**UNIVERSITY OF GHANA**

# ART SIGNIFICANTLY IMPROVES THE COAGULATION PROFILE OF HIV PATIENTS: A CASE-CONTROL STUDY AT MAMPONG MUNICIPAL HOSPITAL, ASHANTI-REGION, GHANA

**BY**

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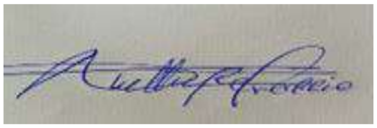
# DECLARATION

By signing this document, I certify that this study is the outcome of my own research and that no part of it has ever been submitted for credit toward another degree at the University of Ghana or anywhere else. All citations have been properly credited.

Any inadequacies are all my responsibility.



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## CERTIFICATION

I hereby attest that the supervision of this thesis followed the guidelines established by the University of Ghana.

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## DEDICATION

I dedicate this work to the Almighty God, Dr. Jacob Manu, Mr. Kwadwo Oteng Akyina, Mr. Enos Kwame Adepa, Ellen Osei, Grace Abena Oppong Kwakyewaa, Elizabeth Diamond Brobbey and to GNPC Foundation.

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# ABSTRACT

**Introduction:** Infection with the Human Immunodeficiency Virus (HIV) causes major morbidity and mortality through a variety of mechanisms, one of which is coagulation problems. ART has considerably lower rates of viral transmission, HIV-related illness, and fatality while simultaneously improving the quality of life of persons living with HIV. The study's goal was to ascertain how ART affected HIV patients' coagulation profiles. **Methods:** One hundred and two (102) HIV patients (52 on ART and 50 newly diagnosed, not on ART as controls) were enrolled in this case-control research from the antiretroviral clinic of Mampong Municipal Hospital. Blood samples were taken to measure the prothrombin time (PT), platelet counts, and activated partial thromboplastin time (APTT), with the INR being computed from the PT values. **Results:** The mean platelet count (ART: 320.10 ±63.29 vs ART-naïve: 238.82 ± 75.18) was significantly higher in participants on ART compared to the ART-naïve participants ($p<0.001$). ART and ART-naïve participants had significantly prolonged PT (>16sec), $p=0.001$ and $p=0.025$ respectively. Both ART and ART-naïve participants had high INR values above the biological reference interval of 0.80 – 1.30 ($p<0.001$ and $p=0.005$ respectively). APTT of ART participants was normal, whereas ART-naïve participants had significantly prolonged APTT (>40sec) [$p=0.001$]. No significant differences were found between the coagulation profiles of ART patients taking drug regimen 1, R1[Tenofovir (TDF)+Lamivudine (3TC)+Efavirenz (EFV)], and those taking drug regimen 2, R2 [Tenofovir (TDF)+Lamivudine (3TC)+Dolutegravir (DTG)]. **Conclusion:** The haemostatic parameter where HIV has the most of an impact is prothrombin time (PT). ART combination (TDF+3TC+DTG) and (TDF+3TC+EFV) can enhance the coagulation profile in HIV-infected patients, by improving platelet count and APTT.

# TABLE OF CONTENT

## Table of Contents

## LIST OF ABBREVIATION

3TC – Lamivudine

AIDS – Acquired Immune Deficiency Syndrome

APTT – Activated Partial Thromboplastin Time

ART – Antiretroviral Therapy

AZT – Zidovudine

CD4 – Cluster of Differentiation-4

CDC – Centre for Disease Control

DTG – Dolutegravir

EFV – Efavirenz

HAART – Highly Active Antiretroviral Therapy

HIV – Human Immunodeficiency Virus

INR – International Normalized Ratio

IV – Intravenous

MMH – Mampong Municipal Hospital

NNRTI – Non-Nucleoside Reverse Transcriptase Inhibitor

NRTI – Nucleoside Reverse Transcriptase Inhibitor

PLT – Platelet

PLWHIV – People Living with HIV

PT – Prothrombin Time

RNA – Ribonucleic Acid

TDF – Tenofovir

TFPI – Tissue Factor Pathway Inhibitor

UNAIDS – Joint United Nations Programme on HIV/AIDS

WHO – World Health Organization

# LIST OF TABLES

# LIST OF FIGURES

# CHAPTER ONE

# 1.0 INTRODUCTION

## 1.1 Background of the Study:

Infection with the Human Immunodeficiency Virus (HIV) has become a global stigma that is spreading. It causes major morbidity and mortality through a variety of mechanisms, one of which is coagulation problems, which occurs often in late-stage HIV infection (Raman et al., 2016).

The defect could be caused by the host, medication, or viral causes. Age, IV drug misuse, CD4 count, opportunistic infections, related malignancies, acquired hypercoagulable condition, and endothelial dysfunction are all host variables (Shankar and Dube, 2004). In advanced stages of HIV infection, with concomitant immunosuppression, these coagulation problems become more severe (Elaine and Groopman, 2013). The virus or antiretroviral therapy medicines can cause liver damage, which can lead to coagulation problems in HIV patients (De Andrade et al., 2007). The development of combined antiretroviral therapy (ART) for the treatment of HIV infection has been widely regarded as the gold standard in HIV patient management (Odunukwe et al., 2005). There has been a dramatic drop in HIV/AIDS-related mortality since the successful introduction of antiretroviral therapy (Palella et al., 2006). Antiretroviral drugs target various processes in replication of HIV, including HIV replication proteins and glycoproteins 41 and 120 (Rathbun, 2013).

According to Ephraim et al. (2018), the type of ART regimen used in HIV patients can impact coagulation and other haematopoietic characteristics in people living with HIV (PLWHIV). According to their findings, ART could enhance the coagulation profile in PLWHIV. PLWHIV's coagulation profiles may respond differently to various ART regimens, where the best combination does seem to be Zidovudine + Nevirapine + Efavirenz. Combining two inhibitors of non-nucleoside reverse transcriptase with one inhibitor of nucleoside reverse transcriptase may increase the likelihood that individuals on ART may regain their disordered coagulation profile.

### 1.2 Problem Statement:

HIV patients' coagulation abnormalities can be linked to the virus's impact, which can result in a range of anomalies that put patients at risk for coagulation problem occurrences (Friel et. al., 2007). The primary causes of the abnormalities have been the virus itself, opportunistic infections linked to the virus, negative effects of antiretroviral therapy (ART), and other related problems (Opie, 2012; Parinitha et al., 2012; Friel et al., 2007). There were no cases of coagulation anomalies in the HIV individuals tested, according to a Dikshit et al. (2009) study. However, due to the virus' deregulatory effects on the coagulation system and haematopoiesis processes, multiple investigations have found that HIV infection can cause severe hematological abnormalities (Seloum et al., 2018). Thrombocytopenia, endothelial cell failure, coagulation factors being activated, with the existence of a phospholipids-specific antibody are some of the problems brought on by HIV infection. Drugs used in effective antiretroviral therapy have side effects such as damage to the liver, which reduces the amount of coagulation factors produced (Raman et al., 2016). HAART medications, in particular protease inhibitors, affect lipid and glucose metabolism, which results in endothelial dysfunction (Funderburg, 2014). However, some researchers have proposed that certain different combinations of the ARTs have beneficial effects to potentially improve the coagulation profile of HIV patients (Ephraim et al., 2018).

### 1.3 Study Aim:

The main aim of the study was to determine the coagulation profile among HIV patients on ART at Mampong Municipal Hospital (MMH) in comparison to newly diagnosed HIV positive treatment-naïve group as control.

### 1.4 Specific Objectives:

I. To determine Platelet (PLT) count in HIV patients at MMH.

II. To determine Prothrombin Time (PT) in HIV patients at MMH [with calculated INRs].

III. To determine Activated Partial Thromboplastin Time (APTT) in HIV patients at MMH.

IV. To determine the impact of ART on HIV patients' coagulation profiles at MMH.

V. To find out the specific combination of ART regimen that could potentially improve coagulation profiles in HIV patients.

### 1.5 Justification:

In order to analyze the coagulation profiles and potential impact of ART on the coagulation profile, the study compared the coagulation profiles of HIV patients on ART at the Mampong Municipal Hospital (MMH) to newly diagnosed HIV positive treatment-naïve group as control. Haemostasis is greatly aided by platelets. Due to autoimmune damage brought by HIV infection as well as consumption coagulopathies present in AIDS, platelets decline (Raman et al., 2016).

Activated Partial Thromboplastin Time (APTT) and Prothrombin Time (PT) are the fundamental tests to respectively determine the intrinsic and extrinsic mechanisms of coagulation (Omoregie et al., 2009). The study evaluated the coagulation anomalies in HIV-infected patients receiving ART at MMH using the aforementioned coagulation parameters together with platelet counts, and it compared these parameters to newly diagnosed HIV patients who had never received ART. This study therefore was to find out the kind of coagulation problems in patients who are HIV positive, and the impact of ART on these coagulation parameters.  The study also expected to find out the specific combination of ART regimen that could potentially improve coagulation profiles in HIV patients. There are six different ART combinations currently being administered at the MMH, which are different from the combinations that was being used about 7 to 8years ago. Currently, ART drug combination (Tenofovir+Lamivudine+Dolutegravir), referred to in this study as regimen 1 (R1) and (Tenofovir+Lamivudine+Efavirenz), referred to in this study as regimen 2 (R2) are the two ART regimens mostly being administered at the Mampong Municipal Hospital for HIV first-line or second-line treatment. No coagulation profile studies have been done in Ghana on these current ART combinations.

# CHAPTER TWO

# 2.0 LITERATURE REVIEW

## 2.1 Structure and Types of the Human Immunodeficiency Virus (HIV)

The genus Lentivirus, which belongs to the retroviridae family of RNA viruses, contains the human immunodeficiency virus (HIV). Subtypes 1 and 2 of HIV are the two subtypes that exist. Even though HIV 2 develops into AIDS slowly, they both cause AIDS. Despite having different genetic makeups, they share several antigens. At least nine genes in HIV are encoded, and the virus is well organized. HIV 1 is the most common strain worldwide and is more usually AIDS-related in the United States of America, Central Africa and Europe, nonetheless, HIV type-2 typically spreads in West Africa and India (Fauci and Lane, 2005). Since HIV are RNA viruses, they need reverse transcription to turn a DNA provirus into a new one in order to proliferate. Figure 1 shows the two primary envelope proteins, exterior gp120 and transmembrane gp41, which are crucial for HIV's attachment to cells, have an icosahedral shape with multiple external spikes. It has a p18 (matrix) inner membrane and a p24 core protein capsid (Pan et al., 2008).

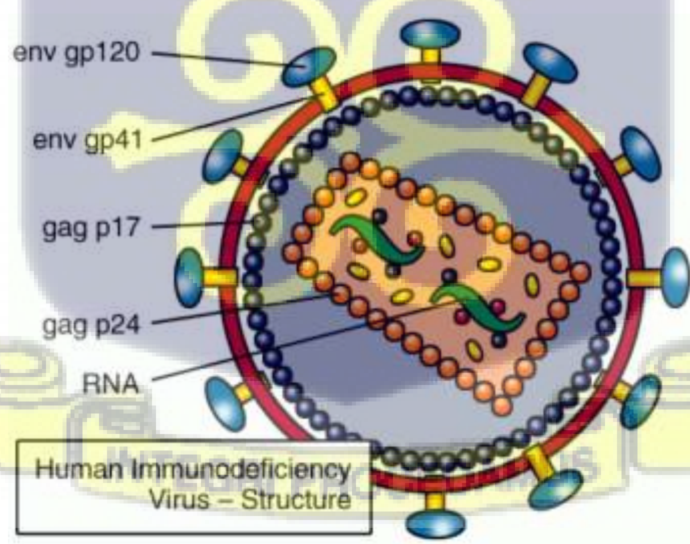



*Figure 2.1: HIV Virus Schematic Illustration*

Source: (Cotran and Richard, 1999)

## 2.2 HIV Genome and Molecular Heterogeneity

These are the structural protein-encoding genes found in HIV:

The Pol gene produces the enzymes protease, RNAses, reverse transcriptase, and integrase. The gag gene produces the proteins that make up the virus's core. The gene Vpu is necessary for the virus development in infected cells, while gene Vpr enhances infection of macrophages with HIV. Vif gene imparts the ability to infect cells. The additional genes are tat, rev, and nef (Fauci and Lane, 2005). There are three categories of HIV-1, and these groupings vary quite a bit.

The first category is m (major) group, in charge of the majority of infections worldwide. A, B, C, D, F, G, H, J, and K are its nine subclasses of genetic relation. It has a wide geographic variety as well. Furthermore, a large number of significant and small circulating recombinant variations (CRV) exist. When two subtypes of a virus infect a person and recombine to form a virus with a selection advantage, CRV are created. Subclasses A and C are more prevalent in Africa, while subclass B is more prevalent in North America and Western Europe. Subclass E is located in Thailand, and other members are dispersed throughout other regions of Africa and Asia. The second is group O (outlier) which is relatively uncommon, is found in France, Gabon, and Cameroon. Then lastly is group N which is found in Cameroon (Seema et al., 2006).

### 2.3 HIV: Mode of Transmission

HIV often spreads via the following routes:

**2.3.1 Sexual Transference:** Heterosexual transmission is the most frequent method of infection in the world. HIV infection susceptibility is increased by combined sexually transmitted diseases, especially those that cause genital ulcers (Fauci and Lane, 2005).

**2.3.2 Blood Transfusion:** This occurs frequently in underdeveloped nations where there are inadequate HIV testing facilities. Patients who have multiple or recurrent blood transfusions, such as those with sickle cell anemia, hemophilia, and pregnant women, are the most vulnerable.

