



**Sudan University of Science and Technology**  
**College of Graduate Studies**



**Assessment of the Effect Plasma Storage Conditions on  
Prothrombin Time**

**تقييم تأثير الظروف التخزينية للبلازما على زمن البروثرومبين**

**A Thesis Submitted for Partial Fulfillment for the Requirements of  
Master Degree in Medical Laboratories Science**

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## الآية

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

﴿ أَقْرَأْ بِاسْمِ رَبِّكَ الَّذِي خَلَقَ ﴿١﴾ خَلَقَ الْإِنْسَانَ مِنْ عَلَقٍ ﴿٢﴾ أَقْرَأْ وَرَبُّكَ الْأَكْرَمُ ﴿٣﴾

الَّذِي عَلَّمَ بِالْقَلَمِ ﴿٤﴾ عَلَّمَ الْإِنْسَانَ مَا لَمْ يَعْلَمْ ﴿٥﴾ ﴾

## **Dedication**

To my great parents who provide invaluable support for me throughout of my life.

To my brothers, cousin, special friends and colleagues who represent strong support and encouragement for me I dedicate this work.

## **Acknowledgement**

All the praise must to Allah Almighty Allah When I started the study I didn't know whether I Could complete it or not but I believed, Fortune favors the brave so I was determined to try my best to make it a success and I most grateful to Almighty Allah.

The second acknowledgement must go to my family members for alwaysinspiration and provided necessary financial support I would like to pay my highest gratitude to my research

I am grateful for my supervisor Dr. Munsorr Mohammed Munsorr for his patience guidance and support throughout the study.

Grateful thanks are extended to my friend and colleague Dr. ALaaHanafe for her true support.

Sincere thanks are also extended to him.

## Abbreviation

PT	Prothrombin Time
APTT	Activated partial thromboplastin time
TT	Thrombin Time
REF	Refrigerator
RT	Room temperature
SPSS	Statistical package for social science
HR	Hour
PPP	Platelet poor plasma
TSC	Tri sodium citrate

## **Abstract**

Prothrombin time is the most frequently ordered test in clinical coagulation laboratory, it is used to assess the integrity of tissue factors pathway of coagulation, more over it is test of choice (reported INR) for monitoring patients on long term anticoagulant therapy. Furthermore it is most sensitive test for assessment of severity of liver disease.

This is cross sectional study carried out in Khartoum state in Advanced Diagnostic laboratory, in the period from April to December 2019 thirty samples collected from normal health individuals.

Plasma sample were collected from volunteers for estimation of, PT using semi- automated analyzer (stago), data was analysed by SPSS version 23.

The sample measure at (0,6,12,24) in RT & REF the PT measured at 6 hr RT, 12 REF and 24 freezing showed in significant difference when compared with measurement at 0hrs while the difference were significant with measurement at 6hrs REF and 12hrs at RT.

INR result show no significant up to 6 hrs at RT/REF and 24 hrs freezing. But significant after 12hrs in RT and REF.

## المستخلص

اختبار البروثرومبين هو الفحص الاكثر طلبا في المعامل الاكلينيكية لتقييم فعالية عوامل التجلط ويستخدم ايضا لمتابعة مرضى الجلطات وحدة امراض الكبد .

اجريت دراسة جزئية في ولاية الخرطوم في المعمل التشخيصي المتطور من ابريل وحتى ديسمبر وتم جمع العينات من المتطوعين الصحيين لقياس البروثرومبين باستخدام جهاز استافو وتم تحليل العينات بواسطة برنامج التحليل الاحصائي اصدار 23 .

تم فحص العينات في اوقات ( 0 ، 6 ، 12 ، 24 ) ساعة ، وتم تخزينها داخل وخارج الثلجة ، ولوحظ انه لا يوجد فرق احصائي في التحليل بعد ستة ساعات في درجة حرارة الغرفة العادية ، وبعد 12 ساعة داخل الثلجة ، 24 ساعة تجميد ، ويوجد فرق احصائي بعد 6 ساعات داخل الثلجة .

المعيار العالمي للتجلط لا يوجد فرق في التحليل الاحصائي بعد ست ساعات داخل وخارج الثلجة و24 ساعة تجميد ولكن يوجد فرق في 12 ساعة داخل وخارج الثلجة .

وقد اخذت النتائج بناء على مقارنة جميع الازمان مع زمن الفحص المباشر .

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# **Chapter One**

## **Introduction**

## **1.1. Introduction:**

Prothrombin time is the most frequently ordered test in clinical coagulation laboratory, it is used to assess the integrity of tissue factors pathway of coagulation, moreover it is test of choice (reported INR) for monitoring patients on long term anticoagulant therapy. Furthermore it is most sensitive test for assessment of severity of liver disease (Mohamed, *et al* 2004).

