Sudan University of Science and Technology

College of Graduate Studies

Measurement of Prothrombin Time and Activated Partial Thromboplstin Time among Johnson and Johnson COVID-19 Vaccine Recipients in The Military Hospital in Khartoum State

قياس زمني البروثرومبين والثرومبوبلاستين الجزئي النشط بين متلقي لقاح كوفيد-19 جونسون وجونسون في مستشفى السلاح الطبي في ولاية الخرطوم

A Dissertation Submitted for Partial Fulfillment of the Requirements for M.Sc. Degree in Medical Laboratory Science (Hematology and Immunohematology)

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الاية

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

{قَالُوا سُبْحَانَكَ لَا عِلْمَ لَنَا إِلَّا مَا عَلَّمْتَنَا إِنَّكَ أَنتَ الْعَلِيمُ الْحَكِيمُ}

صدقاللهالعظيم

سورةالبقرة الاية (32)

Dedication

I dedicate my thesis to my parents for their endless love, support and encouragement throughout my pursuit for education. I hope this achievement will fulfill the dream they envisioned for me.

I also have to dedicate it to my sister Fatima and my brothers Wally, Ahmed and Awab.

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First and foremost, I would like to thank God Almighty for giving me the strength, knowledge, ability and opportunity to undertake this research study and to persevere and complete it satisfactorily. Without his blessings, this achievement would not have been possible.

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Abstract

Johnson and Johnson COVID-19 vaccine although mostly safe and effective, has the potential risk of developing blood clots, alongside other possible side effects.

This was analytical cross-sectional study to analyze the effect of Johnson and Johnson COVID-19 vaccine on prothrombin time (PT) and activated partial thromboplastin time (APTT) among the vaccine recipients. The study was conducted in the vaccination center in the military hospital in Khartoum State, during the period between March 2022 to June 2022.

The study population compromise of 52 recipients of Johnson and Johnson COVID-19 vaccine, samples were taken before and after the administration of the vaccine. The participants' age varies from 19 to 61 years old with 38 males and 14 females.Data was collected using structured questionnaire which included general information about the participants and a venous blood sample 1.8ml was collected in 3.2% tri sodium citrate container from each participant. Then, plasma was separated by centrifugations for 15 minutes and estimation of the PT and APTT was performed by using semi –automated biochemical analyzer (ES-105). The collected data was analyzed by using SPSS ver 26.

The results showed that the pre vaccine mean level of PT and APTT was 13.8 ± SD1.2 and 33.54 ± SD 3.4 respectively. Whereas the post vaccine means level of PT and APTT was 13.9 ± SD 1.4 and 33.46 ± SD 2.75 respectively. Showing that the difference between pre and post mean levels of the two tests were very small and were statistically not significant. The respective p-values were 0.361 and 0.810. The results showed that age, gender, posttest date and the interaction between gender and posttest date had no significant effects on the differences between pre and post levels of each of the two tests (PT& APTT).

The study concluded that the mean level of PT and APTT before and after the administration of Johnson and Johnson COVID-19 vaccine were statistically not significant and that age, gender, posttest date and their interaction had no significant effects on the before and after levels of each of the two tests (PT& APTT).

المستخلص

على الرغم من أن لقاحجونسون وجونسون كوفيد-19 امن وفعال، الا أنه ينطوي على مخاطر محتملة لتطور جلطات الدم جنبا الى جنب مع الاثار الجانبية المحتملة الاخرى.
كانت هذه دراسة مقطعية تحليلية لتحليل تأثير لقاح جونسونوجونسون كوفيد-19 على زمن البروثرومبين (PT) ووقت الثرومبوبلاستين الجزئي النشط (APTT)بين متلقي اللقاح. أجريت الدراسة بمركز التطعيم بمستشفى السلاح الطبي بولاية الخرطوم خلال الفترة من مارس 2022 إلى يونيو2022.

تتكون مجموعة الدراسة من 52 متلقيًا للقاح جونسون وجونسون كوفيد-19 ، تم أخذ العينات قبل وبعد إعطاء اللقاح.تتراوح أعمار المشاركين من 19 إلى 61 عامًا مع 38 ذكرًا و 14 إناثًا. تم جمع البيانات باستخدام استبيان منظم تضمن معلومات عامة عن المشاركين واخذ عينة دم وريدية 1.8 مل في حاوية تحتوي على 3.2% ثلاثي سيترات الصوديوم من كل مشارك. بعد ذلك ، تم فصل البلازما عن طريق جهاز الطرد المركزي لمدة 15 دقيقة وتم قياس (PT and APTT) بواسطة محلل كيميائي حيوي شبهآلي(ES-105). تم تحليل البيانات التي تم جمعها باستخدام الإصدار 26 من برنامج الاحصاءSPSS.

أظهرت النتائج أن متوسط مستوى ما قبل اللقاح لـ PT و APTT كان 13.8 ± SD1.2 و 33.54 ± SD 3.4 على التوالي. في حين أن متوسط مستوى ما بعد اللقاح لـ PT و APTT كان 13.9 ± SD 1.4 و 33.46± SD 2.75 على التوالي. تبين أن الفرق بين المستويات المتوسطة السابقة واللاحقة للاختبارين كان ضئيلاً للغاية ولم يكن معنوياً إحصائياً .كانت قيم p الخاصة بها 0.361 و 0.810 .
أظهرت النتائج أن العمر والجنس وتاريخ الاختبار اللاحق والتفاعل بين الجنس وتاريخ الاختبار البعدي لم يكن لهم تأثير معنوي على الفروق بين المستويات السابقة واللاحقة لكل من الاختبارين (PT،APTT).

خلصت الدراسة إلى أن متوسط مستوى اختبار PTو APTT قبل وبعد إعطاء لقاح جونسون و جونسون كوفيد-19 لم يكن ذا دلالة إحصائية وأن العمر والجنس وتاريخ الاختبار اللاحق والتفاعل بين الجنس وتاريخ الاختبار اللاحق لم يكن لهم آثار معنوية. على المستويات قبل وبعد كل اختبار من الاختبارين (PT and APTT).

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List of Abbreviations

ADP Adenosine diphosphate

ANCOVA Analysis of covariance

APTT Activated partial thromboplastin time

ATP Adenosine triphpsphate

BMI Body mass index

BTG Beta-thromboglobulin

Ca Calcium

CaCl Calcium chloride

CDC Center for disease control

CI Confidence intervals

COVID-19 Coronavirus disease of 2019

CSF Colony stimulating factors

DNA Di-ribonucleic acid

EC Endothelial cell

EDRF Endothelium derived relaxing factor

FDA Food and drug adminstration

GP Glycoprotein

GS Guillain-Barré Syndrome

HMWK High moleculer weight kininogen

IM Intra muscular

INR International normalization ratio

J&J Johnson and Johnson

LAMP Loop mediated isothermal amplification

MERS Middle east respiratory syndrome

mRNA Messenger ribonucleic acid

NA Nucleic acid

NAAT Nucleic acid amplification test

NACI National advisory committee on immunization

NO Nitric oxide

PAI Plasminogen activator inhibitor

PCR Polymerase chain reaction

PF4 Platelet factor 4

PGI2 Prostaglandin I2

PL Phospholipid

PPP Platelet poor plasma

PT Prothrombin time

RE Reticulo-endothelial system

RNA Ribonucleic acid

SARS Severe acute respiratory syndrome

SARS-CoV-2 Severe acute respiratory syndrome coronavirus 2

Scu PA Single chain urokinase plasminogen activator

SD Stander deviation

SPSS Statistical package for the social sciences

tcu PA Two chain urokinase plasminogen activator

TFPI Tissue factor pathway inhibitor

tPA Tissue plasminogen activator

TTS Thrombosis with thrombocytopenia syndrom

TXA2 Thromboxane A2

US United states

VAERSVaccine adverse event reporting system

vWF Von willebrand factor

WHO World health organization

CHAPTER I

Introduction

1.1 Introduction

Coronavirus disease 2019 (COVID-19) is a contagious disease caused by the acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The first known case was identified in Wuhan, China, in December 2019. The disease quickly spread worldwide, resulting in the COVID-19 pandemic. (Wikipedia, 2022)

For most people, symptoms of COVID-19 will be mild and can be managed at home and it tend to show 2 to 5 days after you have been infected but it can take up to 14 days. Some people may develop severe symptoms that may need hospitalization. (WHO, 2020)

To protect yourself and others, it's important to follow the rules restricted by the country and to get vaccinated. Vaccination is one of the most effective ways to protect ourselves and our communities against COVID-19 and there are different types of vaccines which are: mRNA vaccines (Pfizer-BioNTech and Moderna), viral vector (adenovirus) vaccines (Astra-Zeneca, Johnson & Johnson, Rethera and Sputnik), inactivated virus vaccines (Sinovac) and protein subunits vaccines (Novavax). (CDC, 2022)

COVID-19 vaccines are safe and effective and severe reactions after vaccination are rare. CDC recommends COVID-19 vaccines for everyone ages 6 months and older, and boosters for everyone 5 years and older, if eligible. The benefits of COVID-19 vaccination continue to outweigh any potential risks.

