Influence of Temperature And Storage Duration on Measurement of Prothrombin Time.

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Abstract

Objectives:toinvestigate the sample stabilitywith regard to the temperatureand the storage time and to detect influence of temperature and storage duration on Prothrombin time in citrated plasma.

Patients and Method: This study was carried out in the period between April, 2016 and March, 2017 in the department of clinical pathology, Sohag University Hospital, Sohag, Egypt, during the study period whole blood samples were collected from groups of patients: "43"apparently normal healthy (not receiving anticoagulant) as control **(group A)** and"43"receiving anticoagulant (17 on warfarin and 26 on heparin) **(group B)**.Each sample of each group will be analyzed forProthrombin time immediately and after 4hrs, 8hrs, and 24hrs of storage.Also each sample will be tested at room temperature (18-22 °C) and at refrigerator temperature (4 °C).

Results andConclusion:samples for Prothrombin time were acceptable for analyses up to 24 h in those not receiving anticoagulant and up to 8 h in those receiving anticoagulant.

Introduction

Prothrombin time (PT) measurement is one of routine coagulation tests used to assess pathological alterations of the haemostatic and coagulation systems and guide clinical therapy. Pre-analytical factors including specimen collection, transportation, centrifugation, storage and assay method can all affect coagulation testing results (1).

In 1982, it was demonstrated that PT reduction in both normal and patients receiving Coumadin therapy was time and temperature dependent, when samples were collected into borosilicate or siliconized borosilicate tubes, resulting in incorrect adjustment of warfarin dosage (2).

Consequently, the Clinical and Laboratory Standards Institute (CLSI) H21-A5 recommended that specimens should be analysed within 24-h for PT if stored at RT but did not recommend a storage time for refrigerated storage(2–8 °C); furthermore, they recommended that PT samples should not be refrigerated (3).

Many studies have proposed acceptable storage durations for routine coagulation tests at RT and refrigerated temperatures (4). A range of storage times (2, 4, 6, 8 h) and temperatures (4 °C, 25 °C, >30 °C) have been studied with various criteria for acceptability.

Errors occurring within the preanalytical phases are still the prevailing source of concern. Accordingly, lack of standardized procedures for sample collection,

including patient preparation, specimen acquisition, handling and storage, accounts for most of the errors encountered within the total testing process (1).

Moreover, these guidelines do not clearly stipulate whether samples should be kept as whole blood or plasma and to the best of our knowledge, only few investigations have focused on the sample stability with regard to the temperature and the time before centrifugation. Some earlier studies and the current guidelines indicate that the prothrombin time (PT) might be stable for periods much longer, up to 24 h (5,6).

Patients and Method:

This study was conducted at Sohag university hospital, a written consent had been taken from each patient, and the study was approved by Scientific and Ethical Committees of Sohag Faculty of Medicine.During the study period whole blood samples was collected from groups of patients:**Group A** (normal) control patients and **Group B** receiving anticoagulant (like warfarin, heparin whether unfractionated or low molecular weight).

Each sample of each group was analyzed for Prothrombin time immediately and after 4hrs, 8hrs, and 24hrs of storage.

Also each sample was tested at room temperature (18-22 °C) and at refrigerator temperature (4 °C).

Results

PT samples in control group showed Mean ± SD -1.07±4.03 with number of samples with >10% change was zero at room temperature while Mean ± SD 1.07±6.09 with number of samples with >10% change was 3 (6.98%) at 4 C° after 4 hours of storage.

After 24 hours storage Mean ± SD was 1.72±3.78 with number of samples with >10% change was 1 (2.33%) at room temperature while at 4 C° Mean ± SD was 2.08±10.45, with number of samples with >10% change was 8 (18.60%).

According to that the acceptable time for storage of samples of PT from control group not receiving anticoagulant was 24 hours whether at room or 4 C° temperature.

While PT samples in group receiving anticoagulant showed Mean ± SD 2.60±5.81 with number of samples with >10% change was 4 (9.30%) at room temperature while Mean ± SD 0.84±4.03with number of samples with >10% change was 2 (4.65%) at 4 C° after 4 hours of storage.

