ORIGINAL RESEARCH PAPER
Volume - 10 | Issue - 09 | September - 2021 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

COMPARISON OF PROTHROMBIN TIME AND ACTIVATED PARTIAL THROMBOPLASTIN TIME IN PRIMARY TUBES AND SAMPLE ALIQUOTS AFTER 4 HOURS DURATION

Biochemistry

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ABSTRACT

Background: Coagulation tests are widely applied in clinical practice, among which PT and aPTT are the most commonly done. Among many pre-analytical conditions –time, temperature and storage conditions are few of the factors affecting the results of samples. This study aims to compare PT and aPTT values in primary tubes and sample aliquots after 4 hours duration with the initial value.

Materials & Methods : An observational study was done at Central Diagnostic Laboratory, Gandhi Hospital, Secunderabad, T.S. Samples were collected in citrated tubes, centrifuged, PT and aPTT were processed with plasma of primary tubes. Sufficient quantity of plasma was aliquoted immediately from primary tubes and kept at room temperature for 4 hours after which the plasma of primary tubes and aliquots were re-analyzed separately and values were compared.

Results: Pearson correlation was performed to show the relation. PT and aPTT values obtained in the primary tube and aliquot were correlating with the initial value (PT-r =0.833,0.831 respectively) and (aPTT-r =0.97,0.87 respectively) and also with each other (PT-r =0.99) and (aPTT-r =0.90) respectively. There is no statistical significant difference in PT of primary tube and aliquots when compared to initial PT ($p>0.01$). Incase of APTT there is statistically significant difference in primary tube and aliquot when compared to initial value ($p<0.01$) where as there is no significant difference of APTT values in aliquot and primary tube after 4 hours ($p>0.01$).

Conclusion: The present study shows no difference of PT values in aliquots and primary tube when compared to initial value, whereas APTT values differ from initial values in samples stored for more than 4 hours in primary tube and aliquot.

KEYWORDS

PT, aPTT, primary tube, aliquot.

INTRODUCTION

Coagulation tests are widely used in clinical practice among them PT and aPTT are commonly done (1, 2). PT tests are done in patients on warfarin therapy, with history of abnormal bleeding, vitamin K dependent clotting factor deficiency or liver disease; aPTT is done in patients on heparin treatment, hemophilia, lupus anticoagulant antibodies and Von Willebrand disease (2). Both PT and aPTT are used as diagnostic tests as well as for drug dose titration purpose (2). Among various pre-analytical conditions – time, temperature and conditions of storage are few of the factors affecting sample results (1,2,3,4,5) . The Clinical and Laboratory Standard Institute (CLSI) H21-A5 recommends PT and aPTT to be analysed within ≤ 24 hours and ≤ 4 hours respectively, when stored at room temperature (6).

METHODOLOGY:

An observational study with a total of 30 venous blood samples collected in citrate tubes was carried at Central Diagnostic Laboratory, Gandhi Hospital, Sec'bad, T.S. We have estimated PT and aPTT values in plasma of primary tubes and aliquots. Samples were centrifuged at 2500rpm for 15 minutes. Samples up to the mark i.e., 2ml (as per tube) were accepted. Inappropriate volume of samples, lysed samples, lipemic samples and clotted samples were excluded from the study. PT and aPTT were analysed with plasma of primary tubes in Sysmex CA-660 which is based on photo optical method of detection. Sufficient quantity of plasma was aliquoted immediately from primary tubes after initial run and kept at room temperature for 4 hours after which the plasma of primary tubes and aliquots were re-analyzed separately and values were compared with initial values estimated within 1 hour of sample collection.

RESULTS:

All the values of PT and aPTT were compiled and analysed in Microsoft Excel 2016 statistically. The results of primary tube and aliquots were compared with initial results by using paired t-test. P value <0.01 was considered significant. There was no statistical significant difference in PT of primary tube and aliquots when compared to initial PT ($p>0.01$), also PT in primary tube and aliquots after 4 hours were insignificant ($p>0.01$). In case of aPTT there was statistically significant difference in primary tube and aliquot when compared to initial value ($p<0.01$) where as there was no significant difference of aPTT values in aliquot and primary tube after 4 hours ($p>0.01$).

Table. 1 Comparison Of PT And A PTT Showing Mean ± SD With Respect To Time

Mean ± SD			
	Initial value	**1° tube >4hrs**	**Aliquot >4hrs**
PT	15.13± 5.43	16.80± 9.58	16.28± 9.27
INR	1.26± 0.45	1.44± 0.94	1.35± 0.78
aPTT	29.12± 8.57	31.58± 8.24	33.06± 7.72

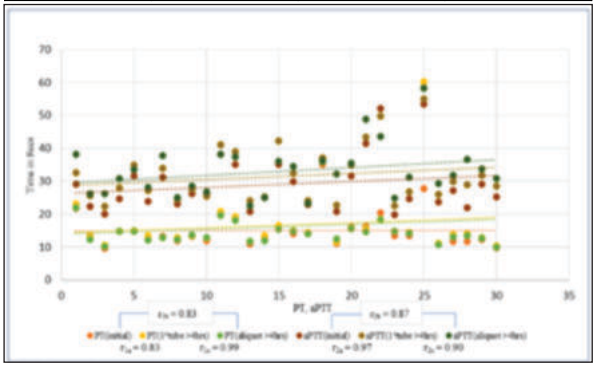

Fig.1 Pearson's Correlation.

DISCUSSION:

PT and aPTT are the very common and frequent coagulation factors determined for assessment of hemolytic function. Accurate results of these laboratory tests depends on many variables, few of which are time, temperature, collection of samples, transportation and storage of samples (7-16). In the present study we have compared the PT and aPTT results after 4 hours in primary tube and also in aliquot with that of the initial values. We have observed PT values were consistent in

both aliquots and primary tubes even after 4 hours with that of initial values, whereas aPTT values showed statistically significant difference in both aliquots and primary tubes when measured after 4 hours with that of initial values. Study by E.J.S Denessen et.al showed that aPTT measurement should be done within 2 hours of sampling whereas PT can be flexibly performed with in 24 hours (3). E.A. Linskens and K.M.J Devreese observed that aliquoted plasma is stable for determination of PT for upto 48 hours whereas aPTT determination is affected by the different reagents used (1). Few reagents can be used for determination of aPTT in clinically acceptable range for 12-24 hours (1). Ying Zhao et.al has put forth from their study that <10% mean percentage change is noted in PT samples stored at -80˚C for 1 year and -20˚C for 1 month, however mean percentage change of aPTT was <10% for samples stored at -20˚C for 1 month was minimally affected by the different analysers used for estimation (4). Xiao-Yun Gao, Xin-Hua Wang and L. Feng et.al observed that storage of upto 24 hours at 4˚C and 25˚C is acceptable for clinically relevant PT values while a storage of 12 hours at 4˚C and 8 hours at 25˚C is acceptable for aPTT determination (5,8).

CONCLUSION:

The present study shows no significant difference of PT values in aliquots and primary tube when compared to initial value, where as aPTT values were significantly different from initial values in samples stored for more than 4 hours in primary tube and aliquot. Early processing of samples with turn around time ≤4hrs at a room temperature has to be preferred for estimation of coagulation tests for best results. Aliquoting plasma sample when sample has to be stored at room temperature is not much useful and not recommended as it increases the cost of storage. However limited sample size and duration of observation is a limitation of our study.

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