Adverse events of the vaccination include: Anaphylaxis which is rare and has occurred at a rate of approximately 5 cases per one million vaccine doses

administered. Anaphylaxis, a severe type of allergic reaction, can occur after any kind of vaccination. (CDC, 2022)

Thrombosis with thrombocytopenia syndrome (TTS) after J&J/Janssen COVID-19 vaccination is rare and has occurred in approximately 4 cases per one million doses administered. TTS is a rare but serious adverse event that causes blood clots in large blood vessels and low platelets. A review of reports indicates a causal relationship between the J&J/Janssen COVID-19 vaccine and TTS.(CDC, 2022)

Guillain-Barré Syndrome (GBS) in people who have received the J&J/Janssen COVID-19 vaccine is rare. GBS is a rare disorder where the body's immune system damages nerve cells, causing muscle weakness and sometimes paralysis. GBS has largely been reported in men ages 50 years and older. Based on a recent analysis of data from the Vaccine Safety Datalink, the rate of GBS within the first 21 days following J&J/Janssen COVID-19 vaccination was found to be 21 times higher than after Pfizer-BioNTech or Moderna (mRNA COVID-19 vaccines). After the first 42 days, the rate of GBS was 11 times higher following J&J/Janssen COVID-19 vaccination. The analysis found no increased risk of GBS after Pfizer-BioNTech or Moderna (mRNA COVID-19 vaccines).(CDC, 2022)

Myocarditis and pericarditis after COVID-19 vaccination are rare. Myocarditis is inflammation of the heart muscle, and pericarditis is inflammation of the outer lining of the heart. Most patients with myocarditis or pericarditis after COVID-19 vaccination responded well to medicine and rest and felt better quickly. Most cases have been reported after receiving Pfizer-BioNTech or Moderna (mRNA COVID-19 vaccines), particularly in male adolescents and young adults.(CDC, 2022)

Reports of death after COVID-19 vaccination are rare. FDA requires healthcare providers to report any death after COVID-19 vaccination to VAERS, even if it's

unclear whether the vaccine was the cause. Continued monitoring has identified nine deaths causally associated with J&J/Janssen COVID-19 vaccination. CDC and FDA continue to review reports of death following COVID-19 vaccination and update information as it becomes available. (CDC, 2022)

1.2 Rationale

A review of reports indicates a causal relationship between the J&J/Janssen COVID-19 vaccine and blood clots with low levels of platelets and these may not be all the possible side effects of the Janssen COVID-19 vaccine. Serious and unexpected effects may occur. The Janssen COVID-19 vaccine is still being studied in clinical trials. (CDC, 2022)(Johnson and Johnson, 2022)

Measuring prothrombin time and activated partial thromboplastin time among Janssen COVID-19 vaccine recipients is an important part of assessing the safety of the vaccine, both tests are used to assess the risk of developing blood clots after receiving the vaccine.

By monitoring these tests, we can ensure that individuals who receive the vaccine are not at an increased risk for developing blood clots. Also this information is essential because of the lack of studies in Sudan about potential serious side effects following the administration ofJanssen COVID-19vaccine.

1.3 Objectives

1.3.1 General Objective

-To measure prothrombin time (PT) and activated partial thromboplastin time (APTT) among Johnson and Johnson COVID-19 vaccine recipients in Khartoum State.

1.3.2 Specific Objectives

-To measure PT and APTT levels among Johnson and Johnson COVID-19 vaccine recipients before and after the administration of the vaccine.

-To compare PT and APTT levels before and after the administration of the vaccine.

-To study the effect of age, gender, posttest date and the interaction between gender and posttest date on the differences between pre and post levels of PT and APTT.

CHAPTER ONE

Introduction

CHAPTER II

Literature review

CHAPTER II

Literature review

2.1 Literature review

2.1.1 Overview of hemostasis

Hemostasis is a complex physiologic process that keeps circulating blood in a fluid state and then, when an injury occurs, produces a clot to stop the bleeding, confines it to the site of injury, and finally dissolves the clot as the wound heals. (Keohane*et al*., 2016) The maintenance of circulatory hemostasis is achieved through the process of balancing bleeding (hemorrhage) and clotting (thrombosis) (Turgeon, 2012) and it depends on five major components: vascular endothelium, platelets, coagulant proteins, anticoagulant proteins, and fibrinolytic proteins. (Perry and Pasi, 1999)

Functionally, several processes are involved in hemostasis following injury to a small blood vessel: blood vessel spasm, formation of a platelet plug, contact among damaged blood vessel, blood platelet and coagulation proteins, development of a blood clot around the injury and finally fibrinolytic removal of excess hemostatic material to reestablish vascular integrity. (Turgeon, 2012)

2.1.2 Components of hemostasis

2.1.2.1 The vascular system

The vascular system forms an extensive distribution system for blood, carrying nutrients to all the body's cells and tissues and transporting waste products for disposal. To perform this function, the system must provide an uninterrupted flow of blood, a nonleaking circuit, and maintain blood in a fluid state. The vascular system consists of three types of blood vessels: arteries, veins, and capillaries. The

arteries carry blood from the heart to the capillaries. The veins return blood from the capillaries to the heart. The vessels in which hemostasis occurs are primarily the smallest veins (venules) and to a lesser degree, the smallest arteries (arterioles). (McKenzie and Williams, 2015)

2.1.2.1.1 Structure of blood vessels

The basic structure of blood vessels can be broken down into three layers: the intima, the media and the adventitia. It is the materials that make up these layers and the size of these layers themselves that differentiate arteries from veins, and indeed one artery or one vein from another artery or vein. The intima is the innermost layer and the surface is covered with a single layer of ECs (the endothelium), which rest on a basement membrane of subendothelialmicrofibrils that are composed of collagen fibres and some elastin. The media or middle layer contains mainly circularly arranged smooth muscle cells and collagenous fibrils, and is divided from the adventitia by the external elastic lamina. The muscle cells contract and relax, whereas the elastin allows vessels to stretch and recoil. The adventitia or outermost layer is composed of collagen fibres and fibroblasts that protect the blood vessel and anchor it to surrounding structures. (Hoffbrand*et al*., 2005)

2.1.2.1.2 Functions of blood vessels in hemostasis

After an injury, the damaged vessels initiate hemostasis. Their first response to injury is constriction or narrowing of the lumen of the arterioles to minimize the flow of blood into the wound area and the escape of blood from the wound site. Vasoconstriction also brings the hemostatic components of the blood (the platelets and the plasma proteins) closer to the vessel wall, facilitating their interactions. Vasoconstriction occurs immediately and lasts a short time and it mechanism is

complex. It is caused in part by neurogenic factors and in part by several regulatory substances that interact with receptors on the surface of cells of the blood vessel wall. These include serotonin and thromboxane A2 (TXA2), both products of activated platelets, and endothelin-1 produced by endothelial cells. In contrast, healthy intact endothelial cells synthesize and secrete prostaglandin PGI2, also called prostacyclin, and nitric oxide (NO), also called endothelium derived relaxing factor (EDRF). Both counteract vasoconstriction by causing vasodilation of the arterioles. Both also inhibit activation and recruitment of platelets. (McKenzie and Williams, 2015)

2.1.2.2 Platelets

2.1.2.2.1 Platelet development and structure

Platelets, or thrombocytes, are small discoid cells (0.5 to 3.0 μm) that are synthesized in the bone marrow and stimulated by the hormone thrombopoietin. They are developed through a pluripotent stem cell that has been influenced by colony-stimulating factors (CSF) produced by macrophages, fibroblasts, T-lymphocytes, and stimulated endothelial cells. The parent cells of platelets are called megakaryocytes. These large cells (80 to 150 μm) are found in the bone marrow. Megakaryocytes do not undergo complete cellular division but undergo a process called endomitosis or endoreduplication creating a cell with a multilobed nucleus. Each megakaryocyte produces about 2000 platelets. Thrombopoietin is responsible for stimulating maturation and platelet release. This hormone is generated primarily by the kidney and partly by the spleen and liver. There is no reserve of platelets in the bone marrow: 80% are in circulation and 20% are in the red pulp of the spleen. Platelets have no nucleus but do have granules: alpha granules and dense granules. These granules are secreted during the platelet release reaction and contain many biochemically active components such as serotonin,

ADP, and ATP. They are destroyed by the reticuloendothelial system (RE) and their development occurs in the following sequence:

Megakaryoblasts are the most immature cell (10 to 15 μm) with a high nuclear to cytoplasmic ratio and two to six nucleoli, promegakaryocyte is a large cell of 80 μm with dense, alpha and lysosomal granules, basophilic megakaryocyte shows evidence of cytoplasmic fragments containing membranes, cytotubules, and several glycoprotein receptors and finally the megakaryocyte is composed of cytoplasmic fragments that are released by a process called the budding of platelets.