Pre-analytical conditions are very important in laboratory assessment of hemostatic and coagulation systems. Pre-analytical variables including specimen collection, anticoagulant type and concentration, hematocrit, filling status of the sampling tube, transportation, centrifugation, as well as storage and assay method can all affect coagulation test and factor analysis results. Activated partial thromboplastin time (APTT), fibrinogen (Fbg), prothrombin time (PT), international normalized ratio (INR, transformed by PT), and thrombin time (TT) measurements are routine coagulation tests used to assess pathological alterations of hemostatic and coagulation systems to guide clinical therapy.

The different storage temperatures and durations affect coagulation test results. For these preanalytical variables, the Clinical and Laboratory Standards Institute (CLSI) H21-A5 has recommended that specimens should be analyzed within 24 h for PT and 4 h for APTT and other assays if stored at room temperature (25°C). However, they have not recommended a storage time for refrigerated storage. (Fenget *al*, 2014)

**Rationale:-**

The prothrombin time is very important test in evaluating the haemostaticextrinsic pathway efficiency. It is also used for calculation of the international normalizing ratio to the evaluation of the anticoagulant therapy. There is no data in the Sudan about the effect of the storage and temperature on the test. Determination of the effect of storage and temperature on the prothrombin time is important in the quality control of test. So the aim of our study is to provide information about the effect of storage time and temperature on plasma of prothrombin time test.

### **1.3.Objectives**

#### **1.3.1. General Objective:-**

To assess the effect of storage conditions (times and temperatures) on plasma prothrombin time.

#### **1.3.2. Specific Objectives:-**

1-To determine plasma prothrombin at 0, 6, 12,24, hour.

2-To correlated prothrombin time at 6 hour in RT and REF.

3-To compare prothrombin time at 12 hour in RT and REF.

4-To determine prothrombin time at 24hour at freezing.

5-To multiple compression between interval (0, 6,12.24hour) in RT and REF.

# **Chapter Two**

## **Literature review**



## **2.1 Literature review:**

Hemostasis derived from Greek meaning the stoppage of blood flow, it is a complex process by which the body maintains blood in fluid state during its circulation and stops bleeding when injury occurs. It contains five components. Blood vessels are formed by three layers. Platelets originate from large cells in the bone marrow called megakaryocytes. The normal range is (150-400 cells/mm<sup>3</sup>). Coagulation is a process whereby, on vessel injury, plasma proteins, tissue factor, and calcium interact on the surface of platelets to form a fibrin clot. Most are referred to both by Roman numerals and by names assigned by the coagulation factor (Martin, 1992).

## **2.2 Stage and mechanism of haemostasis:-**

There are two stages in haemostasis:- Primary haemostasis is a first response to endothelial damage such as normal endothelial turnover or tissue damage which results in the formation of a platelet plug through interactions between platelets and vascular haemostasis is the formation of a stable fibrin clot over the already created platelet plug (Hoffbrand and Pettit, 1993). Secondary haemostasis occurs due to the consecutive activation of various coagulation factors that eventually produce thrombin at the site of vessel harm. (Erne and Mann, 2003; Stokol, 2003).

The mechanism of blood coagulation constitutes a complex and dynamic interaction of platelets, plasma, and blood vessels.

endothelium. Blood coagulation is an important part of the haemostatic process.

It is usually initiated through damage to the vessel wall and subsequent activation of protease enzymes and ends with the transformation of soluble fibrinogen into insoluble fibrin. Natural anticoagulant mechanisms limit and localize haemostatic plug (thrombus) formation at sites of blood vessel injury, and disorders of coagulation can lead to an increased risk of hemorrhage and/or clotting (thrombosis) (Alesci et al, 2008). A total of 128 units of whole blood were pooled in groups of four and split to produce 32 sets of four identical blood units that were processed either within 8 hours of blood collection or after 24 hours storage of whole blood for 24 hours resulted in a 23% decrease in the activity of Factor (F)VIII, but not significant loss of activity of coagulation factors FV, FVII, FXI, FXII, fibrinogen, antithrombin, or von Willebrand factor. There was a small, but significant decrease in levels of FII, FIX, and FX (all <5%) as well as protein C (6%) and free protein S activity (14%). The ability of plasma to generate thrombin after 24-hour storage as whole blood was unaltered, as assessed by real-time thrombin generation tests as was the rate and strength of clot formation by rotational thrombelastometry. Levels of all coagulation factors measured were above 0.50 U/mL in plasma produced from whole blood. These data show that there is minimal effect of storing whole blood at ambient temperature for 24 hours on the coagulation activity of plasma and that this is an acceptable alternative to producing plasma on

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Association of Blood Banks Fibrinolysis is the system whereby the temporary fibrin clot is systematically and gradually dissolved as the vessel heals in order to restore normal blood flow. Damage tissue release substance (plasminogen activator) that activate the inert precursor called plasminogen which is normally circulate in the plasma to its activate which it is capable to degradation fibrin clot. Natural coagulation inhibitor the counterforce's of the naturally occur biochemical coagulation and fibrinolytic inhibitor are necessary to achieve balance between activated clotting factor and fibrinolytic enzyme. (Martin, 1992)

### **2.3 Evaluation of Secondary Hemostases:-**

Test use to evaluation of secondary Hemostasis Prothrombin time, activated thromboplastin, thrombin time, fibrinogen and platelet count (Dacie and Lewis, 2012).

