorus at 1hr compared with 0hr, P-value (0.0001). This agrees with a study done

in China reported by Yusef Kianpoor Kalkhaje et at in 2019 entitled Methods for sample collection, storage, and analysis of freshwater phosphorus [16].

Our study shows a significant decrease in mean concentration levels of glucose at 1 hour compared with 0hr, P-value (0.0001). This result compares with a study done by Melissa Tanner *et al* in the United Kingdom in 2008 [17]. which shows lower concentration levels of glucose compared with baseline in all storage conditions because glucose depletion is dependent on the temperature and time, at higher temperatures there is a higher metabolic rate and glucose is depleted quickly, whereas at lower temperatures it is depleted more slowly. another study reported that an oral phosphorus load can induce elevated postprandial levels of circulating for hours in healthy subjects, despite rapid homeostatic counter reactions [18].

The current study shows a significant decrease in mean concentration levels of glucose at 3hr when compared with 0hr, P-value (0.0001). this agrees with a study done by Glassman AR, *et al*, Plasma separator tubes do not have any overt effects on routine plasma chemistry data in 2021 [19], and other reports by Jones GR. Using analytical performance specifications in a medical laboratory in 2024 [20], which reported clinically concentration even with the use of preservatives and also when tube without preservatives is used and delay in transit to the laboratory is anticipated [19,20].

Our results show a significant increase in mean concentration levels of phosphorus at 3hr compare with 1hr, P-value (0.0001). This agree with study done by Lee JE, Hong M, Park SK, Yu JI, Wang JS, Shin H, Kim JW, Han BG, Shin SY. Inorganic phosphorus and potassium are putative indicators of delayed separation of whole blood. in 2016, which shows that phosphorus levels in the plasma and serum samples were elevated 1.5-fold when whole-blood centrifugation was delayed at room temperature for 48 hours [21].

Phosphorus plays a vital role in diverse biological processes, including intracellular signaling, membrane integrity, and skeletal biomineralization; therefore, the regulation of phosphorus homeostasis is essential to the well-being of the organism [22].

Impact of Glucose is among the most labile analyses, undergoing rapid decline due to glycolysis. Red blood cells consume glucose at an estimated rate of 5–7% per hour at room temperature [23]. Sodium fluoride-containing tubes inhibit glycolysis but only after a lag phase of up to 2 hours, emphasizing the need for rapid centrifugation [24]. Store separated plasma/serum under refrigeration (2–8 °C) to minimize ongoing metabolic activity [3].

In conclusion, delayed separation of specimens introduces clinically significant errors in the measurement of phosphorus and glucose. Ensuring rapid and standardized processing is essential to maintain result accuracy and maintain clinical decision-making.

Strengthening laboratory protocols and staff awareness reduces pre-analytical variability and enhances the overall quality of laboratory services.

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Conflict of interest
The authors declare no conflicts of interest.

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