Original Article

EFFECT OF DELAYED PREPARATION OF PLATELET POOR PLASMA ON COAGULATION STUDIES AT ROOM TEMPERATURE

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ABSTRACT

Objective: To determine the effect of delay in the preparation of platelet poor plasma (PPP) on prothrombin time (PT) and activated partial thromboplastin time (APTT) at room temperature (RT).

Material and Methods: It was a cross sectional study, conducted at Chughtai Institute of Pathology from January 2022 to June 2022. Total 100 healthy individuals, both males and females, participated in the study. Centrifugation of four citrated blood samples was done at 4000 g for 10 minutes at RT; first sample at 0 hour (h), second sample at 4 h, third sample at 8 h and fourth sample at 24 h. PT and APTT were run on each PPP using Sysmex CS-1600.The values at different times were noted and compared through careful statistical analysis of the observed parameters.

Results: There was statistically significant difference for PT at 24h storage time when compared with baseline value at 0h (P value 0.0017**)** but no clinically relevant change up to 24h (<10% percent change). For APTT at RT, statistically significant difference was noted at storage time of 8h (P value 0.0001) and 24h (P value <0.0001**)** but a clinically relevant change was noted only at 24h (>10% percent change).

Conclusion: Blood samples for PT and APTT without centrifugation can be stored for up to 24 hours for PT at RT and for up to 4 hours for APTT at RT. PT and APTT values will not be affected if centrifugation of citrated sample is done within 1 hour of collection and transported at RT in small aliquots.

Key Words: Prothrombin time, Activated partial thromboplastin time, Platelet poor plasma, Pre analytical, Centrifugation.

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INTRODUCTION

Prothrombin time (PT) and activated partial thromboplastin time (APTT) are the two important and baseline coagulation tests used for different diagnostic and therapeutic purposes. These are used for the diagnosis of various hereditary and acquired hemostatic disorders and also to monitor anticoagulant therapy i.e. PT/INR for vitamin K antagonists and APTT for unfractionated heparin [1]. These are also the most requested tests in Emergency Medicine in cases of sepsis and DIC [2].

These coagulation tests are affected by various pre-analytical variables like sample collection method, anticoagulant ratio, hematocrit, sample storage, transportation and sample processing [3]. The CLSI guidelines (H21-A5) recommend that the samples for coagulation studies stored at RT should be transported, processed and tested within 4 hours after sample collection [4]. However, this is practically not possible in remote areas and small towns where specialized testing facilities are not available and the samples have to be transported to central laboratory for testing. Delay in the transport and difficulty in maintaining temperature during transport can affect the test results.

Several studies have demonstrated the effect of prolonged storage of blood samples on coagulation studies at different temperature [5,6,7]. There is difference of opinion regarding the ideal conditions for sample storage like for how long blood sample is stable, either centrifuged or not and at what temperature. Centrifugation of citrated blood sample to prepare platelet poor plasma (PPP) is a mandatory step in the sample processing. Salvagno *et al*. [8] have demonstrated that centrifugation of blood sample improves sample stability by minimizing the chances of platelet activation especially when the blood samples have to be stored at room temperature for prolonged period of time.

We aimed to study the effect of delayed preparation of platelet poor plasma on coagulation studies at room temperature. This study can help in making recommendations about the pre-analytical conditions required for the accurate test results of PT and APTT especially when the samples have to be transported from the remote areas to the central laboratory for testing. In this way, chances of errors and discrepancies can be minimized thus ensuring better patient care.

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MATERIAL AND METHODS

It was a cross sectional study, conducted at Chughtai Institute of Pathology from January 2022 to June 2022. Approval was obtained from the ethical and research committee of the institute (IRB letter No. CIP / IRB / 1026). Total 100 healthy individuals above the age of 18 years, both males and females, took part in the study. Power and sample size calculations software version 2.1.31 for paired test formula was used for sample size calculation with 95% confidence interval[9]. Informed consent was taken from all the participants. Tourniquet was applied almost 4 finger width above antecubital fossa. Four blood samples were drawn from each subject in sitting position within one minute of applying tourniquet from median cubital vein. The blood was collected in tubes with 3.2% tri-sodium citrate in ratio of blood sample: anticoagulant 9:1. Centrifugation was done at 4000 g for 10 minutes at room temperature (18 to 25 °C) to make platelet poor plasma; first sample at 0 h (baseline sample), second sample at 4 h, third sample at 8 h and fourth sample at 24 h. Cut off for platelet count at <10,000 / µl was measured on all the samples. PT and APTT were run on each PPP using Sysmex CS-1600. The controls were also run and checked. The values at different hours were noted and compared through careful statistical analysis of the observed parameters. Healthy subjects both males and females above the age of 18 years were included in the study. Patients with chronic liver disease, pregnancy, hypertension, sepsis, DIC, sickle cell disease, bleeding disorders, or on warfarin and heparin were excluded from the study.