**2.3.3 Mother to Child Transmission (MTCT):** During pregnancy, birth, or breastfeeding, an HIV-positive mother can pass her infection to her unborn child. High maternal plasma viraemia levels are linked to a higher rate of transmission.

**2.3.4 Intravenous Route:** In industrialized nations, intravenous drug abusers exchange needles that have previously been used to inject one person's substance of abuse to the other. One such way that HIV can be transmitted is through the use of unsterilized needles and syringes. HIV can also be found in infected secretions such as saliva, tears, urine, cerebrospinal fluid, and saliva (Fauci and Lane, 2005).

## 2.4 Life Cycle of HIV

As an RNA virus, HIV uses the reverse transcriptase enzyme to convert its genomic RNA to DNA. Viral glycoprotein 120 binds to CD4 on the cell surface to start the process. Gp120 undergoes conformational changes that expose gp41. CCR5 and CXCR4 are chemokine co-receptors with which Gp 41 interacts. The host plasma membrane and the lipid layer of the viral gp41 fuse together. Fusion happens before penetration. Viral genome-containing virus core moves into cytoplasm. Uncoiling follows. Antigen P24 is liberated from where it is surrounding viruses' RNA to facilitate the transfer viruses' genomic information (viruses' RNA and related proteins) into the interior of the affected cell (Costello, 2001).

When the reverse transcriptase enzyme is activated, viral RNA undergoes reverse transcription, yielding double-stranded DNA that is then carried into the host cell's nucleus along with some viral proteins. Viral DNA is incorporated into host DNA in the nucleus. Integrase, a viral enzyme, conducts this activity with the aid of vpr, a viral protein. The freshly created viral RNA is carried into the cytoplasm once the viral DNA has been translated into two strands of RNA. HIV components including reverse transcriptase, structural proteins, proteases, and integrases are translated from one strand of RNA. The genetic material for the new virus is created from the other strand. After the protease enzyme has cleaved the protein, the various components are subsequently separated. The host cell membrane is then contacted during viral assembly and budding, and the nucleocapsid, which contains the integrated viral RNA, is created. The host cell membrane deforms due to the genetic material contained in the nucleocapsid, eventually forming an outer coat. An immature virion is created as a result, and it is then circulated. After maturing, the virion reproduces itself in the cycle (Seema et al., 2006).

## 2.5 Pathogeny of HIV

A subset of T lymphocytes called helper T cells, which are gradually becoming qualitatively and quantitatively deficient, is the defining feature of HIV infection. The presence of the CD4 molecule, the main cellular HIV receptor, on the surface of these helper T cells, defines them phenotypically. HIV fusion and entry require co-receptors; the CXCR4 and CCR5 are the two main co-receptors., which are members of the seven transmembrane G protein family. At various stages of the disease, the multifactorial, multiphasic pathogenic mechanism of HIV disease is present. Direct cell infection, cell death, immunological clearance of infected cells, and immune exhaustion are the mechanisms causing cellular depletion and immune dysfunction (Costello, 2001).

Low CD4 cell counts increase the risk of opportunistic infections and certain neoplasms, which are diseases that define AIDS. Upon entering the bloodstream, HIV is eliminated by the lymphoid organs, including the spleen, where it replicates among CD4 cells. When viraemia spikes, this process accelerates, causing the virus to spread quickly to the brain and other tissues through the lymphoid organs. During the spike in viraemia, symptoms resembling acute mononucleosis are observed. Despite a strong cellular and humoral immune response being established, persistent viral replication persists, preventing the virus from being cleared by the immune system. Almost all patients have viraemia during initial infection, even though some may continue to be asymptomatic or may not remember having any symptoms (Durotoye et al., 2012).

### 2.6 HIV Clinical Presentation

HIV is characterized by severe immunodeficiency, which develops as a subpopulation of T lymphocytes loses both qualitative and quantitative capacity (helper T cells). HIV infection can have a variety of clinical effects, from HIV acute syndrome to a protracted mild state to a serious illness (Fauci and Lane, 2005).

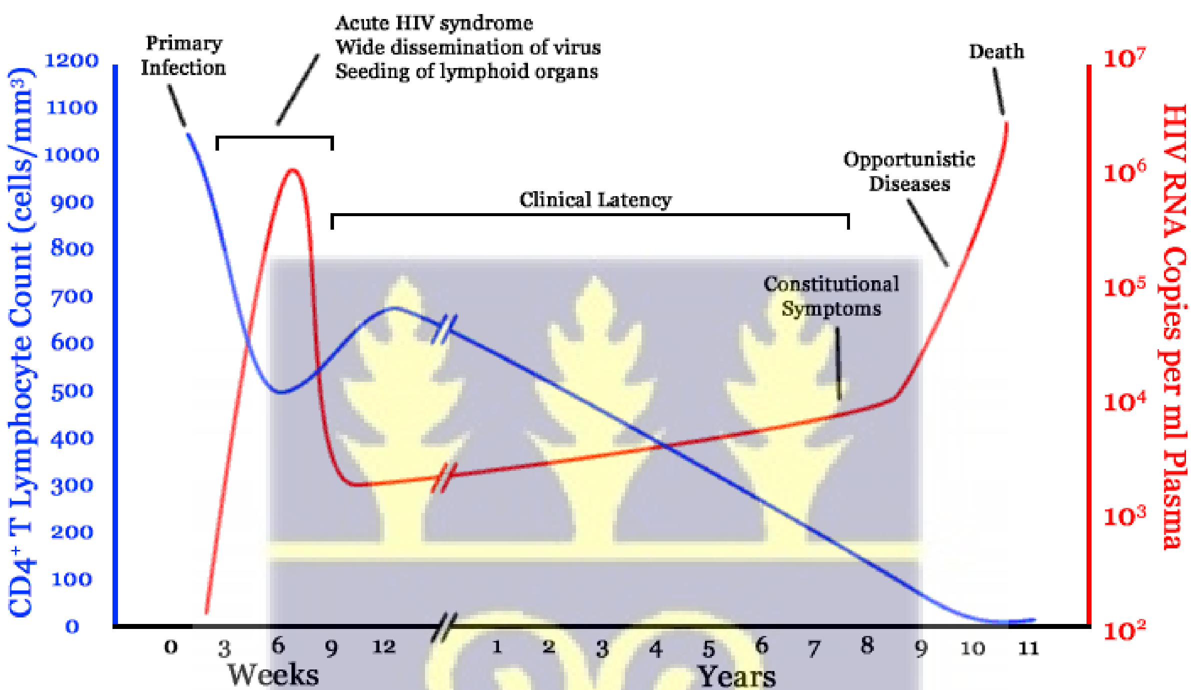



*Figure 2.2: The Evolution of HIV Infection*

Source: (Fauci and Lane, 2005)

#### 2.6.1 Acute Stage of HIV Syndrome

The typical clinical signs with symptomatic seroconversion include headache, lymphadenopathy, pharyngitis, fever, malaise, retroorbital discomfort, myalgia, diarrhea, weight loss, nausea, and vomiting. This happens typically 3–6 weeks after the first infection. At this stage, hepatosplenomegaly, oral candidiasis, and neurological symptoms are possible. The appearance of an immunological response, antibody response, and a decrease in plasma viraemia occur in conjunction with clinical recovery after 2 to 3 weeks of the acute retroviral syndrome (Fauci and Lane, 2005).

### 2.6.2 Asymptomatic Stage of HIV Infection

While the interval between infection and the onset of clinical symptoms differs, the average duration for untreated patients is roughly ten years. Viral replication is continuing at this time, although no clinical symptoms or signs are present. HIV RNA levels are directly linked to the rate of disease development (Costello, 2001).

### 2.6.3 Symptomatic Stage of HIV

HIV symptoms can develop at any point during the infection process. When CD4 is less than 200cells/ul, severe and life-threatening consequences of HIV illness happen. It has been reported that the case definition for AIDS includes indicators, and without medical treatment, the patient often passes away within 1-3 years on average (Fauci and Lane, 2005).

### 2.7 Overview of Haemostasis

An initial haemostatic plug is formed at the site of the vascular damage. Around the platelet clog, a fibrin clot forms, and the clot dissolves once the tissue has healed completely. This intricate web of interactions is known as hemostasis. It consists of several interconnected physiological mechanisms that keep blood in a fluid state, stop bleeding, maintain vascular patency, and restore it after clot breakdown. It is an equilibrium of procoagulant and anticoagulant processes working along with a fibrinolytic process (Durotoye et al., 2012). Haemostasis is made up of five essential elements, including platelets, blood vessels, coagulation factors, anti-clotting agents, and the fibrinolytic system (Hoffbrand et al., 2007).

#### 2.7.1 Important Elements of the Hemostatic System

##### 2.7.1.1 Vessels for Blood Circulation

The intima, media, and adventitia make up the basic three layers of blood arteries. The basal membrane of microfibrillar sub endothelial cells, which is formed of elastin and collagenous fibers, rests on intima, the innermost stratum, which is covered by a single layer of endothelium. Collagen fibres and cells of smooth muscles are primarily organized in circles in the media or middle layer. The exterior elastic lamina separates it from the adventitia; whereas elastin permits vessels to stretch and recoil, muscle cells contract and relax. The collagen fibers and fibroblasts that make up the adventitia, or top layer, keep blood arteries safe and tether them to nearby tissues (Jaiyeola, 2015).

##### 2.7.1.2 Endothelium

The vascular endothelium produces and secretes vital substances that have a strong impact on hemostasis. Due to the substantial inhibitory effects of the molecules the intact endothelium produces and releases, such as prostacyclin and nitric oxide, it is often non-thrombogenic. However, after a vascular injury, endothelial cell activation induces a phenotypic procoagulant state which releases tissue factor (TF) by von Willebrand factor (vWF). This increases localized thrombus development (van Hinsbergh, 2011). The primary prostaglandin with vasodilatory activity that endothelial cells produce is prostacyclin (prostaglandin I2). Increasing cyclic adenosine monophosphate concentration inside of the platelet, prostaglandin I2 prevents platelet and leucocyte aggregation (Barmore et al., 2021). Smooth muscle cells, platelets and endothelial cells all create nitric oxide, which has vasodilatory effects. Because of the inhibition of phosphatidylinositol metabolism, platelet aggregation and the concomitant release of procoagulant substances like vWF and factor V from storage granules are prevented. Alpha granules of platelet, weibel-palade body and endothelial cells stores vWF. At the site of damage, it encourages platelet adherence to the subendothelium. It also carries Factor VIII, inhibiting proteolysis by doing so (Black and Selby, 2013). Two endothelial receptors, thrombomodulin and endothelial cell protein C receptor, are expressed on it and are responsible for the endothelium's anticoagulant properties. Additionally, protein S, a component that deactivates factors Va and VIIIa and stimulates activated protein C, is synthesized and secreted by the endothelial cells. Endothelial cells produce tissue factor pathway inhibitor, which assembles into the quaternary inhibited complex TF- VIIa- Xa-TFPI. It is crucially determined by the balance between endothelial prothrombotic and antithrombotic activity whether a clot forms or is lysed (van Hinsbergh, 2011).

### 2.7.1.3 Platelets

They are essential in the development of the main haemostatic plug, which stops bleeding at the injured site. Additionally, for the construction of complex enzymes like prothrombinase and tennase, they offer the cellular surface. They adhere and aggregate with the creation of a hemostatic plug, release chemicals that activate platelets and promote coagulation, create additional prothrombotic surface for the coagulation system's reactions, among other things. Alpha granules found in the coagulation factors Factor V, VIII, and fibrinogen are present in the platelets (Calverley, 2019). Adenosine triphosphate, calcium, serotonin, and thick granules containing these substances are also present. Vasoconstriction brought on by these medications reduces blood flow to the damaged location. Structures in the sub-endothelium such as micro-fibrils and collagen are vulnerable when a vessel wall is broken, and von Willebrand factor aids platelets in adhering to these components (Kato, 2002).

#### 2.7.1.4 Coagulation Factors

The third part of the hemostatic process is the coagulation cascade, which greatly contributes to thrombosis. A series of proenzymes are transformed into their active enzyme forms in an enzymatic cascade, and the last enzyme, thrombin, converts plasma fibrinogen from soluble to insoluble. Activated platelets usually provide the phospholipid complex on which the activated coagulation components and cofactors are formed. Calcium ions hold this complex together. A common theory of blood coagulation was the waterfall coagulation cascade, in which various intrinsic and extrinsic routes converged to produce factor Xa (Black and Selby, 2013).

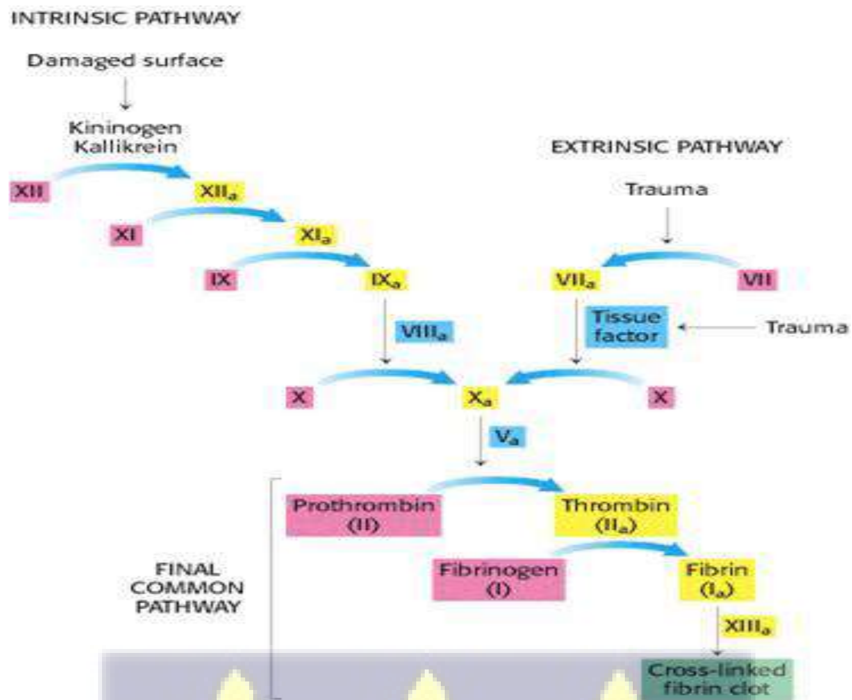



*Figure 2.3: Blood coagulation pathways*

Source: (Black and Selby, 2013)

The role that tissue factor plays in starting coagulation, however, shows that there is no clear distinction between the two pathways.