Platelets have a complex structure comprised of four zones: the peripheral zone is associated with platelet adhesion and aggregation, the sol gel zone provides a cytoskeletal system for platelets and contact when the platelets are stimulated, the organelle zone contains three types of granules: alpha, dense bodies and lysosomes and the membrane system contains a dense tubular system in which the enzymatic system for the production of prostaglandin synthesis is found. (Ciesla, 2007)

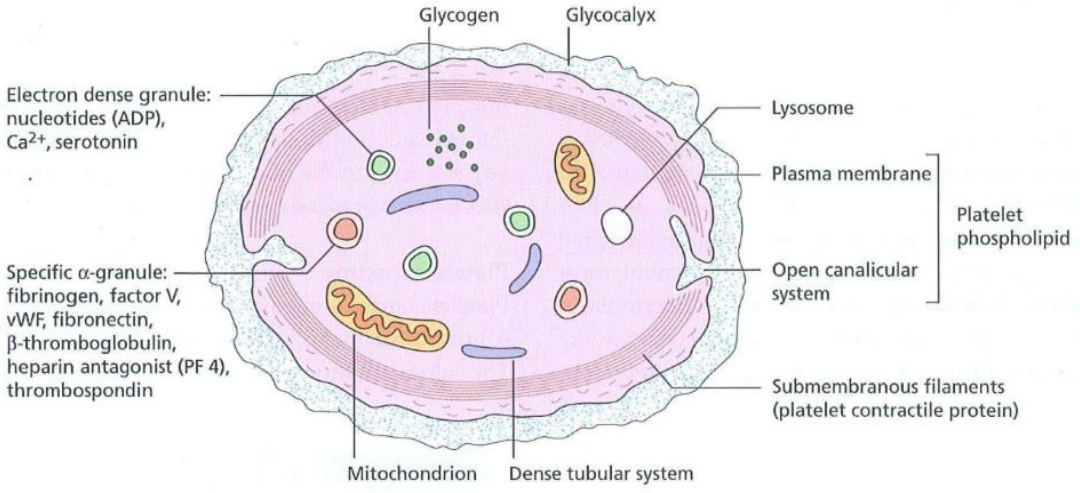


Figure (2.1) Platelet structure (Hoffbrand*et al*., 2006)

2.1.2.2.2 Platelet roles in hemostasis

Platelets are involved in several aspects of hemostasis. it has been shown that platelets maintain the vessels' continuity or integrity by filling in the small gaps caused by the separation of endothelial cells, by attaching to the underlying exposed collagen fibers of the subendothelium and prevent blood from escaping and when injury occurs and an actual break in the continuity of the lining of the vessels occurs, the platelets react by forming the primary hemostatic plug. By sticking first to exposed collagen and other components of the subendothelium and then to each other, the platelets form a mass that mechanically fills openings in the vessels and limits the loss of blood from the injury site. (McKenzie and Williams, 2010)

Following platelet plug formation, membrane phospholipids of the aggregated platelets provide a reaction surface for the proteins that make fibrin. Fibrin stabilizes the initial platelet plug, and the entire mass of fibrin and platelets constitutes the secondary hemostatic plug. (McKenzie and Williams, 2010)

As a fourth role, platelet-derived growth factors stimulate smooth muscle cells and fibroblasts to divide and replace the cells that were damaged, thus promoting healing of the injured tissues. (McKenzie and Williams, 2010)

2.1.2.2.2.1 Formation of the primary hemostatic plug

Platelets circulating in blood vessels do not interact with other platelets or other cell types. Circulating platelets are disc-shaped and inert in the environment of normal endothelium. Injury to the blood vessels causes a change in the normal environment, and in response, activate the platelets. The primary hemostatic plug is the result of the transformation of the platelets from an inactive to an active state. The formation of a platelet plug requires several platelet activation events,

including adhesion, contraction or shape change, secretion, and aggregation. (McKenzie and Williams, 2010)

2.1.2.2.2.1.1 Platelet adhesion

Damage to a blood vessel exposes collagen that is wrapped around the vessel. The exposed collagen reacts with and bind a large multimeric protein known as von willebrand factor. Once it is bound, von willebrand factor changes in conformation at one end so that it can bind to platelet receptor glycoprotein (Gp) Ib. platelet adhesion by von willebrand factor create a platelet monolayer over an injured surface. (De Loughery, 2004)

2.1.2.2.2.1.2 Platelet shape change and secretion

Following adhesion, platelets undergo a shape change from a disc shape to a spherical shape and extend pseudopods. Almost simultaneously the release reaction occurs by which a number of biologically active compounds stored in the platelet granules are secreted to the outside. These released substances, which include ADP, serotonin, Thromboxane A2 (TxA2), βTG, PF4, and VWF, accelerate the reaction of plug formation and also initiate platelet aggregation. (Munker*et al*., 2007)

2.1.2.2.2.1.3 Platelet aggregation

Platelet aggregation is the attachment of platelets to one another. Newly arriving platelets flowing into the area become activated by contact with agonists such as ADP and TXA2 released by the initial adherent and activated platelets, products from the damaged tissue and endothelial cells and thrombin. With activation, the new platelets undergo shape change and exposure of their active GPIIb/IIIa sites. The fibrinogen bound to activated platelets serves as a bridge, cross-linking GPIIb/IIIa molecules on two adjacent platelets. (McKenzie and Williams, 2010)

2.1.2.2.2.1.4 Platelets and secondary hemostasis

The final aspect of platelet activation involves the changes in the platelet membrane that allow platelets to function in secondary hemostasis. Activated platelets accelerate thrombin formation, a function known as platelet procoagulant activity. Coagulation factors bind to the surface of activated platelets by binding either to specific receptors on platelets or nonspecifically to negatively charged phospholipids. The resulting platelet-coagulation factor interactions result in formation of the secondary hemostatic plug. (McKenzie and Williams, 2010)

The primary platelet plug is relatively unstable and is easily dislodged. During secondary hemostasis, fibrin forms amid and around the aggregated platelets. The proteins that interact enzymatically to form fibrin must be assembled on a lipid surface where the reactions take place. The membrane phospholipids of activated platelets are the primary source of this lipid surface. (McKenzie and Williams, 2010)

Fibrin-forming proteins do not bind to resting platelet surfaces in the circulation. In resting platelets, the negatively charged phospholipids are almost exclusively found in the inner half of the membrane bilayer. During the activation of platelets, the membrane phospholipids flip-flop, and the negatively charged phospholipids move to the outer leaflet, perhaps via a Ca2+-activated "scramblase" enzyme that reverses the asymmetric distribution of phospholipids.(McKenzie and Williams, 2010)

This phospholipid rearrangement provides the phospholipid surface allowing coagulation factors to bind, become activated, and initiate fibrin formation. Platelets can assemble both the Xase and prothrombinase complexes. The fibrin-platelet plug is the secondary hemostatic plug. (McKenzie and Williams, 2010)

2.1.2.3 The coagulation system

The main function of the coagulation system is in the event of injury, to produce thrombin, which firstly aids the activation of platelets in hemostasis, secondly forms a stable fibrin network from circulating fibrinogen, and thirdly stimulates coagulation inactivating mechanisms, thus limiting the process to the vicinity of the injury. (Firkin *et al*., 1989)

2.1.2.3.1 Coagulation factors

Coagulation factors are synthesized in the liver and may be categorized into substrates, cofactors, and enzymes. Substrates are the substance upon which enzymes act and fibrinogen is the main substrate. Cofactors accelerate the activities of the enzymes that are involved in the cascade and it include tissue factor, factor V, factor VIII, and Fitzgerald factor. Factors II, VII, IX, X, XI and XII are serine proteases that are inactive as synthesized and acquire enzymatic capability when cleaved (activated) by other proteins. All of the enzymes are serine proteases except factor XIII, which is a transaminase.13. A post synthetic step in the production of factors II, VII, IX, and X and the natural anticoagulant proteins C and S require the activity of a vitamin K–dependent carboxylase that modifies the amino terminus of each factor, enabling it to function. The activity of all clotting factors culminates in a principal event: the generation of thrombin at sites of vascular injury. Thrombin activates platelets (primary hemostasis) and cleaves fibrinogen to form fibrin (secondary hemostasis) at sites of blood vessel compromise. (Rodgers and Young, 2018), (Ciesla, 2007).

2.1.2.3.2 Classification of coagulation factors

There are three groups in which coagulation factors can be classified: The fibrinogen group consists of factors I, V, VIII, and XIII and they are consumed

during coagulation (Factors V and VIII are labile), the prothrombin group consist of factors II, VII, IX, and X and all are dependent on vitamin K during their synthesis. This group is stable and remains preserved in stored plasma and the contact group contain factor XI, factor XII, prekallikrein, and high-molecular-weight kininogen (HMWK) which are involved in the intrinsic pathway, moderately stable, and not consumed during coagulation. (Ciesla, 2007)

2.1.2.3.3 Coagulation pathways

Initiation of clotting begins with either the extrinsic or the intrinsic pathway. Factor X activation is the point of convergence. Factor X can be activated by either of the two pathways and subsequently catalyzes the conversion of prothrombin to thrombin. (Turgeon, 2012)

2.1.2.3.3.1 The extrinsic coagulation pathway

The extrinsic pathway is initiated by the release of tissue thromboplastin that has been expressed after damage to a vessel. Factor VII forms a complex with tissue thromboplastin and calcium. This complex converts factors X to Xa, which in turn converts prothrombin to thrombin. Thrombin then converts fibrinogen to fibrin. (Ciesla, 2007)

2.1.2.3.3.2 The intrinsic coagulation pathway

Activation of contact factors at the site of vascular injury leads to the conversion of factor XII to XIIa and the sequential conversion of XI to XIa and IX to IXa. IXa complexes with VIIIa, PL, and Ca2+, forming the tenase complex, which converts X to Xa. Xa, in a complex with Va, PL, and Ca2+ then cleaves II (prothrombin) to IIa (thrombin). The TF–VIIa complex can also activate IX leading to the subsequent formation of the tenase complex. (Rodgers and Young, 2018)