#### **2.3.1 Prothrombin Time:-**

Prothrombin time (PT) is a blood test that measures how long it takes blood to clot. prothrombin time test can be used to evaluate the extrinsic pathway. PT test may also use for the calculation of international normalized ratio (INR) stands for a way of standardizing the results of prothrombin time tests, regardless of the testing method.

Using the INR system, treatment with blood-thinning medicine (anticoagulant therapy). Prothrombin time is an important test because

it checks to see if five different blood clotting factors (factors I, II, V, VII, and X) are present. The prothrombin time is made longer by:-

Other substances, called inhibitors, which inhibit the clotting factors. An increase in the use of clotting factors. An abnormal prothrombin time is often caused by liver disease or injury or by treatment with blood thinners. Another blood clotting test, called partial thromboplastin time (PTT), measures other clotting factors. Partial thromboplastin time and prothrombin time are often done at the same time to check for bleeding problems or the chance for too much bleeding in surgery. (Turgen, 2012)

Principle of prothrombin time: The PT test measures the clotting time of recalcified plasma in the presence of optimal concentration of thromboplastin and indicates the overall efficiency of the extrinsic clotting system. (Dacie and Lewis 2012)

### **2.3.2 Activated partial thromboplastin time:**

Principle: The test measures the clotting time of plasma after the activation of contact factors and the addition of phospho – lipid and  $\text{CaCl}_2$  but without added tissue thromboplastin and so indicates the overall efficiency of the intrinsic pathway. To standardize the activation of contact factors the plasma is first pre incubated for a set period with a contact activator such as Kaolin, silica or ellagic acid. During this phase of the test factor XIIa is produced which cleaves factor XI to factor XIa but coagulation does not proceed beyond this in absence of calcium. After re calcified, factor XIa activates factor IX and coagulation

follows , A standardized phospholipids is provided to allow the test to be performed on ppp the test depends not only on the contact factors and on factors VIII and IX but also on the reactions with factors X.V. prothrombin and fibrinogen it is also sensitive to the presence of circulating anticoagulants (inhibitors) and heparin.(Dacie and lewis, 2012).