Data were entered into EXCEL worksheets and checked manually and corrected where necessary. Normal value of PT was taken as 10.5-13.2 seconds (s) and a value greater than 13s was considered prolonged. Normal value of APTT was taken as 25-32s and a value greater than 32s was considered prolonged. Statistical package of social sciences (SPSS) version 20:00. was used for statistical analysis. PT and APTT values of PPP made at different time intervals were expressed as mean ± SD. We used paired samples t-test with confidence intervals of 95% for the comparison of test results at different storage times of 4 hours, 8 hours and 24 hours with the results at 0 hour (baseline value). P values of less than 0.05 were considered statistically significant. Clinically significant difference was calculated by mean percentage change as (Test result at storage time X – test result at baseline 0 hour / test result at baseline 0 hour × 100). A mean percentage change of more than 10% was considered as clinically significant according to van geest [10].

RESULTS

Mean age was 25.28 years. 71 participants were males (71%) and 29 were females (29%). There was no statistically significant difference in PT values of PPP that was made immediately (0h baseline value) and PPP made at 4h and 8h (Table-I). Though a statistically significant difference was noted in value at 24h (P value <0.05) with reference to baseline, clinically relevant changes were not noted up to 24h and percent change difference never crossed 10% cutoff value (Table-II).

When compared with baseline value at 0h, APTT of PPP made at 4h showed no statistically significant difference but there was difference in values at 8h and 24h (Table-III). Clinically relevant change was observed in APTT value at 24h when percent change difference crossed 10% cutoff value (Table-IV).

Table-I: PT of PPP made at different hours and comparison with reference value (0h). SD: standard deviation, N: sample size, SE: standard error, CI: confidence interval, *P value <0.05.

Time(s)	Mean	SD	N	Difference	SE	95% CI	t	P value
0h	11.515	0.649	100	0.140	0.085	-0.0283 to 0.0397	1.641	0.1024
4h	11.655	0.553						
0h	11.515	0.649	100	0.160	0.085	-0.0075 to 0.3275	1.88	0.0610
8h	11.675	0.547						
0h	11.515	0.649	100	0.265	0.083	0.1011 to 0.4289	3.1	**0.0017***
24h	11.78	0.518						

Table-II: Mean±SD of PT values of PPP made at different hours and percent change from the value at 0 hour (baseline value).

Time (s)	Mean ± SD	Percent change
0h	11.515±0.649	
4h	11.655±0.553	1.21
8h	11.675±0.547	1.39
24h	11.78±0.518	2.34

Table-III: APTT of PPP made at different hours and comparison with baseline value (0h). *P value <0.05.

Time(s)	Mean	SD	N	Difference	SE	95% CI	t	P value
0h	27.84	0.813	100	0.120	0.115	-0.1072 to 0.3472	1.042	0.2988
4h	27.96	0.816						
0h	27.84	0.813	100	0.480	0.120	0.2429 to 0.7171	3.992	**0.0001***
8h	28.32	0.886						
0h	27.84	0.813	100	7.650	0.142	7.3694 to 7.9306	53.7	**<0.0001***
24h	35.49	1.168						

Table-IV: Mean ± SD of APTT values of PPP made at different hours and percent change from the value at 0 hour (baseline value), *Percent change >10%.

Time (s)	Mean ± SD	Percent change
0h	27.84±0.813	
4h	27.96±0.816	0.43
8h	28.32±0.886	1.72
24h	35.49±1.168	**27.47***

DISCUSSION

There are three phases of laboratory testing: pre-analytical, analytical and post analytical. Out of these phases, pre-analytical phase is the most vulnerable phase to errors. There are various pre-analytical factors that can affect the quality and reliability of coagulation test results like PT and APTT such as collection method, anticoagulant ratio, hematocrit, transportation, sample storage and sample processing. According to The Clinical and Laboratory Standards Institute (CLSI) guidelines, the blood for coagulation studies should be withdrawn slowly in vacuum tube containing 3.2% sodium citrate, using 22-to-19-gauge needle [4]. Fresh samples should be transported for analysis at RT, as transporting the sample on ice or refrigerating the sample before processing can affect the results of PT and APTT either by cold precipitation of von Willebrand (VWF) factor and factor VIII or by cold activation of factor VII [11,12]. Ideally the samples should be processed and analyzed within 4 hours of collection as delay in transportation may falsely prolong the PT and APTT due to in vitro loss of activity of labile factors (FV, FVIII) [6].

