EFFECT OF DELAYED CENTRIFUGATION ON SERUM CHEMISTRY

Nayyar Chaudhry, Asma Hayat*, Tariq Mahmood Ahmad, Numan Majeed*, Sadia Israr*, Amna Saddique***

Armed Forces Institute of Pathology/National University of Medical Sciences (NUMS) Rawalpindi Pakistan, *Army Medical College/National University of Medical Sciences (NUMS) Rawalpindi Pakistan, **Bahria International Hospital, Lahore Pakistan

ABSTRACT

Objective: To assess the effect of delayed centrifugation on integrity of serum chemistry.
Study Design: Quasi experimental study.
Place and Duration of Study: Pak Emirates Military Hospital, Rawalpindi, from Jul 2018 to Sep 2018.
Patients and Methods: A sample of 20 ostensibly healthy adult subjects were recruited in the study. 16 cc of blood was drawn from each individual. It was distributed evenly (2 cc each) in plain tube. One tube was centrifuged immediately after clotting while remaining 7 at the time of expiry of delay period up to 12 hours. All the samples were stored at room temperature (25±1^{o}C). Samples were analyzed for routine chemistry analytes. Data was analyzed by using SPSS 23. Repeated samples ANOVA and Paired t-test was used to access the variation in the levels of different analytes.
Results: Urea and ALT levels showed significant variation over time. While other remained stable up to 12 hours. Urea showed significant change at test on 4th hours (p≤0.001). ALT remained stable up to 6 hours, 8th hour analysis showed significant variation (p=0.001) with sharp increase in the results of both urea and ALT.
Conclusion: Variation in urea levels became significant at 4th hour while ALT, bilirubin and calcium levels were stable up to 6th hour. Cholesterol levels remained within non-significant variation till 8th hour while all other analytes showed stability up to 12 hours.

Keywords: Delayed centrifugation, Delayed processing, Serum chemistry.

INTRODUCTION

Now there is reliable evidence that up to 70% of laboratory errors are attributable to extra-analytical issues[1], especially to inaccurate or inappropriate procedures for collection and management of specimens[2,3]. In routine practice, phlebotomy done for the blood analysis is in OPD labs, wards or clinics and samples are stored prior to sending to the lab for analysis. Usually these samples arrive in the laboratory within 2-3 hours from collection. Among the various steps of biological samples management, centrifugation is a step for obtaining serum or plasma for in vitro diagnostic[4,5]. The increase contact duration of blood cells to serum due to storage can affect the results.

A common problem faced by the laboratories is the integrity of un-centrifuged specimens for analysis. Prolonged contact of serum with cells leads to spurious test results[4,6]. Ideally serum or plasma should be separated in order to prevent ongoing metabolic processes as well as leaking effect of cells[7].

Although there are recommendations by WHO[8] and CLSI[9] but in routine lab practice, they are difficult to apply. As the analyte stability is more often compatible with the time taken to transport the sample from the spot of collection to the lab.

In literature, several studies have reported that prolonged contact time of serum with cells affect stability of several analytes. The instability of these analytes was reported on different times and temperature. In some samples were frozen before analysis which induced bias. In this study sample was drawn in the lab and temperature was kept constant at 25^{O}C (±2^{O}C). In previous studies isolated difference of mean of each individual time was not compared with the time zero levels. Either only ANOVA was applied to

Correspondence: Dr Nayyar Chaudhry, Pathology Dept, Armed Forces Institute of Pathology, Rawalpindi Pakistan
Email: asma.hayat72@gmail.com
Received: 28 Dec 2018; revised received: 31 Dec 2018; accepted: 31 Dec 2018

test overall variance or the time interval between the samples were in days, none of the study tested individual variance of different time intervals with zero time levels within 12-hour time span.

The objective of the current study is to assess the effect of delayed centrifugation on integrity of serum chemistry.

MATERIAL AND METHODS

A sample of 20 ostensibly healthy adult subjects were recruited in the study out of which 12 were males and 8 were females, conducted at Pak Emirates Military Hospital, Rawalpindi, form July 2018 to September 2018. Healthy human subjects, with normal BMI who voluntarily consented for the study were included. 16 cc of blood was drawn from each individual, 2 cc in each tube using BD Vacutainer system TM in clot activator tubes by Vaccute. 1st tube of each individual was centrifuged immediately after clotting (30 minutes standard time for clotting according to manufacturer guidelines) while remaining 7 at the time of expiry of delay period. All the samples were stored at room temperature (25±1°C).

Analysis was done at time 0, 0.5, 1, 2, 4, 6 and 12th hour after drawing the samples, time zero was donated to the 1st sample which was analyzed after 30 minutes, standard time specified by the Vaccute to clot the sample properly before centrifugation. Chemistry analysis was done on Selectra XL.

