Original Article

Effect of Ambient Temperature and Prolonged Storage on Prothrombin Time and Activated Partial Thromboplastin Time During Summers in Pakistan

Nada Sikander[1], Unaiza Qamar[2], Javeria Aijaz[3], Rafeeda Maab[4], Zahid Latif[5] and Muhammad Asif Naveed[6]

ABSTRACT

Objective: The current study was carried out to determine the effect of sample storage duration on PT and aPTT, when samples are stored at ambient temperatures during summers in Lahore, Pakistan.

Study Design: Cross sectional study

Place and Duration of Study: This study was conducted at the Hematology department of King Edward Medical University in June 2021.

Materials and Methods: Eighty blood samples collected in 3.8% sodium citrate vial and PT and aPTT was performed manually at 0, 4, 8, 12 and 24 hours. First test was performed immediately after sample collection (0 hours) which was taken as reference. Paired sample t-test was used for comparison of PT and APTT at 4, 8, 12 and 24 hours with the value at 0 hours while a mean percentage difference of more than 10% from the baseline value was taken as clinically significant.

Results: Initially PT results did not show any clinically significant change until 24 hours and aPTT till 8 hours.

Conclusion: Samples for PT testing give acceptable results up to 8 hours post collection, and for aPTT testing up to 4 hours, when stored at ambient temperatures during summer seasons.

Key Words: Prothrombin time, Activated partial thromboplastin time, ambient temperature

Citation of article: 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.

INTRODUCTION

Prothrombin time (PT) and activated partial thromboplastin time (aPTT) are among the most frequently ordered screening tests in the clinical laboratory.

1. Department of Hematology, PTGD& RI Sir Ganga ram Hospital, Lahore.
2. Department of Hematology, Pakistan Liver & Kidney Institute Lahore.
3. Department of Hematology, Indus Hospital & Health Networks.
4. Department of Hematology, King Edward Medical University, Lahore.
5. Department of Public Health, Azra Naheed Medical College, Lahore.
6. Department of Hematology, University of Health Sciences, Lahore.

Correspondence: Muhammad Asif Naveed, Hematology, University of Health Sciences, Lahore.
Contact No: 0333-2246680
Email: dr_asifnaveed@hotmail.com

Received: June, 2021
Accepted: August, 2021
Printed: November, 2021

These screening tests are used in the evaluation of a wide variety of clinical conditions, either for diagnostic or monitoring purposes of anticoagulant therapy.

They form the basis of many special coagulation tests such as factor assays, and in some locations clot-based specialized assays (e.g., protein C [PC] and protein S [PS] activity assays, activated PC resistance [APCR]).[1]

The reference range for prothrombin time is usually around 10-13 seconds.[2] Prothrombin time can be prolonged as a result of deficiencies in vitamin K, warfarin therapy, malabsorption, liver disease, factor deficiency (II,VII,IX,X), disseminated intravascular coagulation and presence of antiphospholipid antibody syndrome.[3]

The reference range of aPTT is between 25- 39 seconds. Prolonged APTT may indicate use of heparin (or contamination of the sample with heparin), antiphospholipid antibody especially lupus anticoagulant, and coagulation factor deficiency (VIII, IX or XI).[4]

There are numerous preanalytical variables associated with coagulation testing that may impact the diagnostic accuracy of a test result. These variables are categorized into: patient selection (use of anticoagulants, liver disease, bleeding disorder), specimen collection (poor venipuncture, inadequate anticoagulant [ratio of 1:9 is not maintained], collection

in wrong tube, improper fill volume), specimen transport (old sample, improper temperature during transport, inappropriate handling of specimens) specimen processing and storage (inappropriate centrifuge speed and time, stored at warmer than recommended temperature, prolonged storage), others (volume expanders [e.g. crystalloids], antibiotics [lipoglycopeptides], autoimmune disorders, hematin, systemic fibrinolytic drugs) . Therefore, it is integral for each lab technologist and clinician to assess and consider the impact of these variables when interpreting coagulation test data. [1]

According to Clinical and Laboratory Standards Institute (CLSI) H21-A5 specimens should be analyzed within 24-h for PT and 4-h for APTT if stored at room temperature (25ºC).Therefore, the potential impact of pre-analytic factors such as storage temperature and time need to be taken into account when reporting PT and aPTT results.[5] In Pakistan, due to unavailability of temperature moderating systems in most centers, recent expansion in outreach laboratories, and suboptimal adherence to established guidelines, it has been observed that these requirements are often not met with. In summer (April to September), the average temperature in most cities in Pakistan ranges from 30-32ºC, and can even rise up to 45ºC. The current study was carried out to determine the effect of sample storage duration on PT and aPTT, when samples are stored at ambient temperature in summer. All published studies thus far have analyzed time-dependent changes in PT and aPTT by storing samples either at room temperature (20-24ºC), or lower (4ºC, -20ºC, -70ºC). Therefore, the current study is the first one documenting time-dependent changes in PT and aPTT when samples are kept at the prevailing temperature in summer in tropical and sub-tropical countries like Pakistan. Documentation of changes in PT and aPTT occurring as a result of delays in testing will, in turn, assist laboratories in Pakistan and other countries with similar summer climates to determine the course of action to be taken in individual cases – to accept or reject the sample, and the nature of comments, if any, to be included with the results.