2.1.2.3.3.3 The common coagulation pathway

Once factor X is activated to Xa, the extrinsic and intrinsic pathways enter a common pathway. Following the activation of factor Xa, it remains platelet bound and activates factor V. The complex of factors Xa and Va on the platelet surface is formed near platelet-bound factor II molecules and it cleaves it into thrombin, factor IIa. The stage is accelerated by factor V and ionized calcium. (Turgeon, 2012) Thrombin then cleaves fibrinogen to form fibrin, which is then cross-linked via the action of XIII. (Rodgers and Young, 2018)

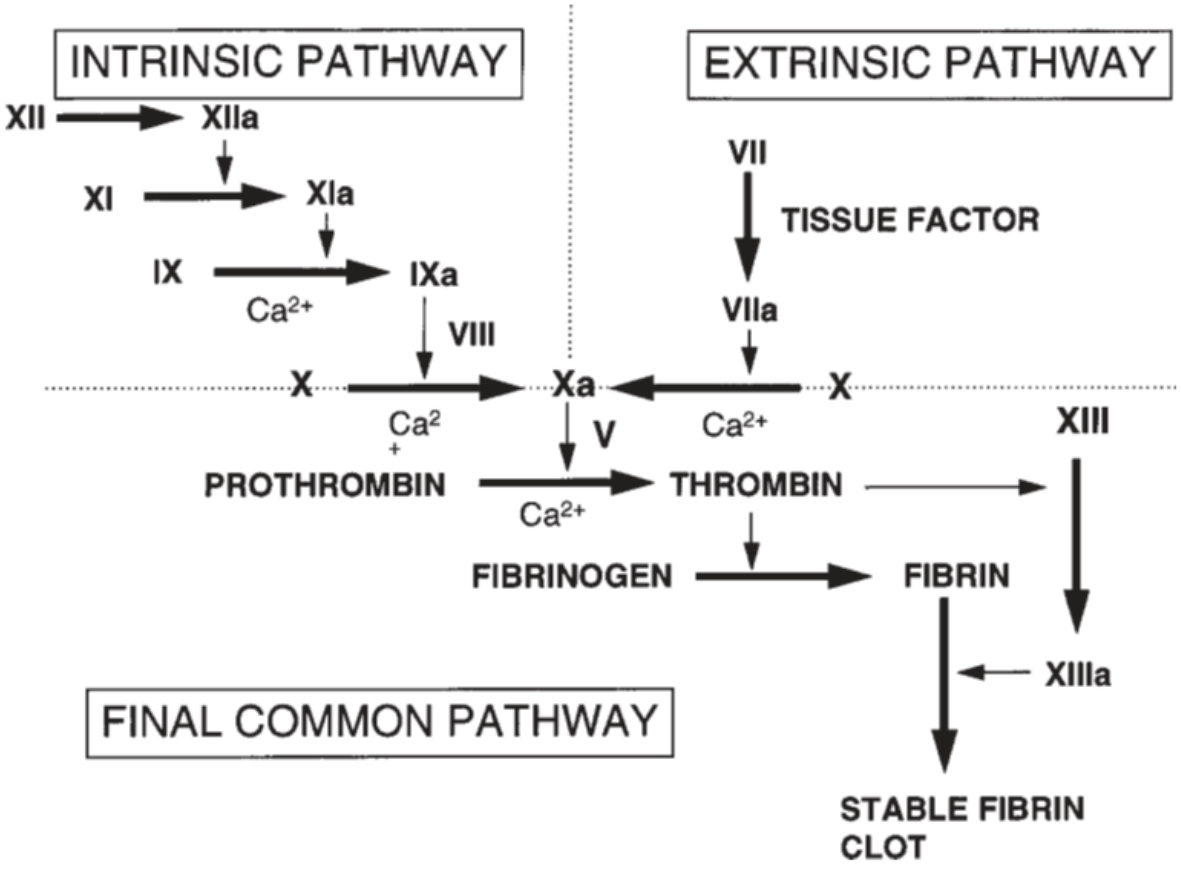


Figure (2.2) Coagulation pathways (Perry and Pasi, 1999)

2.1.2.4 Anticoagulant pathways

2.1.2.4.1 Coagulation factor inhibitors

It is important that the effect of thrombin is limited to the site of injury. The first inhibitor to act is tissue factor pathway inhibitor (TFPI) which is synthesized in endothelial cells and is present in plasma and platelets and accumulates at the site of injury caused by local platelet activation. This inhibits Xa and VIIa and tissue factor to limit the main in vivo pathway by forming the quaternary complex. There is direct inactivation of thrombin and other serine protease factors by other circulating inhibitors of which antithrombin is the most potent. It inactivates serine proteases by combining with them by peptide bonding to form high molecular weight stable complexes. Heparin potentiates its action markedly. Another protein, heparin cofactor II, also inhibits thrombin. CY.2-Macroglobulins, O"2-antiplasmin, C1 esterase inhibitor and CY.l-antitrypsin also exert inhibitory effects on circulating serine proteases. (Hoffbrand*et al*., 2006)

2.1.2.4.2 Protein C system

Protein C is the zymogen of a serine protease. Protein S serves as a co-factor for activated Protein C and like protein C is a vitamin K-dependent protein. Protein S exists in plasma either in a free form or bound to the C4b-binding protein, a component of the complement system. Only the free form of protein S can serve as a co-factor for activated protein C. When thrombin escapes the localized area of vascular injury, it must be kept from freely circulating in blood. This is accomplished by the upregulation of thrombomodulin on the cell surface of the vascular endothelium, primarily in the microcirculation. Thrombomodulin binds thrombin, thus switching off the procoagulant activity of thrombin. Thrombin in this bound form changes its substrate specificity from fibrinogen to protein C. The

thrombomodulin/thrombin-complex activates protein C. Following activation, activated protein C forms a complex with protein S. This complex degrades factor Va and factor VIIIa by limited proteolysis, dramatically reducing the local generation of thrombin. Activated protein C also increases fibrinolysis by inactivating PAI-1. (Munker*et al*., 2007)

2.1.2.5 The fibrinolytic system

The process limiting the extent of clot formation is the fibrinolytic protein system. It consists of the zymogen plasminogen and its naturally occurring activators. Plasminogen is activated to the main clot- lysing enzyme, plasmin, by the endogenous plasminogen activator tissue plasminogen activator (tPA), single - chain urokinase plasminogen activator (ScuPA), and two chain urokinase plasminogen activator (tcuPA). These activators are found in the endothelium as well as in neutrophils and monocytes. Plasminogen activation is regulated by the inhibitor plasminogen activated inhibitor- 1 (PAI- 1). PAI - 1 is mostly found in endothelium and cells; it is not a plasma protein. Formed plasmin degrades fibrinogen, soluble non - cross - linked fibrin, and cross - linked fibrin to liberate fibrin or fibrinogen degradation products. Plasmin degrades insoluble cross - linked fibrin clots to liberate D – dimer (a two D - domain protein fragments held together by a unique bond between them). The plasma serine protease inhibitor, alpha - 2 - antiplasmin, regulates plasmin activity. (Schmaier and Lazarus, 2012)

2.1.3 Screening tests of blood coagulation

Screening tests provide an assessment of the 'extrinsic' and 'intrinsic' systems of blood coagulation and also the central conversion of fibrinogen to fibrin.

2.1.3.1 The prothrombin time (PT)

The PT test measure the clotting time of recalcified plasma in the presence of an optimal concentration of tissue extract (thromboplastin) and indicate the overall efficiency of extrinsic clotting system. Although originally thought to measure prothrombin, the test is now known to depend also on reactions with factors V, VII and X and on the fibrinogen concentration of the plasma. (Bain *et al*., 2011)

2.1.3.2 The activated partial thromboplastin time (APTT)

To perform this coagulation assay, a mixture of a negatively charged surface, phospholipids, and patient plasma is incubated for a few minutes. Plasma is made from whole blood anticoagulated with 3.2 g% sodium citrate. Calcium chloride is added to overcome the calcium chelation of the sodium chloride, and the time required for clot formation is measured. The APTT assesses the coagulation proteins of the intrinsic system and the common pathway. (Schmaier and Lazarus, 2012)

Prolonged clotting times in the PT and APTT because of factor deficiency are corrected by the addition of normal plasma to the test plasma (50: 50 mix). If there is no correction or incomplete correction with normal plasma, the presence of an inhibitor of coagulation is suspected. (Hoffbrand*et al*., 2006)

2.1.4 Coagulation in COVID-19 vaccine

COVID-19 vaccines are safe and effective and severe reactions after vaccination are rare and those related to coagulation include: Thrombosis with thrombocytopenia syndrome (TTS) after J&J/Janssen COVID-19 vaccination and it has occurred in approximately 4 cases per one million doses administered. TTS is a rare but serious adverse event that causes blood clots in large blood vessels and low platelets. (CDC, 2022)

2.2 Coronavirus disease 2019 (COVID-19)

Coronavirus is a kind of common virus that causes an infection in your nose, sinuses, or upper throat. Most coronaviruses aren't dangerous.

