DETERMINATION OF COAGULOPATHY IN TUBERCULOSIS PATIENTS INITIATING ANTI - TB MEDICATION AT THIKA LEVEL FIVE HOSPITAL, KENYA.

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Determination of Coagulopathy in Tuberculosis Patients Initiating Anti - Tb Medication at Thika Level Five Hospital, Kenya.

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DECLARATION

This thesis is my original work and has not been presented in any institution leading to the award of a degree or any other award.

Signature…….. Date……

Nicholas Maina Muraguri.

This thesis has been submitted for examination with our approval as University supervisors.

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UON, Kenya

DEDICATION

To My wife Irene, “Thank you for all your support” And our children Annette, Tracy, Gloria and Zawadi, “Thank you for your patience”

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Thank you God for bringing me this far. My sincere gratitude goes to my supervisors Dr Kibet Shikuku and Dr Mutinda C. Kyama for their Dedication, Guidance and constant supervision during the study. Much appreciation also goes to Mr Samuel Kimiti, Mr Patrick Nyaga and Mr Shadrack Ndeti who assisted in specimen collection and analysis. Mr Gervason Moriasi also appreciated for Data analysis. Finally I appreciate my dear wife Irene Maina and our children for the support during this time.

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ABBREVIATIONS AND ACRONYMS

AFB	Acid Fast Bacilli
AIDS	Acquired Immune Deficiency Syndrome
APTT	Activated Partial Thromboplastin Time
CNR	Case Notification Rate
DIC	Disseminated Intravascular Coagulopathy
DLTLD	Division of Leprosy TB and Lung Diseases
DOTS	Directly Observed Treatment short course
DVT	Deep Venous Thrombosis
EDTA	Ethylene Diamine Tetra- acetic Acid
EPTB	Extra Pulmonary Tuberculosis
FDC	Fixed Dose Combinations
FDP	Fibrin Degradation Products
FSP	Fibrin Split Products
HB	Haemoglobin
HIV	Human immunodeficiency virus
HMWK	High Molecular Weight Kininogen
JKUAT	Jomo Kenyatta University of Agriculture and Technology

KCCT	Kaolin Cephalin Clotting Time
MDR TB	Multi Drug Resistant Tuberculosis
PT	Prothrombin Time
PTB	Pulmonary Tuberculosis
PTI	Prothrombin Time Index
PTT	Prothrombin Time Test
RBC	Red Blood Cells
RHZE	Isoniazid, Rifampicin, Pyrazinamide and Ethambutol
SPSS	Statistical package for social sciences software
TB	Tuberculosis
TF	Tissue Factor
TL5H	Thika Level Five Hospital
WBC	White Blood Cells
WHO	World Health Organization
XDR TB	Extensively Drug resistant TB
ZN	Ziehl Neelsen Staining

ABSTRACT

Coagulopathy is an abnormal blood bleeding disorder in which the blood's ability to form clots is mostly impaired. This condition can cause a tendency toward excessive bleeding occurring spontaneously or resulting from injury. Coagulopathies are common all over the world. Several studies have shown a relationship between coagulopathy with other infections such as HIV viral infection especially after treatment. Determining coagulopathy among TB patients before and after treatment will help in the appropriate therapeutic management of these patients. The study aimed to determine the haemostatic changes in TB treatment naive patients initiating anti TB Medications at Thika Level Five Hospital in Kiambu County of Kenya. These changes were determined by carrying out Prothrombin time (PT), Alternate Partial Thromboplastin Time (aPTT), D - dimer and platelet count tests among TB patients before and after treatment. A total of 197 TB positive patients attending Thika level five hospital TB clinic were recruited in this study upon consenting. Clinical and demographic information of the participants were obtained using a structured questionnaire. With acquisition of eight milliliters of blood, samples were analyzed for Prothrombin time test, Activated partial thromboplastin time test, D-dimer test and platelet count prior to anti TB medication being initiated and after initiation of treatment. Results showed Significant differences in prothrombin time in age groups 42-49, 55-65 and above 66 years with p-values of 0.021, 0.000, and 0.000 respectively at 95 % confidence level. Age group above 66 years old showed significantly lower aPTT after anti TB administration as compared to before treatment (p=0.000), significant increase in D-dimer test in all participant TB positive patients after anti TB administration as compared to those determined before treatment. All the age groups exhibited normal D-dimer concentrations except for the 58-65-year old who presented elevated levels from 448.50±78.20, before treatment to 738.90±32.00 after treatment. (p=0.037; 95 % CI), Age group 66 years and older, there was a significance decrease in platelet count after treatment as compared with the baseline values (p = 0.000). However, there were no significant changes in platelet count in all the age groups. Data was analyzed using the SPSS statistical software version 22, Minitab statistical software version 19 and graph pad prism version 8. Significant haemostatic changes were identified in TB patients initiating TB treatment. This included significant elevation of Prothrombin time and D-Dimer. There was significant association in Prothrombin time test on patients initiating TB treatment. Significant association on D-Dimer test was noted on TB patients initiating treatment. There was no significant association in Activated partial Thromboplastin Time on patients initiating TB treatment. No significant association in Platelets count on patients initiating TB treatment.

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Haemostasis is a process where blood is maintained in the vessels in fluid form (Marieb *et. al..*, 2010). Coagulation, also known as clotting, is the process by which blood changes from a liquid to a gel forming a blood clot resulting in cessation of blood loss from a damaged vessel, followed by repair (Furie *et. Al..*, 2005). A normal haemostatic response to vascular damage is dependent on close linked interaction between the blood vessel walls, platelets, coagulation mechanism and fibrinolytic system (Key *et. al..*, 2017). Coagulopathy blood clotting disorder is a condition where the blood's ability to coagulate is usually impaired causing a tendency toward prolonged excessive bleeding (Hunt *et. al..*, 2014). This may occur spontaneously or as a result of injury or intrusive medical procedures (Hoffman *et. al..*, 2013). Fibrinolysis is a process that prevents blood clots from growing and becoming problematic and fibrin clot, the product of coagulation, is broken down Its main to circulating fragments that are cleared by other proteases or by the kidney and liver. (Ceserman et. al.., 2005)

Haemostasis is the human body's natural response to blood vessel injury resulting to bleeding. A coordinated effort between platelets and blood clotting proteins (or factors), result to the formation of a blood clot and subsequent arresting of the bleeding. (Hoffbrand *et. al..*, 2019). The normal clotting process depends on the interplay of various proteins in the blood. Coagulopathy may be caused by reduced levels or absence of blood-clotting proteins, known as clotting factors or coagulation factors. Genetic disorders, such as haemophilia and Von Willebrand disease, can cause a reduction in clotting factors (Hunt *et. al..*, 2013)

Tissue factor protein is exposed to blood once a blood vessel is injured consequently arresting the bleeding. The process of haemostasis starts when the exposed tissue

factor bind to a certain coagulation protein called factor seven (FVII) which circulates within the blood stream. (Hoffbrand et al., 2019). This will subsequently cause activation of clotting factor FVII to FVIIa. The binding of tissue factor and FVIIa is usually the first step in a process that will in the end lead to the formation of a strong, stable blood clot that will stop and prevent further bleeding. (Hoff brand *et. al..*, 2006).

Disseminated intravascular coagulopathy which is also referred to as consumptive coagulopathy, is a haemostatic pathological condition characterized by the widespread activation of the clotting mechanism that results in the formation of blood clots in the small blood vessels throughout the human body (Toh *et. al..*, 2003). This compromises the tissue blood flow ultimately causing multiple organ damage. In addition, as the coagulation process consumes clotting factors and platelets, norma.l clotting is disrupted and severe bleeding may occur from various body sites (Hoffman *et. al..*, 2013).

Tuberculosis (TB) is a worldwide infectious disease caused by a bacteria called *Mycobacterium tuberculosis* that usually affect the human lungs, and may also affect any other part of the body except the hair and the nails (Chakaya *et. al..*, 2013). TB affecting the lungs is referred to as Pulmonary Tuberculosis and is airborne spread from one person to another through the air droplets. TB that affecting any other part of the body other than the lungs is usually referred to as extra pulmonary TB (Chakaya *et. al..*, 2013).

Tuberculosis continues to be a major problem worldwide. According to the World Health Organization (WHO) approximately one third of the world's population suffer from latent Tuberculosis, meaning they have been infected with the bacteria without exhibiting any clinical symptoms. (WHO, 2011). Only about 10 percent of these people may develop the disease in their lifetime. The other 90 percent may never develop the disease and may not be able to transmit to other people (WHO, 2011).

Tuberculosis treatment involves the use of a combination of several multiple drugs (Chakaya *et. al*.., 2013). This is done importantly to prevent the emergence of anti-microbial drug resistance to any of these types of drugs. Fixed Dose Combinations (FDCs) usually contain more than one medicine within the same tablet or capsule. In the first two months of TB treatment four drugs in combination are used that rapidly reduce the number of bacillary load in the body where dying bacilli are postulated to form a nidus with may predispose coagulopathy (Chakaya *e.t al*.., 2013).

This phase is called the Intensive phase of anti-TB treatment. After two months another two drugs are then used for 4-6 months, this phase is referred to as the Continuation Phase of treatment (Chakaya *et. al*.., 2013). All patients who have not been previously treated should have a two month initial phase of treatment consisting of Isoniazid, Ethambutol, Rifampicin and Pyrazinamide followed by a continuation phase of Ethambutol and isoniazid for the next six months or Isoniazid and Rifampicin for the next four months (Chakaya *et. al*.., 2013).

1.2 Statement of the Problem

Kenya has a large Tuberculosis disease burden ranked position fifteen (15) among the twenty two (22) high TB burden countries that collectively contribute to about 80% of the world's TB cases (Chakaya *et. Al*.., 2013). The TB case notification rate (CNR) in Kenya rose from 305 to 558 per 100,000 populations between 2007 and 2016 and is currently on a slow decline (Enos et. al.., 2016). According to the latest WHO data published in 2017 Tuberculosis Deaths in Kenya reached 8,885 or 3.15% of total deaths (WHO, 2017). A recent prevalence survey indicated more TB cases in Kenya than previously estimated, with a TB prevalence of 558 per 100,000 people (Enos et. al.., 2016). In 2016, 2.5 million people fell ill with TB in the African region, accounting for a quarter of new TB cases worldwide and an estimated 417,000 people died from the disease in the African region (1.7 million globally) in 2016. Over 25% of TB deaths occur in the African Region (WHO, 2016). In year 2018 an estimated 10 Million people contracted TB, half a million

contracted multi drug resistant TB and 1.5 million died from TB (WHO, 2018) .It is also postulated that association between some inflammatory conditions and haemostatic changes arising in pulmonary tuberculosis can result in hypercoagulable state which may consequently predispose to deep vein thrombosis (DVT) (Sharma *et. al..*, 2007). Severe infections by nearly all microorganisms can cause disseminated Intravascular Coagulopathy (DIC) although bacterial infections are the most common cause such as gram negative and gram positive bacteria (Hoffman *et. Al..*, 2013). DIC may occur in 30-50% of patients with sepsis, and it develops in an estimated 1% of all hospitalized patients. DIC occurs at all ages and in all races, and no particular sex predisposition has been noted (Levi et. al.., 2018). Acute phase reactants, haemostatic changes and transient increase in anticardiolipin antibodies have been attributed to link inflammatory conditions with deep vein thrombosis in pulmonary tuberculosis patients. As venous thromboembolism can be very fatal, it is crucial to be proactive in arriving to an early diagnosis and institute prompt treatment. (Shah *et. Al..*, 2011).

1.3 Justification

There is risk of thrombosis developing in patients with severe pulmonary tuberculosis during treatment even in the absence of specific risk factors. Early diagnosis of this underreported phenomenon, need to be established for early institution of prompt treatment for thrombosis while continuing the anti-tuberculosis treatment. Prophylactic therapy can also be stressed to prevent venous thrombosis and its complications in patients with severe pulmonary tuberculosis. It is possible that one of the causes for sudden unexplained deaths in patients with tuberculosis may be undiagnosed pulmonary thromboembolism. According to a retrospective analysis in a South African Hospital, (White *et. Al..*, 1989*)* stated that DVT rate was 3.4% within the first two weeks after initiation of therapy. Another recent study recently, performed a nationwide prospective study comprising a routine evaluation of treatment outcomes in TB patients. This Italian group concluded that the prevalence of VTE was 0.6% in the first month of treatment, one third occurring in

the first week. Furthermore, all cases except one, occurred in hospitalized patients *(*Ambrosetti *et .al..*, 2006*)*

1.4 Research Questions

1. What are the levels of Prothrombin time (PT) among TB patients before and after initiating treatment at Thika level five (5) hospital?
2. What are the levels of Activated Partial Thromboplastin Time (APPT) among TB patients before and after initiating treatment at Thika level five (5) hospital?
3. What are the levels of D-dimer among TB patients before and after initiating treatment at Thika level five (5) hospital?
4. What are the levels of Platelets count among TB patients before and after initiating treatment at Thika level five (5) hospital?

1.5 Hypothesis

1.5.1 Null hypothesis

There are no haemostatic changes in TB patients initiating TB medication.

1.5.2 Alternative hypothesis

There are haemostatic changes in TB patients initiating TB medication.

1.6 Objectives

1.6.1 General Objective

To determine coagulopathy in TB patients initiating anti-TB Medication at Thika level 5 hospital

1.6.2 Specific Objectives

1. To determine the levels of Prothrombin time (PT) among TB patients of different age groups before (TB treatment naive) and after treatment at Thika level five (5) hospital.
2. To determine the levels of Activated Partial Thromboplastin Time (APPT) among TB patients of different age groups before (TB treatment naive) and after treatment at Thika level five
3. To determine the levels of D-dimer among TB patients of different age groups before (TB treatment naive) and after treatment at Thika level five hospital
4. To determine the levels of platelet count among TB patients of different age groups before (TB treatment naive) and after treatment at Thika level five hospital.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction.