Table-I: Mean difference of different variables.

		0 Hr.	0.5 Hr.	1 Hr.	2 Hr.	4 Hr.	6 Hr.	8 Hr.	12 Hr.	ANOVA*
ALP	Mean	218	203	200	205	205	186	199	183	0.24
	SD	94.1	48.85	46.3	58	50.49	56.65	53.18	43.3	
ALT	Mean	36	36.05	35.5	36.8	37.3	36.11	43.22	42.9	0.001
	SD	17.1	17.42	17.76	17.7	18.1	17.75	21.87	23.5	
Total bilirubin	Mean	12.4	12.2	12.05	12.4	12.35	11.95	11.95	12.2	0.14
	SD	8.56	8.7	8.36	9.06	8.84	8.23	8.2	8.22	
Albumin	Mean	53.5	52.55	52.74	51.5	53.95	49.74	49.46	53.8	0.47
	SD	2.44	3.46	3.14	8.37	3.3	9.95	10	1.48	
AST	Mean	27.6	27.8	26.85	27.4	28.73	28.38	28.58	27	0.574
	SD	8.66	8.64	8.54	8.36	10.49	9.46	9.43	11.2	
Total protein	Mean	77.4	77.5	76.9	76.7	79.8	79.05	79.73	82.8	0.18
	SD	6.65	7.51	5.58	6.97	6.39	6.45	5.47	3.7	
Creatinine	Mean	83.6	81.05	80.2	74.4	80.75	78.32	86.44	84.5	0.872
	SD	10.8	10.08	9.67	20.2	11.41	16.52	21.22	10.3	
Urea	Mean	4.34	4.42	4.39	4.38	4.54	4.34	4.39	3.97	0.67
	SD	1.55	1.45	1.58	1.54	1.57	1.61	1.66	1.36	
Uric acid	Mean	292	296	288	293	291	270	275	284	0.02
	SD	92.7	97.68	88.64	90.2	92.42	109.4	82.38	99.9	
TG	Mean	1.74	1.72	1.63	1.75	1.78	1.6	1.69	1.27	0.48
	SD	1.02	0.96	1.03	0.99	0.97	1.02	1.07	0.21	
Cholesterol	Mean	4.67	4.82	4.6	4.61	4.71	4.61	4.31	4.19	0.88
	SD	0.85	1.02	1.17	0.94	0.89	0.95	1.38	0.57	
Calcium	Mean	2.3	2.55	2.19	2.41	2.62	2.34	2.54	3.07	0.27
	SD	0.3	0.56	0.9	0.68	0.32	0.31	0.34	0.19	
Amylase	Mean	67.3	66.92	67.85	68.1	68.92	68.58	72.45	---	0.27
	SD	25.8	24.88	24.9	24.7	24.51	24.01	25.25	---	
Iron	Mean	74	87.62	87.08	82.5	88.46	90.17	139.6	78	0.98
	SD	42.7	45.59	46.06	37.1	39.3	40.21	187.2	32.3	
PO4 (IP)	Mean	1.14	1.13	1.15	1.21	1.22	1.07	1.71	1.29	0.09
	SD	0.25	0.28	0.3	0.29	0.5	0.24	1.23	0.36	

**$p<0.05$ significant (2 tailed analysis using repeated sample ANOVA)

Mean and SD will be calculated for all the samples. T-test and repeated sample ANOVA (non-skewed data) were applied to access significance of difference of means of different analytes between immediate and delayed centrifuge.

RESULTS

Urea and ALT levels showed significant variation over time, while others remained stable up to 12 hours. As predicted by repeated sample ANOVA (table-I).

Urea showed significant change at test on 4 hours ($p \leq 0.001$). While ALT remained stable up to 6 hours, and 8 hour analysis showed significant variation ($p=0.001$) with sharp increase in the results of both urea and ALT. Overall variation of calcium and bilirubin was found non-significant but t-test suggested significant change at 6th hour with significance of <0.001 and 0.004 respectively. Similarly, cholesterol remained stable till 8th hour, on 12th hour the variation became significant ($p=0.002$). As shown in table-II.

Table-II: Variation of different variables.

