بِسْمِ ٱللَّهِ ٱلرَّحْمَٰنِ ٱلرَّحِيمِ

Sudan University of Science and Technology

College of Graduate Studies

Effects of Storage Time and Temperature on The samples of Routine Coagulation Tests

تأثيرات وقت التخزين ودرجة الحرارة على عينات اختبارات التخثر الروتينية

A Dissertation Submitted for partial Fulfillment of the requirements for M.Sc. in Medical Laboratory Science (Haematology and immunohematology)

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DECLARATION

We hereby declare that this work is original research work; under taken under the supervision of **Dr. Munsoor Mohammed Munsoor** and has not been presented elsewhere for award of a degree of certificate. All sources have been cited and appropriately acknowledged.

Name……..... Date…….

الآية

قال تعالى:

(سَنُرِيهِمْ آيَاتِنَا فِي الْآفَاقِ وَفِي أَنْفُسِهِمْ حَتَّىٰ يَتَبَيَّنَ لَهُمْ أَنَّهُ الْحَقُّ ۗ أَوَلَمْ يَكْفِ بِرَبِّكَ أَنَّهُ عَلَىٰ كُلِّ شَيْءٍ شَهِيدٌ)

(سورةفصّلت: الآية53)

DEDICATION

To my wife, parents, brothers, sisters and friends

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First of all thanks to **ALMIGHTY ALLAH** for all benefits and for completion of this study.

I would like to thank my supervisor **Dr. Mansur M. Mansur**, who supported and gave us confidence and his time. We wish him a good health.

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Abstract

The main objective of this study To determine the effects of storage temperature and time for prothrombin time and activated partial thromboplastin time at various intervals at both room temperature and refrigerator. This was case study conducted in Turkish Teaching Hospital in Khartoum State during the period from March to April 2017. After obtaining the consent a total of so samples were collected. The blood specimens were processed and analyzed for prothrombin time and activated partial thromboplastin time.

Coagulation tests (Prothrombin time and activated Partial thromboplastin time) were performed immediately specified times after phlebotomy up to 24 hours (0, 2, 4 and 24 hours) both at room temperature (RT) and refrigerator.

Our data show the means results of Prothrombin time(PT) immediately after phlebotomy at 0 hour was (12.5020 seconds) and after 2 hour storage (12.3800 seconds), and after 4 hour (12.3260 seconds) and after 24 hour (14.6100 seconds) at room temperature (RT), and when samples storage at 4℃ the result after 2 hour (12.4940 seconds), 4 hour (12.4920 seconds) and after 24 hour (11.3480 seconds).

The Means results of activated Partial thromboplastin time (APTT) immediately after phlebotomy at 0 hour (hr) was (35.4900 seconds) and after 2 hour storage (36.2640 seconds), and after 4 hour (37.0400 seconds) and after 24 hour (42.0420 seconds) at room temperature (RT), and when samples storage at 4℃ the result after 2 hour (36.4240 seconds), 4 hour (37.0320 seconds) and after 24 hour (42.2240 seconds).

The PT measurements at 0 hr showed non-significant differences when compared with measurements at 2hr with P-vale (0.614) and 4 hr with P-value (0.467) at RT, also showed non-significant differences when compared with measurements at 2hr with P-vale (0.978) and 4 hr with P-value (0.973) at 4℃, while the differences

were significant with measurements at 24 hr at RT and refrigerator with P-value (0.000) for both. There was an increase in the PT results over time when samples were stored at RT and at 24 hr. On the other hand, when samples were stored in the refrigerator, the PT results obtained decreased over time at 24 hours.

For APTT, comparisons between measurements at 0 hr showed non-significant differences when compared with measurements at 2hr with P-vale (0.431) and 4 hr with P-value (0.364) at RT, also showed non-significant differences when compared with measurements at 2hr with P-vale (0.345) and 4 hr with P-value (0.120) at 4°C, while the differences were significant with measurements at 24 hr at RT and refrigerator with P-value (0.000) for both. The levels of APTT measurements were increased over storage time for samples kept at RT and refrigerator.

This study concluded that a prolonged storage time more than 4 hours could induce statistically significant changes for some routine coagulation test results (both at RT and at refrigerator), but that these changes remained within the desirable limits of variation, and had no clinical relevance.

المستخلص

الهدف الرئيسي من هذه الدراسة هو تحديد آثار درجة الحرارة وزمن التخزين على إختتارات وقت البروثرومبين و وقت الثرومبوبلاستين الجزئي على فترات مختلفة في كل من درجة حرارة الغرفة والثلاجة. وكانت هذه دراسة حالة أجريت في المستشفى التركي التعليمي في ولاية الخرطوم خلال الفترة من آذار/مارس إلى نيسان/أبريل ٢٠١٧. وبعد الحصول على الموافقة تم جمع مجموعه من العينات. وقد تمت معالجة عينات الدم وتحليلها من أجل إختبارات وقت البروثرومبين و وقت الثرومبوبلاستين الجزئي المنشط.

وقد أجريت على الفور إختبارات التخثر (وقت البروثرومبين ووقت الثرومببلاستين الجزئي المنشط) في أوقات محددة بعد عملية سحب الدم لمدة تصل إلى ٢٤ ساعة (٠ و٢ و٤ و٢٤ ساعة) في كل من درجة حرارة الغرفة والثلاجة.