# Coagulation cascade

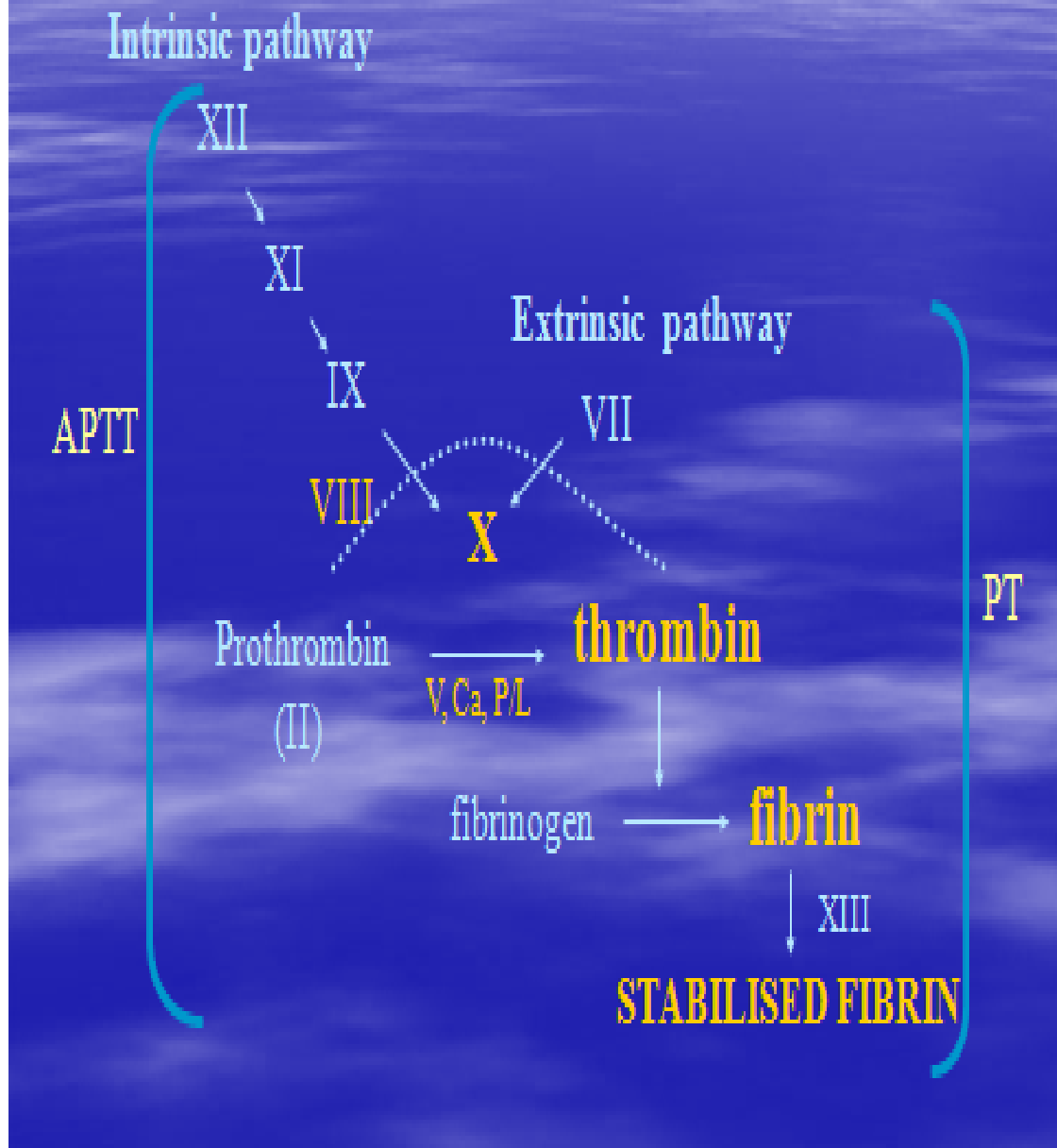


Figure (2-1) shows the blood clotting mechanism

## 2.4 Previous studies

Mohammed Saghiretal(2012),atMalaysia(PT)wasmeasuredat0,4,8and24hoursPartialthromboplastintime(APTT)wasmeasuredatroomtemperature(RT)andrefrigeratorPTshowedno.

significant( $p>0.05$ )differencesatRTat4h,whilesignificant( $p<0.05$ )differencesafter8 hrs 24 hrs RTandafter4h,8hand24hratrefrigeratorwereobserved.

GamalHassan,(2015)wastestPTat0,4,8,12and24hratRTandREFresultRFEacceptableupto24hr( $p>0.05$ )atRTPTat12hr in acceptable( $p<0.05$ ).

Mohamed, A et al (2004)showed that there is no significant change in INR 6,12, and 24 hrs at RT and 4C( $p$  value more than 0.05) multiple comparison have shown there is in significant difference between INR level after 6,12,24 hrs at RT and 4C as compared to 0 value( $p$  value more than 0.05).

Sultan A et al (2010) PT measured at 0 hr showed no significant difference when compared with measurement at 4 hrs while the difference were significant with measurement at 8 and 24hrs at RT on other hand all samples showed statistically difference when stored I RFE for 4,8 and24 hrs when compared with 0 time.

Rao, L, V et al( 2000) was studied stability of prothrombin time and activated partial thromboplastin time tests under different storage condition showed that plasma and whole blood sample can be tested PT up to 24 hrs and APTT for up to 12 hrs when transported either at RT or REF,

There was increased in PT result overtime when samples were stored at RT with maximum level at 24hrs ,on other hand when samples were stored in RFE the PT results obtain decrease over time and the minimum level was at 24 hrs.



# **Chapter Three**

## **Materials and Methods**

### **3.1 Study Design**

This study was analytical cross-sectional study.

### **3.2 Study Area**

This study was carried out at Al kalklaa, Khartoum \_Sudan.

### **3.3 study period**

This period study was from APRIL to DECEMBER 2019

### **3.4 Study population**

Apparently thirty healthy individuals at Alklklaawas enrolled in this study, and the sample size was determine according to availability of resources.

### **3.5 Ethical Considerations:**

This study was done after the researcher took permission from the Sudan University of Science and Technology, faculty of medical laboratories. The researcher took consent from normal volunteer after explanation of study outcome.

### **3. 6 Methodology**

#### **3.6.1 Specimen collection 0and processing**

1.8 ml of venous blood was collected in vacuum tube containing 0.2 tri-sodium citrate. And the sample separate by centrifugation 3000 r\15 min to achieve platelets poor plasma (ppp).

#### **2.6.2 Methods of evaluation of PTand APTT:-**

- 1- Manual method.
- 2- Semi automation.

3- Automation.

### **3.6.3. Data analysis:**

The data was analyzed by SPSS (statistical package for the social sciences), version 23. Paired sample t test and one way anova test were used .