The Global Tuberculosis Report 2017 places Kenya in all the three lists of countries with either a high absolute or per capita burden of TB, TB/HIV and Multi Drug Resistant Tuberculosis (WHO, 2017). Further, findings from the National Tuberculosis Prevalence Survey 2016 suggest that the proportion of patients with tuberculosis who are not notified is unacceptably high at about 55% (Chakaya *et. Al..*, 2013. Therefore, finding and successfully treating all people with tuberculosis remains an important priority for this country (Enos, 2018)

2.2 Blood coagulation and Fibrinolysis mechanism

Blood coagulation is a process where liquid form of blood in certain conditions turn to a gel or fibrin clot. On the other hand fibrinolysis is the process where formed blood clots are broken down and dissolved. Under normal homeostatic conditions, the human body is maintained in a finely tuned balance of both coagulation and fibrinolysis in a balance. (Hoffman *et al.*, 2013). Following body injury the coagulation mechanism is activated yielding thrombin that in turn converts fibrinogen to fibrin; the stable fibrin clot being the final product of haemostasis. (Hoffman *et. al..*, 2013). Activated partial thromboplastin time (aPTT or APTT) is a blood test that characterizes coagulation of the blood while Prothrombin time (PT) is a blood test that measures how long it takes blood to clot. A prothrombin time test can be used to check for bleeding problems. Platelets, also called thrombocytes, are a component of blood whose function (along with the coagulation factors) is to react to bleeding from blood vessel injury by clumping, thereby initiating a blood clot (Laki K. 1972). On the other hand fibrinolytic system then functions to break down fibrinogen and fibrin to fibrin degradation products. Activation of the fibrinolytic system yields plasmin in the presence of thrombin,

which is mainly responsible for the lysis of fibrin clots (Hoffman *et. Al..*, 2013). The breakdown and dissolving of fibrinogen and fibrin results in polypeptides referred to as fibrin degradation products (FDPs) or fibrin split products (FSPs). In a normal state of homeostasis, presence of plasmin is important, as it is the central proteolytic enzyme of coagulation necessary for dissolving and breakdown of fibrin clots (Hoffman *et al*., 2013). D-dimer (or D dimer) is a fibrin degradation product (or FDP), a small protein fragment present in the blood after a blood clot is degraded by fibrinolysis. It is so named because it contains two D fragments of the fibrin protein joined by a cross-link (Adam *et. Al..*, 2009). D-dimer concentration may be determined by a blood test to help diagnose thrombosis. Since its introduction in the 1990s, it has become an important test performed in patients with suspected thrombotic disorders. While a negative result practically rules out thrombosis, a positive result can indicate thrombosis but does not rule out other potential causes. Its main use, therefore, is to exclude thromboembolic disease where

Extrinsic)w (Adar VII 009).

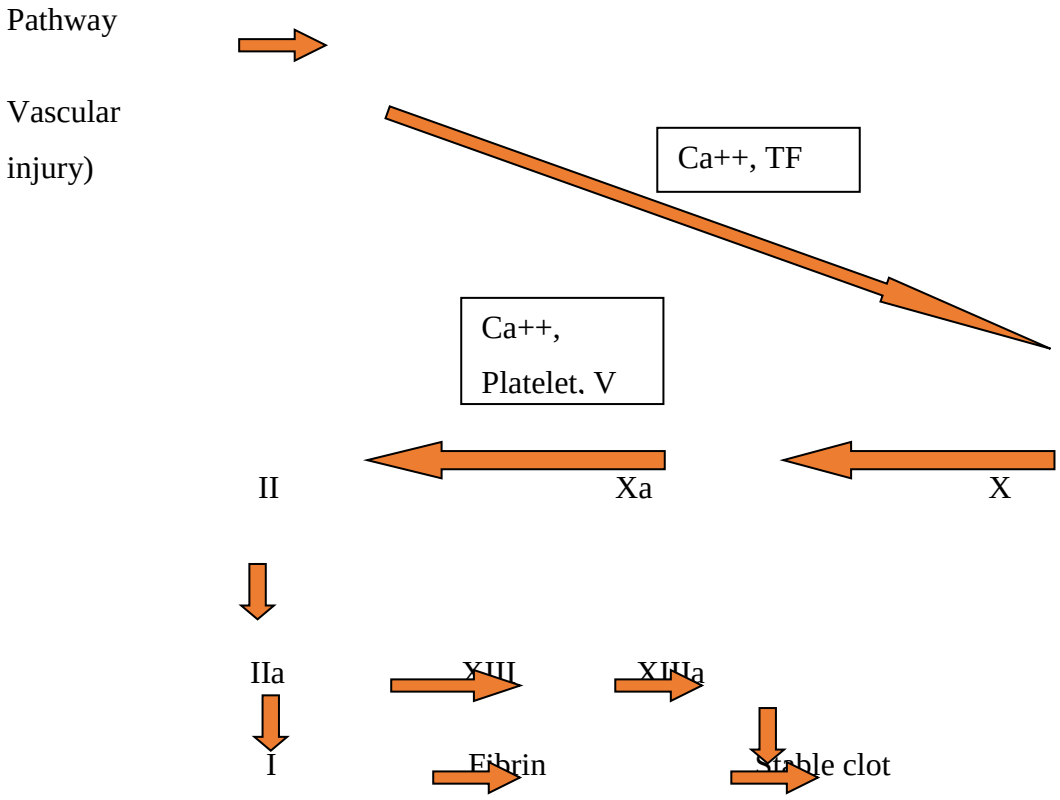

Figure 2.1: Extrinsic blood coagulation mechanism. (Hoffbrand *et. Al..*, 2019).

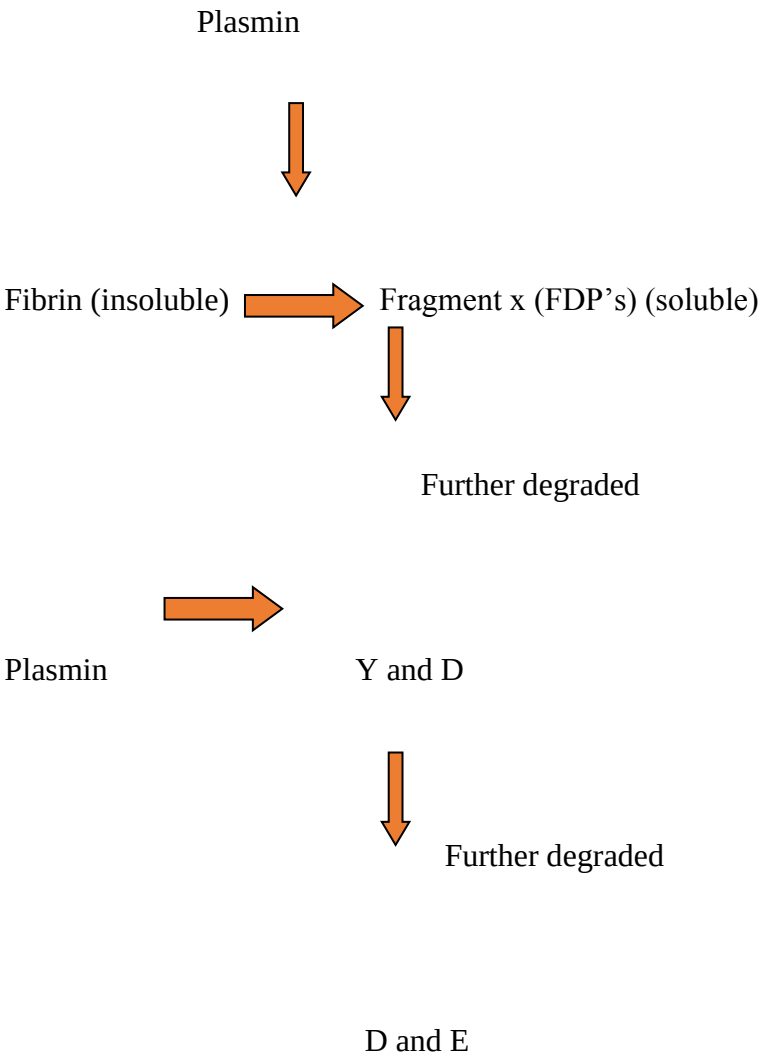

Figure 2.2: Fibrinolysis mechanism (Hoffbrand *et. al..*, 2019).

2.3 Effects of TB on haemostasis.

Coagulation studies in 82 patients with pulmonary tuberculosis clearly showed that latent intravascular coagulation occurs in patients with active tuberculosis as well as in those with marked residual changes. (Priĭmak *et. al..*, 1995). The level of Cl-esterase in activator may serve as an important indicator of the disease prognosis. Low concentration indicates poor prognosis. (Priĭmak *et. a.l.*, 1995). Disseminated

intravascular coagulation (DIC) may develop in patients with active tuberculosis and has a very high mortality rate. (Wang *et. al..*, 2014).

A number of studies have reported the occurrence of blood thrombotic complications, such as disseminated intravascular coagulopathy and deep vein thrombosis, in active tuberculosis

(TB) patients. (Kothari *et. a.l.*, 2012). The magnitude of drug induced haematological abnormalities had been investigated in many parts of the world. For example, leucopenia as a result of rifampicin and isoniazid therapy was reported in Japan (Shishido *et. al..*, 2003). Different study reports showed that after anti-tuberculosis treatment with streptomycin, rifampicin and isoniazid, the Red Blood Cell indices were affected and reached closer to normal value. (Baynes *et. al..*, 1986).

The expression of tissue factor (TF) in the blood circulation, the primary activator of coagulation cascade, is known to be responsible for thrombotic disorders in many diseases conditions including bacterial infections. (Kothari *et. al..*, 2012). However, severe infection and inflammation coupled by treatment reactions almost invariably lead to hemostatic abnormalities, ranging from significant laboratory changes to severe disseminated intravascular coagulopathy (DIC) (Kothari *et. al..*, 2012).

Tuberculosis is a major cause of morbidity and mortality in many countries including Kenya (Chakaya *et. al..*, 2013). It affects all age groups, but mostly affects productive age group of between fifteen (15) to forty four (44) years. Major factor responsible for the large disease burden in Kenya is the concurrent HIV epidemic (Chakaya *et. al..*, 2013). Other factors that have contributed to this large TB disease burden has been increasing concerns about the emergence of drug resistant TB, a threat that would pose major challenges in the fight against TB in resource limited countries like Kenya.

As a major step to address challenges posed by the tuberculosis epidemic in the era of HIV in Kenya, the Ministry of Public Health and Sanitation through the Division

of Leprosy, Tuberculosis and Lung Disease (DLTLD) has identified areas for increased support this includes; strengthening of human resources capacity at all levels of Division of Leprosy, Tuberculosis and Lung Disease (DLTLD) for effective coordination, control activities and decentralization of control services to the community level. Another factor is strong collaboration between TB and HIV control programs to promote delivery of integrated TB/HIV services. Active promotion of private - public partnerships is important in order to increase the number of non - public providers integrated into the TB service provider network. An enhanced sustained public health education campaigns that promote early medical care seeking and adherence to anti TB treatment at community level. Finally sustained health care worker training, continues education and support for better TB case management (Chakaya *et. Al..*, 2013). The World health organization has also declared that TB is only second to HIV/AIDS as the greatest killer disease worldwide out of all infectious diseases conditions caused by a single infectious agent. In the year 2010, approximately 8.8 million people fell ill with TB disease and approximately 1.4 million people died from TB disease. (healthunit.org/infectious/tb/tb)

2.4 Effect of anti TB Drugs on haemostasis

Although frequency of haemostatic changes are rarely reported due to TB medication however hypersensitivity-immune mediated hematologic disorders have been reported and experienced during active treatment with Rifampicin anti TB drug especially on high dosage especially when administered irregularly. This is timely resolved when discontinued on time (Lai *et. Al..*, 2011). An association between inflammation induced by tuberculosis and a hypercoagulable state has been described. Therefore, the occurrence of deep venous thrombosis or pulmonary embolic episodes, should be considered in patients with tuberculosis particularly during the first weeks of treatment. The physician's awareness of these phenomena is important to an early diagnostic suspicion and prompt treatment in order to prevent fatal outcomes (Goncalves *et. Al..*, 2009).