All the blood samples should be centrifuged as soon as possible to get the platelet poor plasma (PPP), preferably within 1 hour of collection. PPP is defined as the plasma containing <10,000 platelets/µL (10×10^9/L) [13]. Centrifugation of citrated blood sample to prepare PPP is a mandatory step in the sample processing. It has been shown that RT by itself significantly activates platelets [14] in whole blood sample, which can alter the coagulation results. In a study by Salvagno *et al*. [8], it was demonstrated that centrifugation of blood sample improves sample stability by minimizing the chances of platelet activation especially when the blood samples have to be stored at RT for prolonged period of time.

It is often very difficult to maintain the standard storage time and temperature given by CLSI guidelines for the coagulation studies. This is particularly important when the blood sampling is being done at collection centers and the samples have to be transported to the central laboratory for processing and analysis. Delay in transport significantly affects the coagulation test results due to platelet activation in whole blood sample and labile nature of coagulation factors like FV and FVIII.

Several studies have demonstrated the effect of prolonged storage at different temperatures on coagulation studies. In a study by Chen *et al*. [15], it was shown that storage time of 72h for PT / INR at RT is acceptable. Ogbenna *et al*. [9] demonstrated that PT result was reliable only within 12h storage of unspun blood and APTT within 6h storage at RT. Some studies suggested a longer storage period contrary to the current CLSI guidelines. Linskens *et al*. [16] processed blood samples to PPP after collection and then stored as aliquots at RT for analysis at different hours. They observed a clinically relevant change of >10% for APTT after 48h of storage at RT and statistically significant, but no clinically relevant differences after 48h storage for PT. In a study by Rimac *et al*. [17], no statistically significant difference between baseline values and results in samples stored at RT and 4°C for 24h was observed for PT and APTT.

We did centrifugation immediately after collection to make PPP (0h) and found that there is no change in values of PT and APTT if this PPP is stored at RT and run at 4h, 8h and 24h. So, we took this value (0h) as baseline value. PPP made by centrifugation of citrated blood sample after 4h of collection and after 8h of collection shows no significant statistical and clinical difference in PT value when compared with baseline 0h value (P value 0.1024 and 0.0610 respectively) and a percentage change <10%. There was statistically significant difference in PT values of PPP made after 24h of collection when compared with baseline value

(P value 0.0017) but there was no clinically relevant change (percentage change <10%). Similarly for APTT at RT, statistically significant difference was noted at storage time of 8h (P value 0.0001) and 24h (P value <0.0001) but a clinically relevant change was noted only at 24h (>10% percent change). So, our study demonstrated that there were no statistically and clinically relevant changes in PT for up to 24h and APTT up to 4h at RT.

CONCLUSION

There are a number of pre analytical variables that can affect the values of PT and APTT, thus doubting the reliability of the results of these most commonly requested tests in medical practice. In a central laboratory like ours, where daily we receive a number of blood samples from the collection centers throughout the country, it is vital to establish the institutional guidelines that can address the pre analytical variables associated with coagulation testing. Due to delay in transport and processing of samples, it is often difficult to strictly follow the CLSI guidelines. Based on the results of our study, we recommend that centrifugation of citrated blood samples to separate PPP should be done immediately after sample collection at collection centers. The aliquots can then be transferred at room temperature. The PT and APTT values of this PPP remain stable for up to 24h at RT. When transported as whole blood, PT is stable for 24h and the APTT is stable for up to 4h at RT.

LIMITATION OF STUDY

We included healthy participants in our study who are not using any anticoagulation therapy. Also, we noted the effect of delayed preparation of PPP for up to 24 hours only. So, we cannot comment on storage conditions of samples from patients on anticoagulation therapy and also on prolonged storage times of more than 24 hours. Additional studies are recommended to address these issues.

AUTHOR CONTRIBUTION

Ayesha Younas: Literature search, data collection, statistical analysis and article writing.
Isma Imtiaz: Literature search and data collection.
Ayisha Imran: Drafted the study design and proof reading.
Nauman Aslam Malik: Overall supervision of the study.
Akhtar Sohail Chughtai: Overall supervision of the study.