تبين بياناتنا نتائج إختبار وقت البروثرومبين مباشرة بعد عملية سحب الدم في الساعة ٠ (١٢.٥٠٢٠ ثانية) وبعد التخزين لمدة ساعتين (١٢.٣٨٠٠ ثانية)، وبعد ٤ ساعات (١٢.٣٢٦٠ ثانية) وبعد ٢٤ ساعة (١٤.٦١٠٠ ثانية) في درجة حرارة الغرفة ، وعند تخزين العينات في الثلاجة كانت النتيجة بعد ساعتين (١٢.٤٩٤٠ ثانية)، و بعد 4 ساعات (١٢.٤٩٢٠ ثانية) وبعد ٢٤ ساعة (١١.٣٤٨٠ ثانية).

متوسط نتائج إختبار ووقت الثرومبوبلاستين الجزئي المنشط مباشرة بعد عملية سحب الدم في الساعة (٠) كانت (٣٥.٤٩٠٠ ثانية) وبعد التخزين لمدة ساعتين (٣٦.٢٦٤٠ ثانية) وبعد ٤ ساعات (٣٧.٠٤٠٠ ثانية) وبعد ٢٤ ساعة (٤٢.٠٤٢ ثانية) في درجة حرارة الغرفة ، وعند تخزين العينات في الثلاجة كانت النتيجة بعد ساعتين (٣٦.٤٢٤٠ ثانية) و بعد ٤ ساعات (٣٧.٠٣٢٠ ثانية) وبعد ٢٤ ساعة (٤٢.٢٢٤٠ ثانية).

وأظهرت قياسات وقت البروثرومبين في الساعة ٠ إختلافات غير هامة عند مقارنتها بالقياسات في الساعة الثانية ذات قيمة P (٠.٦١٤) و ٤ ساعات ذات قيمة P (٠.٤٦٧) في درجة حرارة الغرفة، إختلافات غير هامة أيضا عند مقارنتها بالقياسات في الساعة الثانية ذات قيمة P (٠.٩٧٨) و ٤ ساعات ذات قيمة P (٠.٩٧٣) في الثلاجة، بينما كانت الاختلافات كبيرة حيث كانت القياسات بعد ٢٤ ساعة في درجة حرارة الغرفة والثلاجة ذات قيمة P (٠.٠٠٠) لكلا الفئتين. وكانت هناك زيادة في نتائج وقت البروثرومبين على مر الزمن عندما كانت العينات مخزنة في درجة حرارة الغرفة لمدة ٢٤ ساعة. ومن ناحية أخرى، عندما كانت العينات مخزنة في الثلاجة، انخفضت نتائج وقت البروثرومبين على مدى ٢٤ ساعة.

وفيما يتعلق بنتائج وقت الثرومبوبلاستين الجزئي، أظهرت المقارنات بين القياسات في الساعة ٠ إختلافات غير هامة عند مقارنتها بالقياسات في الساعة الثانية ذات قيمة P (٠.٤٣١) و ٤ ساعات ذات القيمة P (٠.٣٦٤) في درجة حرارة الغرفة، و إختلافات غير هامة عند مقارنتها بالقياسات في الساعة الثانية ذات القيمة P (٠.٣٤٥) و ٤ ساعات ذات القيمة P (١٢٠.٠) في الثلاجة ، بينما كانت الاختلافات كبيرة مع القياسات بعد التخزين لمدة ٢٤ ساعة في درجة حرارة الغرفة والثلاجة ذات القيمة P (٠.٠٠٠) لكلا الفئتين. وقد زادت مستويات قياسات وقت الثرومبوبلاستين الجزئي على مدى وقت التخزين للعينات المحتفظ بها في درجة حرارة الغرفة والثلاجة.

وخلصت هذه الدراسة إلى أن مدة التخزين الطويلة التي تزيد على ٤ ساعات يمكن أن تؤدي الى تغييرات ذات دلالة إحصائية لبعض نتائج إختبارات التخثر الروتينية (في درجة حرارة الغرفة و الثلاجة على حد سواء)، لكن هذه التغييرات بقيت ضمن حدود الاختلاف المرغوب فيها ، وليس لها أي صلة سريرية.

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List of abbreviations

PT	Prothrombin Time
APTT	Activated Thromboplastin Time
RT	Room Temperature
INR	International Normalized Ratio
FFP	Fresh Frozen Plasma
PSGL-1	P-selectin Glycoprotein Ligand
PDGF	Platelet Growth Factor
TT	Thrombin Time
DIC	Disseminated Intravascular Coagulation
ISI	International Sensitivity Index
DVT	Deep Vein Thrombosis
PE	Pulmonary Embolism
PPP	Platelet Poor Plasma
ADP	Adenosine diphosphate

CHAPTER I
INTRODUCTION

CHAPTER I

1. INTRODUCTION

1.1. Introduction

Coagulation tests have been widely applied in clinical practice. Pre-analytical conditions are very important in laboratory assessment (Limin *et al.*, 2014).