In early 2020, after a December 2019 outbreak in China, the World Health Organization identified SARS-CoV-2 as a new type of coronavirus. The outbreak quickly spread around the world.(Nazario, 2021)

COVID-19 is a disease caused by SARS-CoV-2 that can trigger a respiratory tract infection. It can affect your upper respiratory tract (sinuses, nose, and throat) or lower respiratory tract (windpipe and lungs).(Nazario, 2021)

It spreads the same way other coronaviruses do, mainly through person-to-person contact. Infections range from mild to deadly.(Nazario, 2021)

SARS-CoV-2 is one of seven types of coronavirus, including the ones that cause severe diseases like Middle East respiratory syndrome (MERS) and sudden acute respiratory syndrome (SARS). The other coronaviruses cause most of the colds that affect us during the year but aren't a serious threat for otherwise healthy people.(Nazario, 2021)

An early Chinese study of 103 COVID-19 cases found two strains, which they named L and S. The S type is older, but the L type was more common in early stages of the outbreak. They think one may cause more cases of the disease than the other, but they're still working on what it all means. (Nazario, 2021)

It is also normal for a virus to change, or mutate, as it infects people and this virus has done so. There are several variants that are now spreading, some proving to be more contagious as well as more deadly than the original virus. (Nazario, 2021)

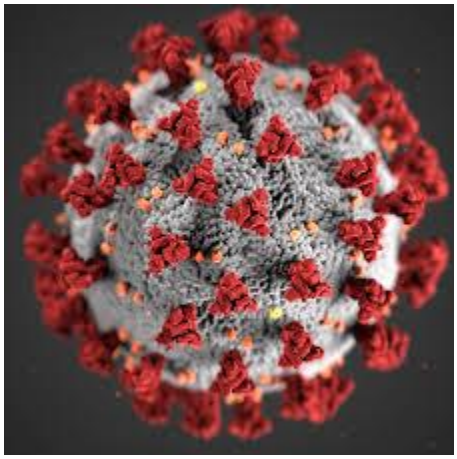


Figure (2.3) Structure of Coronavirus (CDC, 2021)

2.2.1 Coronavirus spreads

Coronavirus disease spreads primarily through contact with an infected person when they cough or sneeze.

The disease can spread from person to person through small droplets from the nose or mouth which are spread when a person with COVID-19 coughs or exhales, these droplets land on objects and surfaces around the person, other people then catch COVID-19 by touching these objects or surfaces, then touching their eyes, nose or mouth. This is why it is important to stay more than 1 meter (3 feet) away from a person who is sick.(Indus health, 2021)

People of all ages can be infected by the new coronavirus (2019-nCoV), older people and people with pre-existing medical conditions (such as asthma, diabetes, and heart disease) appear to be more vulnerable to becoming severely ill with the virus. (Indus health, 2021)

2.2.2 Signs and symptoms

COVID-19 affects different people in different ways. Most infected people will develop mild to moderate illness and recover without hospitalization.

Most common symptoms include: fever, cough, tiredness and loss of taste or smell, less common symptoms include: sore throat, headache, aches and pains, diarrhoea, a rash on skin, or discolouration of fingers or toes and red or irritated eyes and the serious symptoms include difficulty breathing or shortness of breath, loss of speech or mobility, or confusion and chest pain.(WHO, 2020)

People with mild symptoms who are otherwise healthy should manage their symptoms at home. On average it takes 5–6 days from when someone is infected with the virus for symptoms to show, however it can take up to 14 days. (WHO, 2020)

2.2.3 Diagnosis of COVID-19

2.2.3.1 Antigen and molecular tests for COVID-19

The diagnostic testing field for COVID-19 is rapidly evolving, with many tests focused on diagnosing patients with active viral infections. Diagnostics able to detect current, active infections are typically antigen- and molecular-based tests. These tests inform researchers and health providers of the presence of the pathogen, either by amplifying and detecting its genetic material or identifying unique markers of the pathogen itself. The viral genomic material for SARS-CoV-2 is ribonucleic acid (RNA), which remains in the body only while the virus is still replicating, even at very low levels.(Johns Hopkins center for health security, 2022)

Antigen tests detect specific viral proteins (antigens) present in a patient sample taken using nasal or nasopharyngeal swabs and can provide results in under a half hour. Antigens are found on the surface of the virus particle, which are also shed in patient tissues. These rapid tests detect basic levels of antigen already present in a sample, providing only a simple “yes” or “no,” answer.(Johns Hopkins center for health security, 2022)

Molecular tests called nucleic acid amplification tests (NAAT) rely on amplification of existing genetic material in the sample. NAATs include the polymerase chain reaction (PCR) assay in a laboratory to amplify viral genomic material for detection and can provide results in hours to days; other techniques include LAMP and next generation sequencing. These tests require samples from the patient that are likely to contain virus. Most samples are collected using nasopharyngeal swabs or a sputum cup, although the virus may also be detected in feces, urine, or blood. For diseases that are primarily respiratory in nature, like COVID-19, nasopharyngeal swabs have been the most reliable, as they sample an area of the respiratory tract where the virus appears to first infect an individual. This site is relatively easily accessed, compared to the final site of viral infection: the lower respiratory tract. The nasopharyngeal tract likely has: active virus replication and enough virus to be detected in test kits.

Many tests now available or in development can use saliva or nasal swabs that facilitate easier sampling procedures for healthcare providers and patients.(Johns Hopkins center for health security, 2022)

2.2.3.2 Serology tests for COVID-19

Serology testing for SARS-CoV-2 are in high demand because it can help to better quantify the total number of cases of COVID-19 to date. This type of testing is valuable because it can identify those who may have been asymptomatic and recovered. Serology tests measure the levels of specific antibodies in the blood, revealing whether a person has been exposed to a particular pathogen by looking at their immune response. These tests can give greater detail into the prevalence of a disease in a population by identifying individuals who have developed antibodies to the virus.(Johns Hopkins center for health security, 2022)

These tests are not approved for diagnosis of acute, active SARS-CoV-2 infection, but may indicate previous exposure to the virus. Negative results do not rule out prior or current infection. Active infection should be diagnosed using an antigen or molecular diagnostic test. (Johns Hopkins center for health security, 2022)

2.2.4 Prevention

To prevent infection and to slow transmission of COVID-19, the following should be done: vaccination, staying at least 1 metre apart from others, wearing a properly fitted mask, choosing open, well-ventilated spaces over closed ones, open a window if indoors, washing your hands regularly with soap and water or clean them with alcohol-based hand rub, covering your mouth and nose when coughing or sneezing and if you feel unwell, stay home and self-isolate until you recover. (WHO, 2022)

2.3 Vaccination

2.3.1 Designing vaccine

There are three main approaches to designing a vaccine. Their differences lie in whether they use a whole virus or bacterium; just the parts of the germ that triggers

the immune system; or just the genetic material that provides the instructions for making specific proteins and not the whole virus. (WHO, 2021)

2.3.1.1 The whole-microbe approach

Include three ways: inactivated vaccine the first way is to take the disease-carrying virus or bacterium, or one very similar to it, and inactivate or kill it using chemicals, heat or radiation, secondly live-attenuated vaccine and this uses a living but weakened version of the virus or one that's very similar. However, vaccines like this may not be suitable for people with compromised immune systems and lastly viral vector vaccine, this type of vaccine uses a safe virus to deliver specific sub-parts – called proteins – of the germ of interest so that it can trigger an immune response without causing disease.(WHO, 2021)

2.3.1.2 The subunit approach

A subunit vaccine is one that only uses the very specific parts (the subunits) of a virus or bacterium that the immune system needs to recognize. The subunits may be proteins or sugars. (WHO, 2021)

2.3.1.3 The genetic approach (nucleic acid vaccine)

A nucleic acid vaccine uses a section of genetic material that provides the instructions for specific proteins. DNA and RNA are the instructions our cells use to make proteins. In our cells, DNA is first turned into messenger RNA, which is then used as the blueprint to make specific proteins. A nucleic acid vaccine delivers a specific set of instructions to our cells, either as DNA or mRNA, for them to make the specific protein that we want our immune system to recognize and respond to. (WHO, 2021)

2.3.2 A coronavirus disease 2019 (COVID-19) vaccine

COVID-19 vaccine can prevent you from getting COVID-19 or from becoming seriously ill or dying due to COVID-19. Each COVID-19 vaccine causes the immune system to create antibodies to fight COVID-19. COVID-19 vaccines use a harmless version of a spikelike structure on the surface of the COVID-19 virus called an S protein.(Mayo clinic, 2022)

The main types of COVID-19 vaccines currently available in the U.S. or being studied include:

2.3.2.1 Messenger RNA (mRNA) vaccine

This type of vaccine uses genetically engineered mRNA to give your cells instructions for how to make the S protein found on the surface of the COVID-19 virus. After vaccination, your muscle cells begin making the S protein pieces and displaying them on cell surfaces. This causes your body to create antibodies. If you later become infected with the COVID-19 virus, these antibodies will fight the virus. After delivering instructions, the mRNA is immediately broken down. Both the Pfizer-BioNTech and the Moderna COVID-19 vaccines use mRNA. (Mayo clinic, 2022)

2.3.2.2 Vector vaccine

In this type of vaccine, genetic material from the COVID-19 virus is placed in a modified version of a different virus (viral vector). When the viral vector gets into your cells, it delivers genetic material from the COVID-19 virus that gives your cells instructions to make copies of the S protein. Once your cells display the S proteins on their surfaces, your immune system responds by creating antibodies

and defensive white blood cells. If you later become infected with the COVID-19 virus, the antibodies will fight the virus. Viral vector vaccines can't cause you to become infected with the COVID-19 virus or the viral vector virus. The Janssen/Johnson & Johnson COVID-19 vaccine is a vector vaccine. AstraZeneca and the University of Oxford also have a vector COVID-19 vaccine.(Mayo clinic, 2022)