### **3.6.4 Principle of coagulometer (stago):-**

The principle of the device for measuring of the PT by using semi coagulometer method it depend on the apendular movement of the boll throw an electro-magnetic field .the boll still in moving and mixing the mixture of PPP and PT reagent until the clot it formed .when the clot it is formed the boll movement it stop and the time of PT it is record by the device.(stago manual sheet)

### **3.6.5 Procedure:-**

1.8ml from each patient was collected in TSC(9-1) and PPP are prepared by centrifugation of the sample in 40000R for 15 minute .the procedure rune by semi automation coulometer (stago) .the device it is open and incubated until the temperature rice to 37°C, and the cuvate it is putted with boll on the incubation room and then added of ,1ml sample until it warmed and then it transferred to the working area then added ,2ml of PT reagent (cacl<sub>2</sub>+thromboplastine) and start the timer immediatly after added the regent and then recode the result to statically analyses.

# **Chapter Four**

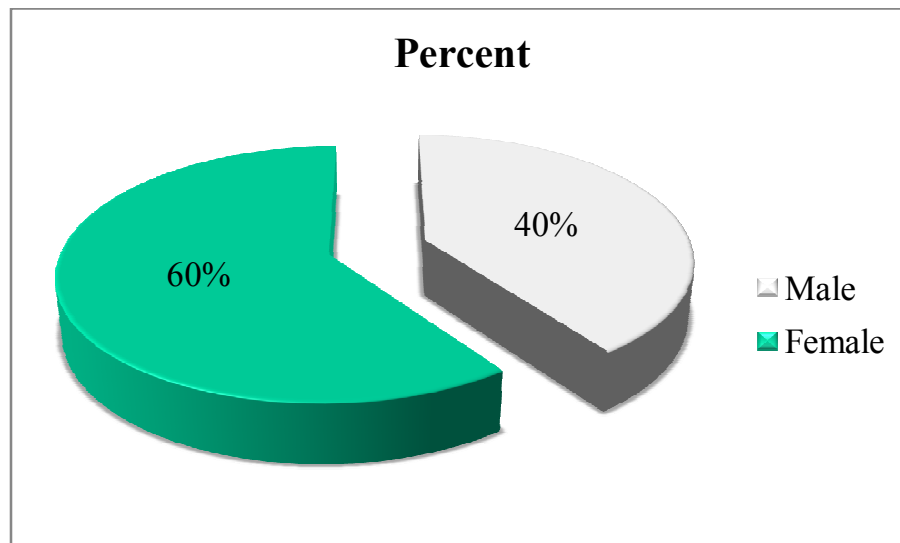
## **Results**

## Demographic data of the participant

30 subjects with mean age ( $30\pm 10$ ) years were included in this study, the results are expressed as percent.

**Table (4.1): Distribution of study population according to gender.**

	Frequency	Percent
Male	12	40%
Female	18	60%
<b>Total</b>	<b>30</b>	<b>100.0</b>



**Figures (4.1): Distribution of study population according to gender.**

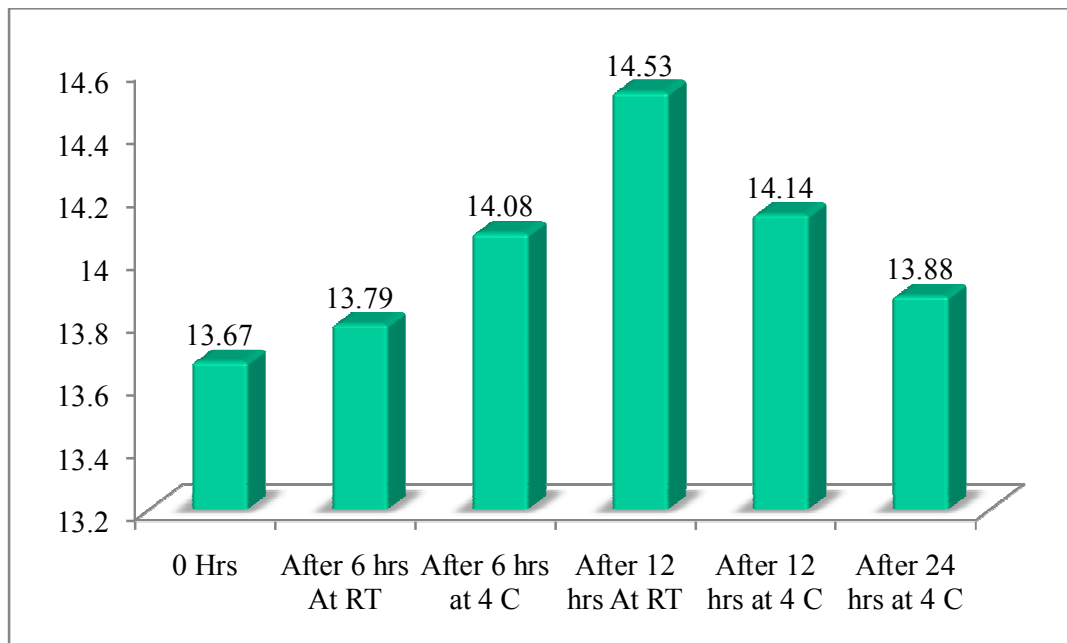
**Table (4.2): Level of the PT at different intervals.**