The term “pre-analytical phase” describes all actions and aspects of the medical laboratory diagnostic procedure that occurs prior to the analytical phase (Walter 2014)**.**

Pre-analytical laboratory errors can arise throughout the pre-analytical phase, because this phase comprises a lot of manual activities and accounts for most of the errors encountered within the testing process as a whole. Unsuitable, inappropriate or wrongly handled procedures during collection and handling of specimens are very likely to lead to pre-analytical errors (Cornes *et al* 2016)**.** Prothrombin time (PT), international normalized ratio (INR) and the Activated partial thromboplastin time (APTT) are common and readily available coagulation tests used to investigate the pathological changes to the haemostatic and coagulation systems and to monitor clinical therapy particularly among patient on anticoagulant therapy (warfarin and Heparin), patients with thromboembolic events, haemorrhage and to monitor coagulopathy (Ikhuenbor *et al*., 2016).

Prothrombin Time (PT) measures the integrity of the extrinsic system as well as factors common to both systems and Partial Thromboplastin Time (PTT) measures the integrity of the intrinsic system. The results of PT and APTT are used to diagnose haemophilia, are often associated with chronic liver disease, risk factors for thrombosis and are used as indicators for use of fresh frozen plasma (FFP) in

hemorrhaging patients and patients having an invasive procedure (Prelipcean *et al*., 2011; Omidkhoda *et al*., 2011)

1.2. Rationale

Coagulation investigations considered one of the most important tests for evaluation of haemostatic disorders its critical in case of emergencies, and common cause of mortality in the world. They are very sensitive tests and highly affected by many factors (intra and extra individual) for example temperature, storage time, anticoagulant blood ratio, etc. Sometimes samples for coagulation profile storage for later or transferred from lab to another lab to analyze, in this situation the storage time and temperature is very important for assurance of reliable results.

The shortage in application of quality control especially pre-analytical lead to difference in results obtained from different laboratories, especially in Sudan. This study was conducted to evaluate the effects of storage time and temperature on some coagulation screening tests.

1.3. Objectives

1.3.1. General objective

To evaluate the effects of storage time and temperature on routine coagulation tests

1.3.2. Specific objectives

-To estimate PT and APTT immediately at time of sample separation (base line) at 37°c.

-To estimate PT and APTT after two-hour storage at room temperature (22°c) and at 4°c.

-To estimate PT and APTT after four-hour storage at room temperature (22°c) and at 4°c.

-To estimate PT and APTT after 24-hour storage at room temperature (22°c) and 4°c.

- To compare all results after storage condition to base line results.

-To identify the effect of storage time and temperature on results of PT and APTT based on the base line results.

CHAPTER II
LITERATURE REVIEW

Chapter II

2.LITERATURE REVIEW

2.1. Overview of Hemostasis

The concept of blood coagulation dates back to 1960's when Davie, Ratnoff and Macfarlane described the "waterfall" and "cascade" theories outlining the fundamental principle of cascade of proenzymes leading to activation of downstream enzymes(Achneck *et al.,* 2010).

Haemostasis is defined as an active process through which blood coagulation is started and completed in a rapid and steady managed style. Normal haemostasis occurs as a result of several managed processes to achieve two functions; it keeps the blood in a fluid state and causes a quick and restricted haemostatic block at the vascular injury site. Haemostasis acts as a reconciler between procoagulant and anticoagulant forces. Procoagulant factors includes tissue factor (TF), serine proteases of the intrinsic and extrinsic pathways, cofactors, fibrinogen, plasminogen activator inhibitor and an activated charged cell surface membrane (Sultan *et al*.,2012).

The maintenance of circulatory hemostasis is achieved through the process of balancing bleeding (hemorrhage) and clotting (thrombosis). Hemostasis, the arresting of bleeding, depends on several components. The four major components are the vascular system, platelets (thrombocytes), blood coagulation factors, and fibrinolysis and ultimate tissue repair. Three other, less important, components are the complement and kinin systems as well as serine proteaseinhibitors (Turgeon, 2005).

There are three main phases of haemostasis, the vascular phase (which incorporates platelet interaction), the coagulation phase (which involves

coagulation proteins and phospholipid complexes), and the natural anticoagulant/fibrinolytic phase (which ensures cessation of the clotting process, the dissolution of the thrombosis, and permits later wound healing). These components work in concert. When any phase of this process is disturbed, adverse clinical events may occur, manifesting as either thrombosis (haemostasis pushed in a procoagulant direction) or bleeding (ineffective haemostasis). In either situation, the clinician must have access to a variety of relevant tests in order to achieve a specific or correct diagnosis and initiate appropriate patient treatment or management(Bonar *et al.*, 2010).

2.1.1. Primary haemostasis

Primary haemostasis refers to the process that involves platelets adhering to each other and to the damaged vasculature subsequent to the initiating event (e.g., tissue injury), followed by platelet activation and aggregation to produce a 'platelet-plug'(Bonar *et al.*, 2010).