		0.5 Hr.	1 Hr.	2 Hr.	4 Hr.	6 Hr.	8 Hr.	12 Hr.
Uric acid	% variance	1.4%	-1.4%	0.3%	-0.3%	-7.5%	-5.8%	-2.7%
	p-value	0.3	0.3	0.9	0.7	0.2	0.3	0.7
Urea	% variance	1.8%	1.2%	0.9%	4.6%	0.0%	1.2%	-8.5%
	p-value	0.22	0.35	0.37	<0.001	0.09	0.023	0.005
Creatinine	% variance	-3.0%	-4.0%	-11%	-3.4%	-6.3%	3.5%	1.1%
	p-value	0.24	0.049	0.041	0.26	0.043	0.65	0.95
AST	% variance	0.7%	-2.7%	-0.7%	4.1%	2.8%	3.6%	-2.2%
	p-value	0.7	0.2	0.8	0.6	0.7	0.3	1.0
ALT	% variance	0.10%	-1.4%	2.2%	3.6%	0.3%	20.1%	19.1%
	p-value	0.94	0.52	0.32	0.07	0.84	0.001	<0.001
ALP	% variance	-6.9%	-8.3%	-6.0%	-6.0%	-14.7%	-8.7%	-16.1%
	p-value	0.36	0.26	0.44	0.40	0.13	0.18	0.26
Total bilirubin	% variance	-1.6%	-2.8%	0.0%	-0.4%	-3.6%	-3.6%	-2.0%
	p-value	0.163	0.049	1.00	0.86	0.004	0.035	0.14
Albumin	% variance	-1.8%	-1.4%	-3.7%	0.8%	-7.0%	-7.6%	0.6%
	p-value	0.09	0.05	0.31	0.48	0.08	0.15	0.18
Total protein	% variance	0.2%	-0.6%	-0.8%	3.2%	2.2%	3.1%	7.0%
	p-value	0.90	0.68	0.60	0.04	0.18	0.11	0.34
Iron	% variance	18.4%	17.7%	11.5%	19.5%	21.9%	88.7%	5.4%
	p-value	0.18	0.30	0.46	0.19	0.12	0.30	0.93
Calcium	% variance	12.3%	-3.5%	6.2%	15.4%	3.1%	11.9%	35.2%
	p-value	0.06	0.66	0.37	<0.001	0.85	0.01	0.03
TG	% variance	-1.1%	-6.3%	0.6%	2.3%	-8.0%	-2.9%	-27.0%
	p-value	0.65	0.09	0.89	0.35	0.33	0.50	---
Cholesterol	% variance	3.2%	-1.5%	-1.3%	0.9%	-1.3%	-7.7%	-10.3%
	p-value	0.02	0.62	0.48	0.60	0.72	0.36	0.002
Amylase	% variance	-0.6%	0.8%	1.1%	2.4%	1.9%	7.6%	---
	p-value	0.71	0.63	0.57	0.06	0.55	0.23	---
IP	% variance	-0.9%	0.90%	6.10%	7.00%	-6.10%	50.0%	13.20%
	p-value	0.93	0.87	0.43	0.48	0.33	0.07	0.20

DISCUSSION

Overall variation bias in analytes

Our study suggested a significant stability in levels of Uric acid, AST, ALP, albumin, iron, TG, amylase and IP up to 12 hours which is similar to the results reported by previously[1,2,7,10-15]. A highly significant raise with ALT and urea levels were observed in a span of 12 hours. These results are similar to those reported earlier[2,6].

Some studies suggest that PO4 levels tend to show significant variations due to the leakage of intracellular ions into serum due to prolonged (48 to 72 hours) contact with cellular component[16-18].

Time based variation of analytes from sample zero

Non-significant variation from time zero samples were observed in uric acid, AST, ALP, Albumin, Iron, TG, Amylase and IP.

Significant variations in creatinine levels at 1st hour and 2nd hour which on further delay became non-significant these findings could not be explained from the available literature. Similarly, urea levels showed significant change at 4th hour. A mean decrease of 0.2 nmol/L was observed which is almost similar to that reported by Balveren. They reported the change to be -0.19 at 4th hour[6]. Calcium showed a significant variation from 4th our and onwards. Study by Daves showed significant variations from 3rd hour and onwards[1]. From this we can infer that calcium levels are stable for 2 hours only.

Most peculiar behavior was seen in cholesterol and bilirubin levels. Analysis with a delay of only half hour showed a significant (p=024, variation=+3.2%). Total protein level in our study showed a significant (p=0.042) increase in levels (by 3.2%) at 4th hour analysis. ALT showed a significant decrease at 8th and 12th hour centrifugation delayed analysis which was -8.0% and 13.4% respectively. Previous published literature dose not corroborate with our findings. Majority studies predicted overall stability of these analytes as were our results of repeated ANOVA, but the individual comparison using statistical tests on one to one test basis like t-test. The studies that applied the tests had different time intervals (in days) or different analytes.

CONCLUSION

Variation in urea levels became significant at 4th hour while ALT, bilirubin and calcium levels were stable up to 6th hour. Cholesterol levels remained within non-significant variation till 8th hour while all other analytes showed stability up to 12 hours.