2.5 Diagnosis and treatment of pulmonary tuberculosis (PTB)

Pulmonary TB diagnosis is primarily based on both clinical and bacteriological evidence. Medical Laboratory diagnosis may require at least two sputum smears stained by either Ziehl-Neelsen direct method or fluorescent microscopy that are required to arrive at a diagnosis i.e. positive for Acid Fast Bacilli (AFB) with microscopy (Chakaya *et. Al..*, 2013). New molecular diagnostic tests such as the Gene xpert test is currently being used for diagnosis as well as culture and sensitivity. Newly confirmed TB patients are treated with the first line drug regimen on a Directly Observed Treatment Short Course (DOTS) of treatment with the right drug regimen. These drugs include Rifampicin (R), Isoniazid (H), Pyrazinamide (Z) and Ethambutol (E) for a two month intensive phase. After this period at least all the mycobacteria are killed and are consequently completely eliminated after a continuation phase of Rifampicin (R) and Isoniazid (H) for at least four months. (Kassa *et. al..*, 2012)

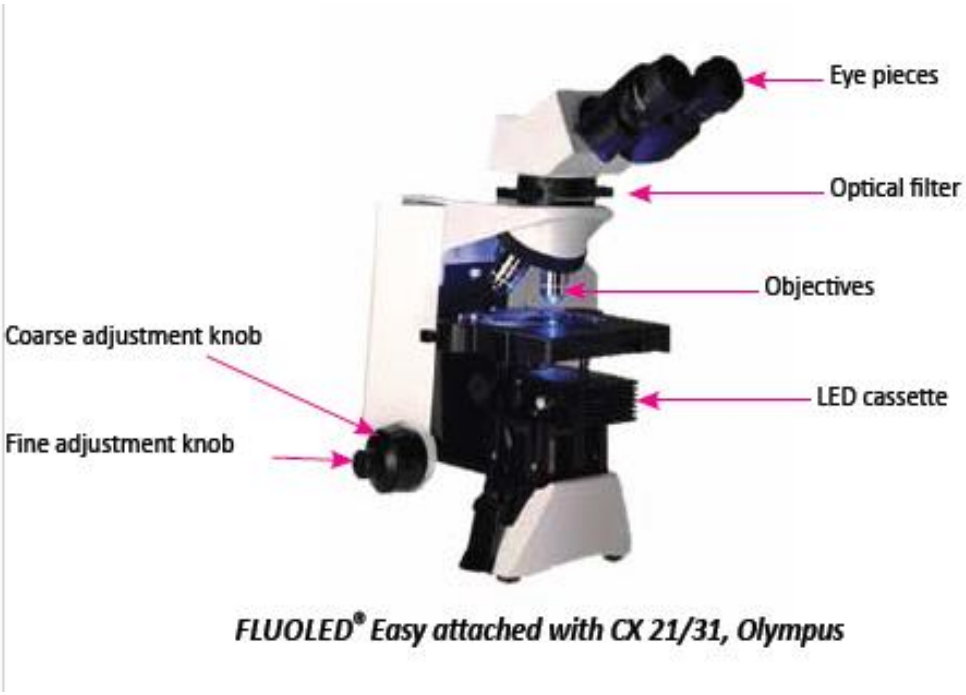

Plate 2.1: Fluorescent microscope for AFB Microscopy: DLTLD Kenya 2015

2.6 Gene Xpert Test

This involves use of a Gene Xpert machine which uses the principle of real-time Polymerase Chain Reaction (PCR) by detection of *Mycobacteria tuberculosis* DNA. The automated test uses a polymerase chain reaction to break apart and identify strips of DNA. The test tests presence of *Mycobacteria tuberculosis* genes as well as the genes associated with rifampicin resistance. Results are obtained after two hours. Sputum samples are used. A four module machine will test sixteen samples in eight hours each talking two hours.

Plate 2.2: Gene Xpert TB testing machine: DLTLD Kenya. 2015

2.7 Platelet count

2.7.1 Principle

Whole blood passes between two electrodes through an aperture so narrow that only one cell can pass through at a time. The impedance changes as a cell passes through. The change in impedance is proportional to cell volume, resulting in a cell count and measure of volume. (Lakomsky *et. al..*, 2000)

2.8 Prothrombin Time test (PTT)

2.8.1 Principle

The addition of pre-warmed (37^{o}c) platelet-poor plasma to thromboplastin - calcium reagent activates the coagulation cascade at factor VII. The time required for clot formation is recorded. Clot formation is detected by optical method. (Capoor et al.., 2015)

Prothrombin time was determined in all the samples by fully automated coagulometer (ACL Elite).

2.9 Activated partial thromboplastin time (aPTT)

2.9.1 Principle

Platelet poor plasma [PPP] when incubated at 37°C then phospholipid (cephalin) and a contact activator (e.g. Kaolin, micronized silica or allergic acid) are added followed by calcium (all pre-warmed to 37°C). Addition of calcium initiates clotting and timing begins. The APTT is the time taken from the addition of calcium to the formation of a fibrin clot. (Chopin *et. al..*, 2006)

2.10 D-Dimer test

2.10.1 Principle

The test uses sandwich immune detection method, the detector antibody in buffer binds to antigen in sample, forming antigen - antibody complexes, and migrate onto nitrocellulose matrix to be captured by the other immobilized antibody on the test strip. The more antigen in sample forms the more antigen-antibody complex and leads to stronger intensity of fluorescence signal on detector antibody, which is processed by instrument for ichroma tests to show D-Dimer concentration in sample. (Koczula *et. al..*, 2016)

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Site

The study was carried out at TB clinic Thika level five (5) hospital located in Thika urban town, Kiambu County, Kenya. It serves rural areas in Kiambu county and neighbouring counties of Muranga, Machakos, Kirinyaga and Nairobi. It has both in patients and outpatients.

3.2 Study Design

A prospective analytical experimental study where allocation or assignments of individuals was under control of the investigator.

3.3 Study Population

Study population was newly diagnosed TB patients from age 18 years and above attending TB Chest clinic at Thika level 5 hospital who have no signs of coagulopathy.

3.3.1 The Inclusion Criteria

Patients were included if one or more of the following findings were present.

1. Positive Acid Fast Bacilli (AFB) on *Ziehl*–Neelsen staining or fluorescent microscopy test.
2. Molecular gene xpert test positive for Mycobacteria tuberculosis with rifampicin sensitive.
3. First time diagnosed TB patients who have never been on any TB treatment (TB treatment naïve).
4. Patients due for first line TB treatment.
5. Patients above 18 years of age.
6. No signs or history of coagulopathy before TB treatment.

3.3.2 The Exclusion Criteria

Cases were excluded if

1. HIV co-infection exists.
2. TB patients below 18 years of age.
3. All TB Retreatment cases.
4. Patients initially on TB treatment(not naïve)
5. Patient has signs of coagulopathy before TB treatment.

6. Molecular gene xpert test positive for Mycobacteria tuberculosis with rifampicin resistance

3.4 Sample Size Determination

The minimum sample size was calculated based on the desired degree of precision and the anticipated prevalence using Cochran's formula (Cochran, 2007).

$$\frac{N = Z2\, p\, (1-p)}{d^2}$$

Where; N = Minimum number of samples required

Z = the X-axis value of the normal curve that cuts off an area α at the tails of the normal curve. Where 1-α is equal to the desired confidence level.

p = prevalence of TB in Kenya

d = desired level of precision

The prevalence (p) of TB in Kenya is 0.00491% which is the estimated TB prevalence in Kenya, in 2017 (Chala *et. Al.*., 2017)

Z is 1.96 and d is 0.01.

$$Therefore;\ N = \frac{1.96^2 \times 0.00491\ (1-0.00491)}{0.01}$$

N = 188 samples.

However, the sample size was increased by a factor of 5% to 197 TB positive samples in order to cover for any discrepancies or attrition of study participants.

3.5 Sampling Method

Simple Random Sampling method was used for all consenting patients. Meaning, every eligible individual in the population had the same chance of being selected or

subjects who met the inclusion criteria were selected until the required sample size was achieved. Blood samples were collected before initiation of treatment and a second set of samples were collected after initiation of treatment. All specimens were collected in the Laboratory.

3.6 Laboratory Procedures.

3.6.1 Sputum specimen collection.

Patients were instructed to collect 2 - 3 millilitres of sputum in a suitable container on an open well ventilated area away from other people.

They were instructed to breathe in deeply three times and then cough out to obtain the sputum sample in the container. Two specimens were collected one when the patient presented in the facility and the other early in the morning the following day for fluorescence microscopy. One early morning specimen was collected in case of Gene Xpert test.

The specimen was delivered to the lab for analysis by fluorescence microscopy or Gene expert test.

3.6.2 Blood Specimen collection

Blood specimens were collected from all the consenting patients.

Eight milliliters of blood was collected from TB patients before treatment. Four milliliters of the blood was dispensed each into both uniquely identified EDTA (purple top) and sodium citrate (blue top) vacutainers. Blood was mixed five to six times by inversion then stood in a rack for analysis, processing or storage.

The EDTA whole blood samples were analyzed for platelet count within 24 hours.

Coagulation screening blood specimens in sodium citrate were centrifuged within four hours and aliquot plasma put in a cyovial for storage in a freezer at -20^{O}c for not more than one month and transportation to Kenyatta National Hospital Laboratory and Shandon diagnostic Laboratory where they were analyzed for Prothrombin time, Activated Partial Thromboplastin Time and D-dimer tests.

A second batch of Samples were collected from the same population after two weeks of treatment and tested in a similar manner.

3.6.3 Sputum Microscopy Test

Sputum Smears were prepared on a slide, stained with fluorochrome stains and examined by fluorescent microscopy.

3.6.4 Sputum Gene Xpert test

Sputum samples were also tested using gene Xpert machine for *Mycobacteria tuberculosis*

3.6.5 Platelet count

Platelet count was done using a fully automated haematology analyzer (Celtac Analyzer). Platelets count results for all the patients were entered into a table.

3.6.6 Prothrombin Time test (PTT)

Prothrombin time was determined on all the samples by fully automated coagulometer (ACL ELITE ^{PRO}).

3.6.7 Activated partial thromboplastin time (aPTT)

Activated partial thromboplastin time was determined on all the samples by fully automated coagulometer (ACL Elite).

3.6.8 D-Dimer test

D-Dimer test was done using ichroma D - Dimer fluorescence Immunoassay Machine

3.6.9 Quality Assurance.

Quality control systems were put in place to avoid occurrence of pre-analytical, analytical and post-analytical errors during the entire process of participants and specimen management. This ensured accurate, reliable and timely results for specimen collected.

Pre-analytical errors were prevented and minimized by involving competent staffs, accurate labeling of specimens, appropriate sample and reagent storage and appropriate sample transportation.

Analytical errors were prevented and minimized by including control specimens whose values were known. All control samples runs passed well before the participant's samples were run. During the testing process Standard Operating Procedures (SOPs) were followed, accurate timing and measurement of the test, use of the right reagents and use of unexpired and properly stored reagent.

Post-analytical errors were prevented and minimized through accurate interpretation of the results and transcription of the right results.

3.7 Data Management, Analysis and Presentation.

Data was collected after the test and entered into the register. Result for all the haematological parameters was entered and grouped according to new cases (baseline) before treatment and after treatment

Data analysis was done by using SPSS statistical software version 22, Minitab statistical software version 19 and graph pad prism version 8. Descriptive statistics - proportions, range, median and mean was done. T test was used to compare groups. (95% CI or ≤ 0.05 was considered significant). Results presentation was done by use of table's bar graphs and charts.

3.8 Ethical Consideration

Ethical approval was obtained from Kenyatta University Ethical Review Committee. Authorization was sort from National Commission for Science and Technology (NACOSTI) and from Thika level five hospital as well as attached in the appendixes. Care and protection of the research participants was enhanced through honesty, trust and respect of the participants. Consent from the participants was sort by signing an informed consent form. Confidentiality of the participant's information was maintained. All information provided by the participants was treated without any bias and appropriate research methodology was used for the benefit of the patients, laboratory personnel and the government.

CHAPTER FOUR

RESULTS

4.1 Demographic characteristics of the study participants

This study involved a total of 197 TB patients attending Thika level 5 hospital in Kiambu County. Ninety-five (48.22 %) of the participants were males while one hundred and two (51.78 %) were females (figure 4.1). The participants' age ranged from 18 years to 66 years old with a mean age of 37.24±0.66 years.

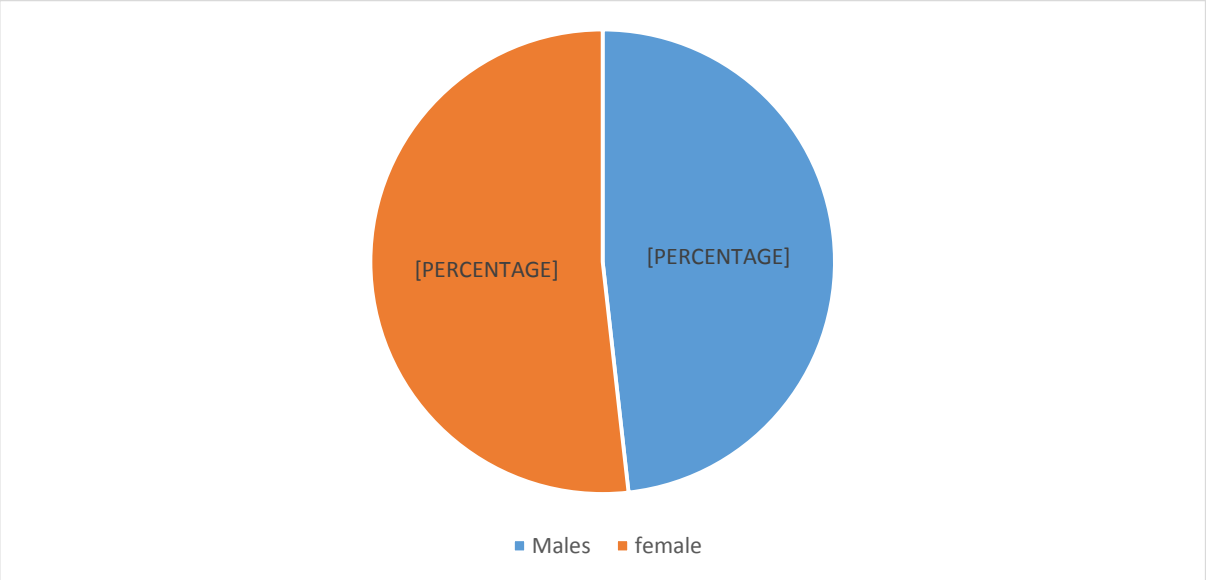

Figure 4.1: Percentage gender composition of participants

Table 4.1: Percentage distribution of TB patients within age groups.