When the endothelium is injured, the procoagulant subendothelial matrix (consisting of proteins, such as collagen, von Willebrand factor (vWF), fibrinogen (FBG), laminin, and fibronectin) is exposed, and the subendothelial matrix proteins bind to glycoprotein receptors on the platelet surface and immediately initiate primary hemostasis, consisting of platelet adhesion, platelet activation, and platelet plug formation (Repetto *et al.*, 2017). Platelet adhesion is mediated by von Willebrand Factor (vWF) that binds to Gp Ib-IX in the platelet membrane. vWF is a blood Gp that serves as an adhesive protein, which could bind to other proteins, especially factor VIII at the wound sites (Umbaut and Thiagarajan, 2010) . Adhered platelets undergo degranulation and release cytoplasmic granules that contain serotonin, platelet activating factors and ADP. Platelets are activated to change shapes into a pseudopodal form upon the adhesion to the injured area which will activate the collagen receptors on their surface membrane, named

GpIIbIIIa, to undergo release reactions. The GpIIbIIIa complex, organized through calcium-dependent association of GpIIb and GpIIIa that is a necessary step in platelet aggregation and endothelial adherence (Periayah *et al.,* 2017). collagen binding to platelet GPVI, and integrin 2 1 receptors trigger a signal transduction process resulting in the local release of platelet activation agonists from dense granules, such as thromboxane A2 and ADP. These agonists, along with thrombin produced from coagulation cascades and activated platelets, bind to platelet surface-bound G-coupled receptors, inducing further platelet activation. Activated platelets release vWF and coagulation factors from granules, which lead to platelet plug formation (vWF and fibrinogen) and fibrin formation (factor V and factor XIII). The granules also contain adhesion molecules, such as P-selectin, which is expressed on the platelet surface after activation and binds in vivo to its ligand P-selectin glycoprotein ligand 1 (PSGL-1) on endothelial cells and leukocytes; thus, leukocytes offer a surface scaffold for fibrin formation. This helps platelets bind firmly to the endothelium and allows leukocytes to be incorporated into developing clots (Repetto *et al.*, 2017).

2.1.2. Secondary haemostasis

Secondary haemostasis involves the activation of the clotting system in a complex interactive fashion with positive and negative feedback, that causes sequential activation of multiple coagulation factors and the eventual conversion of ‘soluble’ fibrinogen (the main clottable protein in plasma) to ‘insoluble’ fibrin. The process of secondary haemostasis in part acts to strengthen the platelet plug, forming a gel-like clot.

This process is kept in check, in part, by the naturally occurring anticoagulant system, e.g., protein C (PC), protein S (PS), antithrombin (AT). The fibrinolytic

system represents a distinct, but inter-related process that is involved in lysis of the clot to facilitate wound healing (Bonar *et al.*, 2010).

Secondary hemostasis refers to the cascade of enzymatic reactions that ultimately results in the conversion of fibrinogen to fibrin monomers. Fibrin monomers are then cross-linked into insoluble strands that serve to stabilize the loose platelet clot formed in primary hemostasis. Secondary hemostasis is triggered by the release of tissue factor from epithelial cells that are exposed to the circulation at the site of vascular injury (John, 2002).

It is required to control bleeding from large wounds and is a continuation of the primary hemostatic mechanisms. Whereas the outcome of primary hemostasis is the formation of the platelet plug, the outcome of secondary hemostasis is the formation of a thrombus (Henry, 2002).

The coagulation cascade of secondary hemostasis has two initial path ways which lead to fibrin formation. These are the contact activation pathway (also known as the intrinsic pathway), and the tissue factor pathway (also known as the extrinsic pathway), which both lead to the same fundamental reactions that produce fibrin. It was previously thought that the two pathways of coagulation cascade were of equal importance, but it is now known that the primary pathway for the initiation of blood coagulation is the tissue factor (extrinsic) pathway. The pathways are a series of reactions, in which a zymogen (inactive enzyme precursor) of a serine protease and its glycoprotein co-factor are activated to become active components that then catalyze the next reaction in the cascade, ultimately resulting in cross-linked fibrin. Coagulation factors are generally indicated by Roman numerals, with a lowercase a appended to indicate an active form (Pallister and Watson 2010).

In the extrinsic pathway exposure of sub endothelial TF (tissue factor) complexed with activated FVII, known as the extrinsic Xase, catalyzes the generation of FXa (activated FX). In the intrinsic pathway initially involving the sequential activation

of FXII, FXI, and FIX leads to formation of the intrinsic Xase, a complex of FVIIIa (activated factor VIII) and FIXa (activated factor IX), that also catalyzes the generation of FXa. FXa in complex with its cofactor FVa forms the prothrombinase complex that catalyzes the cleavage of prothrombin (FII) to thrombin (FIIa). Thrombin as the terminal coagulation protease catalyzes the formation of insoluble fibrin through cleavage of soluble fibrinogen monomers and activation of the transglutaminase FXIII. In addition, thrombin is a potent activator of platelets through cleavage of cell surface protease-activated receptors (Steven and Nigel, 2019).

2.2. Tests and assays within haemostasis

The modern haemostasis laboratory performs a large number of distinct tests, often using a variety of methodologies. All haemostasis laboratories perform routine coagulation tests comprising the prothrombin time (PT)/international normalized ratio (INR) and the activated partial thromboplastin time (APTT), sometimes supplemented by specific fibrinogen assays, and occasionally thrombin time (TT) assays. Most routine test laboratories also perform D-dimer assays. These tests are variably performed to investigate hemostasis in patients suspected of having a potential dysfunction in the secondary hemostasis pathway, either congenital (eg, hemophilia) or acquired (eg, disseminated intravascular coagulation [DIC]) (Emmanuel *et al.*, 2012).