The expression of tissue factor serves as a haemostatic membrane at epithelial surfaces at biological barriers like the skin and vascular adventitia. This makes sure that after vascular integrity is compromised, cells are quickly exposed to blood that express tissue factor, causing blood coagulation to begin. When membrane-bound tissue factor is exposed to zymogen factor VII, blood coagulation begins. Factors IX and X are activated by activated factor VIIa, which has a strong affinity for tissue factor. Factor Xa produces a small quantity of thrombin in the absence of Va (cofactor), which causes factors V and VIIIa to become reactivated (Barmore et al., 2021).

When factor VIIIa and factor IX combine to produce factor VIII-IXa (tenase complex), enough activated factor X (Xa) combine with factor Va to form factor Va-Xa complex (prothrombinase), which causes massive thrombin synthesis, followed by formation of a clot. Inhibitor of the tissue pathway is activated by this thrombin, which quickly inactivates the tissue factor-VIIa complex (Zirlik et al., 2017).

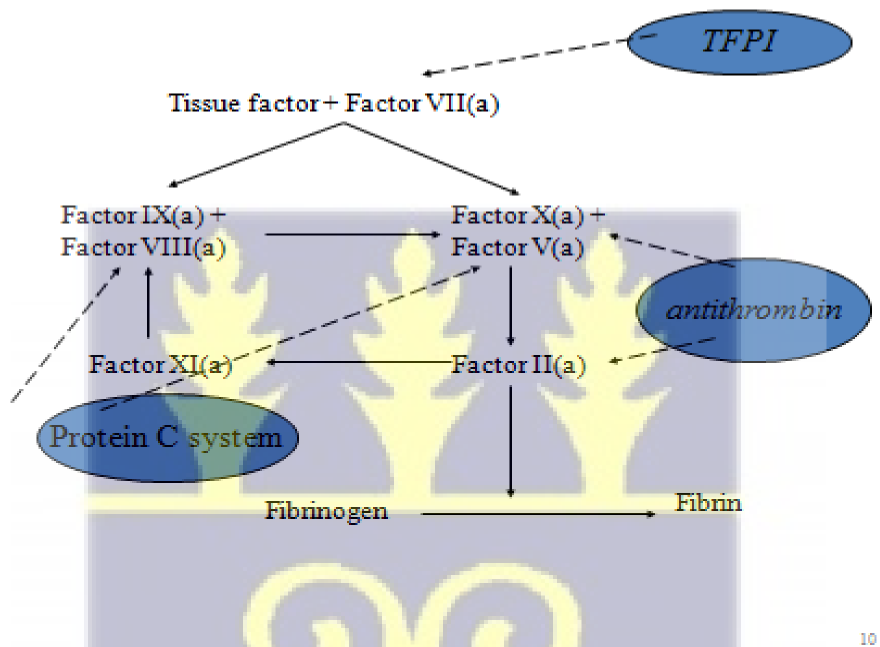



*Figure 2.4: Classical blood coagulation theory*

Source: (Zirlik et al., 2017)

**2.7.1.5 Coagulation Inhibitors**

Once the coagulation cascade is started, they come into play. In order to prevent clotting of the entire vascular tree, this keeps the cascade of clotting contained to the injury's location. The coagulation process needs to be checked, like any other form of defense. This is due to the fact that thrombin and other proteolytic enzymes can be fatal if their function is unchecked. For instance, enough thrombin can be produced by 10ml of plasma to clot the body's entire supply of fibrinogen in about 30 seconds. However, this is avoided because the procoagulant responses are more intense near the platelet block than they are elsewhere, where the clotting-preventive ability of a material is present in the plasma (Black and Selby, 2013). The anticoagulant can be divided into two categories components of the protein C system and inhibitors of serine proteases (Hoffbrand et al., 2007).

**Inhibitors of Serine Protease**

The following includes some serine protease coagulation factor inhibitors that are present in human plasma. The only two that have any bearing on hemostasis are antithrombin and heparin co-factor II. The liver produces antithrombin, which has a molecular weight of 58KDa. It combines 1:1 with thrombin and other serine protease coagulation factors. Thrombin inactivation by antithrombin is elevated by more than 2000 times when using heparin. Co-factor II for heparin is a particular thrombin. It has negligible impact on activated factor X. Heparin increases the extent at which heparin cofactor II neutralizes thrombin by a factor of more than 1000. Heparin and other compounds with heparin-like properties are classified as glucosaminoglycans. It increases the capacity of antithrombin to inactivate thrombin by attaching to the tryptophan residue. Heparin is undetectable in human plasma, however endothelial cells contain heparin-similar compounds such dermatan sulphate and heparin sulphate, which help antithrombotic actions work more effectively. Protein Z and protein Z dependent inhibitor, antitrypsin, C1 esterase inhibitor, antiplasmin, and macroglobulin are further serine protease inhibitors (Schaller and Gerber, 2010; Mohammed, 2021).

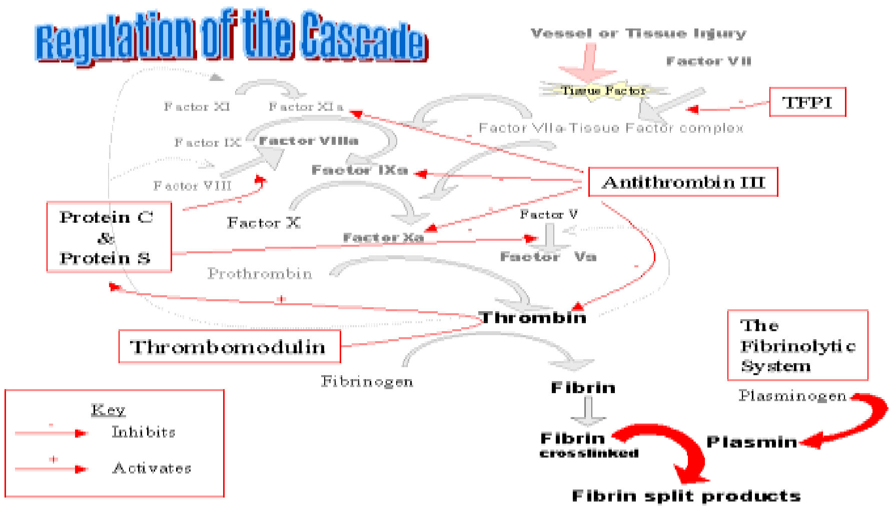



*Figure 2.5: Sequence of the coagulation chain*

Source: (Griffin, 2007)

**Protein C Pathway**

The protein C pathway is a complicated set of events that controls the negative feedback mechanism that limits the procoagulant activity of the turned on versions of factors V and VIII working on the prothrombinase and tennase complex (Hoffbrand, 2016).

Protein C is a serine protease that depends on vitamin K and shares the same unit structure as procoagulant factors VII, IX, and X. Through the action of thrombin, it becomes activated to activated protein C (APC), which then exerts its anticoagulant effect. The anticoagulant activity of activated protein C against Va and VIIIa is increased as a result of an interaction with protein S, which is attached to the surface of active platelets' phospholipids. By interfering with the binding sites for IXa and Xa, respectively, tennase and prothrombinase lose their ability to function. This process is ineffective without protein S. Since it depends on vitamin K, the liver produces a minimal amount of protein C in infants. α-2 antitrypsin with plasminogen activation inhibitor type I (PAI) both work to block activated protein C (Hoffbrand, 2016).

**Thrombomodulin**

All endothelial cells in the body, with the exception of the hepatic sinusoids, lymph nodes, and brain, contain this integral transmembrane receptor. It has a transmembrane, extracellular area as well as cytoplasmic outgrowth. It combines in a 1:1 ratio with thrombin to stop thrombin from attaching to factors XIII, VIII, V, and fibrinogen substrates that are in the aggregation process. Endothelial cells endocytose the thombomodulin-thrombin complex. Thrombomodulin circulates back to the cell membrane and thrombin is broken down by lysosomes (Esmon and Owen, 2004).

**Inhibitor of Tissue Factor Pathway**

It is present on the surfaces of endothelial cells and aids in factor VIIa inhibition. The complex of the protein and factor Xa engages with TF-VIIa to produce an inhibited quaternary complex (FXa-TF-VIIa complex). The majority of inhibitors of tissue factor pathway is found among endothelial cells, particularly those in the microcirculation, while only 5–10% is found in platelets and plasma. Heparin causes a significant rise in TFPI plasma levels (about 10 folds). Due to increased usage, DIC, sepsis, and following surgery can all result in somewhat low TFPI levels (van Hinsbergh, 2011).

**2.7.1.6 Fibrinolytic System**

The fibrinolytic system's basic goal is to either prevent or quickly eliminate excess fibrin deposition that exceeds the amount needed to stop blood loss from injured arteries. Fibrin catalyzes the majority of fibrinolysis events, which means that fibrin facilitates its own destruction. Fibrinolysis is a confined, surface-bound process. The following are the elements of the fibrinolytic system: plasmin, plasminogen, plasminogen activators in the body (produced from plasma or tissue), external activators of plasminogen (derived from bacterial or venom) and plasmin inhibitors.

Venous in addition to arterial thrombosis have been successfully treated with both endogenous and exogenous fibrinolytic agents (Longstaff and Koley, 2015).

Both plasmin and plasminogen are glycoproteins that serve as the zymogens for serine proteases. By enhancing the conversion of plasminogen to plasmin by two to three orders of magnitude, the fibrinolytic response to a fibrin clot is localized with the help of tissue-type plasminogen activator (t-PA), by which the effects of circulatory antiplasmins are mostly shielded from plasmin (Barthel et al., 2012). Numerous substrates, including factors V and VIII, can be hydrolyzed by plasmin. Fibrinogen and fibrin are broken down into fibrin degradation products, which are tiny soluble peptides. A, B, and gamma chains in two sets with disulfide bridges make up fibrinogen, and they are joined by a coiled section, to a central E domain. A succession of soluble degradation products is produced as a result of fibrin and fibrinogen being broken down by plasmin in an orderly sequence. The fibrinogen is digested by plasmin, which produces X fragment, which can still gradually form a clot, by cleaving the fragment B beta and the alpha A polar appendage. As a result of more plasmin activation, D fragment is released from X fragment to produce Y fragment. Fragments D and E are created by further cleaving fragment Y. The Y and D components' smaller pieces prevent typical polymerization of fibrin monomer while the larger X fragments can polymerize into weak clots. Cross-linked fibrin degradation products, also known as D-dimers and oligomers of fragments X and Y, are produced by the action of plasmin. These fibrin degradation products can be identified employing latex beads covered in monoclonal antibodies (Weisel and Litvinov, 2014).

### 2.7.1.7 Activators of Plasminogen

Activators of tissue plasminogen (tPA) are serine proteases produced by endothelial cells. tPA is present in majority of extracellular bodily fluids, including milk, saliva, bile, urine, and cerebrospinal fluid. With only a two-minute half-life, the liver quickly eliminates it from the body. Its affinity for and effect on plasminogen are enhanced after it binds to fibrin. Plasmin breaks down tPA, increasing its performance by revealing its attachment points and enabling tPA more easily form complexes with plasminogen and fibrinogen (Schaller and Gerber, 2010).

Because it was initially isolated from urine, urokinase plasminogen activator (uPA) is also known as urinary plasminogen activator. u-PA is a glycoprotein that is present in urine and has plasma values of 2-4 ng/mL. It is produced by fibroblast-like cells in the gastrointestinal tract as well as the kidney's collecting ducts and tubules. A serine protease known as a zymogen is secreted (prourokinase). Kallikrein and plasmin cleave it to create active uPA. A C-terminal serine protease domain, a kringle domain, and an amino-terminal growth factor domain make up the structure of u-PA. Plasminogen is broken down by urokinase plasminogen activator at the same bond as tPA (Mosnier and Bouma, 2006).

Exogenous plasminogen activators can be found in plants, certain bacteria, vampire bat saliva, and snake venom. The most well-known ones are staphylokinase and streptokinase (SK). Different strains of -hemolytic Streptococci generate the protein streptokinase, which is used to treat life-threatening thrombotic conditions. A non-enzymatic polypepetide called streptokinase binds to plasminogen to form a stable complex. The ability to activate human plasminogen is acquired by SK indirectly through the formation of plasminogen or plasmin 1:1 complex. The Arg560-Val561 link is cleaved intramolecularly to activate the zymogen catalytic site without the use of proteolytic enzymes. This is in contrast to t-PA and u-PA, both of which have inherent proteolytic process.

The Staphylococcus aureus protein staphylokinase has no enzymatic activity, but instead creates a 1:1 combination with fibrin and tiny amounts of plasmin to activate additional plasminogen molecules (Mosnier and Bouma, 2006; Barmore et al., 2021).

**2.7.1.8 Fibrinolysis Inhibitors**

Inhibitors of plasminogen activator (PAIs), the majority are serpins, block the body's whole supply of fibrinogen from being broken down by the plasmin that is produced. Antiplasmins, which are circulating plasmin inhibitors, also stop this (Mohammed, 2021).