Age group	Total Number	Percentage representation
18-25	21	10.66%
26-33	52	26.60%
34-41	63	64.95%
42-49	45	22.84%
50-57	12	6.09%
58-65	3	1.52%
≥66	1	0.51%

In terms of education level, this study noted that more males 16.24% had reached primary level of education as compared to their female counterparts at 13.2%. However, more females had attained secondary 24.37% and tertiary 14.21% level of education as compared to male participants who attained 19.29% and 12.69% respectively. Figure 4.2.

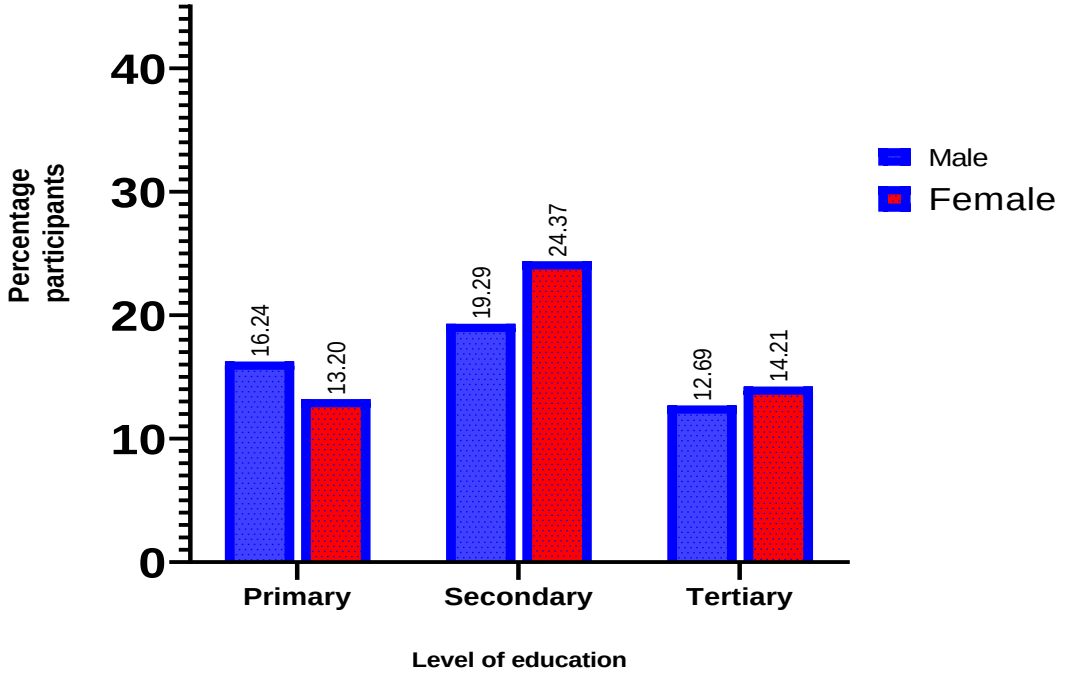

Figure 4.2. Participant's level of education

The current study also sought the occupation status of the included patients. It was found that unemployed female participants were the majority comprising of 17.77 % compared to males 7.61 %. It was also shown that majority of female participants (14.21%) did not disclose their occupation status. It was evident that more males (13.20%) than females (6.60%) were in the formal sector. It was however noted that in the informal employment, there were more females (13.20%) than males (10.15%) figure 4.3.

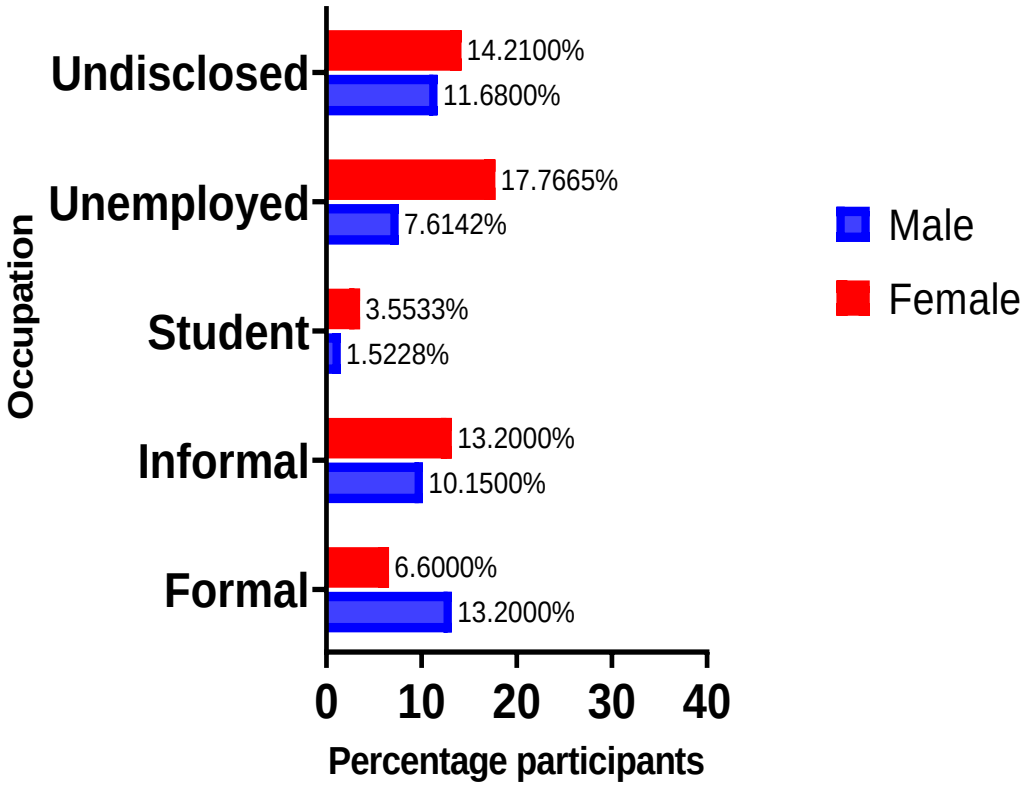

Figure 4.3: Occupation state of participants

Furthermore, the marital status of the study group was assessed. The study revealed that the highest percentage of the participants (64.98 %) were married as opposed to minor cohort that was cohabiting (1.52 %) figure 4.4.

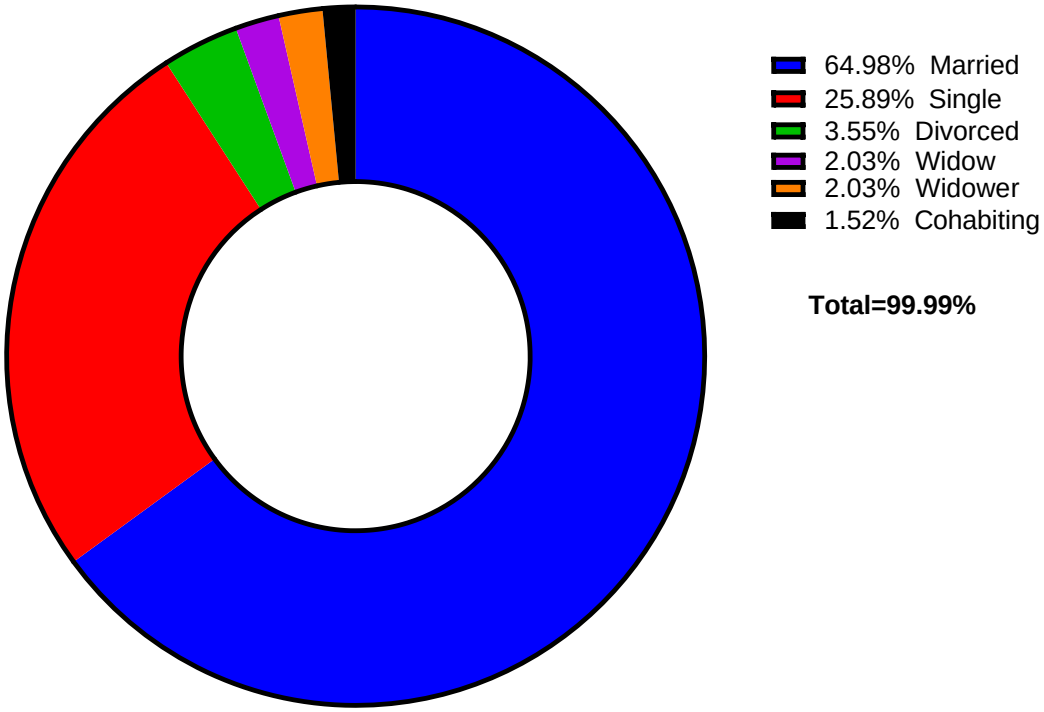

Figure 4.4: Marital status of the participant

In terms of test results for the recruited participants 89% were gene xpert positive and 11% were Fluorescent microscopy (FM) positive.

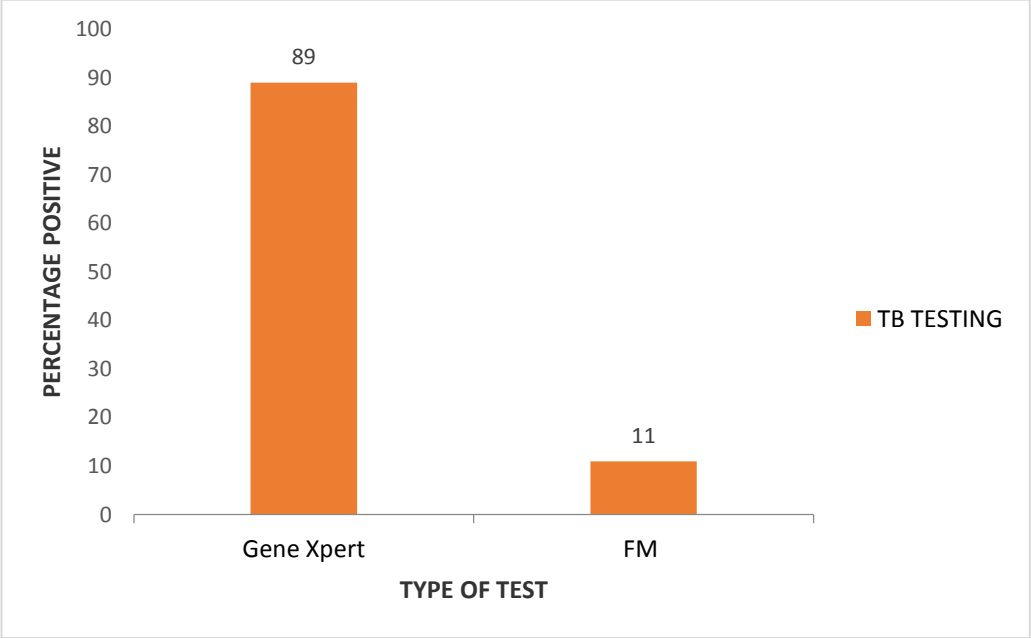

Figure 4.5: Methods of TB testing.

4.2 Determination of Prothrombin time (PT) among TB patients before (TB treatment naive) and after treatment

The mean prothrombin times among TB positive participants before administration of anti-TB drugs were slightly lower in all the age groups except for the 58-65 years group which had 13.700±0.529. The corresponding prothrombin times after treatment were slightly higher in all age groups except for the 58-65 years group which had (11.100±0.586). Significant differences in prothrombin time were witnessed in 42-49, 58-65 and above 66 years age groups with p-values of 0.021, 0.000, and 0.000 respectively at 95 % confidence level. Low p-values indicate strong association and differences with high statistical significance. It was however noted that all the prothrombin times were falling within the normal reference ranges (Table 4.2).

Table 4.2: Association between prothrombin time (PT) with the age of TB patients before and after treatment.

Age group	Before treatment	After treatment	*p*-value
18-25	12.467±0.261	13.133±0.243	0.123
26-33	12.223±0.211	12.737±0.166	0.064
34-41	12.273±0.181	12.637±0.193	0.175
42-49	12.062±0.172	12.689±0.160	0.021*
50-57	12.392±0.495	12.467±0.349	0.916
58-65	13.700±0.529	11.100±0.586	0.000*
≥66	10.352±0.051	12.413±0.010	0.000*

Values expressed as $\bar{X}$ ±SEM; *=significant change at $p \leq 0.05$; Normal reference range: 10-14 seconds

4.3 Determination of Activated Partial Thromboplastin Time (aPPT) among TB patients before (TB treatment naive) and after treatment

Activated partial thromboplastin time of TB participants were not significantly different before and after anti-TB treatment in all age groups except for those aged above 66 years old. Those above 66 years old showed significantly lower alternate thromboplastin times after anti TB administration as compared to when they were naïve (before treatment) (p=0.000). It was however observed that all the determined activated partial thromboplastin times were within the normal reference range (Table 4.3).

Table 4.3: Association between activated partial thromboplastin time (aPPT) with the age of TB patients before and after treatment.

Age group	Before treatment	After treatment	*p*-value
18-25	33.948±0.630	34.829±2.440	0.355
26-33	34.235±0.348	34.667±0.393	0.424
34-41	34.214±0.433	34.225±0.363	0.983
42-49	33.878±0.379	34.371±0.421	0.339
50-57	33.133±0.655	34.533±0.532	0.160
58-65	35.500±2.360	35.500±3.120	1.000
≥66	37.765±0.034	32.185±0.113	0.000*

Values expressed as $\bar{X}$ ±SEM; * = significant change at $p \leq 0.05$; Normal reference range: 30-40 seconds

4.4 Determination of D-dimer among TB patients before (TB treatment naive) and after treatment

The results showed a significant increase in D-dimer test in all participant TB positive patients after anti TB administration as compared to those determined before treatment. Age groups 58 - 65 year old exhibited more elevated D-dimer levels from 448.50±78.20, before treatment to 738.90±32.00 after treatment (p=0.037; 95 % CI) (Table 4.4).

Table 4.4: Association between D-dimer with the age of TB patients before and after treatment.