**Statistics**

	0 Hrs	After 6 hrs At RT	After 6 hrs at 4 C	After 12 hrs At RT	After 12 hrs at 4 C	After 24 hrs at 4 C
Mean	13.67	13.79	14.08	14.53	14.14	13.88
Minimum	11.80	12	13	13.40	13.30	12.90
Maximum	15.10	15.30	15.10	15.90	15.20	15.10

ANOVA

P value = .000\*F value = 5.979



**Figures (4.2): Level of the PT at different intervals.**

**Table (4.3): Compare mean and SD of PT according to gender**

Gender		0 Hrs	After 6 hrs at 4 C	After 12 hrs at 4 C	After 24 hrs at 4 C	After 6 hrs At RT	After 12 hrs At RT
Male	Mean	13.49	14.12	14.16	13.88	13.62	14.64
	Std. Deviation	1.00	0.44	0.51	0.50	1.13	0.45
Female	Mean	13.79	14.05	14.13	13.88	13.90	14.46
	Std. Deviation	0.80	0.56	0.48	0.58	0.91	0.65
Total	Mean	13.67	14.08	14.14	13.88	13.79	14.53
	Std. Deviation	0.88	0.51	0.48	0.54	1.00	0.57

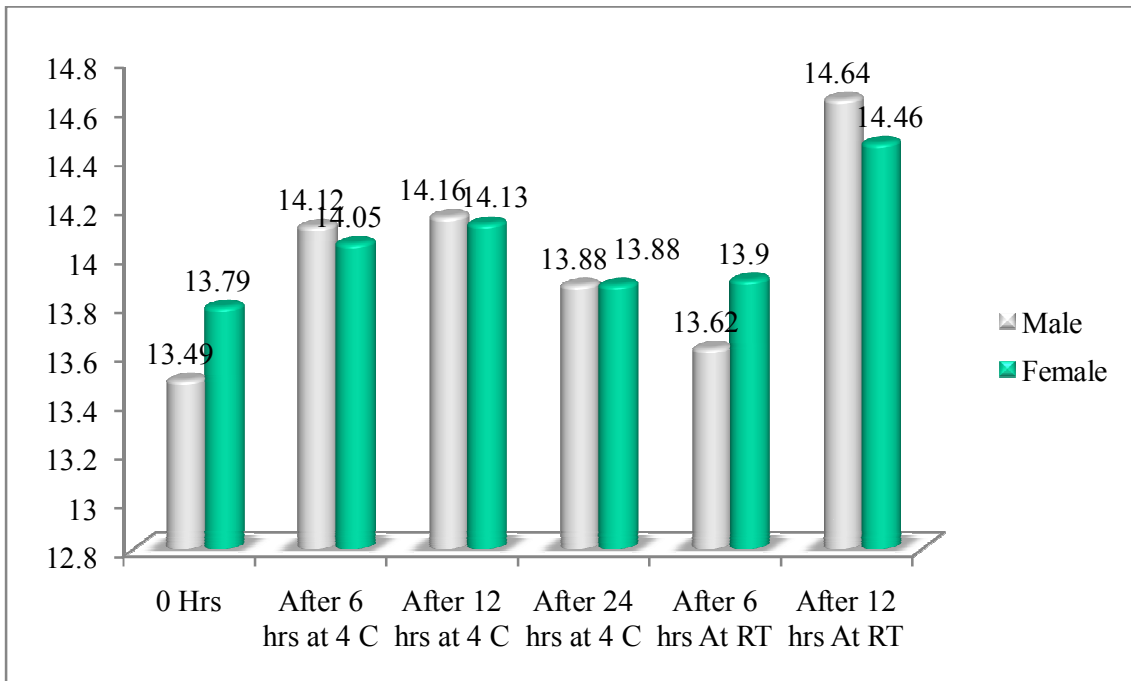
**Table (4.4): Multiple Comparison**

Dependent Variable: sample

LSD

(I) case	(J) case	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
0 hrs	After 6 hrs At RT	-.11667	.17873	.515	-.4694	.2361
	After 6 hrs at 4 C	-.40667 <sup>*</sup>	.17873	.024	-.7594	-.0539
	After 12 hrs At RT	-.86333 <sup>*</sup>	.17873	.000	-1.2161	-.5106
	After 12 hrs at 4 C	-.47000 <sup>*</sup>	.17873	.009	-.8228	-.1172
	After 24 hrs at 4 C	-.21000	.17873	.242	-.5628	.1428
After 6 hrs At RT	0 hrs	.11667	.17873	.515	-.2361	.4694
	After 6 hrs at 4 C	-.29000	.17873	.107	-.6428	.0628
	After 12 hrs At RT	-.74667 <sup>*</sup>	.17873	.000	-1.0994	-.3939
	After 12 hrs at 4 C	-.35333 <sup>*</sup>	.17873	.050	-.7061	-.0006
	After 24 hrs at 4 C	-.09333	.17873	.602	-.4461	.2594
After 6 hrs at 4 C	0 hrs	.40667 <sup>*</sup>	.17873	.024	.0539	.7594
	After 6 hrs At RT	.29000	.17873	.107	-.0628	.6428
	After 12 hrs At RT	-.45667 <sup>*</sup>	.17873	.011	-.8094	-.1039
	After 12 hrs at 4 C	-.06333	.17873	.724	-.4161	.2894
	After 24 hrs at 4 C	.19667	.17873	.273	-.1561	.5494
After 12 hrs At RT	0 hrs	.86333	.17873	.000	.5106	1.2161
	After 6 hrs At RT	.74667 <sup>*</sup>	.17873	.000	.3939	1.0994
	After 6 hrs at 4 C	.45667 <sup>*</sup>	.17873	.011	.1039	.8094
	After 12 hrs at 4 C	.39333 <sup>*</sup>	.17873	.029	.0406	.7461
	After 24 hrs at 4 C	.65333 <sup>*</sup>	.17873	.000	.3006	1.0061
After 12 hrs at 4 C	0 hrs	.47000 <sup>*</sup>	.17873	.009	.1172	.8228
	After 6 hrs At RT	.35333 <sup>*</sup>	.17873	.050	.0006	.7061
	After 6 hrs at 4 C	.06333	.17873	.724	-.2894	.4161
	After 12 hrs At RT	-.39333 <sup>*</sup>	.17873	.029	-.7461	-.0406
	After 24 hrs at 4 C	.26000	.17873	.148	-.0928	.6128
After 24 hrs at 4 C	0 hrs	.21000	.17873	.242	-.1428	.5628
	After 6 hrs At RT	.09333	.17873	.602	-.2594	.4461
	After 6 hrs at 4 C	-.19667	.17873	.273	-.5494	.1561
