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ORIGINAL ARTICLE

Effect of delayed serum separation on various chemistry analytes.

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ABSTRACT… Objective: To observe the effect of delayed serum separation on various chemistry analytes. **Study Design:** Quasi Experimental study. **Setting:** Clinical Chemistry Laboratory, Sheikh Zayed Medical College & Hospital, Rahim Yar Khan. **Period:** 1st December 2021 to 31st January 2022. **Material & Methods:** Fifty healthy volunteers of age 25-55 years from both genders were included in the study using consecutive sampling technique. Informed verbal consent was taken from all the study subjects. 4 cc blood was drawn from each subject and was divided into 3 plain tubes. 1 tube was analyzed at 1 hour for glucose, urea, creatinine, ALT, AST, ALP, sodium, potassium, chloride and calcium. 2nd tube was analyzed for same analytes 2 hours after sample collection and 3rd tube was analyzed at 4 hours. Results were recorded on a predesigned performa. Data was entered and analyzed using SPSS Software 23 for Windows. Data was presented in terms of mean and SD. P value $p < 0.05$ was considered significant. **Results:** Glucose, ALT, sodium, potassium and chloride showed statistically significant variation over time while others remained stable for up to 4 hours. There was decrease in glucose ($p=0.000$) while increase was observed in sodium ($p=0.0001$), potassium ($p=0.0001$), chloride ($p=0.0001$) and ALT ($p=0.002$). **Conclusion:** Blood samples should be transported to laboratory immediately and when received in laboratory samples should be centrifuged and processed within 2 hours as delayed transportation and separation affects many chemistry analytes and may lead to erroneous test results.

Key words: Analyte Stability, Chemistry Analytes, Delayed Transportation, Delayed Separation, Serum-clot Contact Time.

INTRODUCTION

It is proved that extra analytical issues contribute to 70% of laboratory errors[1] that may affect the reliability of test results thereby, influencing the diagnosis, follow-up, or even the treatment plan of the patients.[2] A common problem is to maintain stability of analytes in blood samples during sample transportation from clinical departments to central laboratory, and also after centrifugation if the analysis is delayed.[3] Stability of a sample is its ability to retain the initial property of an analyte within predefined limits for a specified period of time when the sample is stored under predetermined conditions.[4] Specimen stability is affected by ongoing cellular metabolism, air contact and evaporation, exposure to light and variation in temperature.[5] The time between collection of blood sample to its centrifugation and separation of serum is one of the major factors affecting sample stability.[6] The contact time of serum and clot is a unique pre-analytical condition that could alter the analyte stability independent of any physiological condition in the patient.[7]

Prolonged serum clot contact time can cause pre-analytical variation in the specimen.[7] Inorganic phosphorus, glucose and Lactate dehydrogenase (LDH) are few of the biochemical analytes which are strongly affected by serum-clot contact time before centrifugation and delayed sample processing.[2] Increasing trend is seen in all the electrolytes with delay in serum separation and sample processing after centrifugation.[8] Under ISO 15189, pre-analytical process must be controlled by an accredited medical laboratory,

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including the time between sample collection and serum separation.[9] A time lapse of 30 minutes is considered acceptable between specimen collection and serum separation.[10]

This study aims to observe the effect of delayed serum separation on various chemistry analytes in order to educate nursing and laboratory staff regarding immediate sample transportation to central laboratory and earliest possible serum separation so as to prevent reporting of erroneous results due to this pre analytical error.

MATERIAL & METHODS

This Quasi Experimental study was conducted in Clinical Chemistry Laboratory, Sheikh Zayed Medical College & Hospital, Rahim yar khan from 1st December 2021 to 31st January 2022. The study was approved by ethical committee (390/IRB/SZMC/52H). 50 healthy volunteers of age 25-55 years from both genders were included in the study using consecutive sampling technique. Informed verbal consent was taken from all the study subjects. 4 cc blood was drawn from each subject and was divided into 3 plain tubes. One tube was analyzed at 1 hour for glucose, urea, creatinine, ALT, AST, ALP, sodium, potassium, chloride and calcium. 2nd tube was analyzed for same analytes 2 hours after sample collection and 3rd tube was analyzed at 4 hours. Samples were analyzed on Beckman Coulter AU680 fully automated chemistry analyzer based on photometry. Results were recorded on a predesigned performa. Data was entered and analyzed using SPSS Software 23 for Windows. Data was presented in terms of mean and SD. ANOVA and t-test were applied to access significance of difference of means of different analytes between immediate and delayed serum separation. P value < 0.05 was considered significant.