MATERIALS AND METHODS

The study was conducted at King Edward medical University, Lahore in June 2021. It was a cross-sectional study. The study population consisted of 80 asymptomatic adults requiring routine blood investigation. Patients with liver disease, bleeding or thrombotic disorders, pregnancy or anticoagulant therapy were excluded. After obtaining approval from the institutional Ethical Committee, 80 blood samples sent to the laboratory for analysis of PT and aPTT were included in the study. Relevant data were recorded on the pre-designed proformas. PT and aPTT values at the specified time intervals were recorded.

Blood collected from the cephalic vein was transferred, within 20 seconds, into tubes containing 3.8% sodium citrate, and immediately centrifuged. Plasma from each sample was divided into five aliquots to assess PT and aPTT at the specified time intervals. The first aliquot was analyzed immediately after collection i.e. at 0 hour (reference sample), the second at 4 hours, the third at 8 hours, the fourth at 12 hours and the fifth at 24 hours. The samples were kept at ambient temperature throughout before analysis, recorded to be varying between 34ºC and 38ºC, all the times during the study period. Temperature was maintained by placing the samples in incubator to simulate the ambient temperature (daytime) so that fluctuation in temperature would not confound the results. Samples with clotting times of more 180 seconds were, however, excluded as these values depicted disease state (part of exclusion criteria) or improper sampling hampering the results. Each sample was tested in duplicate; the result being taken to be the mean of the two values.

The procedure for prothrombin time consisted of placing 100 µL of citrated plasma in a test tube preheated to 37ºC and subsequently adding 200 µL of tissue factor (PT reagent) in the test tube. Upon the addition of the reagent, a stopwatch was started and the time taken for the sample to clot was measured. The time, expressed in seconds, from the plasma-reagent mixing to a visually detected clot formation was defined as the PT.

The procedure for activated partial thromboplastin time consisted of placing 100 µL of citrated plasma and 100 µL of aPTT reagent (preheated to 37ºC) in a test tube preheated to 37ºC, followed by incubation for 3 min at 37ºC. After incubation, 100 µL of calcium chloride (preheated to 37ºC) was added to the test tube. Upon the addition of calcium chloride, a stopwatch was started and the clotting time was measured. The time, expressed in seconds, from this addition to a visually detected clot formation was defined as aPTT.

Statistical Analysis:

The normal value of PT was taken as 11-16 seconds, a value more than 16 seconds being considered deranged. The normal value for APTT was taken to be 28-40 seconds, while a value more than 40 seconds was taken as deranged. Data was recorded and analyzed in Statistical Package for Social Sciences (SPSS) Program version 23.0. PT and aPTT at different time intervals were expressed as mean ± SD. Paired samples t-test was used to determine the statistical significance of the differences between the initial values at 0 hour (reference value) and the successive ones. P-values of less than 0.05 were considered statistically significant. Clinically significant difference was calculated as the mean percentage change: (PT/aPTT z hr – PT/aPTT 0 hr)/PT/aPTT z hr × 100%. A mean percentage change of more than 10% was taken as clinically significant.

RESULTS

When compared with 0 hour, PT values at 4, 8, 12 and 24 hours showed mean percentage changes of 1.95%, 3.80%, 6.21%, and 8.97% respectively, but never crossed 10% cut off limit. When normal values were analyzed separately, the 10% cutoff was not crossed. (Table 1).

When aPTT values were analyzed, the mean percentage changes at 4, 8, 12 and 24 hours were 3.89%, 4.85%, 25.40 % and 40.40% respectively. The change hence was clinically significant after 8 hours. aPTT results analyzed separately for normal values that showed mean percentage changes less than 10% up to 8. (Table 2).

Table No.1: Comparison of normal values

	Mean ± SD	Percent Change	p-value
0 Hour	11.93±1.2		
4 Hour	12.24±2.4	1.38	<0.5
8 Hour	13.53±2.7	2.67	<0.05
12 Hour	14.20±2.6	5.50	<0.001
24 Hour	15.81±2.4	7.81	<0.001

Table 1: Mean ± SD of PT values, percent change from the value at 0 hour, and p-values of the differences at specified time intervals from the value at 0 hour.