Age group	Before treatment	After treatment	*p*-value
18-25	309.90±32.70	445.90±31.20	0.000*
26-33	300.10±21.00	494.30±26.30	0.000*
34-41	315.40±18.20	489.00±22.60	0.000*
42-49	309.00±18.5	483.40±21.60	0.000*
50-57	329.00±35.20	464.10±49.20	0.001*
58-65	448.50±78.20	738.90±32.00	0.037*
≥66	122.40±0.004	189.70±0.201	0.000*

Values expressed as $\bar{X}$ ±SEM; *=significant change at $p \leq 0.05$; Normal reference range =0-500 ng/ml)

4.5 Determination of platelet count among TB patients before (TB treatment naive) and after treatment

Table 4.5 below presents the results for platelet count in treatment naïve TB positive patients and after treatment. In patients aged 66 years and older, there was a significance decrease in platelet count after treatment as compared with the baseline values. However, there were no significant changes in platelet count in all the other age groups. All the platelet counts were within the normal reference ranges.

Table 4.5: Association between platelet count with the age of TB patients before and after treatment

Age group	Before treatment	After treatment	*p*-value
18-25	294.70±22.5	297.30±22.30	0.933
26-33	304.50±13.80	314.60±16.10	0.666
34-41	308.20±10.8	305.90±13.90	0.899
42-49	316.70±14.10	317.30±15.80	0.973
50-57	291.90±25.00	376.80±33.50	0.068
58-65	272.70±91.80	269.70±61.30	0.983
≥66	400.031±0.05	195.01±0.02	0.000*

Values expressed as $\bar{X}$ ±SEM; *=significant change at $p \leq 0.05$; Normal reference range =150-450 X 10^9 L^{-1})

CHAPTER FIVE

DISCUSSION

5.1 introduction

Tuberculosis is a main public health problem in all developing countries, as it is one of the largest cause of death in the world as an infectious disease (Taramian *et. Al..*, 2012). There is no much literature about the haemostatic changes in pulmonary TB patients in Kenya.

The current study determined the haemostatic changes in TB patients after initiation of anti TB drugs.

Significant differences in prothrombin time were witnessed in age groups 42-49, 58-65 and above 66 years after TB treatment. This agrees with a study done in India showing rifampicin drug may contribute to the hypercoagulable state by decreasing production and increasing clearance of anticoagulant hepatic. Consequently, the initial phase of treatment may result in a higher risk for the development of DVT resulting in prolonged Prothrombin test. DVT is a condition where clots form in the circulation resulting in consumption of the available clotting factors consequently resulting in elevated prothrombin time (Naithani *et. al..*, 2007) Another study at Camlica chest hospital in Istanbul showed that mean PT was significantly increased in the tuberculosis group with respect to the pneumonia group (p=0.0001). Twenty-eight patients in the tuberculosis group (56 %) had an increased PT whereas virtually all measurements in the pneumonia group were within normal range (Kartaloglu *et. Al..*, 2000).

Those patients above 66 years old showed significantly lower activated thromboplastin times after anti TB administration as compared to when they were naïve (before treatment) (p=0.000). It was however observed that all the determined activated partial thromboplastin times were within the normal reference range with no significant values. This did not show any significant changes in activated partial

thromboplastin time. This agreed with a study done in India in comparison analysis for 88 patients for haemostasis parameters, p-value was significant for Prothrombin Time and Fibrinogen studies. Factor VIII and APTT revealed non-significant p-value. (Kutiyal, *et. al..*, 2017)

Significant increase in D-dimer test in all participant TB positive patients after anti TB administration as compared to those determined before treatment ($p<0.05$). However age group 58 – 65 year old exhibited elevated D-dimer concentrations levels from 448.50±78.20, before treatment to 738.90±32.00 after anti TB medication.($p=0.037$; 95 % CI). This corresponds with a study done in Bangladesh which showed that patients with primary TB was associated with activation of coagulation as reflected by elevated plasma concentrations of thrombin - antithrombin complexes (TATc; $P < 0.01$ for primary TB patients versus controls) and D-dimer ($P < 0.001$ for primary TB patients versus controls). D-dimer levels were also significantly elevated in recurrent TB patients compared to controls ($P < 0.001$). D-dimer levels were also significantly elevated in recurrent TB patients compared to controls ($P < 0.001$). (Kager *et. al..*, 2007)

In patients aged 66 years and older, there was a significance decrease in platelet count after treatment as compared with the baseline values ($p=0.000$). However, there were no significant changes in platelet count in all the other age groups. All the platelet counts were within the normal reference ranges. This corresponds to a previous study in Tanzania suggesting no statistically significant difference on platelet count among TB patients before initiation and after completion of the intensive phase tuberculosis treatment. Proportion of TB patients with low platelet count was slightly increased after completion of tuberculosis treatment compared to the corresponding platelet count among tuberculosis treatment naïve patients. (Kassa, *et. al..*, 2016).

Significant changes were witnessed in all parameters in ages above 66 years this could not be considered as it could be attributed to age extremity.

Despite some limitations in the current study, some key contribution to science as well as policy will be of great importance when managing TB patients on treatment. Kenya as one of TB burden countries need to adopt strategies and policies that play an important step towards enhanced good outcomes to treatment regimens.

5.2 Conclusion

Significant haemostatic changes were identified in TB patients initiating TB treatment. This included notable significant elevation of Prothrombin time and D-Dimer. There was significant association in Prothrombin time test on patients initiating TB treatment. Significant association on D - Dimer test was noted on TB patients initiating treatment. There was no significant association in Activated partial Thromboplastin Time on patients initiating TB treatment. No significant association in Platelets count on patients initiating TB treatment.

5.3 Recommedation

Haemostatic tests such as Prothrombin time test need to be done in TB patients initiating treatment.

Activated Partial Thromboplastin Time test not very necessary as no significant association was identified.

D – Dimer test need to be investigated on TB patients initiating treatment.

Platelets count not very necessary as no significant association was identified.

Routine check-up of these haemostatic changes is important for better managements of TB patients.

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APPENDICES

Appendix I: Informed Consent Form

Study title: Determination of Coagulopathy in Tuberculosis Patients Initiating Anti – TB Medication at Thika Level Five Hospital, Kenya.

Principle investigators

Muraguri Nicholas Maina - Student JKUAT

Co-investigators

Dr. Mutinda Kyama - Department of Medical Laboratory sciences (JKUAT).

Dr. Kibet Shikuku - Department of Human Pathology, Haematology and Blood Transfusion. Thematics unit, College of Health Sciences, University of Nairobi.

Dear participant,

My name is..... a student from JKUAT working on the above mentioned project.

The purpose of this study is to determine coagulopathy in TB initiating anti-TB Medication at Thika level 5 hospital.

Introduction

Coagulopathy (also called a clotting disorder) is a condition in which the blood's ability to coagulate (form clots) is impaired. This condition can cause a tendency toward prolonged or excessive bleeding (bleeding diathesis or bleeding disorder), which may occur spontaneously or following an injury. This can arise as a result of medications given for the treatment of TB.

Early diagnosis of this phenomenon, need to be established for early institution of prompt treatment for thrombosis while continuing the anti tuberculosis treatment.

So that I can be sure you are informed about this project, you will or i will read with you this consent Form. This form will inform you why this research study is being done, what will happen in the research study, and possible risks and benefits to you. If there is anything you do not understand, please ask questions.

Procedures to be followed

Once you have agreed to enroll in the study, you will be asked some personal questions concerning the study. This will be done by a professional person and will only take about 10 minutes. The study team will also access your health records to obtain any other information they may need. Five milliliters of blood will be drawn using methods to protect infection by disease causing organisms by a well trained and experienced individual from this institution. This will take about one minute. There will be no attachment of names to the blood samples, but an identification number assigned to you will be used to label the sample. This is to ensure complete confidentiality of the test results. The sample will be taken to the laboratory, analyzed and final report concluded in less than one year. This work will be done at the Kenyatta National Hospital Haematology and blood transfusion Laboratory.

Potential risks, discomforts or inconveniencies

Blood collection will not expose you to any health risks except minimum discomfort associated with puncturing of your skin. The discomfort will last only for few minutes. There might also be some possibility of minimum swelling during withdraw of blood.

Potential benefits

This study will enable you as the participant to know your potential risk during TB Treatment. This information will enable the clinicians to make wise decision in

prescribing anti TB drugs. The study may also assist the government when planning management of problems resulting from TB medication. No monetary benefits will be offered by this study.

Participation and voluntarism

Your participation in this project is free and voluntary. You have a right to decline participation in the project at this time by failing to sign this form. You may also stop at any time. Failure to participate will not affect the services you receive from this facility now or in future.

Confidentiality

The information you give in the questionnaire will be kept confidential and will only be shared by the project staff for analysis. Results of this study may be presented in public talks or written articles, but no information will be presented that identifies you since I will use a special identification codes other than individual names

PARTICIPANT'S CONSENT

A) For participants who informed consent was read for them.

By signing my name below, I confirm the following:

i. This entire consent document has been read to me. All of my questions have been answered to my satisfaction.
ii. The study's purpose, procedures, risks and possible benefits have been explained to me.
iii. I voluntarily agree to participate in this research study. I agree to follow the study procedures as directed. I have been told that I can stop at any time.

Name of participant.....Signature…….....

Date…….....

Name of witness…….Signature…….

B) For participants who read the informed consent on their own

By signing my name below, I confirm the following:

i. I have read the entire consent document. All of my questions have been answered to my satisfaction.
ii. I have understood the study's purpose, procedures, risks and possible benefits.
iii. I voluntarily agree to participate in this research study. I agree to follow the study procedures as directed. I have understood that I can stop at any time.

Name of participant.....Signature…….....

Date…….....

Project contacts

If you have any questions about this project you may contact Principle investigator on Tel: 0722659088, email muragurinicholas2@gmail.com or Co-investigators, Dr Mutinda C. Kyama on Tel: 0711169526, email kcleophas@yahoo.com or Dr Kibet Shikuku on Tel: 0720789843, email: pkibet@uonbi.co.ke or The Kenyatta University Ethical Review Committee Secretariat on P.O. Box 43844 – 0100 Nairobi, Tel: 8710901/12, emails chairman..kuerc@ku.ac.ke, secretary.kuerc@ku.ac.ke, ercku2008@gmail.com.

CONSENT AGREEMENT FORM

I participated in consent process and Acknowledge enrolment of this participant into the study.

Name of Principal investigator/ Assistant…….

Signature……Date……

VIAMBATISHO

Kiambatisho cha II: Fomu Fahamisho ya Kibali.

Mada ya uchunguzi: Uchunguzi wa kuvunja damu katika wagonjwa walio na ugonjwa wa kifua kikuu wanao anzishwa dawa za matibabu ya ungonjwa wa kifua kikuu katika Hospitali ya kiwango cha tano ya Thika Nchini ya Kenya.

Mchunguzi mkuu

Muraguri Nicholas Maina - Mwanafunzi JKUAT

Wachunguzi-wenza

Dr Mutinda C Kyama PhD, idara ya maabara matibabu ya kisayansi, JKUAT.

Dr Kibet Shikuku, Shule ya matibabu na kisayanzi, chuo kikuu cha Nairobi.

Mpendwa Mshiriki,

Jina langu ni …….. Mwanafunzi kutoka JKUAT anayeshughulikia mradi huu uliotajwa hapo juu**.**

Kusudio la uchunguzi huu ni kuweza kutafuta Kiini licha ya kuvunja damu katika wagonjwa walio na ugonjwa wa kifua kikuu wanao anzishwa dawa za matibabu ya ugonjwa wa kifua kikukuu. , katika Hospitali ya kiwango cha tano ya Thika. Kaunti ya Kiambu.

Utangulizi

Kuvunja kwa damu mwilini kwa mda mrefu bila sababu yeyota au kutokana na mathara ya mwili kunaweza sababishwa na dawa zinazo tibu ugonjwa wa kifua kikuu. Kutabua njambo hili mapema ni muhimu ili kulitatua kwenye wagonjwa wanao tumia dawa za kutibu ugonjwa wa kifua kikuu. Ili niwe na hakika kuwa umefahamishwa kuhusu mradi huu, utasoma/mtasoma na muuguzi fomu hii ya kibali. Fomu hii itakufahamisha kwa nini uchunguzi huu wa utafiti unafanywa, ni nini kitafanyika katika uchunguzi huu wa utafiti na hatari pamoja na manufaa yanayowezekana kwako. Kama kunalo lolote usilolielewa, tafadhali uliza maswali.

Taratibu

Punde tu utakapokuwa umekubali kujiunga katika uchunguzi huu, utaulizwa baadhi ya maswali yanayohusiana na uchunguzi wenyewe. Hii itafanyiwa na mshauri muuguzi mtaalamu na itachukua muda wa dakika kumi hivi.Timu ya utafiti itakubaliwa kutumia kumbukumbu za afya za washiriki. Mililita tano za damu zitatolewa kwa njia ya kitaalamu na mtaalamu mwenye tajiriba na mafunzo kutoka katika taasisi hii. Hii itachukua karibu dakika moja. Hakutakuweko na vibandiko vya majina katika sampuli hizo za damu, lakini nambari ya kitambulisho itakayohusishwa na wewe itaweza kutumika katika kutambulisha sampuli hiyo. Hii ni kuhakikisha kuwa ufaragha kamilifu wa matokeo ya kipimo hiki unafikiwa. Sampuli hii itapelekwa kwenye maabara, kwa uchunguzi na ripoti kamilifu kutolewa ndani ya muda usiozidi mwaka mmoja. Kazi hii itafanyiwa katika maabara ya Uchunguzi wa damu kwenye Hospitali Kuu ya Kenyatta.