RESULTS

Glucose, ALT, sodium, potassium and chloride showed statistically significant variation over time. There was decrease in glucose ($p=0.000$) while increase was observed in sodium ($p=0.000$), potassium ($p=0.000$), chloride ($p=0.000$) and ALT ($p=0.002$). Increase was observed in urea, AST, ALP, calcium but this was not significant statistically. Creatinine remained stable for upto 4 hours. Mean difference of various analytes with p value is shown in Table-I.

Analyte		1 Hr.	2 Hr.	4 Hr.	ANOVA	p value
Glucose	Mean	95	90.1	71	0.001	0.0001
	SD	13.7	13.6	13.5		
Urea	Mean	24	25.2	26.3	0.579	.302
	SD	4.9	4.8	4.8		
Creatinine	Mean	0.69	0.69	0.7	0.974	.826
	SD	0.11	0.11	0.11		
ALT	Mean	29.10	34.70	41.90	0.007	.002
	SD	6.95	8.20	9.54		
AST	Mean	29.20	30.40	32.30	0.909	.659
	SD	15.6	15.78	16.37		
ALP	Mean	101.6	103.7	106.9	0.822	.528
	SD	17.6	17.8	21.2		
Sodium	Mean	139.2	143.9	151.5	0.0001	0.0001
	SD	1.87	1.85	2		
Potassium	Mean	4.3	4.8	5.5	0.0001	0.0001
	SD	0.37	0.36	0.34		
Chloride	Mean	101.6	104.3	106.3	0.001	0.0001
	SD	2.36	2.16	2.49		
Calcium	Mean	9.68	9.65	9.8	0.424	.254
	SD	0.37	0.212	1.17		

Table-I. Mean difference of various analytes.

DISCUSSION

Traceability of the pre-analytical phase is essential in laboratory medicine. Storage temperature and the time from sample collection to its processing are two critical aspects of pre-analytical phase.[11] Loss of sample stability is most likely to involve an unacceptable error in the result of an analyte that may lead to incorrect clinical decisions and fallacious management of patient.[11] Prolonged serum-clot contact time can cause pre-analytical variations.[12] We assessed the stability of various analytes in whole blood, that were collected at outpatient collection point and were kept for varying lengths of time (1 hour, 2 hour and 4 hour) prior to centrifugation. Of 10 analytes glucose, ALT, Sodium, Potassium and chloride showed changes in results after 4 hours while urea, creatinine, AST, ALP and calcium were stable for upto 4 hours.

Stability of analytes was observed for 4, 12 and 24 hours before centrifugation by Tanner et al. and they reported decreasing trend in glucose while increase was observed in creatinine.[13] Decrease in glucose can be due to its utilization by glycolysis.[14]

Our study also showed a decreasing trend in glucose on delayed serum separation and analysis. Jaffe's method is routinely used method for creatinine estimation but it shows positive interference from pyruvate and ketones which are increased in stored sample.[15] Spithoven et al. reported that creatinine stability is not affected even after delayed serum separation when enzymatic method is used for analysis.[15] In our study creatinine and urea did not show any significant change even after delay of 4 hours, this can be attributed to the stability of these analytes i.e. non utilization by either enzymatic or non-enzymatic means like auto oxidation and only 4 hours delay in sample separation and processing. Baruah A et al. reported that samples for electrolytes should be analyzed within 1-2 hours of collection and if any delay is expected in analysis, the sample should be stored after centrifugation and separation under predefined condition.[16]

Our study demonstrated a persistent rise in serum electrolytes value with increase in serum clot contact time. The change became highly significant by 4 hours at room temperature. This finding is in agreement as reported by Kalasker.[8] Excellent sample quality and reliable test results can be ensured by adhering to standard operating procedures.[17] Understanding of analyte stability is critical to interpret test results with confidence.[3] Standard operating procedures of stability limits should be designed by clinical laboratories for each analyte, and they should be used as a cause of rejection before sample processing, rather than relying on apparent visible deterioration of sample.[11]

CONCLUSION

Blood samples should be transported to laboratory immediately and when received in laboratory samples should be centrifuged and processed within 2 hours as delayed transportation and separation affects many chemistry analytes and may lead to erroneous test results thereby affecting diagnosis and patient care.

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