2.3.2.3 Protein subunit vaccine

Subunit vaccines include only the parts of a virus that best stimulate your immune system. This type of COVID-19 vaccine contains harmless S proteins. Once your immune system recognizes the S proteins, it creates antibodies and defensive white blood cells. If you later become infected with the COVID-19 virus, the antibodies will fight the virus. (Mayo clinic, 2022)

2.3.3 Johnson & Johnson COVID-19 vaccine

Johnson & Johnson COVID-19 vaccine, is a COVID-19 vaccine that was developed by Janssen Vaccines in Leiden, Netherlands and its Belgian parent company Janssen Pharmaceuticals, a subsidiary of American company Johnson & Johnson. It is a viral vector vaccine based on a human adenovirus that has been modified to contain the gene for making the spike protein of the SARS-CoV-2 virus that causes COVID-19. The body's immune system responds to this spike protein to produce antibodies. (Wikipedia, 2022)

2.3.3.1 Effectiveness

Johnson & Johnson COVID-19 vaccine is 66% effective at preventing the COVID-19 virus with symptoms, 85% effective at preventing the COVID-19 virus with

severe illness and appears to protect against severe COVID-19 due to COVID-19 variants. (Mayo clinic, 2022)

2.3.3.2 Doses

Primary series: 1 dose of Johnson & Johnson's Janssen.

Boosters: 1 booster, preferably of either Pfizer-BioNTech or Moderna COVID-19 vaccine, for most people at least 2 months after J&J/Janssen COVID-19 vaccine and a second booster dose, for adults ages 50 years and older at least 4 months after the 1st booster.(CDC, 2022)

People ages 18 through 49 years who received a J&J/Janssen COVID-19 vaccine for both their primary dose and booster can choose to get a 2nd booster of either Pfizer-BioNTech or Moderna COVID-19 vaccine at least 4 months after the 1st booster. (CDC, 2022)

2.3.3.3 Administration

Intramuscular (IM) injection in the deltoid muscle. (CDC, 2022)

2.3.3.4 Side effects

Side effects that have been reported with the Janssen COVID-19 vaccine include:Injection site reactions: pain, redness of the skin, and swelling.

General side effects: headache, feeling very tired, muscle aches, nausea and fever.(Johnson & Johnson, 2022)

Severe Allergic Reactions: there is a remote chance that the Janssen COVID-19 vaccine could cause a severe allergic reaction. A severe allergic reaction would

usually occur within a few minutes to one hour after getting a dose of the Janssen COVID-19 Vaccine, signs of a severe allergic reaction can include: difficulty breathing, swelling of your face and throat, fast heartbeat, bad rash all over your body and dizziness and weakness.(Johnson & Johnson, 2022)

Blood clots with low levels of platelets: Blood clots involving blood vessels in the brain, lungs, abdomen, and legs along with low levels of platelets, have occurred in some people who have received the Janssen COVID-19 Vaccine. In people who developed these blood clots and low levels of platelets, symptoms began approximately one to two-weeks following vaccination. Reporting of these blood clots and low levels of platelets has been highest in females ages 18 through 49 years. The chance of having this occur is remote and the symptoms of this include: shortness of breath, chest pain, leg swelling, persistent abdominal pain, severe or persistent headaches or blurred vision, easy bruising or tiny blood spots under the skin beyond the site of the injection.

These may not be all the possible side effects of the Janssen COVID-19 Vaccine. Serious and unexpected effects may occur. The Janssen COVID-19 Vaccine is still being studied in clinical trials.(Johnson & Johnson, 2022)

GuillainBarré Syndrome: a neurological disorder in which the body's immune system damages nerve cells, causing muscle weakness and sometimes paralysis has occurred in some people who have received the Janssen COVID-19 Vaccine. In most of these people, symptoms began within 42 days following receipt of the Janssen COVID-19 Vaccine. The chance of having this occur is very low and it symptoms include the following: weakness or tingling sensations, especially in the legs or arms, that's worsening and spreading to other parts of the body, difficulty walking, difficulty with facial movements, including speaking, chewing, or swallowing, double vision or inability to move eyes and difficulty with bladder control or bowel function. (Johnson & Johnson, 2022)

2.4 Previous studies

Elmathani, Hadeel and Shahad (2022) studied a total of 150 sample, 75 blood sample after the first dose and 75 blood sample after the second dose from (AstraZeneca and Pfizer), pre and post from (Janssen) vaccines, from both sex age ranged between 20_80 years old. The result showed insignificant results (P.value 0.2, 0.3, 0.4) respectively in comparison between the mean of prothrombin time in the first and second dose of (Astrazeneca and Pfizer) and pre and post mean of (Janssen) vaccine. (Nasr Eldeen*et al*., 2022)

A total of 11 healthy adult volunteers of both sexes, aged 24–47 years, with a BMI of 21.5–30.0kg/m², were enrolled in this study. SARS-CoV-2 vaccine, inactivated was administered intramuscularly into the deltoid. Volunteers were divided into two cohorts; five participants (cohort A) were vaccinated with a full dose (4μg) of inactivated SARS-CoV-2 Vaccine on days 1 and 14, and six participants (cohort B) received a full dose of the vaccine on days 1 and 28. One of the volunteers in group B was tested positive for anti-SARS-CoV-2 IgM and IgG right before vaccination, suggestive of potential prior infections. However, there was no record of previous positivity by nucleic acid (NA) diagnosis for COVID-19. For all follow-up examinations, data from this individual was marked green to track any possible influences from potential prior infections. Adverse events were monitored daily during the first 7 days after each inoculation and then self-recorded by the participants on diary cards in the following weeks. Overall, adverse reactions were mild (grades 1 or 2) and transient. Blood samples were collected on days 0, 7, 14, 28, 42, 56, and 90, and urine samples were collected on days 0, 14, 28, 42, and 90. They found that coagulation profiles changed significantly after vaccination, in the short-term (7 days) after the 1st inoculation, coagulation profiles were leaning toward shorter Prothrombin Time (PT), whereas the long-term (28 and 42 days)

effect was toward activated partial thromboplastin time (APTT) and PT prolongation. By day 90, the profiles returned back to those before vaccination. (Liu *et al*., 2021)

Thirty subjects who received the Pfizer-BioNTech vaccine were enrolled in this study after informed consent. Blood samples were collected at the specified time points (before the vaccination, 7 days after the first dose of the vaccine, 21 days after the first dose and 14 days after the second dose) into vacuum tubes containing 1/10 vol of 0.109 M trisodium citrate or plain tubes and centrifuged at 3000g for 15 min at controlled room temperature. Supernatant plasma and serum were aliquoted in plastic capped tubes and stored frozen at -70 C until testing. The prothrombin and activated partial thromboplastin time (PT and APTT) were measured by recombiplastin 2G or Synthasil APTT. None of the parameters did show significant variations at different time points before and after vaccination. (Peyvandi*et al*., 2021)

CHAPTER THREE

Material and Methods

CHAPTER III

Materials and Methods

3.1 Study design

This is an analytical cross-sectional study.

3.2 Study area and duration

The study was conducted in the vaccination center in the military hospital in Khartoum State during the period between March 2022 to June 2022.

3.3 Study population

The study population comprises of Johnson and Johnson COVID-19 vaccine recipients.

3.3.1 Inclusion criteria

Healthy recipients of Johnson and Johnson COVID-19 vaccine from both sexes were included.

3.3.2 Exclusion criteria

Smokers, diabeticpatients, hypertensive patients and any condition associated with coagulation profiles affected were excluded.

3.4 Sample size

Fifty-two recipients of Johnson and Johnson COVID-19 vaccine, samples were taken before and after the administration of the vaccine.

3.5 Ethical consideration

The study was approved by Sudan University of Science and Technology. Written permission was obtained from the vaccination center at the Military hospital and verbal consent from the participants, after they had been informed with the objective and benefits of the study. The participants were insured that the collected information will be confidential and will not to be used for any other purpose than this study.

3.6 Data collection

Data were collected using a structured questionnaire. The questionnaire was specifically developed to collect data on age, gender, address, phone number, presence of chronic disease and medication.

3.7 Sample collection

1,8 ml venous blood was collected using sterile disposable plastic syringe after cleaning the venipuncture area with 70% ethanol, the blood was added to the anticoagulant sodium citrate (3.2%) and gently mixed. The sample was centrifuge at 1300 rpm for 15min to obtain platelet poor plasma (ppp).

3.8 Methodology

3.8.1 Principle of semi –automated biochemical analyzer (ES-105)

Its measurement principle of coagulation tests is optical colorimetry. After mixing the reagents with plasma, fibrinogen converts into fibrin and coagulates, thus the optical density of the test sample changes and the analyzer can detect the coagulation end point.