Hatari,usumbufu tarajiwa

Ukusanyaji wa damu hautakuathirii katika hatari yoyote ya kiafya isipokuwa tu maumivu kidogo yanayohusiana na kule kudugwa kwenye ngozi yako. Maumivu haya yatadumu kwa dakika chache tu. Huenda pia kukawa pia na baadhi ya uwezekano wa uvimbe mdogo wakati uondoaji wa damu.

Faida-tarajiwa

Uchunguzi huu huenda ukakuwezesha ukiwa mshiriki kujua hatari unayoweza kuwa nanyo wakati unapopo ungua ugonjwa wa kifua kikuu. Taarifa hii itawezesha wanakliniki kuweza kutoa uamuzi wa busara katika kukushauri ni dawa zipi za ugonjwa wa kifua kikuu. Uchunguzi huu huenda ukasaidia pia serikali wakati inapopangilia shinda zinazo abatana na utumianji wa dawa zinazo tibu ungonjwa wa kifua kikuu. Hakuna manufaa yoyote ya kifedha yatakayokuwepo kwako katika uchunguzi huu.

Kushiriki na Kujitolea mhanga

Kushiriki kwako katika mradi huu ni bila malipo na kwa kujitolea mhanga. Unayo haki ya kukataa kushiriki katika mradi huu wakati huu kwa kukataa kupiga saini fomu hii. Unaweza pia kusita kuendelea na mradi huu wakati wowote. Kushindwa kushiriki hakutaathiri huduma zozote unazopokea kutoka katika huduma hii sasa au hata katika siku za usoni.

Ufaragha

Taarifa unayotoa katika hojaji (questionnaire) hii itahifadhiwa kwa ufaragha na itaonyeshwa tu wafanyikazi wa mradi huu kwa kusudio la uchambuzi. Matokeo ya uchunguzi huu huenda yakawasilishwa katika mikutano ya umma ua makala yaliyoandikwa, lakini hakuna taarifa yoyote itakayowasilishwa itakutambulisha

KIBALI CHA KUSHIRIKI

A) Kwa washiriki ambao fomu fahamishi ya kibali imesomwa kwao

Kwa kupiga saini na kuliandika jina langu hapa chini, nathibitisha yafuatayo:

Waraka huu wote wa kibali umesomwa kwangu. Maswali yangu yote yameweza kujibiwa na nikatosheka.

i. Kusudio, taratibu, hatari na manufaa yanayowezekana katika uchunguzi huu yameweza kuelezewa kwangu.

ii. Ninajitolea kwa hiari yangu kukubali kushiriki katika uchunguzi wa utafiti. Nakubali kufuata taratibu za uchunguzi huu kama nilivyoelekezwa. Nimeelezewa pia kuwa naweza kusitisha kushiriki kwangu wakati wowote.

Jina la mshiriki…….....
Saini……

Tarehe……..

Jina la shahidi…….
Saini……

B) Kwa washiriki ambao wamejisomea fomu Fahamisho ya kibali

Kwa kupiga saini na kuliandika jina langu hapa chini, nathibitisha yafuatayo:

Nimeusoma waraka huu wote wa kibali. Maswali yangu yote yameweza kujibiwa na nikatosheka.

Nimejisomea kusudio, taratibu, hatari na manufaa yanayowezekana katika uchunguzi huu.

Ninajitolea kwa hiari yangu kukubali kushiriki katika uchunguzi wa utafiti. Nakubali kufuata taratibu za uchunguzi huu kama nilivyoelekezwa. Nimeelezewa pia kuwa naweza kusitisha kushiriki kwangu wakati wowote.

Jina la mshiriki…….....
Saini……

Tarehe……..

Anwani za mradi

Kama una swali lolote unaweza kuwasiliana na Mchunguzi Mkuu katika nambali ya simu: 0722659088, baruapepe: muragurinicholas2@gmail.com. au Wachunguzi-

wenza, Dr Mutinda C. Kyama, nambali ya simu: 0711169526, baruapepe: kcleophas@yahoo.com, Dr Kibet Shikuku, nambali ya simu: 0720789843, baruapepe: pkibet@uonbi.co.ke au Kamati ya Maadili ya Kimatibabu na Taasisi ya Chuo Kikuu cha Kenyatta, anwani 43844 – 0100 Nairobi, nambali ya simu: 8710901/12, baruapepe: chairman..kuerc@ku.ac.ke, secretary.kuerc@ku.ac.ke, ercku2008@gmail.com.

FOMU YA MKATABA WA KIBALI

Nilishiriki katika mchakato huu wa kibali na nina dhibitisha kujiunga kwa mshiriki huyu katika uchunguzi huu.

Jina la Mchunguzi mkuu/Msaidizi……

Saini……Tarehe……
….

Appendix II: Questionnaire

Date of hospital visit …….

Unique study number.....

TEL……..

A: Socio-Demographic Characteristics

1. Date of birth.....
2. Sex: Male ( ) Female ( )
3. Employment status: Employed_____ Unemployed _____
4. Occupation…….
5. Which is your country of origin? .....
6. Level of education

   a. None ☐
   b. primary ☐
   c. secondary ☐
   d. tertiary ☐

7. Marital status

   ☐
   a. Single
   b. Married ☐
   c. Divorced ☐
   d. Widow/widower ☐
   e. Co-habiting ☐
   f. **B: Clinical Characteristics**

8. Date Diagnosed with PTB……...
9. List of symptoms

a. …….

b. …….

c. …….

10. Are you on TB therapy? _____since when? _____

11. Have you ever been on TB medication? YES ☐ NO ☐

If yes when_____

12. Have you ever suffered from any bleeding disorder YES ☐ NO ☐

13. Are you intending to finish the whole course of treatment YES ☐ NO ☐

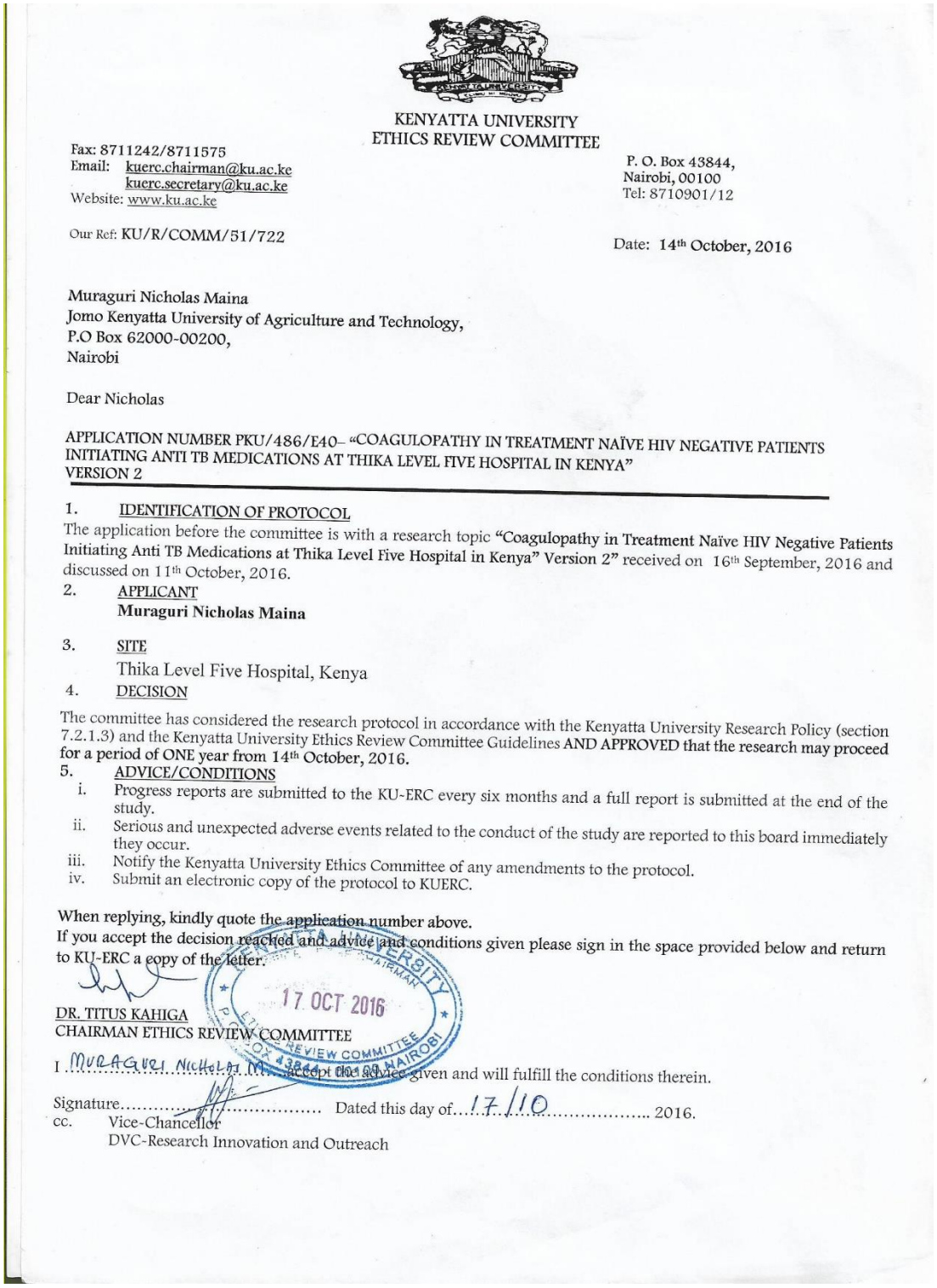
14. Are you willing to come for all the drugs as instructed YES ☐ NO ☐

15. Have you ever been done a HIV test? YES ☐ NO ☐

a. If yes what were the results? POSITIVE ☐ NEGATIVE ☐

b. If no would you like to be done the test? YES ☐ No ☐

Appendix III: Ethical approval



KENYATTA UNIVERSITY
ETHICS REVIEW COMMITTEE

Fax: 8711242/8711575
Email: kuerc.chairman@ku.ac.ke
kuerc.secretary@ku.ac.ke
Website: www.ku.ac.ke

P. O. Box 43844,
Nairobi, 00100
Tel: 8710901/12

Our Ref: KU/R/COMM/51/722

Date: 14th October, 2016

Muraguri Nicholas Maina
Jomo Kenyatta University of Agriculture and Technology,
P.O Box 62000-00200,
Nairobi

Dear Nicholas

APPLICATION NUMBER PKU/486/E40– "COAGULOPATHY IN TREATMENT NAÏVE HIV NEGATIVE PATIENTS INITIATING ANTI TB MEDICATIONS AT THIKA LEVEL FIVE HOSPITAL IN KENYA" VERSION 2

1. IDENTIFICATION OF PROTOCOL
The application before the committee is with a research topic **"Coagulopathy in Treatment Naïve HIV Negative Patients Initiating Anti TB Medications at Thika Level Five Hospital in Kenya" Version 2"** received on 16th September, 2016 and discussed on 11th October, 2016.

2. APPLICANT
Muraguri Nicholas Maina

3. SITE
Thika Level Five Hospital, Kenya

4. DECISION
The committee has considered the research protocol in accordance with the Kenyatta University Research Policy (section 7.2.1.3) and the Kenyatta University Ethics Review Committee Guidelines **AND APPROVED that the research may proceed for a period of ONE year from 14th October, 2016.**

5. ADVICE/CONDITIONS
i. Progress reports are submitted to the KU-ERC every six months and a full report is submitted at the end of the study.
ii. Serious and unexpected adverse events related to the conduct of the study are reported to this board immediately they occur.
iii. Notify the Kenyatta University Ethics Committee of any amendments to the protocol.
iv. Submit an electronic copy of the protocol to KUERC.

When replying, kindly quote the application number above.
If you accept the decision reached and advice and conditions given please sign in the space provided below and return to KU-ERC a copy of the letter.