REFERENCES

1. Gilbert BW, Reeder JA, Reynolds TR, Tabaka CA, Rech MA. Anticoagulation monitoring in the intensive care unit. Crit Care Nur Q. 2022; 45(2): 108-18. DOI: https://doi.org/10.1097/CNQ.0000000000000394
2. Iba T, Levy JH, Raj A, Warkentin TE. Advance in the management of sepsis-induced coagulopathy and disseminated intravascular coagulation. J Clin Med. 2019; 8(5): 728. DOI: https://doi.org/10.3390/jcm8050728
3. Favaloro EJ, Funk DM, Lippi G. Pre-analytical variables in coagulation testing associated with diagnostic errors in hemostasis. Lab Medicine. 2012; 43(2): 1-10. DOI: https://doi.org/10.1309/LM749BQETKYPYPVM
4. Gosselin RC, Marlar RA. Preanalytical variables in coagulation testing: setting the stage for accurate results. Semin Thromb Hemost. 2019; 45 (5): 433-48. DOI: https://doi.org/ 10.1055/s-0039-1692700
5. Mohammed KOM. Effects of Storage Time and Temperature on The samples of Routine Coagulation Tests (Doctoral dissertation, Sudan University of Science & Technology). 2020: 1-30.
6. Sikander N, Qamar U, Aijaz J, Maab R, Latif Z, Naveed MA. Effect of ambient temperature and prolonged storage on prothrombin time and activated partial thromboplastin time during summers in Pakistan. Med Forum. 2021; 32 (11): 11-15.
7. Putri AE, Indrasari YN, Hernaningsih Y. The effect of storage and time of blood specimen examination of plasma prothrombin time and activated partial thromboplastin time stabilities. Indian J Forensic Med Toxicol. 2020;14(3):1721-6. DOI: https://doi.org/10.37506/ijfmt.v14i3.10664
8. Salvagno GL, Lippi G, Montagnana M, Franchini M, Poli G, Guidi GC. Influence of temperature and time before centrifugation of specimens for routine coagulation testing. Int J Lab Hematol. 2009; 31(4): 462-7. DOI: https://doi.org/10.1111/j.1751-553X.2008.01058.x
9. Ogbenna AA, Oyedele OA, Adeyemo TA, Oyewole KM. Effect of varying storage time and temperature on unspun blood samples for prothrombin time and activated partial thromboplastin time in a tertiary hospital laboratory in the tropics. Sahel Med J. 2022; 25(2): 41. DOI: 10.4103/smj.smj_44_21
10. van Geest-Daalderop JH, Mulder AB, Boonman-de Winter LJ, Hoekstra MM, van den Besselaar AM. Preanalytical variables and off-site blood collection: influences on the results of the prothrombin time/international normalized ratio test and implications for monitoring of oral anticoagulant therapy. Clin Chem. 2005; 51(3): 561-8. DOI: https://doi.org/10.1373/clinchem.2004.043174
11. Adcock DM, Hoefner DM, Kottke-Marchant K, Marlar RA, Szamosi DI, Warunek DJ. CLSI document H21-A5. collection, transport, and processing of blood specimens for testing plasma-based coagulation assays and molecular hemostasis assays; Approved guideline. 2008: 1-33.
12. Kim YA, Lewandrowski KB, Lucien FA, Van Cott EM. The effects of transport temperature and time on routine and specialized coagulation assays. Blood Coagul Fibrinolysis. 2018; 29(2): 184-8. DOI: 10.1097/MBC.0000000000000700
13. Magnette A, Chatelain M, Chatelain B, Ten Cate H, Mullier F. Pre-analytical issues in the haemostasis laboratory: Guidance for the clinical laboratories. Thrombosis J. 2016; 14(1): 1-4.

DOI: https://doi.org/10.1186/s12959-016-0123-z
14. Maurer-Spurej E, Pfeiler G, Maurer N, Lindner H, Glatter O, Devine DV. Room temperature activates human blood platelets. Lab Invest. 2001; 81(4): 581-92. DOI: https://doi.org/10.1038/labinvest.3780267
15. Chen RM, Wee YS, Lucas R. Influence of storage time on stability of routine coagulation parameters (international normalised ratio, activated partial thromboplastin time and fibrinogen) at room temperature. N Z J Med Lab Sci. 2021; 75(3): 177-84.
16. Linskens EA, Devreese KM. Pre-analytical stability of coagulation parameters in plasma stored at room temperature. Int J Lab Hematol. 2018; 40(3): 292-303. DOI: https://doi.org/10.1111/ijlh.12784
17. Rimac V, Coen Herak D. Is it acceptable to use coagulation plasma samples stored at room temperature and 4° C for 24 hours for additional prothrombin time, activated partial thromboplastin time, fibrinogen, antithrombin, and D-dimer testing?. Int J Lab Hematol. 2017; 39(5): 475-81. DOI: https://doi.org/10.1111/ijlh.12664