Table No.2: Differences

	Mean ± SD	Percent Change	p-value
0 Hour	31.95±3.6		
4 Hour	33.95±2.4	2.98	<0.1
8 Hour	35.29±2.1	4.50	<0.01
12 Hour	45.184±2.3	24.45	<0.001
24 Hour	55.35±2.1	38.30	<0.001

Table 2: Mean ± SD of aPTT values, percent change from the value at 0 hour, and p-values of the differences at specified time intervals from the value at 0 hour.

DISCUSSION

The study was aimed at determining the effect of delays in testing on the validity of PT and aPTT results, when samples are stored at summer temperatures (34ºC-38ºC), commonly witnessed in tropical and sub-tropical countries like Pakistan. Samples for PT and aPTT are mostly drawn in wards or collection centres, and later transferred to laboratories. Plasma clotting factors have limited half-lives, and due to delays in testing as a result of sample transfers, PT and aPTT test results might be rendered unreliable.[5] According to BCSH guidelines, only a limited time frame (4 hours) at optimal temperatures (20-24ºC) is all that can be allowed for the performance of these tests reliably.[6] This, unfortunately, is not the case in most situations in Pakistan; due both to delays in testing, and storage of samples at sub-optimal temperatures.

The results of the current study suggest that PT tests do not affect clinical interpretation till 24 hours even when they are kept at a temperature as high as 38ºC. This, however, holds true only for samples in which PT falls within the normal range at 0 hour. For aPTT tests that are normal at 0 hour, valid results may be obtained uptil 8 hours, after which a marked deviation in results is seen which may be attributed to loss of labile factors..

The current study was carried out at temperatures ranging from 34ºC-38ºC. No published study analysing changes in PT and aPTT over time has been carried out at such high temperatures. All previous studies have been conducted either at room temperature (20ºC - 24ºC), or lower (4ºC, -20ºC, -70ºC). [7, 8]

According to a study conducted by Sajjad A Geelani et al[9], analyzing samples stored at 2-8ºC and at RT (room temperature), PT results remained reliable for upto 24 hours at RT and at 2-8ºC. APTT remained reliable upto 4 hours when tested at RT and at 2-8ºC A study conducted by Osta Manish et al[7] in India analysed PT and aPTT at RT (18-25 ºC) which revealed no clinically significant changes in PT for upto 24 hours and upto 4 hours for aPTT. According to a study conducted by Toulon P et al[10] PT/INR, and aPTT, can be reliably evaluated in tubes stored unspun at room temperature for up to 8 hours after blood collection. However some older studies conducted by Zhao et al[8] and Yao et al[11] demonstrated that storage time interval upto 24 hrs for PT and 8hrs for APTT at RT is acceptable. Similar studies have been done in early 2000 which revealed variable results. There results were analysed for the sake of comparison with our findings. M.A. Awad et al[12], analyzed samples stored at 4ºC and at 24ºC. He concluded that if samples are kept at 4ºC, results do not change clinical interpretation till 24 hours, but if kept at 24ºC then the results are valid only uptil 6 hours. The study conducted by GL Salvaign et al[13] concluded that whatever the temperature conditions, results for PT and APTT are valid only uptil 6 hours. The study conducted by Matthes B, Fischer R and Peetz D[14] reported that both PT and APTT can be reliably be tested uptil 8 hours post-collection. The storage temperature in this study is, however, not mentioned. According to another similar study conducted by Rao LV, Okorodudu AO, Petersen JR, Elghetany MT[15] either plasma or whole blood samples can be accepted for PT testing up to 24 hours post-collection and for aPTT testing up to 8 hours only, when transported at room temperature (20ºC -24ºC). Saghir S et al[16] concluded that when samples are stored at 24ºC, PT can only be performed till 4 hours and aPTT till 2 hours.

The wide variation in reported results may be attributed to the divergent preanalytical and analytical variables under which the studies were carried out, the most important of which is the storage temperature. Moreover, some studies have used statistical

significance of differences with initial values as an index of validity of results, while others have used the clinical significance for this index.

In the present study, we have reported both the mean percentage changes from initial values, as well as the statistical significance of the differences from the initial values. In our opinion, however, for all practical purposes, the mean percentage change from intial values can more reliably determine sample validity. The present study showed that though differences from initial values became statistically significant early on in the course of the study, clinically significant differences became apparent later on.

Therefore, PT can be reliably reported till 24 hours post-collection, and aPTT till 8 hours, even if samples are kept at a temperature of up to 38ºC. In situations where a reanalysis of samples is required, and the initial values are known, PT can be reliably reported even until 24 hours post-collection if the value obtained at 0 hour is known to be within normal limits. On the other hand, aPTT results remain valid until 8 hour if the initial results are within normal limits.