17 OCT 2016

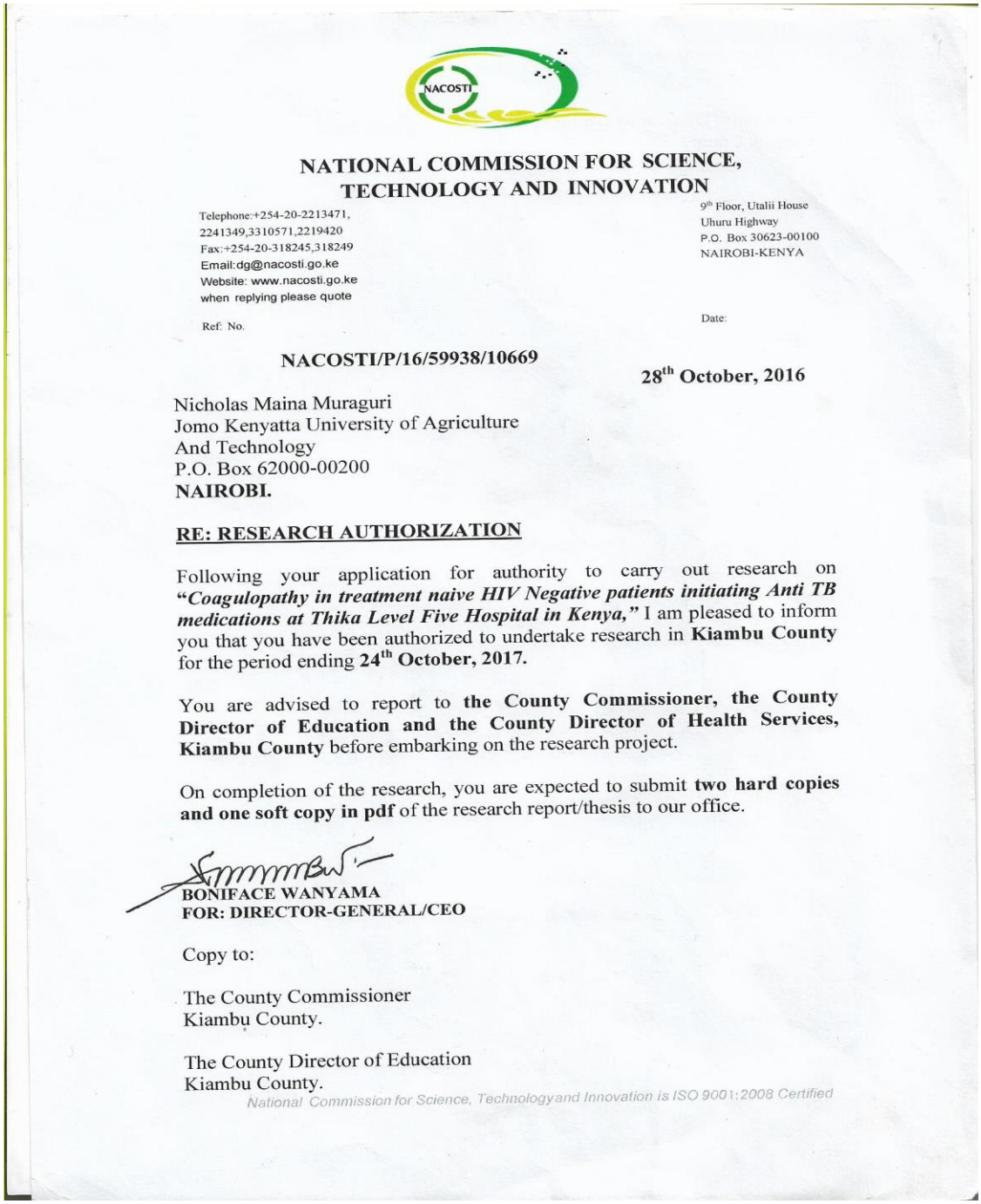
DR. TITUS KAHIGA
CHAIRMAN ETHICS REVIEW COMMITTEE

I MURAGURI NICHOLAS MAINA accept the advice given and will fulfill the conditions therein.

Signature..... Dated this day of 17/10 2016.

cc. Vice-Chancellor
DVC-Research Innovation and Outreach

Appendix IV: National Commission of Science and Technology Institute Approval



NATIONAL COMMISSION FOR SCIENCE, TECHNOLOGY AND INNOVATION

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9th Floor, Utalii House
Uhuru Highway
P.O. Box 30623-00100
NAIROBI-KENYA

Ref: No. NACOSTI/P/16/59938/10669

Date: 28th October, 2016

Nicholas Maina Muraguri
Jomo Kenyatta University of Agriculture
And Technology
P.O. Box 62000-00200
NAIROBI.

RE: RESEARCH AUTHORIZATION

Following your application for authority to carry out research on ***"Coagulopathy in treatment naive HIV Negative patients initiating Anti TB medications at Thika Level Five Hospital in Kenya,"*** I am pleased to inform you that you have been authorized to undertake research in **Kiambu County** for the period ending **24th October, 2017.**

You are advised to report to **the County Commissioner, the County Director of Education and the County Director of Health Services, Kiambu County** before embarking on the research project.

On completion of the research, you are expected to submit **two hard copies and one soft copy in pdf** of the research report/thesis to our office.

BONIFACE WANYAMA
FOR: DIRECTOR-GENERAL/CEO

Copy to:

The County Commissioner
Kiambu County.

The County Director of Education
Kiambu County.

National Commission for Science, Technology and Innovation is ISO 9001:2008 Certified

Appendix VII: Publication

JMSCR Vol||07||Issue||08||Page 514-519||August 2019

http://jmscr.igmpublication.org/home/
ISSN (e)-2347-176x ISSN (p) 2455-0450
crossref DOI: https://dx.doi.org/10.18535/jmscr/v7i8.87

IGM Publication
Journal Of Medical Science And Clinical Research
An Official Publication Of IGM Publication

Research Article

Coagulopathy on TB treatment naive HIV negative patients initiating anti TB medications at Thika level five hospital in Kenya

Authors

Nicholas Maina Muraguri[1*], Dr Mutinda C. Kyama[2], Dr Kibet Shikuku[3]

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[2]Department of Medical Laboratory Sciences, Jomo Kenyatta University of Agriculture and Technology (JKUAT), P.O. Box.2435 -00100, Nairobi, Kenya
[3]Department of Human Pathology, Haematology and Blood Transfusion - Thematics Unit, College of Health Sciences, University of Nairobi, P.O. Box 19676 – 00202 Nairobi, Kenya
*Corresponding Author
Nicholas Maina Muraguri
County Government of Kiambu, Department of Health Services, P.O. Box 6068 – 01000 Thika, Kenya

Abstract

Coagulopathyis an abnormal bleeding disorder in which the blood's ability to clot is impaired resulting to prolonged bleeding. Studies have shown a relationship between coagulopathy with many infections such as HIV viral infection after treatment.

Specific Objective: *The study aims to determine the haemostatic changes* in TB treatment naive HIV Negative patients initiating Anti TB Medications at *Thika Level Five Hospital in Kiambu County of Kenya.*

***Design**: Prospective study design was used by carrying out Prothrombin time (PT) and Alternate Partial Thromboplastin Time (aPTT) among TB patients before and after treatment.*

Subjects: *A total of 197 TB positive patients attending Thika level five hospitals TB clinic were recruited.*

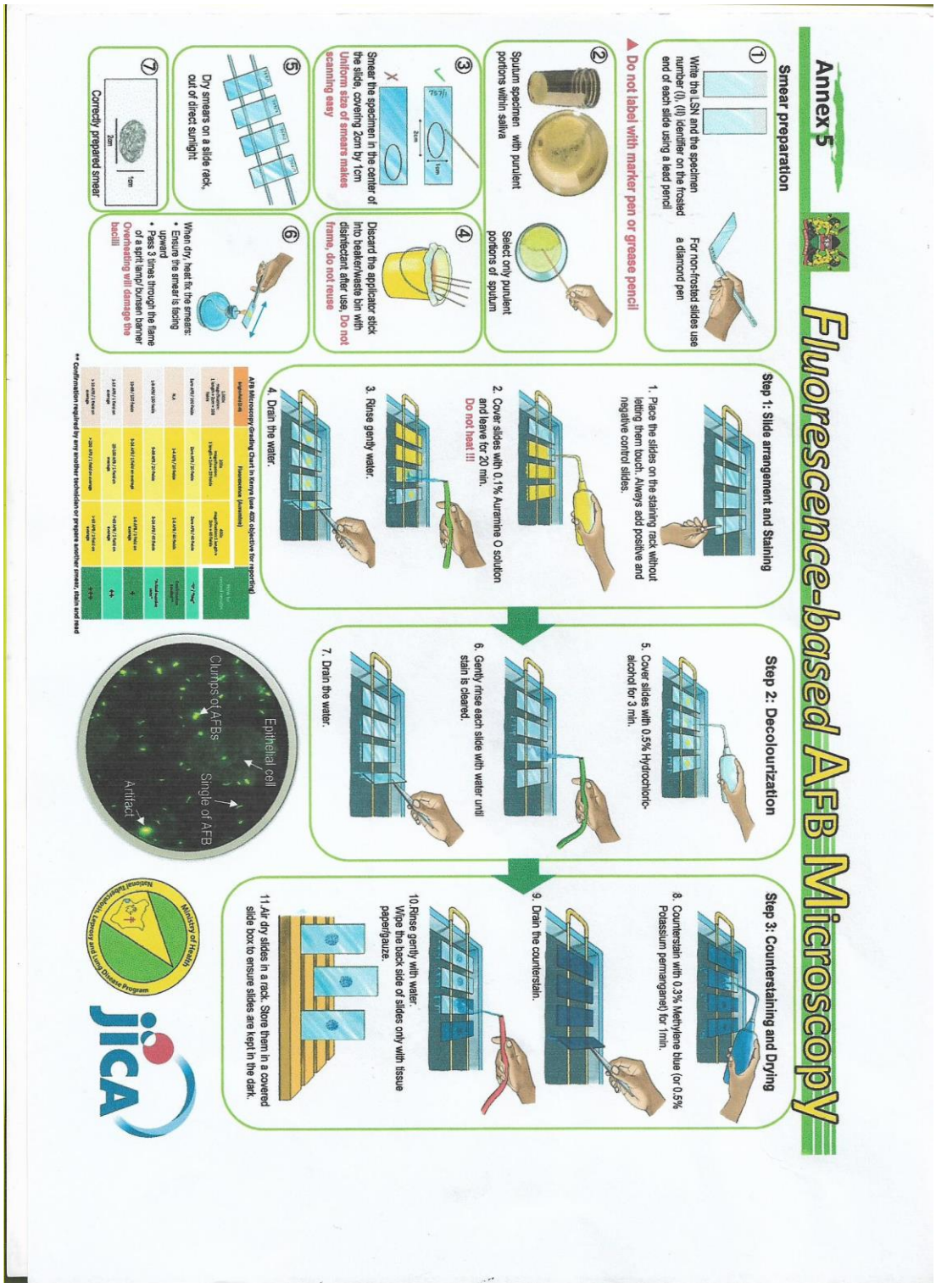
Method: *With acquisition of eight milliliters of blood, samples were analyzed for Prothrombin Time Test and Activated Partial Thromboplastin Time Test prior to initiation of anti TB medication and after initiation of treatment.*

Results: *showed Significant differences in prothrombin time in age groups 42-49, 55-65 and above 66 years with p-values of 0.021, 0.000, and 0.000 respectively at 95 % confidence level before and after treatment. Age group above 66 years old showed significantly lower Activated Partial Thromboplastin Time after anti TB administration as compared to when they were naïve (p=0.000).*

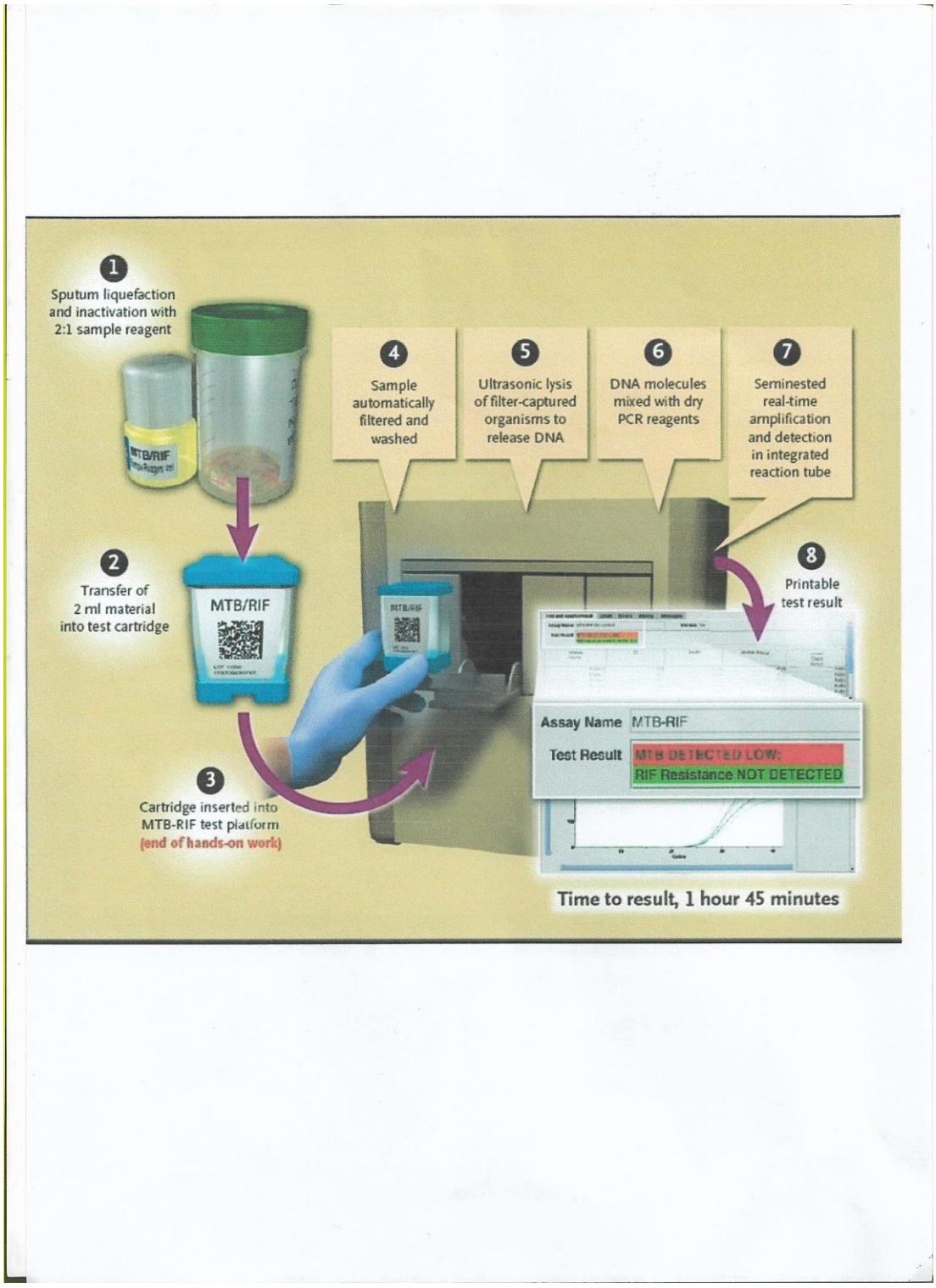
Conclusion: *The study concluded that significant hemostatic changes occur after anti TB medication and recommended. Health Care providers should be aware of the haemostatic Changes that occur in TB Patients initiating treatment and carry out these haemostatic tests and initiate treatment as soon asany problem is identified.*