Type-1 Plasminogen Activator Inhibitor (PAI-I): It functions as a significant quick plasmin serpin inhibitor, u-PA, then to a lessening degree, tPA. Endothelial cells secrete it, and platelet alpha granules also contain it. A 1:1 complex is created when tPA, u-PA, and plasmin, the 3 primary profibrinolytic serines proteases, all PAI-1 enzymes split the same bond and as a result blocked. The liver quickly eliminates these compounds (Zirlik et al., 2017). While its local concentration in platelet-rich thrombi can be substantial due to its continual synthesis in platelets, its typical plasma concentration of PAI-1 is only about 0.4nM. Due to interactions with vitronectin, it can build up at the sites of vascular damage to prevent early lysis of the forming thrombus (Mosnier and Bouma, 2006).

Type-2 Plasminogen Activator Inhibitor: The placenta primarily produces this serpin inhibitor of tPA, and it helps to prevent fibrinolysis during pregnancy. Monocytes and epidermal cells both generate it. It is believed to be a regulator of non-fibrinolytic intracellular proteolytic processes (Zirlik et al., 2017).

Plasmin inhibitors: Serpin alpha-2 antiplasmin is the most effective plasmin inhibitor, and there are many others as well. Plasminogen levels in plasma are higher than those of 2 antiplasmin, although the inhibitor quickly neutralizes only a little amount of plasmin produced. The main physiologic inhibitor of plasmin is 2-antiplasmin (2-AP), or 2-plasmin inhibitor, which circulates at a concentration of around 70 g/mL. It can be chemically cross-linked to fibrin to increase its resistance to lysis and creates a very stable, inactive plasmin-2-AP complex. Due to the exposed lysine residues, it can block plasminogen interactions with fibrin in a competitive manner (Schaller and Gerber, 2010).

### 2.8 Global HIV Epidemiology and Statistics:

Since the peak of HIV infection in 2004, HIV remains a significant global public health issue, and about 36.3 million people have lost their lives (WHO, 2020). The World Health Organization (WHO) projected that an estimated 680,000 individuals will die from HIV-related causes worldwide in 2020. As of the end of 2020, just about 37.7 million were HIV-positive individuals, and 1.5 million people becoming newly infected with HIV (UNAIDS, 2021). The prevalence of HIV worldwide, according to the Centers for Disease Control and Prevention (CDC), among persons aged 15 to 49 years old was expected to be 1.7 percent by the end of 2020. By the end of 2020, UNAIDS set a global aspirational objective of 90-90-90, which meant that 90 percent would be cognizant of their condition, 90 percent would be receiving therapy, and 90 percent would have viral suppression. By the end of 2020, however, 84 percent of all PLWHIV knew their status, 73 percent were taking treatment, and 66 percent were virally suppressed (WHO, 2020). Ninety percent of those who received therapy were virally suppressed. This demonstrates the medications' efficacy while also revealing that there is additional work to be done in terms of HIV surveillance to completely achieve these goals.

Africa has the greatest HIV infection rate, accounting for roughly 60% of all new HIV infections worldwide (UNAIDS, 2021). According to the Ghana AIDS Commission - HIV fact sheet (2019), there were 342,307 PLHIV in Ghana, with 122,321 (36%) males and 219,986 (64%) females, indicating that females are more likely than males to be infected with HIV. Adults aged 15 and up made up about 92 percent of the total. As of 2019, 20,068 people in Ghana had been newly infected with HIV, with the infection rate among adults 15 and over (85 percent) being higher than that of children under 14 years old (15 percent).

**2.9 Antiretroviral Therapy (ART):**

ART is one of the treatments used to help people with HIV to suppress viral replication (Tanle et al., 2017). By boosting CD4 counts and lowering viral loads, ARTs greatly improve the quality of life of HIV/AIDS patients (Osonuga et al., 2010). Only over 6 million of the 9 million HIV infection diagnoses in 2010 requiring antiretroviral medication (ART) in Sub-Saharan Africa were able to receive it (Osonuga et al., 2012). Globally, 27.5 million people had access to ART by the end of 2020, up from 7.8 million in 2010. As of the end of 2020, 208,811 PLWHIV were reported to be receiving ART in Ghana, up from 81,987 in 2015 and 40,575 in 2010 (WHO, 2021).

Effective viral suppression to minimize non-AIDS-related morbidity and mortality, preventing irreversible immune system damage, and preventing HIV transmission to others are only a few of the advantages of using ART (Jain and Deeks, 2010). Fusion inhibitors (FI), Protease inhibitors (PIs), Nucleoside reverse transcriptase inhibitors (NRTIs), Non-nucleoside reverse transcriptase inhibitors (NNRTIs), and Integrase inhibitors are some of the antiretroviral drugs used to treat HIV infection (AVART, 2021). Currently, the WHO recommends highly active antiretroviral therapy (HAART) for the treatment of HIV infection. It combines at least three retroviral medicines into a single set dose. HAART has considerably reduced mortality, morbidity from HIV co-infections, and viral transmission rates while simultaneously improving the quality of life of persons living with HIV (Delaney, 2006).

The Ministry of Health (MoH), Ghana Health Service (GHS) guidelines for ART in Ghana (2008), recommends a triple therapy – having three drugs combination and discourages monotherapy or dual therapy – treatment with one or two drugs only; for treatment of PLWHIV. The recommended combinations for the triple therapy are listed in the recommendation:

o One Non-Nucleoside Reverse Transcriptase Inhibitor (NNRTI), two Nucleoside Reverse Transcriptase Inhibitors (NRTIs),

o Two NRTIs plus one Protease Inhibitor (PI)

o Two NRTIs plus two PIs. The two PIs are regarded as one since the second PI, typically ritonavir, is given at modest doses and is used to raise the first PI's blood level.

Antiretroviral drugs available in Ghana for the triple therapy combinations include: Zidovudine, Lamivudine, Nevirapine, Efavirenz, Stavudine, Abacavir, Tenofovir, Nelfinavir, Lopinavir/r, and Didanosine (Ministry of Health, Ghana Health Service, 2008).

### 2.9.1 Nucleotide/Nucleoside Reverse Transcriptase Inhibitors (NRTIs)

Their initial approval came in 1987. Zidovudine is the first agent in this class to be created. Others include didanosine, stavudine, lamivudine, abacavir, tenofovir. They undergo intracytoplasmic phosphorylation transformed by cellular enzymes into active forms that HIV reverse transcriptase inhibition through competition. Additionally, they could be included in chain termination and growth of the viral DNA chain (Osonuga et al., 2012).

#### 2.9.1.1 Zidovudine (AZT)

Available in Ghana as Combivir (with Lamivudine), which is taken orally twice daily in the form of one tablet. The most widely used antiretroviral medication in Sub-Saharan Africa, it was discovered first. It is a thymidine analog given at a dose of 300 mg for adults. Zidovudine is quickly absorbed and widely disseminated after oral treatment. Low plasma protein binding is seen. Zidovudine is largely excreted by the liver, therefore dosage must be changed in cases of liver failure (Katzung, 2007).

Zidovudine is phosphorylated intracellularly to produce its active 5'-triphosphate metabolite, zidovudine triphosphate (ZDV-TP). ZDV-TP primarily inhibits reverse transcriptase (RT) by causing DNA chain termination upon inclusion of the nucleotide analogue. Early prenatal use of AZT lowers viral transmission rates (particularly to mothers with HIV RNA 1,000 copies/mL) and serves as prophylaxis for high-risk healthcare personnel (Osonuga et al., 2012).

Severe anemia, gastrointestinal distress (such as diarrhea, vomiting, and flatulence), lactic acidosis, lipodystrophy, neutropenia, and gonad toxicity are among the hazardous side effects (Osonuga et al., 2010).

#### 2.9.1.2 Lamivudine (3TC)

Hepatitis B infection was its original indication. It is given to adults in doses of 150 mg twice daily. Lamivudine is quickly absorbed and widely absorbed after oral treatment. Most of it is eliminated in urine undisturbed. It has fewer hazardous side effects. However, there are a few recorded unusual incidences of neutropenia (Katzung, 2007).

#### 2.9.1.3 Abacavir

When combined with other anti-retrovirals, it can stop the spread of HIV infection. The active ingredient in abacavir is abacavir. Abacavir slows down the generation of new viruses, but it does not totally eradicate the HIV virus or treat AIDS. Additionally, it raises the blood's CD4 cell count (Ziagen, 2008). Abacavir therapy has not been shown to lower the likelihood of infection transmission. Sexual contact, blood transfusion, and sharing of needles are all still ways that infection can spread. Some individuals who use abacavir for HIV are more vulnerable to severe adverse effects. The usage of abacavir has been linked to side effects such as nausea, headaches, muscle soreness, or decreased energy. A significant hypersensitivity reaction can happen on occasion. When taking abacavir for the first six weeks, this reaction typically happens. Many people will experience skin rashes, but not everyone. Additionally, this reaction may cause flu-like symptoms like fever, chills, aches in the muscles and joints, exhaustion, and an overall feeling of being poorly. Along with these symptoms, abacavir may also cause low blood pressure, mouth ulcers, skin blistering, swelling of the neck, eyes, and tongue, red eyes, shortness of breath, coughing, and sore throat (Ziagen, 2008).

#### 2.9.1.4 Stavudine

Stavudine is also known by its chemical name, 2',3'-didehydro-3'-deoxythymidine. In vitro, the nucleoside analogue of thymidine stavudine prevents HIV from replicating in human cells. Cellular kinases phosphorylate stavudine to produce the active metabolite stavudine triphosphate. Both by competing with the natural substrate deoxythymidine triphosphate and by incorporating into viral DNA, stavudine triphosphate prevents HIV reverse transcriptase from enlarging DNA chains because it lacks the necessary 3'-OH group. Stavudine triphosphate significantly slows down the synthesis of mitochondrial DNA and blocks cellular DNA polymerase beta and gamma (Zerit, 2007). In vitro studies have shown that stavudine had additive and synergistic effects when combined with didanosine and zalcitabine, respectively (Zerit, 2002). Depending on the molar ratios of the drugs examined, the combination of stavudine and zidovudine exhibits either additive or antagonistic activity in vitro. Stavudine's ability to reduce HIV replication in humans in vitro does not appear to be correlated with this ability. Stavudine is quickly absorbed after oral treatment, with peak plasma concentrations occurring one hour after dosage. Following administration of stavudine as a solution or as capsules, the same amount of the drug enters the bloodstream. Stavudine is distributed equally between plasma and red blood cells.

Peripheral neuropathy, a side effect of stavudine, can result in tingling, burning, or numbness in the hands and feet. If these symptoms are quickly identified, the medicine's dosage is lowered, or the drug is stopped, they will go away. Rarely, the drug stavudine might result in pancreatic inflammation (pancreatitis). Severe abdominal discomfort is one of the symptoms, along with or without nausea and vomiting. Other negative effects include difficulty sleeping, nausea, vomiting, abdominal pain, and diarrhea. Stavudine can very infrequently lead to leucopenia (a drop in white blood cell count that increases your risk of bacterial infection) and impaired liver function. These side effects typically appear after using the medication for a long time (Zerit, 2007).

**2.9.1.5 Tenofovir (TDF)**

The family of nucleotide reverse transcriptase inhibitors includes the antiretroviral (anti-HIV) medication tenofovir. An acyclic nucleoside phosphonate diester analog of adenosine monophosphate is tenofovir disoproxil fumarate. Tenofovir disoproxil fumarate must first undergo diester hydrolysis in order to be converted to tenofovir, and then cellular enzymes must phosphorylate tenofovir to create tenofovir diphosphate. By competing with the natural substrate deoxyadenosine 5'-triphosphate and, after incorporation into DNA, by DNA chain termination, tenofovir diphosphate reduces the activity of HIV-1 reverse transcriptase (Viread, 2009). Mammalian DNA polymerases, and mitochondrial DNA polymerase are only moderately inhibited by tenofovir diphosphate. In lymphoblastoid cell lines, primary monocyte/macrophage cells, and peripheral blood lymphocytes, the antiviral efficacy of tenofovir against laboratory and clinical isolates of HIV-1 was evaluated. In cell culture, tenofovir exhibits antiviral efficacy against HIV-1 clades A, B, C, D, E, F, G, and O as well as HIV-2 strain-specific activity (Viread, 2007).

### 2.9.1.6 Didanosine

Since 1990, the nucleoside reverse transcriptase inhibitor didanosine has been used to treat HIV infections (Lambert et al., 1990). Didanosine is transformed to dideoxyadenosine-triphosphate (ddATP), which is in competition with endogenous nucleotides for inclusion into viral DNA and, if inserted, results in chain termination because it lacks a 3' OH group. Although resistance eventually arises, viral resistance to didanosine develops most slowly. Didanosine is prone to acid hydrolysis and breaks down quickly at pH levels below 3. Didanosine has a plasma half-life of 0.8 to 1.9 hours following a single dose and 1.1 to 2.7 hours following several doses. For 30–50% of the body's total clearance of didanosine, renal clearance is responsible. The remaining is expelled through biliary excretion and metabolism.

The renal clearance appears to be attributed to active tubular excretion. Didanosine has side effects just like other antiretroviral medications. Didanosine therapy is linked to gastrointestinal issues, pancreatitis, hyperamylasemia, hyperuricemia, and tingling, burning, or numbness in the hands and feet (peripheral neuropathy). Additionally, observed were hepatic damage, uncommon electrolyte abnormalities, and cardiac arrhythmias. With the buffered formulation, dry mouth, changed taste, decreased palatability, and nausea are more typical (Videx, 2009).