2.2.1. Routine coagulation tests

Laboratories usually offer a set of tests (the coagulation screen) that aims to identify most clinically important hemostatic defects. Invariably this includes the PT, APTT, fibrinogen, and usually thrombin time. It is important to perform a full blood count to quantify the platelet count, but assessment of platelet function is not usually offered or performed in the initial tests (Nigel *et al.*, 2009).

2.2.1.1. Prothrombin time (PT)

PT is a widely used test to evaluate secondary hemostasis. In this test, platelet poor plasma from a patient (collected in a blood collection tube containing sodium citrate) is mixed with thromboplastin and calcium, and then clotting time is determined at 37℃ using a variety of methods, including photo optical and electromechanical. Automated coagulation analyzers are commercially available for measuring PT along with other coagulation parameters. PT is a functional measure of the extrinsic pathway and the common pathway, and the reference range is 10.0-15.0 sec. Therefore, PT is a useful test to detect inherited or acquired defects in coagulation related to the extrinsic pathway (Amer and Amitava 2015).

The PT is sensitive to and thus prolonged in patients with Liver disease and deficiencies of Vitamin K, factors VII, X, V, and II and fibrinogen. It is particularly useful in monitoring anticoagulation in patients on warfarin (Nigel Key *et al.,* 2009).

2.2.1.2. International Normalized Ratio (INR)

Because thromboplastins are produced using different methods and from different sources, the sensitivity of an individual thromboplastin to another can vary greatly between and within lots. Variance can even occur within a single batch depending on shelf time of the reagent. To help standardize the difference in sensitivity in individual thromboplastin reagents and the effect on PT assays, two approaches have been developed to standardize results. The first was the International Sensitivity Index (ISI) and the second was the International Normalized Ratio (INR). The INR was developed to incorporate the ISI values and attempt to make prothrombin results uniformly useable.

INR use has been recommended for monitoring oral anticoagulant therapy. It is important to emphasize that the INR is not a new laboratory test. It is simply a mathematical calculation that corrects for the variability in PT results caused by

variable sensitivities (ISI) of the thromboplastin agents used by laboratories. INR = (Patient PT ÷ Control PT) ^{ISI} (Mary, 2012).

2.2.1.3. Activated partial thromboplastin time (APTT)

The APTT is performed by first adding a surface activator (eg, kaolin, celite, ellagic acid, or silica) and diluted phospholipid (eg, cephalin) to citrated plasma. The phospholipid in this assay is called partial thromboplastin because tissue factor is absent. After incubation to allow optimal activation of contact factors (factor XII, factor XI, prekallikrein, and high-molecular-weight kininogen), calcium is then added, and the clotting time is measured. Although the clotting time varies according to the reagent and coagulometer used, the aPTT typically ranges between 22 and 40 seconds. The aPTT may be prolonged with deficiencies of contact factors; factors IX, VIII, X, or V; prothrombin; or fibrinogen. Specific factor inhibitors, as well as nonspecific inhibitors, may also prolong the aPTT. Fibrin degradation products and anticoagulants (eg, heparin, direct thrombin inhibitors, or warfarin) also prolong the aPTT, liver disease and DIC (Shannon, 2005).

2.2.1.4. Thrombin time (TT)

The thrombin time test determines the rate of thrombin induced cleavage of fibrinogen to fibrin monomers and the subsequent polymerization of hydrogen-bonded fibrin polymers to form an insoluble fibrin clot. The normal value is less than 20 seconds. Prolonged results will be seen if the fibrinogen concentration is less than 100 mg/dL. Abnormal results will also be encountered in the presence of thrombin inhibitors or substances that interfere with fibrin formation (e.g., heparin, fibrin degradation products), or high concentrations of immunoglobulins that interfere with fibrin monomer polymerization such as in cases of multiple myeloma (Mary, 2012).

2.2.1.5. Fibrinogen

Fibrinogen assays are useful in detecting deficiencies of fibrinogen and alterations in the conversion of fibrinogen to fibrin. Fibrinogen can be quantitated by various methods including precipitation or denaturation methods, turbidimetric or fibrin clot density method, coagulable protein assays, immunological assays, and the modifi ed thrombin time. The normal value of 200 to 400 mg/dL may be decreased in liver disease or the consumption of fibrinogen owing to accelerated intravascular clotting. Fibrinogen titers may be useful. The normal titer of fibrinogen is 1:128 to 1:256; a titer less than 1:64 is abnormal (Mary, 2012).

2.2.1.6. D-dimer

This represents a specific breakdown fragment of fibrin, and so is a measure of fibrinolysis. This test is important in order to assess the possibility of clot dissolution as a marker of thrombosis; e.g., deep vein thrombosis (DVT) or pulmonary embolism (PE) or aberrant clot activation processes such as DIC (Roslyn *et al*., 2010)

2.3. Pre-analytical Issues

Errors within the pre-analytical phase, which far exceeds those occurring during both the analytical and the post-analytical phase, account for around two- third of the total errors recorded in medical laboratories, particularly for coagulation testing (Toulon *et al.*, 2017).