	After 12 hrs At RT	-.65333 <sup>*</sup>	.17873	.000	-1.0061	-.3006
	After 12 hrs at 4 C	-.26000	.17873	.148	-.6128	.0928





**Figures (4.3): Comparison between males and females on different intervals.**

**Table (4.5): INR Analysis results**

	0 Hrs	after 6 hrs At RT	after 6 hrs At 4 C	after 12 hrs At RT	after 12 hrs At 4 C	after 24 hrs At 4 C
Mean	.9367	.9433	1.0033	1.0233	.9967	.9633
Minimum	.80	.80	.90	.90	.90	.90
Maximum	1.00	1.10	1.30	1.20	1.10	1.10

**Table (4.6): Hypothesis test summary**

**Hypothesis Test Summary**

	Null Hypothesis	Test	Sig.	Decision
1	The distributions of 0 Hrs, after 6 hrs At RT, after 6 hrs At 4 C, after 12 hrs At RT, after 12 hrs At 4 C and after 24 hrs At 4 C are the same.	Related-Samples Friedman's Two-Way Analysis of Variance by Ranks	.000	Reject the null hypothesis.

Asymptotic significances are displayed. The significance level is .05.

**Table (4.7): Anova results.**

		Mean Square	F	Sig.
after 6 hrs At RT * 0 Hrs	Between Groups (Combined)	.071	36.749	.000
	Within Groups	.002		
	Total			
after 6 hrs At 4 C * 0 Hrs	Between Groups (Combined)	.003	.331	.721
	Within Groups	.009		
	Total			
after 12 hrs At RT * 0 Hrs	Between Groups (Combined)	.006	1.667	.208
	Within Groups	.004		
	Total			
after 12 hrs At 4 C * 0 Hrs	Between Groups (Combined)	.002	.425	.658
	Within Groups	.004		
	Total			
after 24 hrs At 4 C * 0 Hrs	Between Groups (Combined)	.012	3.863	.033
	Within Groups	.003		
	Total			

**Table (4.8): Paired samples test**

	Paired Differences					t	df	Sig. (2-tailed)	
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference					
				Lower	Upper				
Pair 1	0 Hrs - after 6 hrs At RT	- .00667	.04498	.00821	-.02346	.01013	-.812	29	.423
Pair 2	0 Hrs - after 6 hrs At 4 C	- .06667	.12685	.02316	-.11403	-.01930	- 2.878	29	.007
Pair 3	0 Hrs - after 12 hrs At RT	- .08667	.08604	.01571	-.11879	-.05454	- 5.517	29	.000
Pair 4	0 Hrs - after 12 hrs At 4 C	- .06000	.09322	.01702	-.09481	-.02519	- 3.525	29	.001
Pair 5	0 Hrs - after 24 hrs At 4 C	- .02667	.07849	.01433	-.05598	.00264	- 1.861	29	.073

**Chapter Five**  
**Discussion &**  
**recommendations**

## 5.1 Discussions

The quality of any laboratory result is dependent on the quality of sample collection, transportation and storage. This is due to the fact erroneous result may arise from avoidable mistake such as inappropriate storage temperature. This pre-analytical source of error can be eliminated by taking precaution through confirming to set-down guideline during blood sample collection and storage. My result finding for PT that storage time interval up to 6hr in RT are acceptable. This suggested that sample for PT should be analyzed within 6hr at RT30C in order to ensure reliable result.

