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Original Research Paper

Pathology

EFFECT OF STORAGE TIME ON PT, APTT AND FIBRINOGEN AT ROOM TEMPERATURE

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ABSTRACT **Background:** Coagulation parameters are essential part of clinical and laboratory workup, Pre-Analytical variables like collection, anticoagulation, transportation, storage and hematocrit all effect coagulation parameters tests and accuracy of results, like PT, APTT and fibrinogen. As transportation and storage effecting time and temperature are very variable, they can interfere with the results of coagulation parameters. **Aim:** To study the effect of time on coagulation parameters PT, APTT, and Fibrinogen at Room temperature. **Materials and Methods:** The study population consisted of 70 adult asymptomatic patients 18 years or older. Sample in 3.2% citrate vials with blood to citrate ratio 9:1 were run in automatic analyser (Stago compact) and results were recorded. Statistical analyses were performed using GraphPad QuickCalcs .**Results:** PT and Fibrinogen did not have clinically relevant changes up to 24 hours and APTT upto 4 hours at Room temperature **Conclusion:**, PT and Fibrinogen measurement could be safely stored for upto 24 hours at Room temperature, while APTT can only be stored upto 4 hours at Room temperature.

KEYWORDS : PT (Prothombin time), APTT (Activated Partial Thromboplastin Time), RT (Room temperature), Hours (hrs), TEMP (Temperature), Storage Time

INTRODUCTION

Coagulation parameters are essential part of clinical and laboratory workup. Pre-Analytical variables are very important in work up of coagulation and thrombotic disorders [(1)].Factors like collection technique, hematocrit, transportation, storage [(2-5)], anticoagulation [(6)], processing [(7)] all effect coagulation parameters tests and accuracy of results, like PT, APTT and Fibrinogen. In countryside particularly in villages, towns and hilly areas, where proper 24 hours laboratory service are not available, much time is lost in transportation and proper temperature is also hard to maintain, so there is a need to know how temperature and delay in time affect coagulation tests results particularly required in Emergency Medicine in cases of DIC, sepsis and bleeding disorders. The Clinical and Laboratory Standards Institute (CLSI) H21-A5 has recommended samples should be analyzed within 24 hrs for PT, and 4 hrs for APTT [(8)], Fibrinogen and other assays if stored at RT .However few studies have suggested that PT,APTT and Fibrinogen could be stored for longer periods [(5,9)]. Therefore this study is to determine the effect of storage time on PT, APTT and Fibrinogen at Room Temperature upto to 24 hrs.

MATERIALS AND METHODS

The study population consisted of 70 adult asymptomatic individuals 18 years of age or older who required blood investigations but not having clinical manifestations of any bleeding or thrombotic disorders, and was conducted in Medical College, Kolkata after taking consent and obtaining permission from ethical committee.

Procedure: Blood sample was drawn aseptically from individuals in proper light after identifications and labeling of coagulation tubes, preferably in fasting status with no vigorous activity in last 30 minutes. Samples were taken from the cubital vein in sitting position with minimum or no tourniquet pressure, with tourniquet applied 4 to 5 finger width above antecubital fossa, with minimum or no fisting and blood drawn within one minute of applying tourniquet. The blood sample collected in 3.2%citrated vials in the ratio of blood to citrate 9:1 and then gently the samples were mixed. The samples were then centrifuged to make platelet poor plasma and then coagulation parameters PT, APTT and Fibrinogen measured at 0, 2,4,12 and 24 hrs at Room temperature (18 to 25 °C).

Instrument: Automatic Analyzer (Stago compact)-The results were expressed in seconds (PT, APTT), g/l (Fibrinogen level).

Reagents: PT reagents: STA®-Neoplastine®, APTT reagents: STA®-C.K. Prest®, Calcium Chloride for APTT: STA® CaCl2 0.025M, Fibrinogen assays: STA® Liquid FIB. Diluent Buffer: STA® Owren- Koller.

Quality Controls: STA®-System Control N & P.

STATISTICAL ANALYSES:

The results were reported as mean and standard deviation. By using paired t-tests results of storage time at 2, 4, 12, and 24 hours at RT were compared with the baseline (0 hours). Percentage changes compared to the baseline results were calculated as [(result at storage time T– result at baseline)/result at baseline]. In this study the numbers of individual samples with greater than 10% percentage changes in more than 25% of total samples was taken as clinically relevant changes. Statistical analyses were performed using GraphPad QuickCalcs.

RESULTS

There were no clinical relevant changes for PT and Fibrinogen for upto 24 hours at RT and upto 4 hrs for APTT at RT. Mean age of individuals were 42 years with 57% male and 43% female.