Laboratory data must be accurate and reliable as erroneous results may lead to patient misdiagnosis and therefore therapeutic misadventures. Inaccuracy in laboratory reporting can arise due to problems introduced at any point in the testing process, from sample collection to report results. Due to significant advances in instruments and informatics, the analytical and reporting phases of testing do not contribute significantly to laboratory error. Rather, the pre-analytical phase of testing represents the major source of inaccurate laboratory results. The pre-analytical phases of testing include specimen collection, transportation of whole

blood specimens to the laboratory, specimen processing and storage (Roslyn *et al*., 2010).

2.3.1. Sample collection

One of the most critical aspects of specimen collection is proper patient and also sample identification. Specimens must be collected in a manner to avoid in vitro clot development. Samples should be collected in a relatively atraumatic fashion and blood should flow freely into the specimen container. Samples for haemostasis testing should be anticoagulated with sodium citrate. The World Health Organization (WHO) and CLSI recommend 3.2% sodium citrate, although 3.8% might also be acceptable (Roslyn *et al*., 2010).

Coagulation samples should preferably be collected before other test samples are drawn, if these contain stronger anticoagulant agents such as ethylenediaminetetraacetic acid (EDTA) (for a complete blood count), lithium-heparin (for clinical chemistry testing), as well as clot activators (ie, thrombin), since these materials may contaminate a subsequent coagulation test sample. A specific sequence of tube collections (so-called “order of draw”) is provided by the CLSI (Emmanuel *et al.*, 2012).

The phlebotomy procedure provided by the CLSI requires wearing gloves, cleansing the venipuncture site and drying of skin before applying the tourniquet and selecting the venipuncture site and vein. The venipuncture should be ideally collected directly from a peripheral vein (antecubital vein). If a tourniquet needs to be used, it should immediately be released when the first tube starts to fil. The diameter of the needle should preferably be comprised between 19 and 22 gauge (Magnette *et al.*, 2016).

It is important to take samples so as to reduce platelet activation in-vitro and to restrict the use of the tourniquet. Extended tourniquet application might produce unnecessary venous stasis or in-vitro haemolysis,

which could introduce spurious and clinically meaningfulbiases in the measurement of several haematologic parameters. Hence, the tourniquet should be placed tightly but less than one minute in order to prevent haemoconcentration, increased fibrinogen and factors VII, VIII, XII as well as activation of endothelial cells and therefore fibrinolysis. Some special coagulation assays, such as those that measure thrombin generation markers (e.g., thrombin antithrombin complex (TAT) and prothrombin fragment 1+2), should be drawn without the use of a tourniquet because that may lead to spurious elevation of these markers, particularly if the tourniquet is left in place for more than one minute.(Magnette *et al.,* 2016).

2.3.2. Transportation of samples

Samples should be transported non-refrigerated at ambient temperature (15–25 °C) in as short a time as possible. Ideally, testing for routine coagulation tests like the PT and the APTT should be accomplished within 4 hours of collection, although allowable tolerances may be greater than this. However, APTT testing for unfractionated heparin monitoring should preferably be processed within 1 hour due to the potential for heparin neutralization by platelet releasates. Extremes of temperature (ie, both refrigerated or high) should be avoided. Delays in transport may affect in particular the labile factors (FV, FVIII), leading to prolonged clotting times and *in vitro* loss of factor activity. In such cases, local centrifugation and separation of plasma followed by freezing and frozen transport of the plasma should be considered (Emmanuel *et al.,* 2012).

2.3.3. Sample Processing and Storage

This should also in general proceed as per current CLSI guidelines, noting limitations according to which test is being performed. Most coagulation-based tests, including PT, APTT, and clotting factor assays, are performed on plasma derived from once-centrifuged samples. Centrifugation should ideally be at 1500 g

for a minimum of 10-15 minutes.16 Shorter centrifuge times might be acceptable for routine coagulation tests performed immediately post-centrifugation when there are no subsequent test requirements (ie, plasma not to be frozen or processed for additional assays). Using centrifugal forces greater than 1500 g are not recommended as this may induce platelet activation and lysis of RBCs (Emmanuel *et al.,* 2012).

The stability of coagulation samples varies depending on a number of variables such as the blood collection system, whether the samples are stored as whole blood or centrifuged, the temperature at which samples are maintained during storage, the reagent/instrument system used for analysis, and the test parameter to be analyzed. In general, to afford the greatest sample integrity, samples should be processed as quickly as possible (ideally within 1 hour of collection) and testing performed within 4 hours of procurement (or else be processed by centrifugation and plasma frozen). During this short-term storage, whole blood samples should be kept capped and maintained at room temperature. If testing is not to be performed within about 4 hours for the APTT and 24 hours for the PT, the plasma should be separated from the cellular fraction of the once or twice-centrifuged sample, without disturbing the cell pellet. For many tests of hemostasis, the separated plasma can be safely frozen for later testing. Separated plasma can generally be maintained at room temperature or refrigerated for a few hours without an adverse effect on coagulation. Otherwise, separated plasma samples should be frozen (Emmanuel *et al.*, 2012).

CHAPTER III

MATERIALS AND METHODS

Chapter III

3. Materials and Methods

3.1. Study design

This is a descriptive case study.

3.2. Study area

This study was conducted in Turkish Teaching Hospital in Khartoum State.