Other negative side effects include rash, muscle and joint aches, nausea, dizziness, headaches, fever, sleeplessness, and moderate disorientation. Didanosine can very infrequently lead to leucopenia (a drop in white blood cell count that increases your risk of bacterial infection) and impaired liver function. These side effects typically appear after using the medication for a long time (Videx, 2009).

### 2.9.2 Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs)

Their use was authorized in 1996. They disrupt RNA and DNA-dependent DNA polymerase by directly attaching to HIV-1 reverse transcriptase. The binding location is different from NRTIs' binding site. They are not integrated into the expanding viral DNA chain and do not require phosphorylation. Examples include delavirdine, efavirez (used to treat HIV/TB co-infection in Ghana), nevirapine, and rilpivirine (Osonuga et al., 2012).

#### 2.9.2.1 Nevirapine

In 1996, it received use approval. HIV-1 infections are resistant to it, but not HIV-2 infections. This is because nevirapine (and other NNRTIs), which are given orally at an adult dose of 200mg, cannot bind to the allosteric site of the virus since it has a different structure (WHO, 2020). Metabolized substances are eliminated in urine (80%) and feces (10%) via the liver's CYP450 system. In many underdeveloped countries, including Ghana, it is recommended as prophylaxis as a low-cost method of minimizing mother-to-child transmission.

The primary adverse effect of this medication is hepatotoxicity, which can be fatal. However, nevirapine's toxicity rises with a larger patient CD4 level, unlike other hepatotoxic ARTs. Rashes and Steven Johnson's condition are more examples (Osonuga et al., 2012).

#### 2.9.2.2 Efavirenz (EFV)

A non-nucleoside reverse transcriptase inhibitor (NNRTI) for human immunodeficiency virus type 1 is efavirenz (EFV). Most of the time, noncompetitive suppression of HIV-1 reverse transcriptase (RT) mediates EFV action. Human cellular DNA polymerases, and as well as HIV-2 RT are not inhibited by EFV. It can be used by adults, teenagers, and kids who are at least 3 months old and weigh at least 3.5kg. Children under 3 months of age or weighing less than 3.5kg are not advised to take efavirenz since these patients have not been sufficiently examined (Sustiva, 2022).

### 2.10 Coagulation Abnormalities in HIV Infection

HIV patients' coagulation abnormalities can be related to the virus' effects, which can lead to a number of irregularities that put the patients at risk for developing coagulation disorders. Thrombocytopenia, endothelial cell failure, activation of coagulation factors, and the existence of an anti-phospholipids antibody are some of the problems brought on by HIV infection (Friel et al., 2007; Raman et al., 2016). Studies conducted in vitro and in vivo demonstrated that HIV can attach to host cells by using their surface-bound receptors. Glycoprotein 120, for example, mediates the interaction of HIV with host cells that have the CD4 receptor, CXC chemokine receptor or CXCR4, and chemokines receptor 5, or CCR5 (gp120). This relationship decreases nitric oxide expression, which promotes endothelial cell dysfunction and impairs vascular endothelial cell immune function (Jiang et al., 2010; Taremwa et al., 2015). Endothelial cells create many substances that are involved in the coagulation and fibrinolysis processes. The compounds produced by endothelial cells include protein S, tissue plasminogen activator, plasminogen activator inhibitor, and von Willebrand factor (vWF) (Rajendran et al., 2013). Endothelial dysfunction in HIV-infected people is a frequent consequence brought on by the virus's activity and the immune response to it. Such endothelial dysfunction activates the coagulation system and causes coagulation factors to be consumed (Cotter, 2006). Additionally, the presence of antiphospholipid antibodies, lupus anticoagulant, protein C, protein S, heparin cofactor II, and antithrombin deficiencies, as well as elevated levels of vWF and D-dimers, are all abnormalities brought on by HIV infection that predispose a person to a hypercoagulable state (Shen and Frenkel, 2004). Additionally, HIV infection can result in complications connected to AIDS, such as liver and renal disorders. Due to these challenges, it is difficult to manufacture the majority of coagulation factors, natural anticoagulants, fibrinolytic factors, and hematopoietic growth hormones that may contribute to hemostatic illnesses (Al-Ghumlas and Abdel-Gader, 2003; Poordad, 2007). Furthermore, highly active antiretroviral therapy (HAART) drugs cause liver damage that has undesirable side effects and decreases the production of coagulation factors (Raman et al., 2016). HAART medications, in particular protease inhibitors, affect lipid and glucose metabolism, which results in endothelial dysfunction (Funderburg, 2014). Since ART is known to cause hepatotoxicity, coagulation factors—particularly those that depend on Vitamin-K—are similarly impacted, which in turn affects their ability to be synthesized (Oguntibeju et al., 2006). Prothrombin time (PT) and partial prothrombin time (PPT) are the main assays frequently employed for the evaluation of coagulopathy (PTTK). Platelets also play a crucial part in hemostasis. Due to platelet dysfunction and decreased thrombopoiesis brought on by cytokines and platelet activation factor (PAF) produced during HIV infection, thrombocytopenia develops (Levi et al., 2003).

### 2.10.1 Thrombocytopenia in HIV Infection

A diagnostic test called a platelet count counts the amount of platelets in a patient's blood. The process of blood clotting depends on platelets, also known as thrombocytes, which are tiny disk-shaped blood cells made in the bone marrow. Each microliter of blood typically contains between 150,000 and 450,000 platelets. Bleeding problems are related with low platelet levels or irregularly shaped platelets (Henry, 2001).

Another hematological issue that affects HIV patients is thrombocytopenia. HIV-associated thrombocytopenia restricts platelet's ability to perform hemostasis, particularly primary hemostasis (Babina et al., 2015). In various study contexts, the prevalence of thrombocytopenia ranges from

4 to 40%, and it is connected to all illness stages (Sloand et al., 1992; Firnhaber et al., 2010; Enawgaw et al., 2014; Deressa et al., 2018). Due to its link to potential dangers of tissue bleeding, it has also been associated to an increased morbidity and death in HIV patients. In the setting of HIV infection, immune-mediated platelet destructions, the harmful side effects of HIV drugs, and decreased hematopoiesis are some of the mechanisms that lead to the development of thrombocytopenia (Li et al., 2005; Alexaki et al., 2008). Increased viral load has been linked to a drop in platelet count, which has been used to predict a rapid drop in CD4 cell count (Nicolle et al., 1998). Highly active antiretroviral therapy (HAART), according to several studies, has decreased the frequency of thrombocytopenia (Servais et al., 2001; Ambler et al., 2012). There are, however, several cases that demonstrate that this hematological anomaly continues to exist even in patients using HAART (Vannappagari et al., 2011). According to Deressa et al. (2018), Ethiopia has a 6.3% prevalence of thrombocytopenia, which is lower than the 26% incidence in Australia (Nicolle et al., 1998), the 17.4% rate in Uganda (Taremwa et al., 2015), and the 20% rate in Iran (Alaei et al., 2000). A portion of the low occurrence of thrombocytopenia is related to HAART's ability to increase platelet count (Montaner et al., 1990). People over 40years old are known to have a high prevalence of thrombocytopenia (Enawgaw et al., 2014; Shen et al., 2015). This is connected to the rise in myelodysplastic instances in elderly people (Nicolle et al., 1998; Shen et al., 2015). When HAART is used to treat HIV in adults, the degree of thrombocytopenia is reduced by 29% compared to when HAART is not used to treat HIV in adults (Getawa et al., 2021).

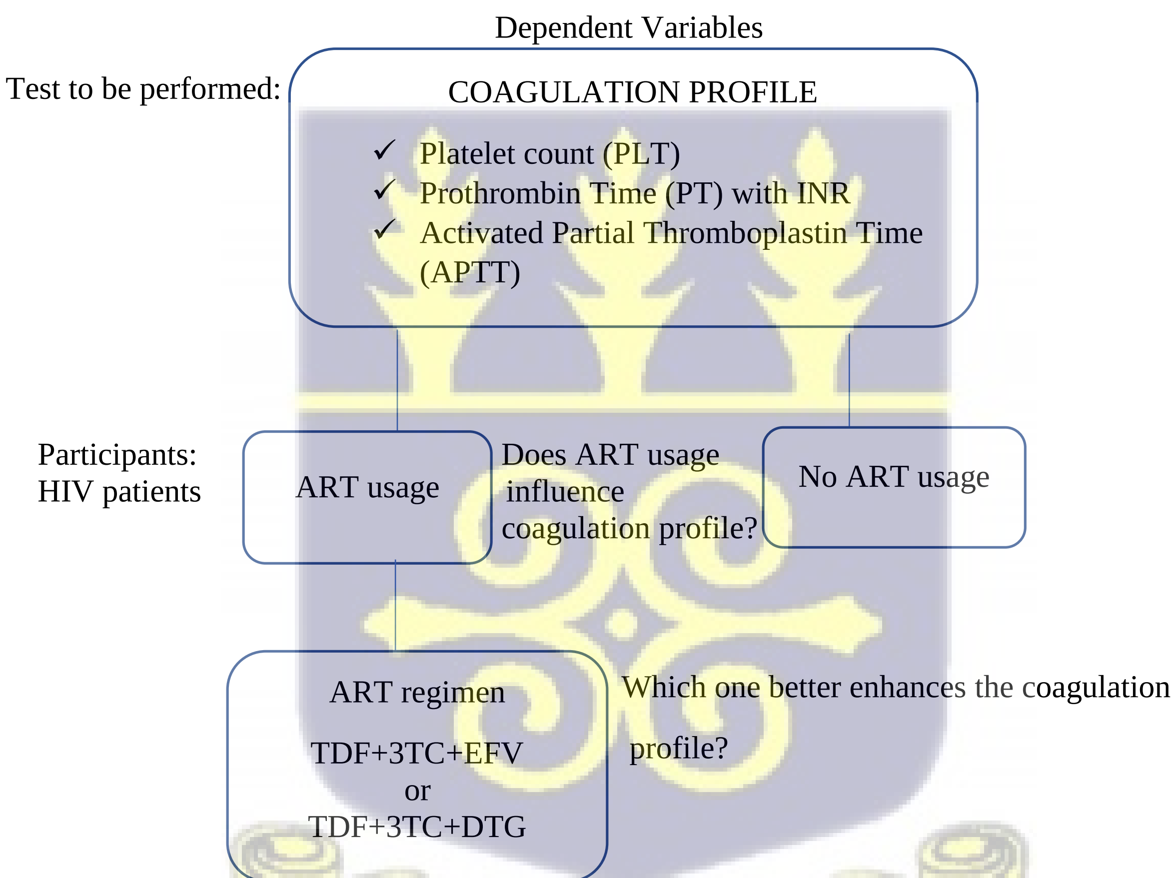
### 2.10.2 Prothrombin Time/International Normalized Ratio (INR) in HIV Infection

Prothrombin time (PT) is a screening test to assess the extrinsic pathway. It detects deficiency or inhibition of clotting factors in the extrinsic pathway. Endothelial dysfunction and liver damage develop with HIV infection, and this may cause significant clotting impairment (Obragu et al., 2015). One of the coagulation factors made by the liver is thrombin. The cascade's conversion of prothrombin (factor II) to thrombin is one of its final steps. The extrinsic and common routes are evaluated by the prothrombin time test, which measures the combined function of the coagulation factors. To normalize PT results, the International Normalized Ratio (INR) is utilized (Horsti et al., 2005). When compared to non-ART patients, the PT and INR of HIV patients on CART have been observed to be 13.83 seconds and 1.2 respectively. Most HIV patients using CART have normal PT compared to non-ART patients who have extended PT (>16 seconds), suggesting that CART may play a role in normalizing the PT (Ephraim et al., 2018). PT is higher in non-ART HIV patients than in HIV-negative people. In contrast to 10.9% of HIV-negative patients, abnormal PT is seen and reported in 37.3% of non-ART HIV patients. Patients with CD4 counts below 200 cells/µl are found to have a 1.155 chance of having an aberrant PT value. When the CD4 count is below 200 cells/l, there is no increased risk of abnormalities for the APTT, fibrinogen, or platelet counts (Jaiyeola, 2015). When compared to HIV-negative people, several investigations have found that HIV positive subjects have greater PT values (Omoregie et al., 2009; Ifeanyi and Obeagu, 2015).

### 2.10.3 Activated Partial Thromboplastin Time (APTT) in HIV Infection

An evaluation of the intrinsic route is done using the activated partial thromboplastin time. A screening test called the APTT is used to assess a person's capacity to produce blood clots. It evaluates the quantity as well as the function of the haemostatic coagulation components XII, IX, VII, X, V, II, and I. (Pagana and Pagana, 2006). According to studies, circulating anticoagulants cause roughly 35% of HIV-positive people to have abnormal partial thromboplastin time activated with kaolin (PTTK), which is associated with thrombotic diseases (Kasthuri et al., 2006; Ephraim et al., 2018). Patients on CART have an estimated APTT within the reference range. Prolonged APTT (APTT >45seconds) have been reported in about 78.5% of CART patients, whereas non-ART patients have prolonged APTT rate of 86.7% (Ephraim et al., 2018). APTT among non-ART HIV patients is higher compared to HIV-negative patients (Jaiyeola, 2015). Studies have reported higher PT value in HIV positive subjects when compared with HIV-negative subjects (Omoregie et al., 2009; Ifeanyi and Obeagu, 2015).

## 2.11 Conceptual Framework



# CHAPTER THREE

# 3.0 METHODOLOGY

## 3.1 Study plan:

This case-control study compared the coagulation profiles of HIV patients receiving ART to newly diagnosed HIV patients who had not yet initiated ART as the control group (referred to as ART-naïve group). The control group (ART-naïve) were newly diagnosed HIV patients whose samples were taken before they were put on any drugs.