According to that the acceptable time for storage of samples of PT from group receiving anticoagulant was 8 hours whether at room or 4 C° temperature.

Table (1): Percentage change in PT measurement over time of studied population in control group.

PT	At room temperature	Number (%) of samples with >10% change	At 4 C°	Number (%) of samples with >10% change
At 4 hours Mean ± SD Median (range)	-1.07±4.03 -0.89 (-8.45-8.00)	0	1.07±6.09 0.89 (-16.26-12.12)	3 (6.98%)
At 8 hours Mean ± SD Median (range)	-1.36±4.29 -0.79 (-11.38-5.74)	2 (4.65%)	1.53±6.65 2.65 (-16.26-11.81)	5 (11.63%)
At 24 hours Mean ± SD Median (range)	1.72±3.78 0.96 (-3.82-10.08)	1 (2.33%)	2.08±10.45 2.33 (-30.08-36.61)	8 (18.60%)
Acceptable time	24 hours		24 hours	

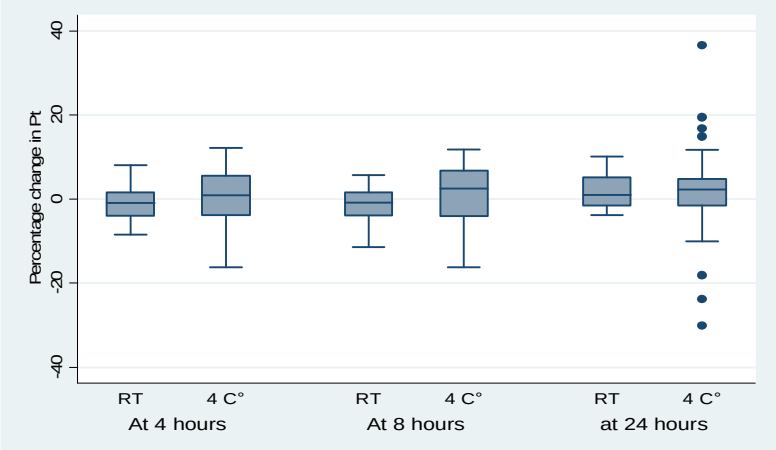

Figure (1): Percentage change in PT measurement over time of studied population in control group.

Table (2): Percentage change in PT measurement over time of studied population in Anticoagulant group.

PT	At room temperature	Number (%) of samples with >10% change	At 4 C°	Number (%) of samples with >10% change
At 4 hours Mean ± SD Median (range)	2.60±5.81 0.73 (-9.47:20.14)	4 (9.30%)	0.84±4.03 0.66 (-8.38:12.35)	2 (4.65%)
At 8 hours Mean ± SD Median (range)	3.55±4.21 3.31 (-10.53:12.28)	3 (6.98%)	5.29±4.45 4.65 (-8.42:15.07)	4 (9.30%)
At 24 hours Mean ± SD Median (range)	27.39±51.40 14 (1.24:303.68)	33 (76.74%)	19.10±9.80 17.39 (1.86:52.72)	36 (83.72%)
Acceptable time	8 hours		8 hours	

Discussion

In coagulation studies, the diagnosis of coagulation disorders and monitoring of anticoagulant therapy usually depend mainly on PT values (7).

In general, coagulation tests should be carried out as soon as possible after collection of the blood samples.(Wang B) demonstrated that after as little as 1 h, samples stored at RT may show significant differences compared with baseline results, although these differences would not have a significant impact on clinical diagnosis or treatment decisions. Thus, a statistically significant difference need not be the same as a clinically relevant difference(12).

Identification of a clinically relevant difference is difficult because there is no consensus defining clinical relevance. Most studies use percentage change (Kemkes-Matthes) decided that sporadic changes exceeding 15% in more than 10% of samples defined clinical relevance(15).(Adcock et al.,) defined a change by >15% as a clinically relevant difference while (Geest-Daalderop et al.,) proposed that if the number of individuals with a greater than 10% percentage change was less than 25% of the total sample number, the effect should be termed moderate and clinically relevant (3,16).

