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RESEARCH ARTICLE

The Effect of Freezing Storage of Citrated Plasma on Prothrombin Time and Activated Partial Thromboplastin Time

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ABSTRACT

Prothrombin time (PT) and activated partial thromboplastin time (APTT) are important investigations used for evaluation of coagulation system and monitoring of anticoagulant therapy.

This study was conducted at Faculty of Medical laboratories Science, Elneelain University, Khartoum, Sudan. It is aimed to study the effect of freezing storage (at -20c) of citrated plasma on PT and APTT.

Blood samples were collected from 50 healthy volunteer, and platelet poor plasma was separated. PT and APTT were performed immediately after plasma separation using part of the plasma; the rest of the plasma stored at -20^{o}C in 3 different plastic containers and PT and APTT were performed after 1, 2, and 3 weeks on these samples.

The results revealed that PT and APTT results for the samples stored for 1, 2, and 3 weeks were significantly different (P.value> 0.05) from the result of fresh plasma and from each others. PT and APTT were raised with increased storage duration.

In conclusion, freezing storage at -20^{o}C affect PT and APTT, so it is preferably that PT and APTT performed immediately after sample collection.

Keywords: Prothrombin; Thromboplastin; freezing; storage.

1. INTRODUCTION:

Prothrombin time (PT) and activated partial thromboplastin time (APTT) are important coagulation tests used for the evaluation of extrinsic and intrinsic coagulation pathways respectively. Furthermore, they are used for monitoring of anticoagulant therapy [(1)].

Although specimen integrity is important for every laboratory test, coagulation testing that requires plasma specimens seems to be particularly sensitive to even minor deviations from standard practices regarding anticoagulant concentration, container materials, collection technique, centrifugation, and storage [(2)].

Recommended guidelines for coagulation tests that, these tests should be performed within 2 hours if samples are kept at room temperature, 4 hours if stored at 2°-4°C, and 2 weeks if preserved at −20°C [(3)].

Researches concerned with the effect of sample storage on PT and APTT at different temperature and durations revealed conflict results [(4, 5, 6)].

This study was conducted to evaluate the effect of sample freezing storage at -20 on PT & APTT.

2. MATERIALS AND METHODS

Blood samples were collected from 50 healthy volunteer in a plastic test tubes containing 3.2% trisodium citrate anticoagulant; the volume of blood: anticoagulant was 9:1 (4.5: 0.5 milliliter).

The samples were then mixed carefully and centrifuged at 4000 revolution per minute for 15 minute to provide platelets poor plasma. The plasma was harvested and divided evenly into four tubes, each tube containing 400 µl and the tubes were labeled as A, B, C, and D.

Tubes B, C, and D were stored at -20^{o}C. PT and APTT were performed for sample A immediately, sample B after 1 week, sample C after 2 weeks, and sample D after 3 weeks.

Data was analyzed using statistical package for social sciences (SPSS). Normality of results was tested by skweness and kurtosis. As results were normally

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distributed means of PT and APTT for the four samples were compared by ANOVA test.

Informed consent was taken from each volunteer before sample collection.

3. RESULTS

This study was conducted to evaluate the effect of freezing storage (at-20^{o}C) of citrated plasma on PT and APTT. Blood samples were collected from 50 healthy individual, platelets poor plasma was separated, and PT and APTT were performed immediately, after 1week, 2weeks, and 3 weeks on parts of the plasma stored at -20^{o}C.

Our results showed that PT and APTT were prolonged on freezing storage and the results of both tests after 1week, 2 weeks, and 3 weeks were significantly different from the results of the fresh plasma and the results of each other's (Table 1 and 2).

Storage duration	Mean	S.D	*P.value
Fresh	12.9	1.5	
1week	14.9	1.2	
2week	18.1	3.3	0.000
3week	21.01	4.2	

- **P.value considered significant if < 0.05**

Table 1: Comparison of PT results in different storage periods

Storage duration	Mean	SD	*P.value
Fresh	35.3	3.1	
1week	39.2	1.2	
2week	54.08	9.6	0.000
3week	57.9	10.1	

- **P.value considered significant if < 0.05**

Table 2: Comparison of PT results in different storage periods

4. DISCUSSION

The results of the current study showed that PT and APTT results after 1 week, 2weeks and 3 weeks storage at -20^{o}C were significantly different from the results of fresh sample and from each others. It is observed that the results of both PT and APTT were increases with increasing storage duration.

This finding was consistent with the finding of Alsci *et al* who concluded that prothrombin time and APTT should be measured in fresh samples, since freezing has an inconstant and unpredictable effect on the results [(7)].

Our results was disagree with results of Woodham *et al* who study the stability of many coagulation proteins in frozen plasma and reported that these factors are stable for at least 18 months at -74^{o}C[(8)]. The difference between this finding and our finding may be because of the difference in storage temperature.

Our results also was differ from that reported by Bennett *et al* who reported that PT & APTT are stable at -20^{o}c for 2 weeks [(2)].

We suggest that the negative effect of freezing storage of PT & APTT due to the fact that some coagulation factors are labile & others are storage labile, so can be consumed on storage & results prolonged PT & APTT.

5. CONCLUSION

In summary, freezing storage at -20^{o}C affect PT and APTT results, so it is preferably that PT and APTT performed immediately after sample collection.

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