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Original Research Article

Effect of Time and Storage Condition on Prothrombin Time and Activated Partial Thromboplastin Time

Karthikeyan T.M.[1], Shivapriya R.[2], Umamageswari M.S.[3], Sharanya K.[4], Priya Fedric[5]

[1]Professor and Head, Department of Pathology, KMCHIHSR, Coimbatore, Tamilnadu, India
[2]Associate Professor, Department of Pathology, KMCHIHSR, Coimbatore, Tamilnadu, India
[3]Professor and Head, Department of Pharmacology, Palakkad Institute of Medical Science, Walayar, Tamilnadu, India
[4]Associate Professor, Department of Pathology, KMCHIHSR, Coimbatore, Tamilnadu, India
[5]KMCH Institute of Allied Health Sciences, Coimbatore, Tamilnadu, India

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Corresponding author: Dr. R. Shivapriya
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Abstract

Background: Prothrombin Time (PT) and Activated Partial Thromboplastin Time (aPTT) are vital coagulation tests done to evaluate the extrinsic and intrinsic pathways, respectively. Pre-analytical variables such as delay in time to process and storage conditions can significantly affect the test results, influencing the clinical decision-making.

Objective: To assess the effect of time and storage conditions (room temperature and refrigeration) on PT and aPTT values in blood samples.

Materials and Methods: This prospective cross-sectional study was conducted on 30 healthy volunteers between the age group of 18–25 years. Blood samples were collected in 3.2% sodium citrate tubes. Two sets of samples were processed: one set of samples are centrifuged immediately and the obtained plasma is stored in refrigerator, and another set of samples are kept as whole blood at room temperature. PT and aPTT were measured at the time interval of 0, 4, 12, and 24 hours using a fully automated coagulation analyzer (Elite Pro ACL). Statistical analysis was performed using repeated measures ANOVA.

Results: PT showed no difference in values between centrifuged and uncentrifuged samples significantly but there was a gradual decline over time but remained relatively stable upto 24 hours. In contrast, aPTT values showed a significant decrease over time, specifically after 4 hours, in both centrifuged and uncentrifuged samples. Statistically significant differences were observed at the interval of 12 and 24 hours ($p < 0.05$).

Conclusion: PT is stable up to 24 hours under both room temperature and refrigerated conditions. However, aPTT is time-sensitive and should ideally be processed within 4 hours for accuracy. If there is a delay for analysing the sample, separation of plasma and refrigeration are recommended to retain the sample integrity.

Keywords: Prothrombin Time, Activated Partial Thromboplastin Time, storage.

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Introduction

Coagulation (clotting) of blood is a physiological mechanism that transforms fluid plasma into a gel by converting soluble fibrinogen into insoluble fibrin. Maintenance of blood in fluid state depends on an integrity of vascular endothelium, quiescent platelets, and inactive clotting factors. Conversely, blood loss is controlled by rapid activation of platelets and coagulation factors to achieve hemostasis. [1]

Hemostasis is a complex, tightly regulated physiological process involving the interaction of three components-blood vessels, platelets, and the coagulation cascade which finally leading to the formation of fibrin clot. [2] It consists of two major phases: primary and secondary hemostasis. Primary hemostasis is the early phase involving platelet adhesion, activation, and aggregation, ultimately leading to the formation of a primary platelet plug. Secondary hemostasis is characterized by activation of the coagulation cascade through intrinsic and extrinsic pathways, which converge into a common pathway leading to formation of stable fibrin clot.

The extrinsic pathway is activated by the release of tissue factor from damaged endothelial cells, whereas the intrinsic pathway is initiated by

exposure of blood to subendothelial collagen, leading to activation of factor XII. [3]

Prothrombin Time (PT) and Activated Partial Thromboplastin Time (aPTT) are critical laboratory assays used to evaluate the function of coagulation factors. PT assesses the integrity of extrinsic and common pathways, while aPTT assesses the intrinsic and common pathways. Both tests measure the time required to form clot in seconds. PT is determined after the addition of tissue factor, phospholipid, and calcium to blood sample, with a normal range of 9–13 seconds. aPTT is measured following the addition of a contact activator, phospholipid, and calcium; the term "partial" refers to the absence of tissue factor, and "activated" refers to the use of contact activators to accelerate clot formation. The normal aPTT range is approximately 30–35 seconds. [4,5]

Pre-analytical variables, specifically, delay in processing the samples and storage conditions, can significantly influence the outcome of coagulation tests. The Clinical and Laboratory Standards Institute (CLSI) guideline H21-A5 recommends that PT samples can be analyzed within 24 hours and aPTT samples within 4 hours when stored at room temperature (20–25°C). However, there are limited ecommendations regarding the stability of these parameters under refrigerated conditions (2–8°C) and whether samples should be stored as whole blood or plasma.