(Wang B) defined a change >10% in PT as significant. There are no guidelines defining acceptable percentage preanalytically for coagulation assays(12).We therefore used the same approach as (Zürcher) and defined a percentage change >10% from baseline results as a clinically relevant difference(11).

In our study we found that the acceptable time for storage of PT ,in those who didn't receive anticoagulant, whether at room or 4 °C temperature was up to 24 hours, as the number of samples with >10% change was more than 25% of the sample. While in those who received anticoagulant (with prolonged PT) we found that the acceptable time for storage of PT whether at room or 4 °C temperature was up to 8 hours, as the number of samples with >10% change was more than 25% of the sample.

This findings were partly consistent with A previous study carried out by (Adcok et al.,) on plasma samples reported different findings; they exposed plasma to three different types of storage conditions (RT, refrigerator and frozen) (3).

At both RT and Following refrigerated storage, (Wang Xiao) and (Rao) found that PT results were increased with duration of storage(9,7).(Salvagno et al.,) found that PT results decreased initially before increasing(10).(Kemkes-Matthes) found that PT results after 8 and 24 h storage were all statistically different when compared with baseline results at RT. They further demonstrated that the differences exceeded the analytical quality specifications for desirable bias following 24 h storage(15).

Earlier studies vary in the acceptable time interval they identify. (Wang Xiao) found PT could be analysed up to 8h following collection at 4 °C and up to 4 h at 25 °C. Wang B found PT samples were acceptable up to 8 h following collectionat RT(9,12).

In our study variation in measurement of PT in control samples was insignificant up to 24 hours in those not receiving anticoagulant while variations were significant at 24 hours with more than 25% of samples had more than 10% change in measurement in those receiving anticoagulant.

The variation in the PT values during the first few hours of storage was too small. Although statistically significant differences were observed for PT tests after 24 h and 8 h at both room temperature and refrigerator, the differences might not alter the clinical interpretation of the results.

Our findings are partially comparable to those reported by (Koepke et al.,) and (Neofotistos et al.,) who concluded that no changes were noted in PT up to 6 h, and no changes in PT up to 8 h respectively.(13,14)

Conclusion

To conclude, we found that samples for PT were acceptable for analyses up to 24 h in those not receiving anticoagulant and up to 8 h in those receiving anticoagulant.

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المخلص العربي

زمن البروثرومبين عبارة عن اختبارمن اختبارات التخثر الروتينية المستخدمة لتقييم التغيرات المرضية لنظم تخثر الدم والتي تستخدم لتوجيه طرق العلاج اللازم. ان عوامل ما قبل التحليل والتي قد تؤثر علي نتائج الاختبارات تشمل جمع العينات، النقل، الطرد المركزي، التخزين وطرق الفحص. المعهد المعني بمعايير الاختبارات والتحاليل اكد علي ان العينات يجب ان يتم فحصها وتحليلها في خلال ٢٤ ساعة لزمن البروثرومبين لو تم تخزينها في درجة حرارة الغرفة ولكنه لم يحبذ وقت تخزيني لدرجات حرارة من ٢-٨ درجة سيلزية علاوة علي ذلك فإنه فضل عدم وضع عينات زمن البروثرومبين في درجة حرارة المبرد. العديد من الدراسات وضعت مدي للزمن التخزيني لعينات اختبارات تخثر الدم الروتينية في درجة حرارة الغرفة وكذلك المبرد فمدي تخزيني (٢-٤-٦-٨ ساعات) في درجات حرارة (٤-٢٥-٣٠ او اكثر) تم دراستها مع نتائج مرضية الي حد ما. تم ايضا فحص عوامل ماقبل التحليل مثل العوامل الميكانيكية والتي تتعرض لها العينات كطرق النقل، تم فحصها وتبين انها تؤثر الي حد كبير علي زمن البروثرومبين بعض الدراسات الاولية والتوجيهات الحديثة **اشارت الي ان زمن البروثرومبين قد** يظل مستقرا لفترة ليست اكثر من ٢٤ساعة