Strengths

Application of multiple statistical tools (t-test, ANOVA, percentage bias and difference of means)

LIMITATIONS OF STUDY

All the subjects ranged from 28 to 45 years of age

Samples should be analyzed beyond 12 hours

Further Studies

Probing into unusual pattern of variation in analytes like creatinine, AST, ALT, Albumin, and calcium which showed unusual rise and falls over the course of 12 hours duration.

ACKNOWLEDGEMENT

We are thankful to the laboratory staff for assisting in analyzing the samples, especially Mr. Majeed Ali, Mr. Azam, Mr Waseem Ahmed and Mr. Noorullah for their cooperation.

CONFLICT OF INTEREST

This study has no conflict of interest to be declared by any author.

REFERENCES

1. Daves M, Roccaforte V, Giacomi M, Riva M, Leitner M, Platzgummer S, et al. Effect of delayed centrifugation of whole blood on serum samples stability. La Rivista Italiana della Medicina di Laboratorio - Italian J Lab Med 2017; 13(1): 41-4.
2. Clark S, Youngman LD, Palmer A, Parish S, Peto R, Collins R. Stability of plasma analytes after delayed separation of whole blood: implications for epidemiological studies. Int J Epidemiol 2003; 32(1): 125-30.
3. Rioja RG, Espartosa DM, Segovia M, Ibarz M, Llopis MA, Bauça JM, et al. Laboratory sample stability. Is it possible to define a consensus stability function? An example of five blood magnitudes. Clin Chem Lab Med 2018; 56(11): 1806-18.
4. Lippi G, Guidi GC, Mattiuzzi C, Plebani M. Preanalytical variability: the dark side of the moon in laboratory testing. Clin Chem Lab Med 2006; 44(4): 358-65.
5. Grankvist K, Gomez R, Nybo M, Lima-Oliveira G, von Meyer A. Preanalytical aspects on short-and long-term storage of serum and plasma. Diagnosis 2018.
6. van Balveren JA, Huijskens MJ, Gemen EF, Péquériaux NC, Kusters R. Effects of time and temperature on 48 routine chemistry, haematology and coagulation analytes in whole blood samples. Annals Clin Biochem 2017; 54(4): 448-62.
7. Boyanton BL, Blick KE. Stability studies of twenty-four analytes in human plasma and serum. Clin Chem 2002; 48(12): 2242-7.

8. (CLSI) CaLSI. Procedures for the handling and processing of blood specimens for common laboratory tests 2010.
9. Medicine GSfCCaL. Quality of diagnostic samples. Recommendations of the working group on pre-analytical quality of the german society for clinical chemistry and laboratory medicine 2009.
10. Henriksen LO, Faber NR, Moller MF, Nexo E, Hansen AB. Stability of 35 biochemical and immunological routine tests after 10 hours storage and transport of human whole blood at 21 C. Scandinavian J Clin Lab Inves 2014; 74(7): 603-10.
11. Drammeh BS, Schleicher RL, Pfeiffer CM, Jain RB, Zhang M, Nguyen PH. Effects of delayed sample processing and freezing on serum concentrations of selected nutritional indicators. Clin Chem 2008; 54(11): 1883-91.
12. Tanner M, Kent N, Smith B, Fletcher S, Lewer M. Stability of common biochemical analytes in serum gel tubes subjected to various storage temperatures and times pre-centrifugation. Annals Clin Biochem 2008; 45(4): 375-9.
13. Tapper MA, Pethick JC, Dilworth LL, McGrowder DA. Pre-analytical Errors at the Chemical Pathology Laboratory of a Teaching Hospital. J Clin Diag Res: JCDR 2017; 11(8): BC16.
14. Taylor E, Sethi B. Stability of 27 biochemistry analytes in storage at a range of temperatures after centrifugation. Br J Biomed Sci 2011; 68(3): 147-57.
15. Zwart SR, Wolf M, Rogers A, Rodgers S, Gillman PL, Hitchcox K, et al. Stability of analytes related to clinical chemistry and bone metabolism in blood specimens after delayed processing. Clin Biochem 2009; 42(9): 907-10.
16. Oddoze C, Lombard E, Portugal H. Stability study of 81 analytes in human whole blood, in serum and in plasma. Clin Biochem 2012; 45(6): 464-9.
17. Lee JE, Hong M, Park SK. Inorganic phosphorus and potassium are putative indicators of delayed separation of whole blood. Osong public health and research perspectives 2016; 7(2): 90-5.
18. Fagerberg L, Oksvold P, Skogs M, Algenäs C, Lundberg E, Pontén F, et al., Contribution of antibody based protein profiling to the human chromosome centric proteome project (C HPP). J. Proteome Res 2013; 12: 2439-48.