The Mampong Municipal Hospital's Retroviral Clinic served as the recruitment site for the study participants. Using a questionnaire (Appendix II), further clinical and demographic data were gathered. ART participants were on two different ART combinations. ART regimen 1 (R1), made up of (Tenofovir+Lamivudine+Dolutegravir) and ART regimen 2 (R2), made up of (Tenofovir+Lamivudine+Efavirenz). The coagulation profile of ART participants on regimen 1 was compared to those on regimen 2 to assess the impact of the two ART regimens.

## 3.2 Study area:

The research was done in Ghana's Ashanti Region at the Retroviral Clinic of Mampong Municipal Hospital (MMH).

As a result of the division and upgrading of the previous Sekyere West District into Mampong Municipal and Sekyere Central District, Mampong Municipality is one of the six Municipal Assemblies in the Ashanti region. Sekyere South, Sekyere Central, and Ejura Sekye-Dumasi districts form its southern, eastern, and northern boundaries, respectively. Mampong is the capital of the Municipal, has a population of more than 42,000 people. The Municipality contains several important towns, including Mampong, Krobo, Dadease, Asaam, Kofiase, Adidwan, and Apaah.

According to the Population Housing Census, the Municipality has an 88,051 inhabitants in 2010 compared to 78,056 in 2000. (Ghana Statistical Service, 2014).

In 1973, the Mampong Municipal Hospital (MMH) was founded, serving the entire municipality. The general wing and the maternity wing are the MMH's two divisions. Malaria is by far the most common reason for admission to the hospital's 98 beds in the general wing, followed by anemia, pneumonia, diarrhea, typhoid, hypertension, and diabetes (Volunteer Base Camp, 2021). One kilometer away, in the maternity wing, there are 56 extra beds; nevertheless, the maternity wing actually serves as a distinct hospital.

### 3.3 Sampling method:

Convenience sampling was used in this study. Sample collection was done at the Mampong Municipal Hospital (MMH) – Retroviral clinic.

**3.3.1 Platelet Count:** Under aseptic conditions, 1ml of venous blood was drawn into a vacutainer containing ethylene diamine tetraacetic acid (EDTA). With a focus on the platelet count, samples were examined using the automated hematology analyzer RAYTO RT-7300, which is subject to rigorous internal and external quality control and assurance procedures on a regular basis.

**3.3.2 PT, INR, APTT:** 4ml of venous blood was drawn into a vacutainer containing 3.8% tri sodium citrate in a ratio of 1:9, processed using a semi-automated coagulation analyzer, and then strictly subjected to ongoing internal and external quality control and assurance checks. The samples were spun for 10 minutes at 3000 rpm in order to extract the clear plasma into a dry, clean plastic container (Eppendorf tubes). The Rayto semi-automated coagulation analyzer, model RT-2204C (Rayto life and analytical sciences, China), was used for the test. INR was calculated from the PT results for each participant.

A modified Kaolin technique was used to measure the activated partial thromboplastin time (APTT). When pre-incubated with kaolin, factor XIIa forms and cleaves to factor XI and XIa in the presence of calcium, causing coagulation to occur. 4ml of distilled water were used to reconstitute the APTT reagent (Thermo Scientific™, Canada), which was then mixed by inversion and left to remain at room temperature for 30 minutes. A test cuvette was filled with 50µl of the sample. The sample was then mixed with 50µl of the reconstituted APTT reagent, and the reaction was allowed to sit at 37ºC for 3 minutes. Rapidly adding 50µl of calcium chloride, the time it took for the blood to coagulate in seconds was noted.

**3.4 Sample size:**

Using Raosoft sample size calculator, a sample size of 102 participants were used for this study, considering an average attendance of 137 at a 95% confidence level, a 5% margin of error, and a 50% response distribution. Fifty-two (52) participants on ART were the main subjects and 50 newly diagnosed HIV patients who had not yet received ART as the control group were used for this study.

The sample size ***n*** and margin of error ***E*** are given by the following formula:

$$x = Z(^{c}/_{100})^{2} r(100-r)$$

$$n = {}^{N x}/_{((N-1) E^{2} + x)}$$

$$E = \mathrm{Sqrt}[^{(N - n)x}/_{n(N-1)}]$$

where ***N*** is the population size, ***r*** is the fraction of responses, and Z(c/100) is the critical value for the confidence level c.

**3.5 Inclusion and exclusion criteria:**

Criteria for inclusion and exclusion applied to participants. Patients who refused to participate, those who had bleeding problems, those who were receiving anticoagulant therapy, those who had myeloproliferative disorders, those who had liver diseases, and those who were taking medications other than ART were all excluded from the study. Both patients on ART and non-ART who are HIV positive and voluntarily agree to participate in the study were included.

**3.6 Ethical considerations:**

The School of Biomedical and Allied Health Sciences (SBAHS), University of Ghana, and Ghana Health Service Ethics Review Committee (GHS-ERC) were consulted for ethical clearance and permission (Appendix VII & VIII). Before collecting the participants' data and samples, we obtained their written informed consent (Appendix I). Confidentiality and privacy of participants was upheld by using self-generated identification code number for each participant to conceal their identity. Participants were carefully oriented on the nature and purposes of the study as well as the data and sample collection procedures.

**3.7 Analysis of Data:**

IBM Statistical Package for Social Sciences (SPSS) statistics for Windows, version 21.0 (Armonk NY; IBM Corporation) and STATA MP/14.1 were used to analyze the data after it was imported into Microsoft Excel (2016). For various variables, statistical analysis was done using the means, percentages, standard deviation, standard error of means. Microsoft Excel and the statistical software (SPSS & STATA) was used to create the graphs and tables.

The coagulation parameters of both ART-experienced and non-ART HIV-infected patients was analyzed. Between HIV patients on ART and those not on ART, platelet count, PT, INR, and APTT was compared. The means of scores from more than two groups was analyzed using one-way ANOVA. The variation between HIV patients on ART and those not on ART was analyzed using an unpaired T-test for continuous variables. The impact of the two ART regimens of the ART participants was comparatively analyzed. Statistical significance was set at $P<0.05$.

# CHAPTER FOUR

# 4.0 RESULTS AND DISCUSSION

## 4.1 Results

### 4.1.1 General Characteristics of Study Participants

Table 4.1 provides demographic summary of the study participants. One hundred and two (102) participants were included in this study. 52 ART participants and 50 ART-naïve participants. Of the ART participants, 17 (32.7%) were males and 35 (67.3%) were females. 10 (19.2%) were between 26-35years, 17 (32.7%) were between 36-45years, and 25 (48.1%) were above 45years. Of the ART-naïve participants, 21 (42%) were males and 29 (58%) were females. 7 (14%) were below 18years, 10 (20%) were between 19-25years, 8 (16%) were between 26-35years and 36-45years, and 17 (34%) were above 45years.

**Table 4.1: Sex and age of ART and ART-naïve respondents**

| Demographic | ART | | ART-naïve | |
|---|---|---|---|---|
| **Sex** | **Frequency** | **Percentage (%)** | **Frequency** | **Percentage (%)** |
| Male | 17 | 32.7 | 21 | 42 |
| Female | 35 | 67.3 | 29 | 58 |
| Total | 52 | 100 | 50 | 100 |
| **Age (years)** | | | | |
| Below 18 | None | | 7 | 14 |
| 19-25 | None | | 10 | 20 |
| 26-35 | 10 | 19.2 | 8 | 16 |
| 36-45 | 17 | 32.7 | 8 | 16 |
| Above 45 | 25 | 48.1 | 17 | 34 |
| Total | 52 | 100 | 50 | 100 |

### 4.1.2 Coagulation Profile of ART and ART-naïve participants

Table 4.2 summarizes the coagulation profile of ART and ART-naïve study participants. The mean platelet count (ART: 320.10 ±63.29 vs ART-naïve: 238.82 ± 75.18) was significantly higher in patients on ART compared to the ART-naïve participants ($p<0.001$). Prothrombin time (PT) was prolonged and INR higher in patients on ART compared to ART-naïve patients. However, this did not reach statistical significance (ART-PT: 23.36 ± 15.77, ART-naïve-PT: 18.61 ± 7.97, p=0.059; ART-INR: 2.06 ± 1.41, ART-naïve-INR:1.62 ± 0.76, p=0.054). APTT was lower in patients on ART compared to ART-naïve patients. This was not statistically significant (ART: 40.60 ± 8.01, ART-naïve: 43.06 ± 6.08, p=0.084).

**Table 4.2: Comparing coagulation profile of ART participants against ART-naïve participants**

| Parameters | ART Status | Number (N) | Mean | Standard deviation | P-value |
|---|---|---|---|---|---|
| **PLT ($x10^9/L$)** | ART | 52 | 320.10 | 63.29 | |
| | ART-naïve | 50 | 238.82 | 75.18 | 0.00 |
| **PT (sec)** | ART | 52 | 23.36 | 15.77 | |
| | ART-naïve | 50 | 18.61 | 7.97 | 0.059 |
| **INR** | ART | 52 | 2.06 | 1.41 | |
| | ART-naïve | 50 | 1.62 | 0.76 | 0.054 |
| **APTT (sec)** | ART | 52 | 40.60 | 8.01 | |
| | ART-naïve | 50 | 43.06 | 6.08 | 0.084 |

Independent Samples T-Test: PLT=Platelet count; PT=Prothrombin Time; INR=International Normalized Ratio; APTT=Activated Partial Thromboplastin Time

ART and ART-naïve participants had significantly prolonged PT (>16sec), p=0.001 and p=0.025 respectively. Both ART and ART-naïve participants had high INR values above the biological reference interval of 0.80 – 1.30 (p<0.001 and p=0.005 respectively). APTT of ART participants was normal, whereas ART-naïve participants had significantly prolonged APTT (>40sec) [p=0.001] (Table 4.3).

**Table 4.3: Coagulation profile of study participants against reference standards**

| Profile | Number (N) | Mean±SD | Reference Range | P-value | Diagnostic Flag |
|---|---|---|---|---|---|
| **ART: PLT ($x10^9/L$)** | 52 | 320.10±63.29 | 150 - 450 | 0.000 | Normal |
| **ART-naïve: PLT ($x10^9/L$)** | 50 | 238.82±75.18 | | 0.000 | Normal |
| **ART: PT (sec)** | 52 | 23.36±15.77 | 11 - 16 | 0.001 | Prolonged |
| **ART-naïve: PT (sec)** | 50 | 18.61±7.97 | | 0.025 | Prolonged |
| **ART: INR** | 52 | 2.06±1.41 | 0.80 – 1.30 | 0.000 | High |
| **ART-naïve: INR** | 50 | 1.62±0.76 | | 0.005 | High |
| **ART: APTT (sec)** | 52 | 40.60±8.01 | 30 – 40* | 0.594 | Normal |
| **ART-naïve: APTT (sec)** | 50 | 43.06±6.08 | | 0.001 | Prolonged |

One-Sample T-Test; significance (p<0.05)
Reference range: ALMA Diagnostic Laboratory
*Reference range: Mosby's Diagnostic & Laboratory Test Reference (Pagana et al., 2019)

### 4.1.3 Effects of different ART regimen on coagulation profile

The effects of different combination of ART regimen on platelets, prothrombin time, INR and APTT were also investigated, presented in Table 4.4. Participants on ART regimen 1, R1 (TDF+3TC+EFV) had a higher mean platelet count compared to those on ART regimen 2, R2 (TDF+3TC+DTG) [322.88±58.39 vs 318.74±66.32]. However, this difference did not reach statistical significance [t(50)=0.219, p=0.827]. Participants on ART regimen 1, R1 (TDF+3TC+EFV) had a lower mean PT and INR when compared to those on ART regimen 2, R2 (TDF+3TC+DTG) [PT: 21.56±16.74 vs 24.24±15.45; INR: 1.87±1.46 vs 2.15±1.39]. This did not reach statistical significance (p=0.571 and p=0.509; for both PT and INR respectively). Participants on ART regimen 1, R1 (TDF+3TC+EFV) also had a lower mean APTT when compared to those on ART regimen 2, R2 (TDF+3TC+DTG). Again, this did not reach statistical significance [39.53±5.75 vs 41.11±8.93, p=0.508].

**Table 4.4: Effects of different ART regimen on coagulation profile of ART participants**

| Parameters | ART Regimen | N | Mean | Standard Deviation | P-value |
|---|---|---|---|---|---|
| Platelet Count ($x10^9/L$) | TDF+3TC+EFV | 17 (32.69%) | 322.88 | 58.39 | 0.827 |
| | TDF+3TC+DTG | 35 (67.31%) | 318.74 | 66.32 | |
| Prothrombin Time (sec) | TDF+3TC+EFV | 17 (32.69%) | 21.56 | 16.74 | 0.571 |
| | TDF+3TC+DTG | 35 (67.31%) | 24.24 | 15.45 | |
| INR value | TDF+3TC+EFV | 17 (32.69%) | 1.87 | 1.46 | 0.509 |
| | TDF+3TC+DTG | 35 (67.31%) | 2.15 | 1.39 | |
| Activated Partial Thromboplastin Time (sec) | TDF+3TC+EFV | 17 (32.69%) | 39.53 | 5.75 | 0.508 |
| | TDF+3TC+DTG | 35 (67.31%) | 41.11 | 8.93 | |

One-Way ANOVA: TDF-Tenofovir; 3TC-Lamivudine; EFV-Efavirenz; DTG-Dolutegravir

### 4.2 Discussion

This study determined the effect of ART on the coagulation profile of HIV patients. The results show that participants on ART had significantly higher platelet count when compared to ART-naïve HIV patients. This may indicate that ART helps improve the platelet count of HIV-infected people. However, thrombocytopenia was noted in reports from other research. HIV-positive patients' thrombocytopenia is similar to that of people who have immune thrombocytopenia purpura. Deposition of circulating immune complexes on platelets results in enhanced platelet destruction, which is the reason of the decreasing platelet count. It is also hypothesized that specialized antiplatelet antibodies exist and that HIV can directly infect megakaryocytes (Akinbami et al., 2010). However, neither the ART-naïve control group nor the ART HIV-infected participants in this study met the criteria for being thrombocytopenic or displayed any symptoms consistent with the condition.