Keywords: *Coagulopathy, Haemostatic changes, Prothrombin Time test, Activated Partial Thromboplastin time.*

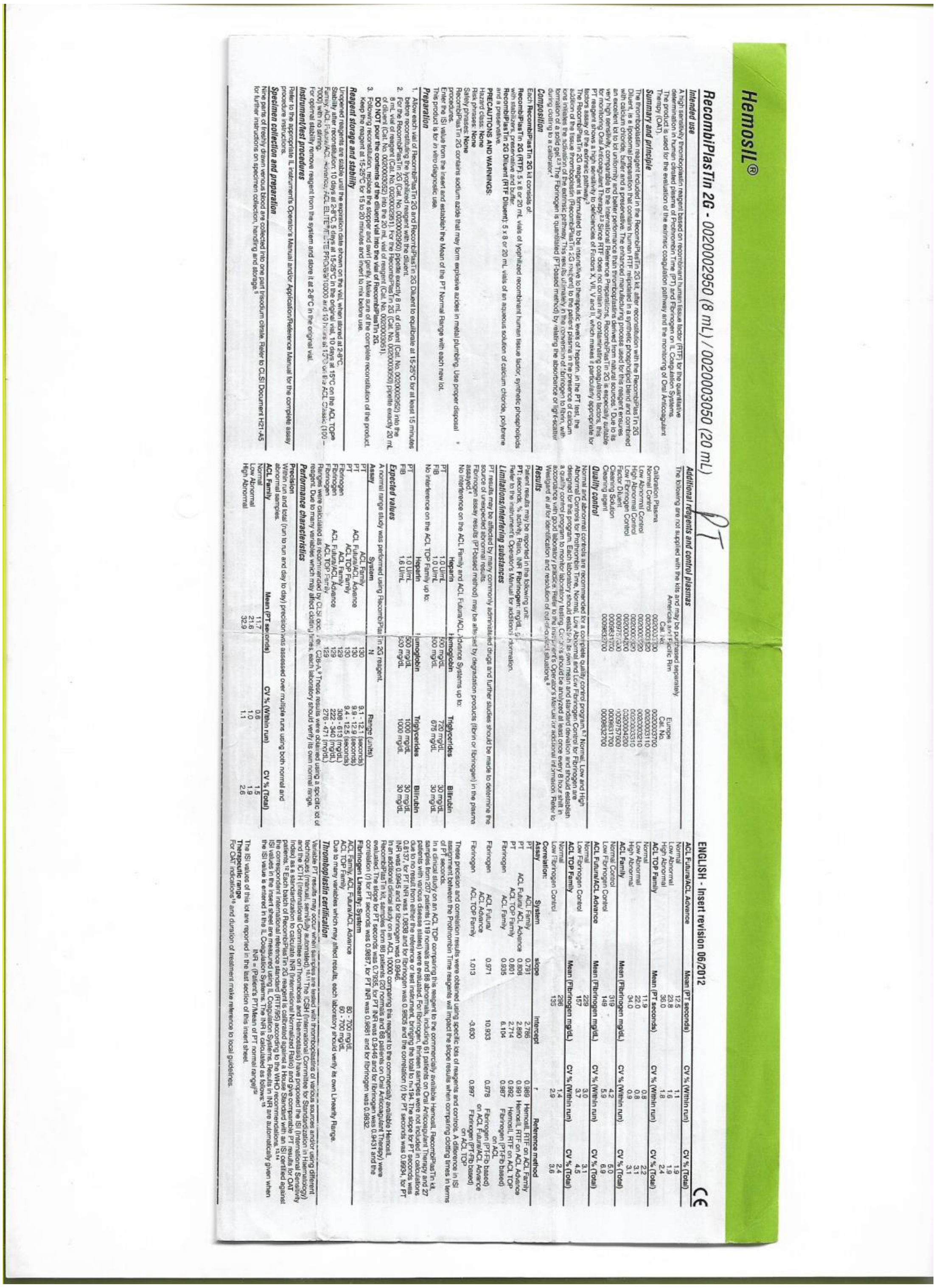
Appendix VIII: Job aid of Fluorescence Microscopy staining



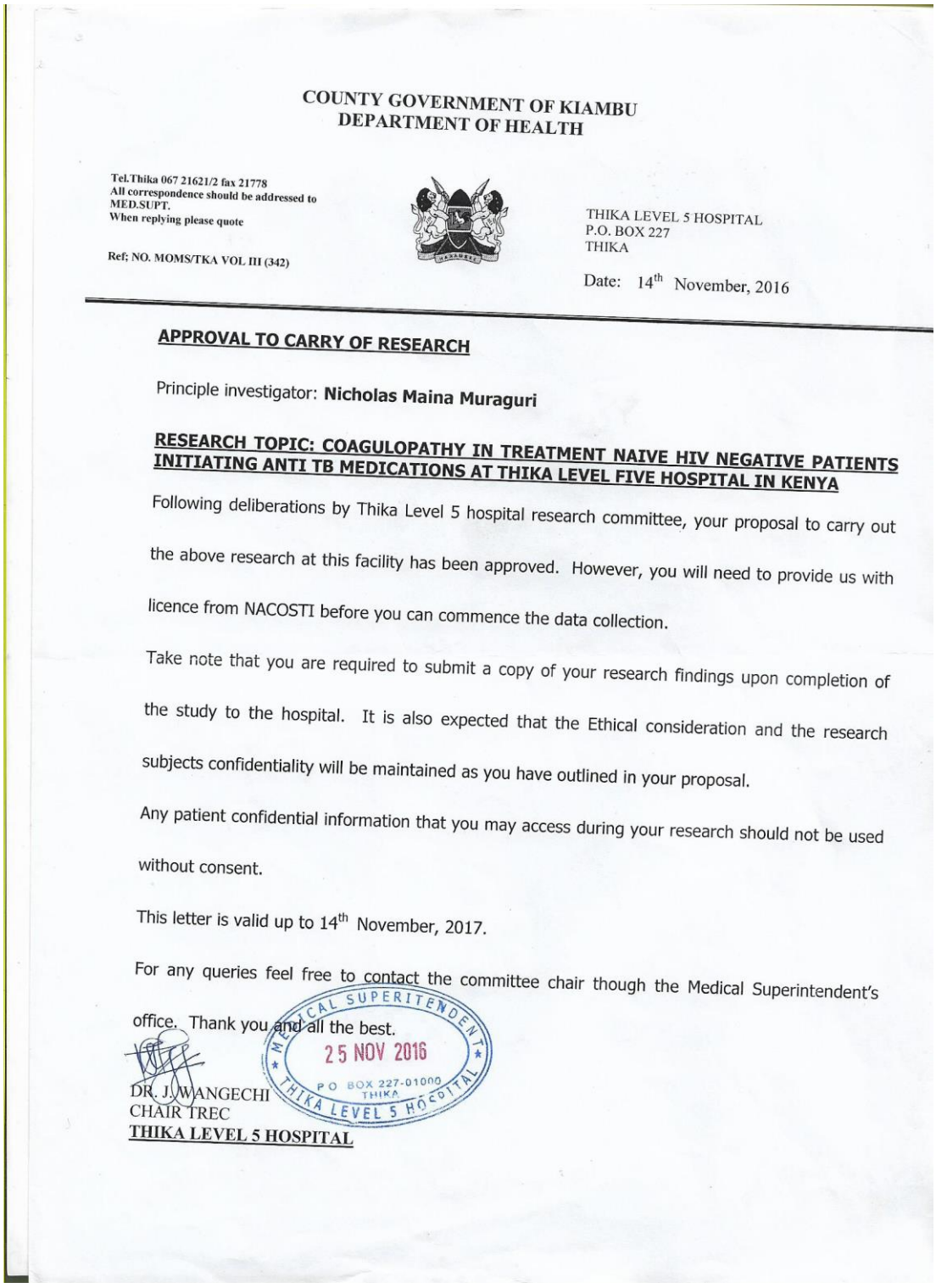
Appendix IX: Procedure for Gene Xpert test



Appendix X: Procedure for Prothrombin test



Appendix V: Thika Level 5 Hospital Approval



COUNTY GOVERNMENT OF KIAMBU
DEPARTMENT OF HEALTH

Tel.Thika 067 21621/2 fax 21778
All correspondence should be addressed to
MED.SUPT.
When replying please quote

Ref; NO. MOMS/TKA VOL III (342)

THIKA LEVEL 5 HOSPITAL
P.O. BOX 227
THIKA

Date: 14th November, 2016

APPROVAL TO CARRY OF RESEARCH

Principle investigator: **Nicholas Maina Muraguri**

RESEARCH TOPIC: COAGULOPATHY IN TREATMENT NAIVE HIV NEGATIVE PATIENTS INITIATING ANTI TB MEDICATIONS AT THIKA LEVEL FIVE HOSPITAL IN KENYA

Following deliberations by Thika Level 5 hospital research committee, your proposal to carry out the above research at this facility has been approved. However, you will need to provide us with licence from NACOSTI before you can commence the data collection.

Take note that you are required to submit a copy of your research findings upon completion of the study to the hospital. It is also expected that the Ethical consideration and the research subjects confidentiality will be maintained as you have outlined in your proposal.

Any patient confidential information that you may access during your research should not be used without consent.

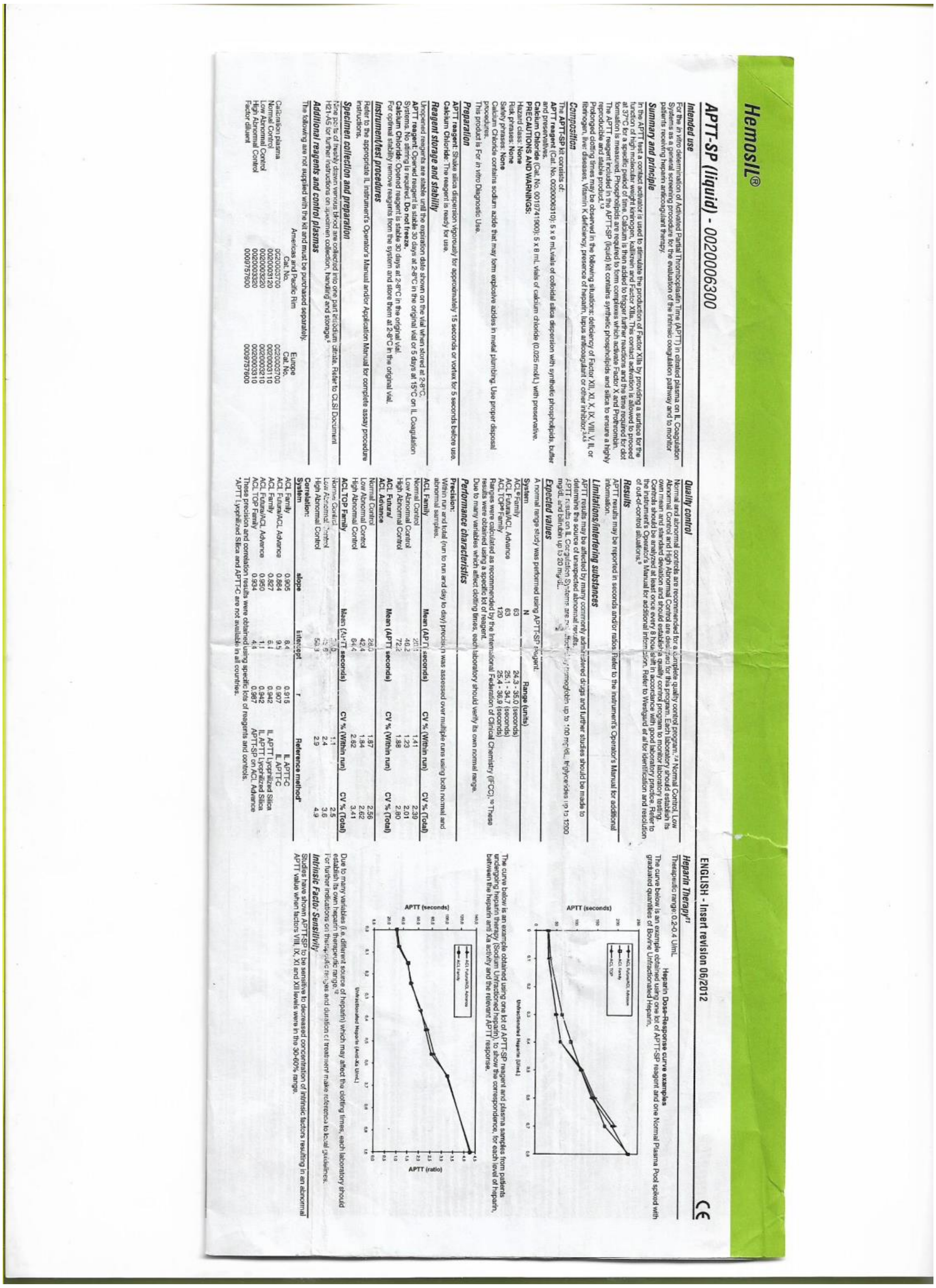
This letter is valid up to 14th November, 2017.

For any queries feel free to contact the committee chair though the Medical Superintendent's office. Thank you and all the best.

MEDICAL SUPERINTENDENT
25 NOV 2016
P O BOX 227-01000 THIKA
THIKA LEVEL 5 HOSPITAL

DR. J. WANGECHI
CHAIR TREC
THIKA LEVEL 5 HOSPITAL

Appendix XI: Procedure for Activated Partial Thromboplastin Time test



Appendix XII: Procedure for D-Dimer test