

**PREVALENCE OF VITAMIN D DEFICIENCY AMONG TB PATIENTS  
AT A KENYAN HEALTH FACILITY**

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**A DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE  
REQUIREMENT FOR A MASTER OF SCIENCE IN MEDICAL MICROBIOLOGY.**

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**DECLARATION**

**I declare that this proposal is my original work and has not, to the best of my knowledge been presented anywhere else for the award of Master of science degree in Medical Microbiology.**

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**H56/80668/2015**

**Signed : .....**

**Date: .....**

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This study is self – funded.

## **SUPERVISOR'S DECLARATION**

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Signed ..... Date : .....

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## ABBREVIATIONS AND ACRONYMS

<b>APC</b>	Antigen Presenting Cells
<b>CD</b>	Cluster of Differentiation
<b>CAMPs</b>	Cathedine antimicrobial peptides
<b>ELISA</b>	Enzyme linked immunoassay.
<b>ECLIA</b>	Electro chemiluminescent assay
<b>GOK</b>	Government of Kenya
<b>HIV</b>	Human Immunodeficiency Virus
<b>MOH</b>	Ministry of Health
<b>PAMPAs</b>	Pathogen Associated Molecular Patterns
<b>TB</b>	Tuberculosis
<b>TLRs</b>	Toll – like receptors
<b>UVB</b>	Ultraviolet B rays
<b>VDR</b>	Vitamin D receptor
<b>WHO</b>	The World Health Organization.

## ABSTRACT

**Background:** Tuberculosis (TB) is a chronic illness of global importance, caused by a bacterium known as *Mycobacterium tuberculosis*. Vitamin D is known to play a central role as an immune regulator especially in active TB infections. Hypovitaminosis D decreases macrophage activation and production of cathelicidin both of which are key active players in the host's immune response to TB infection. Although there are conflicting conclusions as to whether or not vitamin D supplementation is beneficial in the treatment of TB, this study was conducted to further provide evidence of its importance, as the need for research in new and improved treatment strategies continue.

**Hypothesis:** Vitamin D deficiency is associated with increased risk and progression of tuberculosis infection.

**Objective:** To determine the prevalence of vitamin D deficiency in newly diagnosed patients with active pulmonary tuberculosis.

**Methodology:** A comparative descriptive study was conducted by recruiting newly diagnosed treatment naive TB patients and a control group consisting of TB negatives individuals (blood donors) from Malindi Hospital in Kilifi County. Their serum vitamin D level were evaluated using a COBAS platform. Their weight/BMI, hemoglobin, marital status, education level and location of household were also collected and evaluated. The prevalence of vitamin D deficiency was determined in the newly diagnosed TB patients and the blood donors. Serum vitamin D levels of less than 20ng/dl was classified as severe deficiency while those less than 49ng/dl as deficient. Mean and median were used to measure central tendency while Wilkinson Mann – Whitney statistical test was used to measure association.

**Results:** A total of 170 participants were recruited, 80 (TB cases) and 90 (non-TB cases). Median age was 32(23-40) years and male to female ratio was 2:1. Overall serum vitamin D levels were significantly lower in the TB cases (P value = 0.002) compared to the non-TB cases, with a mean of 44.6ng/dl. Among the TB cases 87% recorded deficient levels of vitamin D while 11% were severely deficient. 97.5 % of the TB cases were anemic with a mean BMI of 20.7. 18% of the TB cases were rifampicin resistant according to GeneXpert results. Sociodemographic characteristics demonstrated 22.5 % unemployment rate with majority (70%) of the cases residing in the urban areas. Higher levels of vitamin D (median 36.1) were observed among TB cases who worked in the informal sector.

**Conclusion:** Vitamin D insufficiency was highly prevalent among the TB cases. Lower levels of vitamin D were recorded among the young men who resided in the urban centers. 18 % of the newly diagnosed treatment naive TB patients were Rifampicin resistance.

## 1.0 CHAPTER ONE: INTRODUCTION

Tuberculosis is a chronic infection caused by bacteria known as *mycobacterium tuberculosis*. It is one of the most important diseases in the world infecting one-third of the world's population (Dye & Borgdorff, 2008). Ever since its discovery in the early 19<sup>th</sup> century by Dr Koch it has continued being relevant despite modern advances in medicine (Dye & Borgdorff, 2008).

The World Health Organization ranks Tuberculosis among the top 10 high disease burdens of the world (WHO global tuberculosis report, 2018). This has led to its eradication being included as a global priority agenda, depicted in the sustainable development goals of 2030 and was adopted by the United Nations world health assembly in 2014. One of the global TB eradication strategies includes research and development in diagnostics, drug regimens and vaccines (Schmit, Zimy and Pratt, 2017). So far slow progress has been seen in the development of a vaccine, but more research is needed especially in the development of new drug strategies and regimes.

Vitamin D has recently been in the forefront of research in the last few years ever since the discovery of the role it plays as an immune modulator especially in respiratory bacterial infection (Ellner, 2017). Several studies worldwide have demonstrated the important role vitamin D plays in the innate immunity against Tuberculosis (Ernst, 2012). The daily maintenance dose of vitamin D varies by age, but most adults require 600 – 2000 IU of vitamin D daily. Higher doses are however recommended during vitamin D deficiency.