In this study, PT was prolonged in both ART and the control (ART-naïve) HIV patients. INR in both subjects and controls were high above the biological reference interval. In addition, APTT in ART participants was normal, but ART-naïve participants had a significantly prolonged APTT. This may suggest that the effects of ART can normalize or improve APTT in HIV patients. Prolonged PT observed in this study is not in agreement with a study by Ephraim et al (2018), which gives the assertion that combined ART has a potential role in restoring or correcting coagulation factors disorders. A recent study in Nigeria (Okoroiwu et al., 2022) have reported that HIV seropositive patients have prolonged PT and APTT, contrary to a normal APTT found in ART patients in this current study. Endothelial dysfunction has been suggested as the underlying defect in the prolonged PT in these patients. The normal APTT seen amongst the participants on ART in this current study could be due to the effect of ART improving the hepatic functional ability to synthesize some coagulation factors of the intrinsic pathway that are indirectly assessed through the APTT assay (Omoregie et al., 2009). Similarly, significantly high PT has also been reported among HIV positive subjects (Jaiyeola, 2015).

Tests for the extrinsic and intrinsic pathways are the prothrombin time (PT) and activated partial thromboplastin time (APTT), respectively. Normally, one would anticipate that HIV's hypercoagulable state would result in shorter PT and APTT readings. However, in this investigation, the PT values in the HIV patients receiving ART and the ART-naïve controls showed a haemorrhagic tendency to be significantly greater. In this investigation, APTT was normal in ART patients, in contrast to past investigations that evaluated these haemostatic characteristics in HIV-positive individuals (Lafeuillade et al., 1992; Gross and Aired, 2000; Omoregie et al., 2009). This study is consistent with a study by Abdollahi et al. (2013) that found that PT in the study group was considerably greater than in the control group, but that APTT did not differ in this way. Prolonged PT in both subjects and control could be due to hepatic damage, which has been documented to occur frequently in HIV patients (Crane et al., 2012). HIV can influence liver function directly or indirectly. HIV RNA has also been found in vivo in hepatocytes and sinusoidal cells (Housset et al., 1990; Cao et al., 1992). Additionally, it has been demonstrated that primary human sinusoidal cells are in vitro susceptible to HIV infection (Schnitt et al., 1990). All of these result in increased fibrosis and apoptosis within the liver.

All of these illnesses will have an impact on the hepatic synthetic processes, including the production of clotting factors. Factors II, VII, IX, and X, as well as procoagulant Proteins C and S, all require the final terminal carboxylation process, which is dependent on vitamin K and occurs in the liver. The endothelium, particularly that of the liver, produces tissue plasminogen activator (tPA), von Willebrand factor (vWF), and factor VIII (Gomez et al., 2005). The majority of clotting factors and fibrin breakdown products are also processed by the liver's reticuloendothelial system. All of them demonstrate the crucial role the liver plays in coagulation and how a problem therein may cause PT and APTT to be prolonged, predisposing HIV patients to bleeding tendencies. This study's findings about the lengthening of PT are consistent with a study by Awodu et al. (2009) in which endothelial activation was suggested as the cause.

Antiphospholipids or lupus anticoagulants, which were shown to occur in HIV patients as indicated in studies in Benin and Ife, Nigeria (5.7% and 2.9%, respectively), as high as 46% and 72% of those in the USA and France, have been linked to a prolonged APTT (Arnaud et al., 1992; Bloom et al., 1996; Ndakotsu et al., 2009; Awodu et al., 2010). This is consistent with the present study's findings that ART-naïve HIV patients had prolonged APTT but normal for ART patients, and it strongly suggests that ART can improve APTT in HIV-infected individuals.

No significant differences were found between the coagulation profiles of ART patients taking ART regimen 1, which contains tenofovir (TDF)+lamivudine (3TC)+efavirenz (EFV), and those taking ART regimen 2, which contains tenofovir (TDF)+lamivudine (3TC)+dolutegravir (DTG). This study shows that there is no significant difference in the coagulation profile of HIV patients depending on the kind of ART regimen or drug combination. Patients on ART regimen 1, (TDF+3TC+EFV), appeared to have a better coagulation profile than those taking ART regimen 2 (TDF+3TC+DTG), as seen by their higher platelet counts, lower mean PT, INR, and APTT, though this was not statistically significant (Table 4.4). Therefore, it implies that ART combinations (TDF+3TC+DTG) and (TDF+3TC+EFV) can enhance the coagulation profile in HIV-infected patients by improving platelet count and APTT.

At least two nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs), such as lamivudine (3TC), emtricitabine (FTC), and a third drug from any of the following medication class have served as the cornerstone of HIV treatment: Protease inhibitors (PIs), such as lopinavir/ritonavir (LPV/r) and atazanavir; Non-nucleoside reverse transcriptase inhibitors (NNRTIs), such as nevirapine and efavirenz (EFV); and Integrase strand transfer inhibitors (INSTIs), such as dolutegravir (DTG) and raltegravir (RAL). Because this combination of three medications offers three different ways to combat the virus, it has been the key to the success of antiretroviral therapy. Both ART regimen 1 (R1) and ART regimen 2 (R2) contain two NRTIs, and one integrase strand transfer inhibitor (DTG) for R2 and one NNRTI (EFV) for R1. Currently, the optimal first-line and second-line ART regimen for all populations, including pregnant women and women of childbearing age, is ART regimen 2 (TDF+3TC+DTG) (WHO, 2018).

# CHAPTER FIVE

# 5.0 CONCLUSION AND RECOMMENDATION

## 5.1 Conclusion

HIV infection has a negative impact on a person's coagulation profile. Platelet count and APTT is improved by taking ART. The results of this study show that both ART-treated and ART-naïve HIV patients had prolonged prothrombin time (PT), with high INRs; normal APTTs in ART-treated patients, but prolonged APTTs in ART-naïve participants, and significantly higher platelet counts in ART participants than in ART-naïve individuals. Moreover, this study shows that the particular ART regimen has no apparent selective advantage in enhancing the coagulation profile in HIV patients receiving ART. This implies that ART combinations (TDF+3TC+DTG) and (TDF+3TC+EFV) can enhance the coagulation profile in HIV-infected patients by improving platelet count and APTT. However, HIV patients on ART regimen that includes the drugs tenofovir, lamivudine, and efavirenz (TDF+3TC+EFV) appear to have a better coagulation profile. The haemostatic parameter where HIV has the most of an impact is prothrombin time.

## 5.2 Recommendations

The following are some recommendations based on the aforementioned findings: HIV patients should have their hemostatic parameters evaluated at baseline before starting treatment and before undergoing any invasive procedures or major surgeries. In order to prevent potentially fatal complications including pulmonary embolism and hemorrhage, clinicians must be aware of the elevated risk of haemostatic abnormalities in these patients. There should be more HIV awareness campaigns to promote voluntary testing and early diagnosis in order to prevent late presentations with AIDS. Future research on the relationship between coagulation factors (factor assays), liver function, combined ART, and coagulation profiles of HIV-infected people is anticipated to yield fascinating results. Future research should consider a longitudinal research to assess the impact of the different ART combinations on haemostatic functions in HIV patients.

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# APPENDICES

## Appendix I: Consent Form

**STUDY TITLE: Coagulation Profile of HIV Patients on Combined Antiretroviral Therapy (ART) at the Mampong Municipal Hospital, Ashanti Region, Ghana**

PARTICIPANTS' STATEMENT

I acknowledge that I have read or have had the purpose and contents of the Participants' Information Sheet read and all questions satisfactorily explained to me in a language I understand (Asante Twi). I fully understand the contents and any potential implications as well as my right to change my mind (i.e. withdraw from the research) even after I have signed this form. I voluntarily agree to be part of this research.

Name of Participant: ………………………………………………………………………………

Participants' Signature: ……………………… OR Thumb Print: ……………………………

Date: ……………………………………

INTERPRETERS' STATEMENT

I interpreted the purpose and contents of the Participants' Information Sheet to the afore named participant to the best of my ability in the (Asante Twi/Hausa) language to his proper understanding.

All questions, appropriate clarifications sort by the participant and answers were also duly interpreted to his/her satisfaction.

Name of Interpreter: ……………………………………………………………………………

Signature of Interpreter: ……………………… OR Thumb Print: ……………………………

Date: ………………………

Contact Details

STATEMENT OF WITNESS

I was present when the purpose and contents of the Participant Information Sheet was read and explained satisfactorily to the participant in the language he/she understood (Asante Twi)

I confirm that he/she was given the opportunity to ask questions/seek clarifications and same were duly answered to his/her satisfaction before voluntarily agreeing to be part of the research.

Name: ……………………………………………………………………………………………

Signature…………………………….. OR Thumb Print ……..................................

Date: ………………………………

INVESTIGATOR STATEMENT AND SIGNATURE

I certify that the participant has been given ample time to read and learn about the study. All questions and clarifications raised by the participant have been addressed.

Researcher's name: ………………………………………………………………………………

Signature: ……………………………………………………

Date: …………………………………………………………………

## Appendix II: Questionnaire

Hello, I am **FREDDIE BOATENG OPOKU**, pursuing a **Master of Science (M.Sc.)** program in **Medical Laboratory Sciences from the University of Ghana, School of Biomedical and Allied Health Sciences, Department of Medical Laboratory Sciences**, working on a research project.

We are conducting a research study on the **Coagulation Profile of HIV Patients on Combined Antiretroviral Therapy (ART) at the Mampong Municipal Hospital**. By filling this questionnaire, we hope to learn about the specific kind of ART combination and how it affects the coagulation profile and how to improve them.

You have been selected to participate in this research and would be very much grateful to receive your responses. Before we get started, I would like to explain to you how the interview works.

**ID Number: _ / _ / _**

**Your Name Initials Only:**

**ID CODE: _ / _ / _ / _ / _ / _ / _** **(i.e. ID# followed by your initials, e.g. 001FBO)**

Please tick ☐ if you have any of the following conditions:

i. Do you have any bleeding disorder? Yes ☐ No ☐

ii. Are you taking any anticoagulant therapy? Yes ☐ No ☐
If Yes, (please specify): ……………………………………………………………

iii. Are you taking other drugs apart from cART? Yes ☐ No ☐
If Yes, (please specify): ……………………………………………………………

**SOCIODEMOGRAPHICS**

**Q1.** Sex of the respondent? Male ☐ Female ☐

**Q2.** Age of respondent.

1). Below 18 2). 19-25yrs 3). 26-35yrs 4). 36-45yrs 5). Above 45yrs

**Q3.** What was the last level of schooling that you completed?

☐ no formal education
☐ primary
☐ junior high school (JHS)
☐ senior high school (SHS)
☐ tertiary
☐ other (please specify):
☐ declined to answer

**Q4.** Marital status.

☐ never married
☐ married or cohabiting
☐ divorced or separated
☐ widowed
☐ declined to answer

In the next section of this interview, I would like to ask you about your experiences with taking ART.

**Q5.** Just to confirm, are you taking ART to manage your HIV?

☐ yes [Continue from Q6]

☐ no [End your responses here]

**Q6.** How long ago did you first start taking antiretroviral therapy to manage your HIV? Record the number of months and years that have passed.

number of years ago... ..........................................

number of months ago ..........................................

**Q7.** If married or cohabitating: Does your partner / spouse know that you are taking ART?

☐ yes
☐ no
☐ don't know
☐ declined to answer
☐ not applicable

**Q8.** If respondent lives with other people: Do all the other adults living in your household know that you are taking ART?

☐ yes
☐ no
☐ don't know
☐ no other adults live in the household
☐ declined to answer

**Q9.** Is it ever difficult for you to take your ART when someone from your family can see you?

☐ yes
☐ no Go to Q11
☐ declined to answer

**Q10.** (If Yes) Please tell me about this.

**Q11.** Is it ever difficult for you to take your ART when someone from your community or your workplace can see you?

- ☐ yes
- ☐ no       Go to Q13
- ☐ declined to answer

**Q12.** (If Yes) Please tell me about this.

**Q13.** Is there anyone who regularly reminds you to take your ART?

- ☐ yes
- ☐ no       Go to Q15
- ☐ declined to answer

**Q14.** Please tell me about how this person reminds you.

**Q15.** During the past month, have you ever not taken your ART because you did not want someone to find out?

- ☐ yes
- ☐ no       Go to Q17
- ☐ declined to answer

**Q16.** (If Yes) Please tell me about this.

**Q17.** In general, do you find it easy, not very easy or difficult to take your ART?

- ☐ easy       Go to Q19
- ☐ not very easy
- ☐ difficult
- ☐ it depends
- ☐ declined to answer

**Q18.** Why?

**Health Status**

Now I would like to ask you some questions about your health before and after starting your ART, and then I will ask about any side-effects or body changes you experienced after starting ART.