3.3. Study duration

During the period from March to April 2017 the study was done.

3.4. Study population

The study involved individuals who visited the Turkish Teaching Hospital in Khartoum State.

3.5. Ethical consideration

Verbal consent was obtained from each patient participated in this study.

3.6. Collection of specimens

About 3.7 milliliters of blood sample was collected from each subject aseptically using the venepuncture technique. The blood was collected into tubes containing sodium citrate anticoagulant.

3.7. Data Collection

From internet, published research, scientific paper, books and references.

3.8. Sample processing

The citrated samples were centrifuged at 3000 rpm for 5 minutes to obtain clear non-haemolysed citrated plasma. The plasma was transferred into sterile labeled test tubes and assayed (in batches) for PT and APTT.

3.8.1. Sample technique

3.8.1.1. Prothrombin time

Calcified thromboplastin reagent was pre-warmed to 37°C. 0.1ml of plasma (PPP) was placed into labeled coagulation cup using the automatic pipette and pre-warmed to 37°C; 0.2 ml of Calcified thromboplastin reagent was forcibly added to each sample cup, using the automatic pipette (switch in the "on" position); and the timer was automatically started. The timer was stopped when clot formation occurred. The time taken for clot formation was recorded. Each sample was performed in duplicate.

3.8.1.2. Activated partial thromboplastin time

Calcium chloride reagent was Pre-warmed to 37°C. 0.1 mL of patient sample was pipetted into labeled coagulation cup using the automatic pipette; 0.1 mL of partial thromboplastin reagent was added to each sample cup, using the automatic pipette. The contents of each cup were mixed carefully. Sample-partial thromboplastin reagent mixture were allowed to pre-warm to 37°C for 2 minutes. 0.1 mL of pre-warmed calcium chloride reagent was added into coagulation cup containing sample-partial thromboplastin reagent mixture using the automatic pipette (switch in the "on" position); the calcium chloride reagent was dispensed and the timer was automatically started. The timer was stopped when clot formation occurred. The time taken for clot formation was recorded. Each sample was performed in duplicate.

3.8.2. Reagent

BioMed-Liquiplastin (PT) and BioMed-Liquicellin-E (APTT) kits , was used for quantitative determination of PT and APTT.

BioMed-Liquiplastin is a highly sensitive, ready to use liquid calcified liquiplastin reagent for quantitative determination of PT,which is derived from rabbit brain.

BioMed-Liquicellin-E is liquid ready to use activated cephaloplastin reagent for quantitative determination of APTT, it is phospholipids preparation derived from rabbit brain with ellagic acid as an activator.

3.8.3. Quality Control

Quality control was applied in all steeps from sample collection to sample analysis .

3.9. Data analysis

3.9.1. Statistical analysis

Statistical analysis was performed using statistical package for social sciences (SPSS) version 21. ANOVA test were used for comparison of data. The results were presented as mean. A p-value of ≤ 0.05 was considered as significant in all statistical comparisons.

CHAPTER IV
RESULTS

Chapter IV

RESULTS

This study evaluated the effects of time and temperature variables on routine Prothrombin Time test and Activated Partial Thromboplastin Time (APTT) test among subjects of Sudanese, Khartoum state.

Fifty samples were collected, made up of 26 (50.5%) male and 24 (49.5%) female subjects (Table 1). In this study, PT and APTT samples were analyzed and compared to the initial result tested at 0 hr. The room temperature (RT) range was 20 - 24°C and the average was 22°C. The range temperature of the refrigerator was 3 - 5°C and the average was 4°C. Coagulation tests were performed immediately specified times after phlebotomy up to 24 hours (0, 2, 4 and 24 hours at room temperature of 22°C, and at refrigerator of 4°C.PT results obtained at 0 hr was compared with those obtained at 2, 4, and 24 hours at RT and refrigerator.

All PT results for 50 subjects were within the normal range (11.348 - 14.61 seconds).INR calculated for each PT result at (0, 2, 4 and 24 hours at room temperature of 22°C, and at refrigerator of 4°C. All the INR results were within the normal range (1.0573 - 1.1249). On the other hand, the measurements of APTT at 0 hr were compared with measurements at 2, 4, and 24 hours at RT and refrigerator. All the measurements were within the normal range (35.49 – 42.224 seconds). (Table 1) show the mean of PT and APTT at RT and 4°C.

PT measurements when samples were stored in RT at 0 hr (12.5020) showed non-significant differences when compared with measurements at 2hr(12.3800) P-value (0.614) and at 4 hr (12.3260) P-value (0.467), while the differences were significant with measurements at 24 hr (14.6100) P-value (0.000). Also PT

measurements when samples were stored in 4℃ at 0 hr (12.5020) showed non-significant differences when compared with measurements at 2hr(12.4940) P-value (0.978) and at 4 hr (12.4920) P-value (0.973), while the differences were significant with measurements at 24 hr (11.3480)P-value (0.000). There was an increase in the PT results over time when samples were stored at RT and at 24 hr. On the other hand, when samples were stored in the refrigerator, the PT results obtained decreased over time at 24 hours (Table 2).