Although statistical signifance were noted for PT at RT with reference to baseline, at 2, 4, 12, and 24 hrs the result were not clinically relevant, that is clinically relevant changes did not occur up to 24 hrs **(Table no 1).**

PT at RT (2, 4, 12 and 24 hrs)

PT(s)	Baseline	TEMP		2hours	4 hours	12hours	24 hours
	Mean-13.7	RT	Mean	13.5	13.5	13.6	14.4
	SD-1.61		SD	1.61	1.54	1.6	1.74
s=seconds			Mean P% change	<10%	<10%	<10%	<10%
			> 10% change in >25% of samples	NO	NO	NO	NO
P=PERCENTAGE			Statistically significant=P<.05 compared to baseline	YES 0.001	YES 0.004	YES 0.005	YES <0.0001

Although statistically significant result were noted for APTT at 4, 12, and 24 hrs with reference to baseline, clinically relevant changes did not occur upto 4 hrs **(Table no 2)**

APTT at RT (2, 4, 12 and 24 hrs)

APTT(s)	Baseline	TEMP		2hours	4 hours	12hours	24 hours
	Mean-28.8	RT	Mean	29	29.8	30.9	32.3
	SD-3.42		SD	3.38	3.42	3.54	3.96
s=seconds			Mean P% change	<10%	<10%	<10%	<10%
			> 10% change in >25% of samples	NO	NO	YES	YES
P=PERCENTAGE			Statistically significant=P<.05 compared to baseline	NO 0.075	YES <0.0001	YES <0.0001	YES <0.0001

Although statistically significant result Fibrinogen at room temperature at 2, 4, 12, and 24 hrs with reference to baseline they were not clinically relevant **(Table no 3)**

Fibrinogen at RT (2, 4, 12 and 24 hrs)

Fibrinogen(g/L)	Baseline	TEMP		2hours	4 hours	12hours	24 hours
	Mean-2.87	RT	Mean	2.94	2.97	3.02	3.03
	SD-0.64		SD	0.75	0.75	0.80	0.80
			Mean P% change	<10%	<10%	<10%	<10%
			> 10% change in >25% of samples	NO	NO	NO	NO
			Statistically significant=P<.05 compared to baseline	YES 0.049	YES 0.007	YES <0.0001	YES <0.0001

Clinically relevant change => 10% individual change in >25% of samples

DISCUSSION

Many times it is very difficult to maintain standard time given by Clinical and Laboratory Standards Institute (CLSI) guidelines, and therefore many studies have suggested that time interval for coagulation tests can be extended [5, 9] .Study done by Zhao et al [10] and Yao et al [11] demonstrated that storage time interval upto 24 hrs for PT and Fibrinogen and 8hrs for APTT at RT is acceptable. Rao et al [12] studied effect of PT and APTT at different storage time, and found that statistically significant result were observed for PT after 12 hrs but based on clinical relevant changes, either plasma or whole blood samples can be accepted for PT for 24 hrs, when transported at RT while APTT samples upto 12hrs at RT could be accepted. Kemkes-Matthes et al [13] found that the acceptable storage time can be extended to 24 hrs for PT at ambient temperature. Zurcher et al [14] observed that PT, APTT and Fibrinogen time could be extended to 24 hrs without observing clinically relevant changes at ambient temperature. Different Studies used of mean percentage change to evaluate the effect of storage time on coagulation parameters. Kemkes-Matthes et al[13] and Van Geest-Daalderop et al [15] both considered individual percentage change of more than 10% in more than 15% and 25% of sample respectively as clinically relevant changes. Similarly Feng et al [5] considered clinically relevant changes if individual percentage change of more than 10% occurred in more than 25% of samples compared to baseline and found that although statistically significant difference were noted in their study with PT/INR at 2, 4,8,12 and 24 hrs and Fibrinogen upto 12hrs compared to baseline, but values were not clinically relevant and samples for PT/INR and Fibrinogen can be stored for upto 24 hrs at 25°C, while samples for APTT can be stored upto 8hrs at 25°C.

In our study the numbers of individual samples with greater than 10% percentage changes occurring in more than 25% of total samples compared to baseline (0 hrs) was taken as clinically relevant difference. Although statistically significant difference was noted in PT, APTT and Fibrinogen at 2, 4 12 and 24 hrs compared to baseline but based on clinically relevant changes of more than 10% in greater than 25% of samples, PT and Fibrinogen can be stored up to 24 hrs at RT, and APTT can be stored upto 4 hrs at RT, which was similar to findings of studies already mentioned above.

CONCLUSION

In this study we concluded that PT and Fibrinogen could be stored up to 24 hrs and APTT for 4 hrs at RT, although limitation of the study was that it was a single centre small study, done only on asymptomatic persons. Therefore it should be further validated by multicentre studies based on different study populations, and on larger sample size, including more coagulation parameters and at different Time and Temperatures.

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