\*Mohammed Saghiret al (2012), at Malaysia (PT) was measured at 0, 4, 8 and 24 hours (h). Partial thromboplastin time (APTT) was measured at room temperature (RT) and refrigerator. PT showed insignificant ( $p < 0.05$ ) differences at RT at 4 h, while significant ( $p < 0.05$ ) differences after 8 h and 24 h at RT and after 4 h, 8 h and 24 h at refrigerator were observed. This result agrees with our results.

\*Gamal Hassan, (2015) was test PT at 0, 4, 8, 12 and 24 hr at RT and REF. Result REF acceptable up to 24 hr ( $p < 0.05$ ) at RT. PT at 12 hr non acceptable ( $p < 0.05$ ) this result is agree with our result.

\*Mohamed A. Awad et al (2004) at Egypt was studied influence of storage time and temperature on INR level and plasma activities of vitamin K dependent factors showed no significant different change of INR level after 6, 12 hrs at RT and REF, and at 24 hrs at freezing.

This result is partially agree with our result non-significant up to 6 hrs in RT and REF.

\*Rao, L, V et al( 2000) was studied stability of prothrombin time and activated partial thromboplastin time tests under different storage condition showed that plasma and whole blood sample can be tested PT up to 24 hrs and APTT for up to 12 hrs when transported either at RT or REF, This result is partially accepted with our result .

\* Sultan A et al (2010) PT measured at 0 hr showed no significant difference when compared with measurement at 4 hrs while the difference were significant with measurement at 8 and 24hrs at RT on other hand all samples showed statistically difference when stored I RFE for 4,8 and24 hrs when compared with 0 time. This result is partially agree with our resut.

## **5.2 CONCLUSION:-**

Sample for PT testing can be accepted for 6 RT, 12 REF, 24 freezing while the sample cannot be accepted 12 RT 6 REF.

INR result show no significant up to 6 hrs at RT/REF and 24 hrs freezing. But significant after 12 hrs in RT and REF.



### **5.3Recommendation:-**

1. Plasma for PT better tested immediately or within 6hr at RT.
2. I recommended further study with increase sample size and use of abnormal sample .the necessity for sample storage.
3. The study can be done for all coagulation routine study.

# References

## References

- Alesci S, Borggrefe M, Demple C: 2008, Effect of freezing method and storage at 220uC and 270uC on prothrombin time, aPTT and plasma fibrinogen levels. *Thromb Res* 124:121–126.
- \*Anne ,stiene-martin , Cheryl ,A , lotspeich-Steininger , john A. Koepke ,( 1992). *Clinical hematology* ,second edition ,j.BLippinctt company ,(58)
- \* Erne JB, Mann FA. Surgical haemostasis. *Compend Contin Educ Pract Vet* 2003; 25:732-40.
- \* Hoffbrand A, Vand Pettit JE, editors. *Essential haematology*. 3<sup>rd</sup>ed. London: *Blackwell Science Ltd*; 1993.
- \*LiminFeng ,Yingzhao, Hongcon Zhao and ZhexinShoa. Effect of storage time and temperature on coagulation tests and factors in fresh plasma. *Scientific Reports* 2014.
- \* Mary louse Turgeon, *clinical hematology theory and procedures*, 5<sup>th</sup> edition ,2012, page 413.
- \*Mohamed A. Awad, TarekE.Selim and FatmaA.Al-Sabbagh. Influence of storage Time and Temperature on international Normalized Ratio (INR) Level and plasma activates of vitamins K dependent clotting factors .*tandfonline*2013;9:5-6,333-337.
- \* MohamedSaghiretaloptimization storage condition for coagulation tests.*Journal of College of Physions and SurogenPakistan*(2012).

\* Sultan. A five minute preparation of platelet poor plasma for routine coagulation testing, *Eastern Mediterranean Health Journal* (2010) 16(2) 233-236

# Appendixes

## إقرار

أقر انا/ ..... بكامل قواي العقلية على موافقتي على أخذ عينة من الدم وذلك من أجل إجراء بحث الدكتور / سلوان عادل بابكر ، التابع لجامعة السودان للعلوم والتكنولوجيا ، وذلك في المعمل التشخيصي المتطور ، وهذا مني للاعتماد.



Stago Instrument Image