One billion people worldwide are estimated to be vitamin D deficient. The most common causes of vitamin D deficiency in adults include Inadequate Sunlight exposure and Vitamin D malabsorption problems e.g. cystic fibrosis, celiac sprue disease etc. (Belay, 2017). However, despite several studies on Vitamin D status levels worldwide, it has been difficult to establish any conclusion as to whether or not vitamin D supplementation is beneficial in the treatment of TB. This has been mainly because of multiple confounding factors in different populations and regions i.e. genetics, diet, exposure to sunlight and or skin color (Holick, 2016).

Recent data regarding vitamin D levels in TB patients in Kenya is not available. There is a need for these studies in Kenya, a country considered among the high TB burden countries of the world (WHO Global TB report, 2018). Some countries e.g. India have adopted vitamin D supplementation as part of their Tuberculosis treatment regimens (Hassanein, 2016). However, these countries have done several studies in order to support recommendation of vitamin D supplementation in their TB treatment regimes. (Kipruto, 2013).

This study recruited newly diagnosed treatment naive TB patients from Kilifi County; a county considered among the top 5 highest TB burden areas in Kenya (Kenya Tuberculosis prevalence survey, 2017) and compared their vitamin D levels to a control group comprising of healthy blood donors. Their sociodemographic and other clinical characteristics were also recorded. Statistical comparison was done to find the prevalence of vitamin D deficiency among the TB cases.

## **2.0 CHAPTER TWO: LITERATURE REVIEW**

### **2.1 Epidemiology**

Tuberculosis (TB) continues to be one of the major infectious diseases of public health concerns globally (United Nations Report on TB, 2018). It is caused by bacillus bacteria called *mycobacterium tuberculosis*. The transmission of the bacteria is through infectious aerosolized droplets making it easy to spread from person to person (Korb, 2016). Globally the TB epidemic is larger than previously estimated. In 2018 there were an estimated 10.4 million new (incident) TB cases worldwide of which 5.9 million (56%) were among men, 3.5million (34%) among women and 1 million (10%) among children (WHO, 2018). People living with HIV accounted for 0.3million of all new TB cases. In 2018 world health organization estimated 1 million TB deaths worldwide. This translated to over 2740 deaths every day. Sub-Saharan Africa accounts for more than one in four of the globally TB reported cases annually. This translates to 2.6 million cases annually which results to 41% of the total global TB deaths (WHO Global TB report, 2018).

Kenya is an East African country with an estimated population of 52 million people. It is considered among the 30 high TB burden countries of the world (Schmit, 2018). WHO TB report-2018 indicated more TB cases in Kenya than previously estimated with a prevalence rate of 558 per 100,000 people. N/B 83% of these reported cases were HIV negative. TB is considered the fourth leading cause of death in Kenya with a mortality rate of 7.2 per 100,000 with the majority of the deaths occurring between the ages of 15years to 55years.

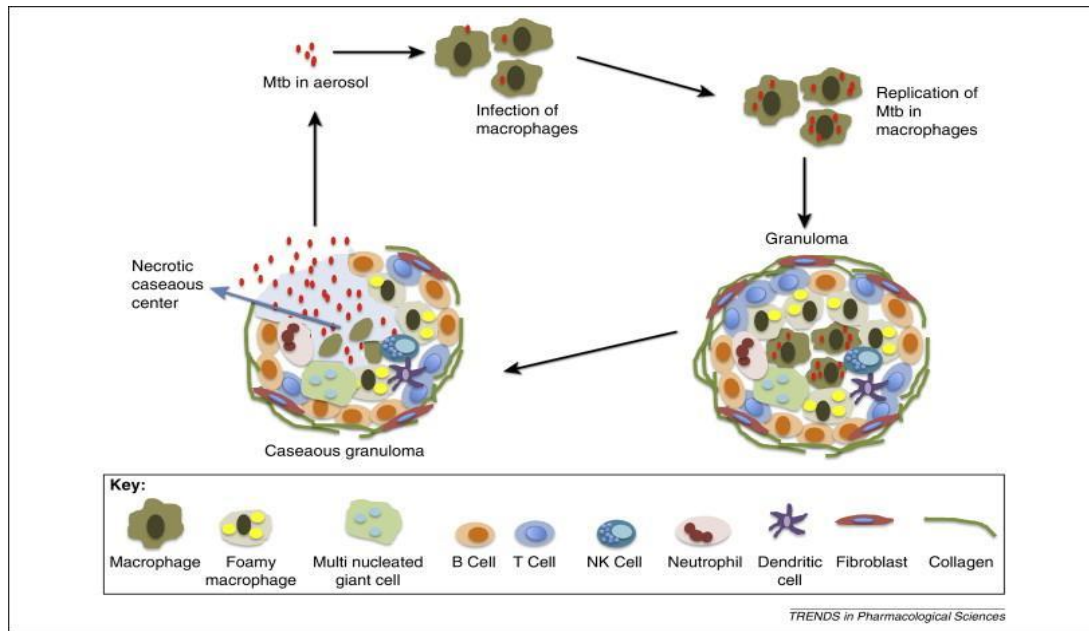
The major factor responsible for the large TB disease burden in Kenya is the concurrent HIV epidemic (Oketch, 2018). Other factors include poverty and social deprivation which in turn brings about malnutrition and overcrowding in the form of slums such as the ‘famous’ Kibera slum in Nairobi city.