3.8.2 Prothrombin time (PT)

The PT is a clot -based test of the extrinsic and common coagulation pathways. (Marchant and Davis, 2012)

3.8.2.1 Principle of Prothrombin Time

In this test, platelet poor plasma from the patient is collected in a tube containing sodium citrate is mixed with thromboplastin and calcium, and then clotting time is determined at 37C using a variety of methods, including photo-optical and electromechanical. (Wahed and Dasgapta, 2015)

3.8.2.2 Reagents and material

The kit reagent (TEClot PT-S) used contain an extract of rabbit brain (thromboplastin) with buffer, stablizers and calcium chloride.

3.8.2.3 Assay procedure

Open the device, check the system, put samples and reagent in incubation station, chose PT test, sample identification code entered, remove cuvette to measuring well, pipet the sample in the cuvette, start the test and pipet the reagent (thromboplastin) and the device will record the time.

3.8.2.4 Normal range

Normal value depends on thromboplastin used, the exact technique and whether visual or instrumental end point reading is used. With most rabbit thromboplastin the normal range of the PT is between 11 to 16 sec; for recombinant human thromboplastin, its somewhat shorter 10 – 12 sec. (Bain *et al.*, 2011)

3.8.2.5 Interpretation

The common causes of prolonged PT include: administration of oral anticoagulant drugs, liver disease practically obstructive jaundice, vitamin k deficiency, disseminated intravascular coagulation and rarely, factor VII, X, V, II deficiency or defect. (Bain *et al.*, 2011)

3.8.3 Activated partial thromboplastin time

The APTT is a clot -based test of the intrinsic and common coagulation pathways. (Marchant and Davis, 2012)

3.8.3.1 Principle of the activated partial thromboplastin time

This test is performed by adding a surface activating agent, such as kaolin or ellagic acid, and phospholipid to citrate-anticoagulated plasma. After a standardized incubation time to allow optimal activation of the contact factors, the plasma is recalcified and the clotting time recorded. (Munker*et al.*, 2007)

3.8.3.2 Reagent and material

The kit reagent (TEClot APTT-S) used contains colloidal silicate with phospholipids, buffer and preservatives. The $CaCl_2$ contain sodium azide.

3.8.3.3 Assay procedure

Open the device, check the system, put samples and reagent in incubation station, chose APTT test, sample identification code entered, put the sample and the activator in the cuvette and incubated for 3min, remove cuvette to measuring well, start the test and pipet the calcium and the device will record the time.

3.8.3.4 Normal range

The normal range is typically 26 – 40 sec. The actual time depend on the reagent used and the duration of pre-incubation period which varies in manufacturer recommendation for different reagents. (Bain *et al.*, 2011)

3.8.3.5 Interpretation

The common causes of prolonged APTT include: administration of or contamination with heparin or other anticoagulant, a circulating anticoagulant (inhibiter), liver disease, massive transfusion with plasma depleted red blood cell, disseminated intravascular coagulation and rarely, deficiency or defect of coagulation factors other than factor VII. It can also be moderately prolonged in the presence of vitamin K deficiency and in patients taking oral anticoagulant drug. (Bain *et al.*, 2011)

3.9 Data analysis

The collected data was entered, checked and processed by using SPSS (Statistical Package for Social Science) Version 26. The data were analyzed using descriptive statistical methods including arithmetic mean, standard deviation, standard errors, coefficient of variation and bar plots. In addition, paired t-test and analysis of covariance were used to test whether there were significant between pre and post levels of PT and APTT. In the two test 0.05 level of significance were used.

CHAPTER FOUR

Results

CHAPTER IV

Results

This study was carried out in Khartoum state during the period from March 2022 to June 2022 to analyze the effect of Johnson and Johnson COVID-19 vaccine on prothrombin time (PT) and activated partial thromboplastin time (APTT) among the vaccine recipients. This was done by measuring PT and APTT before and after the administration of the vaccine. The total number of participants in this study was 52.

4.1 Demographic characteristics of participants

Gender and place of residence:

Table 4.1: Distribution of participants with respect to place of residence and gender

Residence/Gender	Male	Female	Total	Percent
Omdurman	24	8	32	61.5
Khartoum	9	4	13	25.0
Bahri	4	2	6	11.5
White Nile	1	0	1	1.9
Total	38	14	52	100%
Percent	73.1	26.9	100%	

The results showed that most of the participants were males with 73.1% and 26.9% were females. The majority of the participants reside in Omdurman city with 61.5%, 25.0% reside in Khartoum city, 11.5% reside in Bahri city and 1.9% reside in White Nile state.

Age:

Table 4.2: Distribution of participants with respect to age group

Age group (years)	Frequency	Percent
<= 25	18	34.6
26 – 35	21	40.4
36 – 45	7	13.5
46 – 55	3	5.8
56+	3	5.8
Total	52	100%

Mean = 31.5, SD=10.2 years

The participants' age varies from 19 to 61 years old. The participants were divided into 5 age groups and most of them were between 26 to 35 years old. The mean age of the participants was 31.5 ±10.2 years.

Date of posttest:

Table 4.3: Distribution of participants with respect to date of posttest

Date of posttest	Frequency	Percent
7 days after pretest	25	48.1
> 7 days after pretest	27	51.9
Total	52	100

The results show that almost half of the participants' posttests were taken after seven days with 48.1 percent and 51.9 percent of participants' posttests were taken after more than seven days from the pretest date.

4.2 Main results

Descriptive statistics of PT and APTT pre and post vaccine:

Table 4.4: Mean, standard deviation and coefficient of variation of pre and post readings of PT and APTT

Test	Mean		Std. Deviation		Coefficient of Variation	
	Pre	**Post**	**Pre**	**Post**	**Pre**	**Post**
PT	13.80	13.99	1.24	1.44	9.0	10.3
APTT	33.54	33.46	3.40	2.75	10.1	8.2

Table 4.4 shows that the pre vaccine mean level of PT was 13.8 ± SD1.2, whereas the post vaccine level was 13.9 ± SD 1.4 and the pre and post vaccine mean levels of APTT were 33.54 ± SD 3.4 and 33.46 ± SD 2.75 respectively. The values of the coefficient of variation were small, showing slight differences in both pre and post readings for the two tests. Figures 4.1 and 4.2 demonstrate that there were slight differences in the levels of pre and post mean levels of the two tests.

Figure 4.1: Mean and 95% CI for Pre and Post PT readings

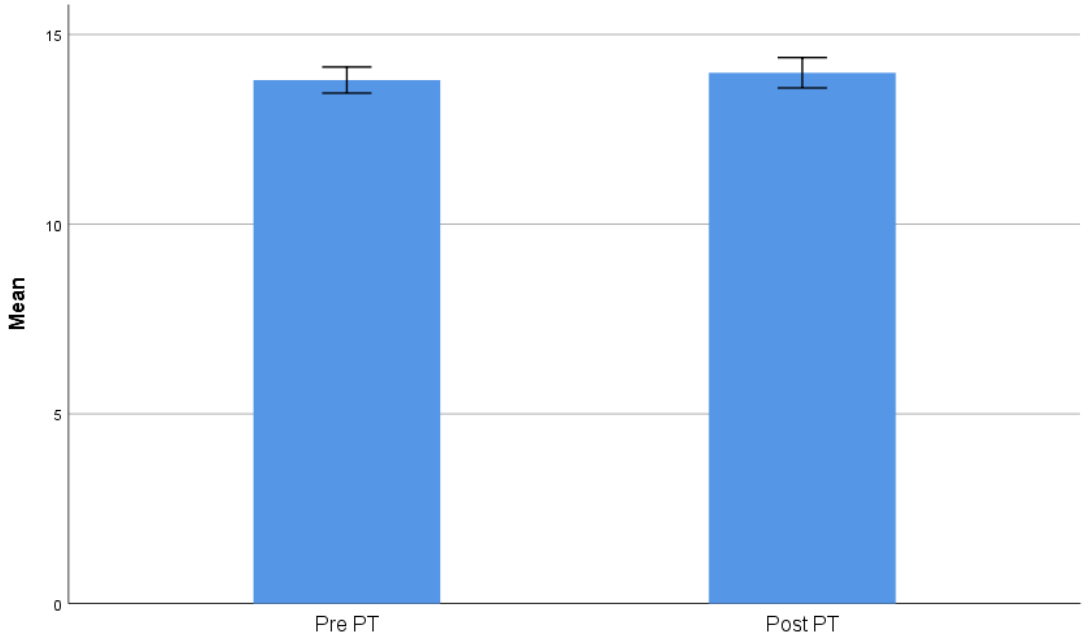
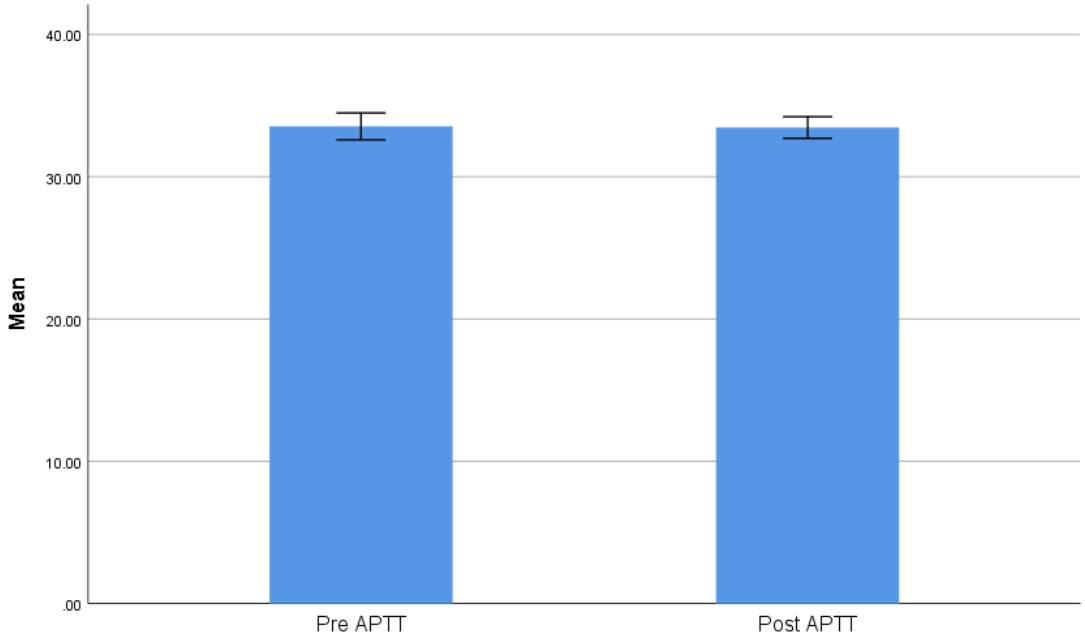