Ideally, PT and aPTT tests should be performed soon after sample collection, and it is desirable that we achieve complete adherence to established guidelines for sample storage. The present study demonstrates, however, that higher temperatures do not appreciably reduce the recommended time frames for testing of PT and aPTT. We would therefore recommend other comparative studies, carried out in similar conditions, validating or refuting the present one. This would enable laboratories to determine validity of samples that have been kept under less stringent conditions, especially in hot tropical and sub-tropical climates like those found in Pakistan.

CONCLUSION

Samples for PT testing give acceptable results up to 8 hours post collection, and for aPTT testing up to 4 hours, when stored at ambient temperatures during summer seasons.

Acknowledgment: The authors would like to extend their gratitude to the phlebotomist and lab technicians of hematology department of King Edward Medical University for assisting us in bench work.

Author's Contribution:

Concept & Design of Study:	Nada Sikander
Drafting:	Unaiza Qamar, Javeria Aijaz
Data Analysis:	Rafeeda Maab, Zahid Latif, Muhammad Asif Naveed
Revisiting Critically:	Nada Sikander, Unaiza Qamar
Final Approval of version:	Nada Sikander

Conflict of Interest: The study has no conflict of interest to declare by any author.

REFERENCES

1. Gosselin R, Marlar R. Preanalytical Variables in Coagulation Testing: Setting the Stage for Accurate Results. Seminars in Thrombosis and Hemostasis 2019;45(05):433-448.
2. Winter W, Flax S, Harris N. Coagulation Testing in the Core Laboratory. Laboratory Med 2017;48(4): 295-313.
3. Yang R, Moosavi L. Prothrombin Time [Internet]. Ncbi.nlm.nih.gov. 2021 [cited 29 October 2021]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK544269/
4. Abnormal PT and PTT - causes [Internet]. Pathologyoutlines.com. 2021 [cited 29 October 2021]. Available from: https://www.pathologyoutlines.com/topic/coagulationabnormalPTPTT.html
5. Dacie and Lewis Practical Haematology. 12th ed. 2017.
6. Baker P, Platton S, Gibson C, Gray E, Jennings I, Murphy P, et al. Guidelines on the laboratory aspects of assays used in haemostasis and thrombosis. Br J Haematol 2020;191(3):347-362.
7. Osta M, Bose D, Chakraborty J, Shirure V, Maity P. Effect of storage time on PT, APTT and fibrinogen at room temperature. Global J Res Analysis 2020;9(11):1-3.
8. Zhao Y, Lv G. Influence of temperature and storage duration on measurement of activated partial thromboplastin time, D-dimers, fibrinogen, prothrombin time and thrombin time, in citrate-anticoagulated whole blood specimens. Int J Laboratory Hematol 2013;35(5):566-570.
9. Geelani S, Wani G, Khan S, Qadri S, Rasool J, Quadri S, et al. Effect of storage time on prothrombin time and activated partial thromboplastin time: study at a tertiary care center in Kashmir valley. Int J Sci Reports 2018;4(7):182.
10. Toulon P, Metge S, Hangard M, Zwahlen S, Piaulenne S, Besson V. Impact of different storage times at room temperature of unspun citrated blood samples on routine coagulation tests results. Results of a bicenter study and review of the literature. Int J Laboratory Hematol 2017; 39(5):458-468.
11. Yao J, Lv G. Effect of pre-analytical variables on coagulation tests in hepatitis B patients. Blood Coagulation Fibrinolysis 2014;25(7):761-764.
12. Awad M, Sharaf Eldeen O, Ibrahim H. Stability of activated partial thromboplastin time (APTT) test under different storage conditions. Hematol 2006;11(5-6):311-315.

13. Salvango G, Lippi G, Montagnana M, Franchini M, Poli G, Guidi G. Influence of temperature and time before centrifugation of specimens for routine coagulation testing. Int J Laboratory Hematol 2009;31(4):462-467.
14. Kemkes-Matthes B, Fischer R, Peetz D. Influence of 8 and 24-h storage of whole blood at ambient temperature on prothrombin time, activated partial thromboplastin time, fibrinogen, thrombin time, antithrombin and D-dimer. Blood Coagulation Fibrinolysis 2011;22(3):215-220.
15. Rao L, Okorodudu A, Petersen J, Elghetany M. Stability of prothrombin time and activated partial thromboplastin time tests under different storage conditions. Clinica Chimica Acta 2000;300(1-2):13-21.
16. Saghir S, Hassan F, Alashi O, Manaf F, Baqir H. Optimization of the storage conditions for coagulation screening tests. J Coll Physicians Surg Pak 2012; 22(5): 294-7.