In this context, the present study was done to evaluate the effect of time and storage conditions on PT and aPTT values, with the aim of improving laboratory sample handling and ensuring reliable coagulation assays.

Objectives

1. To study the effect of time on Prothrombin Time (PT) and Activated Partial Thromboplastin Time (aPTT).
2. To evaluate the effect of storage conditions (room temperature and refrigeration) on PT and aPTT.

Materials and Methods

This prospective cross-sectional study was conducted on blood samples collected from 30 healthy volunteers. The study was carried out in the Department of Pathology, KMCH Institute of Health Sciences and Research, Coimbatore, after obtaining approval from the Institutional Human Ethics Committee (Approval No.: 02/IHEC/2022).

Inclusion Criteria

- Healthy volunteers aged 18–25 years
- Individuals not on anticoagulant or antiplatelet therapy

Exclusion Criteria

- Pregnant women
- Inadequate or improperly collected samples

Equipment

- Fully automated coagulation analyzer: Elite Pro ACL

Methodology: After obtaining informed consent from healthy volunteers, venous blood samples were collected under aseptic conditions using 3.2% sodium citrate anticoagulant containing vacutainers (blood: anticoagulant ratio 9:1).

Two sets of samples were processed to evaluate the effects of time and storage conditions:

- **Set 1 (Centrifuged samples):** Samples were centrifuged and plamsa separation done immediately and stored under refrigerated conditions (2–8°C). PT and aPTT were measured at the intervals of 0, 4, 12, and 24 hours.
- **Set 2 (Uncentrifuged samples):** Whole blood samples were stored at room temperature (20–25°C) and centrifuged just prior to analysis at 4, 12, and 24 hours.

All measurements were performed using a fully automated coagulation analyzer (Elite Pro ACL) following standard laboratory protocols.

Activated Partial Thromboplastin Time (aPTT): To perform this common coagulation assay, a mixture of a negatively charged surface, phospholipid, and anticoagulated patient plasma is incubated for several minutes. The recommended anticoagulant is 3.2 g% sodium citrate, because less variation is seen in blood specimens from normal patients and those on anticoagulants collected in this concentration of anticoagulant. Sodium citrate is a reversible chelator of calcium that prevents coagulation protein activation. When whole blood is collected, the ratio of anticoagulant to whole blood is 1 part anticoagulant to 9 parts whole blood. After incubation of patient plasma with reagent for a prescribed time, depending on the assay, the sample is recalcified with excess calcium chloride, and the time required for clot formation is measured. The APTT assesses the coagulation proteins of the so-called intrinsic system and common pathways. This assay is commonly referred to as the partial thromboplastin time (PTT), but it is really an "activated" PTT in that its reagents contain a negatively charged surface that accelerates the rate of the reaction. [6–8]

Prothrombin Time (PT): To perform this common coagulation assay, tissue thromboplastin (recombinant human or isolated animal tissue factor) and patient plasma are incubated for several minutes, after which the citrated plasma mixture is

recalcified by the addition of excess CaCl2, and the time required for clot formation is measured. The PT assesses the coagulation proteins of the so-called extrinsic system and common pathway.

Tissue thromboplastin traditionally has been a crude preparation of animal brain TF. Presently, recombinant TF is used in the preparation of several commercial PT reagents. In general, the range of prolongation of time of an abnormal PT increases when recombinant TF is used. This fact makes for a more sensitive assay. The PT serves as the basis for the international normalized ratio (INR) value used to monitor anticoagulation with warfarin or other vitamin-antagonists.

The INR is the ratio of patient PT divided by geometric mean normal PT for the local laboratory (based on a population of normal individuals assessed with identical sample collection, reagents, and machines), raised to the power of the international sensitivity index (ISI). Although the INR is clearly the most appropriate measure to use in conjunction with oral anticoagulant monitoring, for hemostatic evaluation of the non warfarinized patient, actual PT values in seconds may be used, referencing the laboratory's locally established reference interval for the PT test. [9]

Statistical Analysis: Statistical analysis was performed using repeated measures ANOVA and two-way ANOVA to evaluate the effects of time and storage conditions on PT and aPTT values. A p-value of <0.05 was considered statistically significant.

Results:

The mean Prothrombin Time (PT) values in centrifuged samples showed a gradual decline from baseline over time, with statistically significant changes observed on repeated measures ANOVA (Table 1). In uncentrifuged samples, PT values remained relatively stable upto 4 hours, after which a decline in values was noted. Significant differences were observed at 12 and 24 hours, while no significant change was seen at 4 hours (Table 2).The mean Activated Partial Thromboplastin Time (APTT) values in centrifuged samples showed a mild decreasing trend over time; however, these changes were not statistically significant on repeated measures analysis (Table 3).In contrast, uncentrifuged samples demonstrated a significant progressive decline in APTT values over time, with statistically significant differences observed at 4, 12, and 24 hours compared to baseline (Table 4).Comparison between centrifuged and uncentrifuged samples for PT revealed no statistically significant difference between the two groups, although both showed a reduction from baseline values over time (Table 5).Similarly, comparison of APTT values between centrifuged and uncentrifuged samples showed no significant group difference overall; however, a significant decline over time was evident, particularly at 12 and 24 hours (Table 6).