Figure 4.2: Mean and 95% CI for Pre and Post APTT readings



Analysis of pre and post vaccine levels of PT and APTT:

Table 4.5: Paired t-test results of pre and post readings of PT and APTT

Test	Paired Differences					t-test results	
	Mean	SD	Std. Error	95% Confidence Interval			
				Lower	Upper	T	P. value
Pre - Post PT	0.19	1.52	0.211	-0.23	0.62	0.922	0.361
Pre -Post APTT	-0.08	2.30	0.319	-0.72	0.56	-0.241	0.810

The results show that the difference between pre and post mean levels of the two tests were very small; amounting 0.19 and 0.08 for PT and APTT respectively. Moreover, the results show that these differences were statistically not significant at 0.05 for the two tests. The respective p-values were 0.361 and 0.810; each was greater than 0.05.

Table 4.6: Paired t-test results of pre and post readings of PT and APTT according to date of posttest

Test	Pre	Post	Paired Differences					t-test results	
			Mean	SD	Std. Error	95% Confidence Interval			
						Lower	Upper	T	P. value
Posttest at 7 days after pretest:									
PT	13.68	14.07	0.38	1.12	0.22	-0.08	0.85	1.713	0.100
APTT	33.45	33.48	0.03	2.55	0.51	-1.02	1.08	0.055	0.957
Posttest after 7 days from the pretest date:									
PT	13.90	13.92	0.02	1.82	0.35	-0.70	0.74	0.053	0.958
APTT	33.62	33.44	-0.17	2.09	0.40	-1.00	0.65	-0.433	0.669

Table 4.6 shows the results of pre and post readings of PT and APTT according to date of posttest, the result of tests on 7 days' difference shows that the pre vaccine

mean level of PT was 13.68, whereas the post vaccine level was 14.07. The pre and post vaccine mean levels of APTT were 33.45 and 33.48 respectively. Whereas the result on more than 7 days' difference shows that the mean level of PT pre vaccine was 13.90 and 13.92 for the post vaccine while The mean level of APTT for the pre vaccine was 33.62 and 33.44 for the post vaccine.

The results show that posttest date has no significant effects on the differences between pre and post levels of each of the two tests (PT & APTT) at 0.05 level of significance.

The effect of age, gender and posttest date on the levels of PT and APTT:

Analysis of covariance (ANCOVA) was carried to test the effect of age, gender and posttest date on the differences between pre and post levels of PT and APTT. Tables 4.7 and 4.8 show the results of ANCOVA of differences between pre and post levels of the two tests.

The results show that age, gender, posttest date and the interaction between gender and posttest date had no significant effects on the differences between pre and post levels of each of the two tests (PT& APTT) at 0.05 level of significance. This reveals the differences between pre and post levels of the two tests were similar between male and females, similar across posttest dates, and were also similar between different age groups.

Table 4.7: Results of ANCOVA of differences between pre and post PT levels

Source	**Type III Sum of Squares**	**Mean Square**	**F**	**P. value**
Corrected Model	7.211a	1.803	0.767	0.552
Intercept	1.335	1.335	0.568	0.455
Age	1.558	1.558	0.663	0.420
Gender	0.022	0.022	0.009	0.924
Posttest date	0.13	0.13	0.055	0.815
Gender × Posttest date	1.852	1.852	0.788	0.379
Error	110.458	2.35		
Total	119.63			
Corrected Total	117.668			

Table 4.8: Results of ANCOVA of differences between pre and post APTT level

Source	**Type III Sum of Squares**	**Mean Square**	**F**	**P. value**
Corrected Model	17.975a	4.494	0.839	0.507
Intercept	14.994	14.994	2.799	0.101
Age	12.612	12.612	2.355	0.132
Gender	10.571	10.571	1.974	0.167
Posttest date	4.813	4.813	0.899	0.348
Gender × Posttest date	3.216	3.216	0.6	0.442
Error	251.738	5.356		
Total	270.02			
Corrected Total	269.712			

CHAPTER FIVE

Discussion, Conclusion and Recommendations

CHAPTER V
Discussion, Conclusion and Recommendations

5.1 Discussion

Johnson and Johnson COVID-19 vaccine although mostly safe and effective, has the potential risk of developing blood clots, alongside other possible side effects.

The study investigated the effect of Johnson and Johnson COVID-19 vaccine on prothrombin time (PT) and activated partial thromboplastin time (APTT) among the vaccine recipients. This was done by measuring PT and APTT before and after the administration of the vaccine. The total number of participants in this study was 52 with 38 males and 14 females and their ages ranged from 19 to 61 years old.

The result revealedthat the pre vaccine mean level of PT was 13.8 ± SD1.2 and the post vaccine level was 13.9 ± SD 1.4 showing slight statically insignificant increase (p-value 0.361) which agreed with Nasr Eldeen*et al* (2022) whoshowed insignificant result (p-value 0.4) in comparison between the mean of prothrombin time pre and post from (Jansen) vaccines. Whereas the post vaccine level at 7 days after pretestwas 14.07 ± SD 1.33 and 13.92 ± SD 1.55 after more than 7 days from the pretest date both showing no significant difference from the pre vaccine level (p- value 0.100 and 0.958) respectively.

The pre, post vaccine means levels of APTT at 7 days after the pretest and after more than 7 days from the pretest were 33.54 ± SD 3.4, 33.48 ± SD 2.33 and 33.44 ± SD 3.13 respectively, which show no significant difference (p-value 0.957 and 0.669).

The results show that age, gender, posttest and the interaction between gender and posttest had no significant effects on the differences between pre and post PT

levels (p-value 0.420, 0.924, 0.815 and 0.379 respectively) andno significant effectson the differences between pre and post APTT levels (p-value 0.132, 0.167, 0.348 and 0.442 respectively)at 0.05 level of significance.

This reveals that the differences between pre and post levels of the two tests were similar between male and females, similar across posttest dates, and were also similar between different age groups.

Previous studies on other types of vaccines showed similarities and differences. Peyvandi*et al* (2021) whose study was on Pfizer, showed that the mean level of PT pre vaccine was 13, at 7 days after pretest was 13 and 14 after 21 days from the pretest which didn't show significant variations at different time-points before and after vaccination (p-value 0.354)and that the mean level of APTT didn't show significant variations at different time-points before and after vaccination pre vaccine 30, 31 at 7 days after pretest and 30 after 7 days from the pretest (p-value 0.793).

Liu *et al* (2021) whose study was on inactivate vaccine found that coagulation profiles changed significantly after vaccination, in the short-term (7 days) after the 1st inoculation, coagulation profiles were leaning toward shorter Prothrombin Time (PT), whereas the long-term (28 and 42 days) effect was toward prothrombin(PT) andactivated partial thromboplastin time (APTT) prolongation.

5.2 Conclusion

The study concluded that the mean level of PT and APTT before and after the administration of the Johnson and Johnson COVID-19 vaccine were statistically not significant and that age, gender, posttest date and the interaction between gender and posttest date had no significant effects on the before and after levels of each of the two tests (PT& APTT).

5.3 Recommendations

- The study showed no significant difference from the before levels of PT and APTT after the administration of Johnson and Johnson COVID-19 vaccine reveling that it is mostly safe and can be used.

- Another study should be done on different times point after the vaccination.

- Another study should be done on the other types of the vaccine.

- Completion of the other coagulation profile tests such as platelet count, fibrinogen level and D-dimer test.

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Appendix I

Semi –automated biochemical analyzer (ES-105)

APPENDIXES

Appendix II

Sudan University of Science and Technology

Faculty of Graduate Studies

College of Medical Laboratory Sciences

Department of Hematology and Immunohematology

Questionnaire

(Measurement of Prothrombin time and Activated partial thromboplastin time among Johnson & Johnson COVID-19 Vaccine Recipients in Khartoum State)

Sample number:.....

Name:.....

Gender: Male ( ) or Female ( )

Age:.....

Phone number:.....

Address:.....

Result:

Before the administration of COVID vaccine Date of pretest: \ \ 2022

PT:.....

APTT:.....

After the administration of COVID vaccineDate of posttest: \ \ 2022

PT:.....

APTT:.....