For APTT, comparisons between measurements when samples were stored in RT at 0 hr (35.4900), 2 hr (36.2640) P-value (0.431) and 4 hr (37.0400) P-value (0.364) showed non-significant differences. However, there were statistically significant differences with measurements at 24 hr(42.0420)P-value(0.000). Also APTT measurements when samples were stored in 4℃ at 0 hr (35.4900) showed non-significant differences when compared with measurements at 2hr (36.4240) P-value (0.345) and at 4 hr (37.0320) P-value (0.120). However, there were statistically significant differences with measurements at 24 hr(42.2240)P-value(0.000) (Table 3).

The means for APTT measurements were increased slightly when samples were stored for 2 hours at RT and refrigerator. Maximum level was observed at 24 hours at RT and refrigerator. Moderate increase was noted at 4 hours at both RT and refrigerator. The levels of APTT measurements were increased slightly over storage time for samples kept at RT and refrigerator.

Table 1. Mean of PT and APTT at RT and 4℃

Conditions	PT		APTT	
	4℃	RT	4℃	RT
Base(0hr)	12.5020		35.4900	
2hr	12.4940	12.3800	36.4240	36.2640
4hr	12.4920	12.3260	37.0320	37.0400
24hr	11.3480	14.6100	42.2240	42.0420

Table 2. Comparison between PT measurements at different conditions

Time (hr)	RT (22℃)	P. value	4℃	P. value
Base(0hr)	12.5020	-	-	-
2hr	12.3800	0.614	12.4940	0.978
4hr	12.3260	0.467	12.4920	0.973
24hr	14.6100	0.000	11.3480	0.000

Samples (n = 50) were stored at room temperature & refrigerator and tested at 0, 2, 4 and 24 hr. Values are represented as mean; Oneway ANOVA test has been used. 0.05 considered significant.

Table 3. Comparison between APTT measurements at different conditions

Time (hr)	RT (22℃)	P. value	4℃	P. value
Base(0hr)	35.4900	-	-	-
2hr	36.2640	.431	36.4240	.345
4hr	37.0400	.364	37.0320	.120
24hr	42.0420	.000	42.2240	.000

Samples (n = 50) were stored at room temperature & refrigerator and tested at 0, 2, 4 and 24 hr. Values are represented as mean; Oneway ANOVA test has been used. 0.05 considered significant.

CHAPTER V
DISCUSSION

Chapter V

DISCUSSION

The diagnosis of coagulation disorders and monitoring of anticoagulant therapy usually depend on Prothrombin Time and Activated Partial Thromboplastin Time values. Because reliable measurements of coagulation function and coagulation factor activity could be affected by sample storage temperature and time from sample collection to testing, coagulation tests should be carried out as soon as possible after collection of the blood samples.

In this present study, PT and APTT tests were performed immediately at specified times after phlebotomy up to 24 hours (0, 2, 4 and 24 hours at 4℃ and room temperature of 22 degrees C).

In this study, our data demonstrate PT measurements at 0 hr showed non-significant differences when compared with measurements at 2 and 4 hr, while the differences were significant with measurements at 24 hr at RT and 4℃. There was an increase in the PT results over time when samples were stored at RT at 24 hr. On the other hand, when samples were stored in the refrigerator, the PT results obtained decreased over time at 24 hours. These results agree with results of Sultan *et al*. (2012) and Toulon *et al*. (2017) whom concluded that PT measurements at 0 hr showed non-significant differences when compared with measurements at 4 hr, while the differences were significant with measurements at 8 and 24 hr at RT, also there was an increase in the PT results over time while the samples which stored in the refrigerator obtained a decreased PT results over time and the minimum level was at 24 hours. Also agree with results of Zhao and Lv (2013) whom revealed that PT results following 4, 8 and 24 hr storage were statistically significantly differences at RT and 4 °C.

For APTT, comparisons between measurements at 0, 2 and 4 hours showed non significant differences, when samples stored at RT or in refrigerator. However, there were statistically significant differences with measurements at 24 hours at both RT and Refrigerator. The means for APTT measurements were increased slightly when samples were stored for 2 hours at RT and refrigerator. Maximum level was observed at 24 hours at RT and refrigerator. Moderate increase was noted at 4 hours at both RT and refrigerator. These results agreed with results of Toulon *et al*. (2017) and Adcock *et al.* (1998) whom found that APTT test results obtained after a 4, 6, or 8-hours storage vs a <2-hours storage demonstrated statistically significant differences. However, it could be mentioned that differences between APTT results evaluated after storages longer than 2 hours, that is, 4, 6, and 8 hours, were not statistically significant. These results disagree with Sultan *et al*. (2012) study which revealed a statistically significant differences with measurements at 6 hours, and 8 hours at both RT and Refrigerator.

5.2. Conclusion

This study concluded that a prolonged storage time more than 4 hours could induce statistically significant changes for some routine coagulation test results (both at RT and at refrigerator), but that these changes remained within the desirable limits of variation, and had no clinical relevance.

5.3. Recommendations

1. PT and APTT should be analyzed as soon as collection of blood.

2. Samples for PT and APTT should not stored more than 4hr.

3. . PT and APTT samples should be accepted only up to 4 hr when kept at RT and at refrigerator, but cannot be accepted for more than 4 hr and above when kept at PT and at refrigerator.

4. More studies should be carried out to validate these results.

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