Overall, both PT and APTT values tend to decrease with time, with more pronounced and statistically significant changes observed after 12 hours, emphasizing the importance of performing coagulation tests within 4 hours of sample collection (Tables 1–6).

Table 1: Mean and SD of PT level at 0, 4,12 and 24 hrs with centrifuge

Assessment	Mean (seconds)	SD	Anova repeated measure test result		Simple contrast test result with 0 hour		
			F-value	P-value	Comparisons	F-value	P-value
At 0 hour	13.540	1.0309	5.274	0.017			
AT 4th hour	12.820	0.9175			At 0 hour with At 4th hour	4.603	0.060
AT 12th hour	12.350	0.7200			At 0 hour with At 12th hour	20.265	0.001
At 24th hour	12.800	0.7134			At 0 hour with At 24th hour	9.888	0.012

Table 2: Mean and SD of PT level at 0,4,12 and 24 hrs without centrifuge

Assessment	Mean	SD	Anova repeated measure test result		Simple contrast test result with 0 hour		
			F-value	P-value	Comparisons	F-value	P- value
At 0 hour	15.380	5.9299	12.200	<0.001			
AT 4th hour	15.140	5.6232			At 0 hour with At 4th hour	1.097	0.322
AT 12th hour	13.740	5.7604			At 0 hour with At 12th hour	23.222	0.001
At 24th hour	13.980	6.1555			At 0 hour with At 24th hour	16.333	0.003

No change till fourth hour

Table 3: Mean and SD of APTT level at 0,4,12 and 24 hrs with centrifuge

Assessment	Mean	SD	ANOVA repeated measure test result		Simple contrast test result with 0 hour		
			F-value	P-value	Comparisons	F-value	P-value
At 0 hour	35.220	3.2169	2.515	0.127			
AT 4th hour	35.890	3.8751			At 0 hour with At 4th hour	--	--
AT 12th hour	32.470	2.7968			At 0 hour with At 12th hour	---	---
At 24th hour	32.030	4.7355			At 0 hour with At 24th hour	---	---

Table 4: Mean and SD of APTT level at 0,4,12 and 24 hrs without centrifuge

Assessment	Mean	SD	Anova repeated measure test result		Simple contrast test result with 0 hour		
			F-value	P-value	Comparisons	F-value	P- value
At 0 hour	35.570	3.0244	12.096	<0.001			
AT 4th hour	32.590	2.0190			At 0 hour with At 4th hour	9.408	0.013
AT 12th hour	30.200	3.2833			At 0 hour with At 12th hour	36.288	0.000
At 24th hour	31.280	2.8448			At 0 hour with At 24th hour	14.435	0.004

Discussion

Koepke JA et al. reported that there was no significant difference in PT and aPTT values between centrifuged and uncentrifuged samples when analysis was performed within 4 hours, supporting the stability of coagulation parameters in the early pre-analytical period. [10] These findings are consistent with the present study, where no significant difference was observed between sample types at 4 hours.

Rao LV et al. evaluated 36 samples of whole blood and plasma and demonstrated that PT values remained stable without statistically significant variation when stored at room temperature for up to 24 hours. They also observed that aPTT remained stable for up to 6 hours. [11]

In comparison, the present study showed that aPTT stability was limited to 4 hours, beyond which statistically significant changes were observed, indicating slightly reduced stability under similar conditions.

Vinod Kumar Goyal et al. assessed the effect of delayed analysis and storage conditions on coagulation parameters. They reported that PT remained stable under room temperature, refrigerated, and frozen conditions for up to 6 hours, whereas aPTT showed significant variability even within this period. Fibrinogen levels were stable up to 48 hours. Their findings emphasize the need for prompt analysis of aPTT for reliable results. [12] Overall, the findings of the present study are in agreement with previous literature, confirming that PT is relatively stable over time, whereas aPTT is more sensitive to pre-analytical variables, particularly delay in processing.

Conclusion

The present study demonstrates that PT values remain stable for up to 24 hours irrespective of storage conditions or centrifugation status, making it a reliable parameter even with delayed processing. In contrast, aPTT is highly time-dependent and should ideally be analyzed within 4 hours of sample collection to ensure accuracy.

Centrifugation and refrigeration of plasma samples help preserve aPTT values when immediate analysis is not feasible. These findings are important for establishing laboratory sample acceptance criteria and improving the reliability of coagulation testing.

Further studies with larger sample sizes and inclusion of patients on anticoagulant therapy are recommended to validate these observations.

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