**Q19.** How would you rate your health before starting ART? Would you say it was:

- ☐ excellent
- ☐ very good
- ☐ good
- ☐ fair
- ☐ poor

**Q20.** Now that you are taking ART, how is your health? Would you say it is:

- ☐ excellent
- ☐ very good
- ☐ good
- ☐ fair
- ☐ poor

**Perceived Side-Effects:**

**Some people experience side-effects when they take ART. This varies a great deal. Some people have a few, while others have more.**

**Q21.** Have you experienced any side-effects since you started taking ART?

- ☐ yes
- ☐ no
- ☐ don't know
- ☐ declined to answer

**Q22.** (If Yes) Which side-effects have been the most bothersome for you?

**Antiretroviral Therapy (ART) Regimen (Prescribed Doses)**

**Now I want to go over your ART medications: I will need to ask you about your prescribed doses of all ART medications, and then I will ask you how many doses of your medication you actually took at different time periods.**

**Q23.** The following should be completed according to the prescribed ART dosing, not the respondent's actual behaviour. The information may be obtained from the patient, the provider or the health care facility. Cross out all non-applicable boxes.

| COMBINATION OF MEDICATION | MORNING DOSE<br>NO. OF PILLS | MID-DAY DOSE<br>NO. OF PILLS | EVENING DOSE<br>NO. OF PILLS | DAILY TOTAL<br>NO. OF PILLS |
|---|---|---|---|---|
| **Tenofovir + Lamivudine + Efavirenz** | | | | |
| **Tenofovir + Lamivudine + Dolutegravir** | | | | |
| **Abacavir + Lamivudine + Dolutegravir** | | | | |
| **Abacavir + Lamivudine + Efavirenz** | | | | |
| **Tenofovir + Lamivudine + Lopinavir** | | | | |
| **Abacavir + Lamivudine + Lopinavir** | | | | |

Source of information above (patient, clinic, medical records, etc) .......................................

**Q24.** During the past six months, did you ever stop taking your ART for 48 hours or longer?

☐ yes
☐ no
☐ declined to answer

**Q25.** How long did you stop taking your antiretroviral therapy? Probe for a guess if respondent does not remember very clearly.

☐ for more than 48 hours and less than a week
☐ from one to two weeks
☐ for more than two weeks and less than one month
☐ for more than one month
☐ declined to answer

**Q26.** What were the reasons you stopped taking your ART?

**Q27.** Overall, how do you feel about being on ART?

## Appendix III: Coagulation profile of ART participants (Descriptive & One-way ANOVA)

Descriptive

| ART participants | N | Minimum | Maximum | Mean | Std. Deviation |
|---|---|---|---|---|---|
| Platelet Count | 52 | 198.00 | 428.00 | 320.0962 | 63.28871 |
| Prothrombin Time | 52 | 11.80 | 85.00 | 23.3615 | 15.77259 |
| INR value | 52 | 1.03 | 7.40 | 2.0594 | 1.40687 |
| Activated Partial Thromboplastin Time | 52 | 32.00 | 76.00 | 40.5962 | 8.00554 |
| Valid N (listwise) | 52 | | | | |

ART Regimen on coagulation profile (One-way ANOVA)

ANOVA Report

| ARTRegimen | | Platelet Count | Prothrombin Time | INR value | Activated Partial Thromboplastin Time |
|---|---|---|---|---|---|
| TDF+3TC+EFV | Mean | 322.8824 | 21.5588 | 1.8724 | 39.5294 |
| | N | 17 | 17 | 17 | 17 |
| | Std. Deviation | 58.39187 | 16.74373 | 1.46446 | 5.74584 |
| TDF+3TC+DTG | Mean | 318.7429 | 24.2371 | 2.1503 | 41.1143 |
| | N | 35 | 35 | 35 | 35 |
| | Std. Deviation | 66.31668 | 15.45371 | 1.39057 | 8.93036 |
| Total | Mean | 320.0962 | 23.3615 | 2.0594 | 40.5962 |
| | N | 52 | 52 | 52 | 52 |
| | Std. Deviation | 63.28871 | 15.77259 | 1.40687 | 8.00554 |

ANOVA Table

| | | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| Platelet Count * ARTRegimen | Between Groups (Combined) | 196.069 | 1 | 196.069 | .048 | .827 |
| | Within Groups | 204082.450 | 50 | 4081.649 | | |
| | Total | 204278.519 | 51 | | | |
| Prothrombin Time * ARTRegimen | Between Groups (Combined) | 82.080 | 1 | 82.080 | .326 | .571 |
| | Within Groups | 12605.423 | 50 | 252.108 | | |
| | Total | 12687.503 | 51 | | | |
| INR value * ARTRegimen | Between Groups (Combined) | .884 | 1 | .884 | .442 | .509 |
| | Within Groups | 100.059 | 50 | 2.001 | | |
| | Total | 100.943 | 51 | | | |
| Activated Partial Thromboplastin Time * ARTRegimen | Between Groups (Combined) | 28.741 | 1 | 28.741 | .444 | .508 |
| | Within Groups | 3239.778 | 50 | 64.796 | | |
| | Total | 3268.519 | 51 | | | |

## Appendix IV: Coagulation profile of ART-naïve participants (Descriptive)

Profile Descriptive Statistics of Non-ART Participants

| | N | Minimum | Maximum | Mean | Std. Deviation |
|---|---|---|---|---|---|
| Platelet Count of Non-ART Respondents | 50 | 88.00 | 428.00 | 238.8200 | 75.18133 |
| Prothrombin Time of Non-ART Respondents | 50 | 11.00 | 68.70 | 18.6140 | 7.97445 |
| International Normalized Ratio (Non-ART) | 50 | 1.12 | 6.50 | 1.6204 | .76191 |
| Activated Partial Thromboplastin Time (Non-ART) | 50 | 33.00 | 58.00 | 43.0600 | 6.07910 |
| Valid N (listwise) | 50 | | | | |

## Appendix V: Coagulation profile of participants against reference standards (One-Sample T-Test)

### Va: Platelet Count (150 – 450 x $10^9$/L)

One-Sample Statistics

| | N | Mean | Std. Deviation | Std. Error Mean |
|---|---|---|---|---|
| Platelet Count of ART Participants | 52 | 320.0962 | 63.28871 | 8.77657 |
| Platelet Count of ART-naive Respondents | 50 | 238.8200 | 75.18133 | 10.63225 |

One-Sample Test

| | Test Value = 450 | | | | | |
|---|---|---|---|---|---|---|
| | t | df | Sig. (2-tailed) | Mean Difference | 95% Confidence Interval of the Difference | |
| | | | | | Lower | Upper |
| Platelet Count of ART Participants | -14.801 | 51 | .000 | -129.90385 | -147.5235 | -112.2842 |
| Platelet Count of ART-naive Respondents | -19.862 | 49 | .000 | -211.18000 | -232.5463 | -189.8137 |

**Vb: Prothrombin Time (11 – 16 sec)**

One-Sample Statistics

| | N | Mean | Std. Deviation | Std. Error Mean |
|---|---|---|---|---|
| Prothrombin Time of ART Participants | 52 | 23.3615 | 15.77259 | 2.18726 |
| Prothrombin Time of ART-naïve Respondents | 50 | 18.6140 | 7.97445 | 1.12776 |

One-Sample Test

| | Test Value = 16 | | | | | |
|---|---|---|---|---|---|---|
| | t | df | Sig. (2-tailed) | Mean Difference | 95% Confidence Interval of the Difference Lower | 95% Confidence Interval of the Difference Upper |
| Prothrombin Time of ART Participants | 3.366 | 51 | .001 | 7.36154 | 2.9704 | 11.7527 |
| Prothrombin Time of ART-naïve Respondents | 2.318 | 49 | .025 | 2.61400 | .3477 | 4.8803 |

**Vc: INR (0.80 – 1.30)**

One-Sample Statistics

| | N | Mean | Std. Deviation | Std. Error Mean |
|---|---|---|---|---|
| INR value of ART Participants | 52 | 2.0594 | 1.40687 | .19510 |
| INR of ART-naïve Respondents | 50 | 1.6204 | .76191 | .10775 |

One-Sample Test

| | Test Value = 1.3 | | | | | |
|---|---|---|---|---|---|---|
| | t | df | Sig. (2-tailed) | Mean Difference | 95% Confidence Interval of the Difference Lower | 95% Confidence Interval of the Difference Upper |
| INR value of ART Participants | 3.893 | 51 | .000 | .75942 | .3677 | 1.1511 |
| INR of ART-naïve Respondents | 2.974 | 49 | .005 | .32040 | .1039 | .5369 |

**Vd: APTT (30 – 40 sec)**

**One-Sample Statistics**

| | N | Mean | Std. Deviation | Std. Error Mean |
|---|---|---|---|---|
| APTT of ART Participants | 52 | 40.5962 | 8.00554 | 1.11017 |
| APTT of ART-naïve Respondents | 50 | 43.0600 | 6.07910 | .85972 |

**One-Sample Test**

| | Test Value = 40 | | | | | |
|---|---|---|---|---|---|---|
| | t | df | Sig. (2-tailed) | Mean Difference | 95% Confidence Interval of the Difference | |
| | | | | | Lower | Upper |
| APTT of ART Participants | .537 | 51 | .594 | .59615 | -1.6326 | 2.8249 |
| APTT of ART-naïve Respondents | 3.559 | 49 | .001 | 3.06000 | 1.3323 | 4.7877 |

## Appendix VI: Coagulation profile of ART vrs ART-naïve participants (Independent Samples T-Test)

**Group Statistics**

| | ART Status | N | Mean | Std. Deviation | P-value |
|---|---|---|---|---|---|
| Platelet Count of Participants | ART | 52 | 320.0962 | 63.28871 | 0.000 |
| | ART-naïve | 50 | 238.8200 | 75.18133 | |
| Prothrombin Time of Participants | ART | 52 | 23.3615 | 15.77259 | 0.059 |
| | ART-naïve | 50 | 18.6140 | 7.97445 | |
| INR value of Participants | ART | 52 | 2.0594 | 1.40687 | 0.054 |
| | ART-naïve | 50 | 1.6204 | .76191 | |
| Activated Partial Thromboplastin Time | ART | 52 | 40.5962 | 8.00554 | 0.084 |
| | ART-naïve | 50 | 43.0600 | 6.07910 | |

## Appendix VII: Ethical clearance – Ghana Health Service

**GHANA HEALTH SERVICE ETHICS REVIEW COMMITTEE**

*In case of reply the number and date of this Letter should be quoted.*

*My Ref. GHS/RDD/ERC/Admin/App* |22|509
*Your Ref. No.*

Research & Development Division
Ghana Health Service
P. O. Box MB 190
Accra
Digital Address: GA-050-3303
Mob: +233-50-3539896
Tel: +233-302-681109
Email: ethics.research@ghs.gov.gh
2nd November, 2022

Freddie Boateng Opoku
P. O. Box 40,
Asante-Mampong

The Ghana Health Service Ethics Review Committee has reviewed and given approval for the implementation of your Study Protocol.

| | |
|---|---|
| GHS-ERC Number | **GHS-ERC: 042/09/22** |
| Study Title | Coagulation Profile of HIV Patients on Combined Antiretroviral Therapy (ART) at the Mampong Municipal Hospital, Ashanti Region |
| Approval Date | 2nd November, 2022 |
| Expiry Date | 1st November, 2023 |
| GHS-ERC Decision | **Approved** |

**This approval requires the following from the Principal Investigator**

- Submission of a yearly progress report of the study to the Ethics Review Committee (ERC)
- Renewal of ethical approval if the study lasts for more than 12 months,
- Reporting of all serious adverse events related to this study to the ERC within three days verbally and seven days in writing.
- Submission of a final report after completion of the study
- Informing ERC if study cannot be implemented or is discontinued and reasons why
- Informing the ERC and your sponsor (where applicable) before any publication of the research findings.

**You are kindly advised to adhere to the national guidelines or protocols on the prevention of COVID -19**

Please note that any modification of the study without ERC approval of the amendment is invalid.

The ERC may observe or cause to be observed procedures and records of the study during and after implementation.

Kindly quote the protocol identification number in all future correspondence in relation to this approved protocol

SIGNED.............................................
Dr. Naa-Korkor Allotey
(Ag. Head, Ethics & Research Management Department)

Cc: The Director, Research & Development Division, Ghana Health Service, Accra

**Appendix VIII: Ethical clearance – SBAHS, UG**

UNIVERSITY OF GHANA
SCHOOL OF BIOMEDICAL AND ALLIED HEALTH SCIENCES

**OFFICE OF THE SCHOOL ADMINISTRATOR**

October 24, 2022

**Mr. Freddie Boateng Opoku**
**Medical Laboratory Sciences**
**SBAHS, Korle–Bu.**
Dear Mr. Opoku,

**ETHICS CLEARANCE**

Ethics Identification Number: SBAHS/AA/MLAB/1803789/2021-2022

Following a meeting of the Ethics and Protocol Review Committee of the School of Biomedical and Allied Health Sciences held on August 29, 2022, I write on behalf of the Committee to approve your research proposal entitled: **"COAGULATION PROFILE OF HIV INFECTED PERSONS ON COMBINED ANTIRETROVIRAL THERAPY (ART) AT THE MAMPONG MUNICIPAL HOSPITAL, ASHANTI-REGION, GHANA".**

This clearance is valid for three years and requires that you submit three-monthly review reports of the protocol to the Committee and a final full review to the Committee on completion of the research. The Committee may observe the procedures and records of the research during and after implementation.

You are required to report all serious adverse events related to this research to the Committee within seven (7) days verbally and fourteen (14) days in writing.

Please note that any significant modification of the research must be submitted to the Committee for review and approval before its implementation.

As part of the review process, it is the Committee's duty to review the ethical aspects of any manuscript that may be produced from this research. You will, therefore, be required to furnish the Committee with any manuscript for publication.

Please always quote the ethics identification number in future correspondence regarding this protocol.

Thank you.

Yours sincerely,

**David Nana Adjei (PhD)**
Chairman, Ethics and Protocol Review Committee

CC: Dean, SBAHS
Head, Dept. of Medical Laboratory Sciences
School Administrator, SBAHS

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