## 2.2 TB Pathogenesis and Immunology

*Mycobacterium tuberculosis* (Mtb) is an intracellular pathogen, belonging to a group of genetically closely related variants called Mycobacterium tuberculosis complex. All cause a chronic granulomatous disease affecting humans and many other mammals e.g. *M. bovis* in cattle, *M. caprae* in goats, *M. pinnipedii* in seals (Martineau, 2016). *Mycobacterium tuberculosis* bacteria are non-motile, non-sporing, non-capsulated, straight or slightly curved rods that are about  $3 \times 0.3\mu\text{m}$  in size. They are obligate aerobes hence their predilection for lung tissue rich in oxygen. The tubercle bacillus owes its virulence to its ability to survive within macrophages. The immune response to the bacilli is of a cell mediated type and tissue destruction caused by a secondary hypersensitivity reaction is the hallmark of disease progression (Owolabi, 2016).

The site of primary infection is usually the lung tissue following inhalation of the bacilli. The bacilli are engulfed by alveolar macrophages in which they replicate to form the initial lesion. Some are carried to the hilar lymph nodes together forming the primary complex known as the Ghon focus (Talat, 2011).

During infection the mycobacterium tuberculin antigen are processed by antigen-presenting cells activated by bacterial components and presented to antigen – specific T lymphocytes which undergo proliferation (Jo, 2011). The activated T cells release cytokines i.e. interferon- $\gamma$  which together with calcitriol activate macrophages and cause them to form a compact cluster or granuloma around the foci of infection. Activated human macrophages inhibit the replication of the tubercle bacilli but their ability to kill the bacilli is limited. Programmed cell death or apoptosis of the bacteria – laden macrophages by cytotoxic T cells and Natural Killer cells contribute to the individuals' protective immunity by generating a metabolic burst that kills tubercle bacilli (Lodge, 2017). Below is a summary diagram showing the different cells involved during the host immunological response following an acute TB infection.



**Figure 2.1:** Diagram showing TB pathogenesis and host immunological response. (TRENDS in pharmacological science, 2008)

In most infected individuals the primary infection resolves, but in a minority of cases one of the infective foci may progress and give rise to serious manifestations. If the focus ruptures into a blood vessel, bacilli are disseminated throughout the body giving rise to the formation of numerous granulomata clinically known as miliary tuberculosis. Clinically acute pulmonary TB case definition according to MOH include persistent cough for more than 2 weeks, fever, night sweats and weight loss. However, HIV co infection may complicate the symptoms because of its immunosuppressive effect. Currently the Kenyan treatment strategy for acute pulmonary TB involves both preventive measures in the form of vaccines, Public education and curative measures in the form of drugs. Current drug regime used in Kenya include 2 months of Rifampicin(R),Isoniazid(H),Pyrazinamide(Z) and Ethambutol (E) followed by 4months of Rifampicin(R) and Isoniazid(H). i.e. 2RHZE/4RH

Multi drug resistant TB (MDR-TB) is a form of TB that is resistant to two of the above first line drugs. This has proven to be a big challenge especially in the developing world. This is because treatment options are limited and often very expensive. A more severe form is called extensively drug resistant TB (XDR-TB) which is a nightmare for clinicians to manage even in the best medical facilities. Currently Kenya has about 3000 MDR / XDR TB patients

(Oketch, 2018).

### **2.3 Vitamin D and TB infection**

Vitamin D (25-hydroxycholecalciferol) is a secosteroid hormone (Jo E K 2011). It can be obtained from two sources;( diet source and sunlight ).The two diet sources are vitamin D3 (cholecalciferol) which is found in foods of animal origin e.g. cod –liver oil , butter, cream and egg yolk and vitamin D2 (ergocalciferol) derived from plants (fungi/yeast). Secondly it can be obtained through exposure to sunlight as UVB rays absorbed through the epidermal layer of the skin as pre-vitamin D3. Both vitamin D3, obtained from pre-vitamin D3 in the skin and/or by intestinal absorption of dietary components, bind to vitamin D binding protein (DBP) in circulation and is transported to the liver. Here vitamin D3 is hydroxylated by liver 25-hydroxylase (CYP2R1). The resulting 25(OH) D3 is then hydroxylated in the kidney by 1- $\alpha$ -hydroxylase (CYP27B1), generating the bioactive hormone of vitamin D 1, 25(OH)<sub>2</sub> D3 ( calcitriol).(Bikel, 2017 )

25(OH) D3 is the major circulating form of vitamin D. It has a half-life of about 2/3 weeks (17 days) hence a good indicator of the level of vitamin D in the body. It is thus considered the primary indicator of vitamin D status in clinical practice.

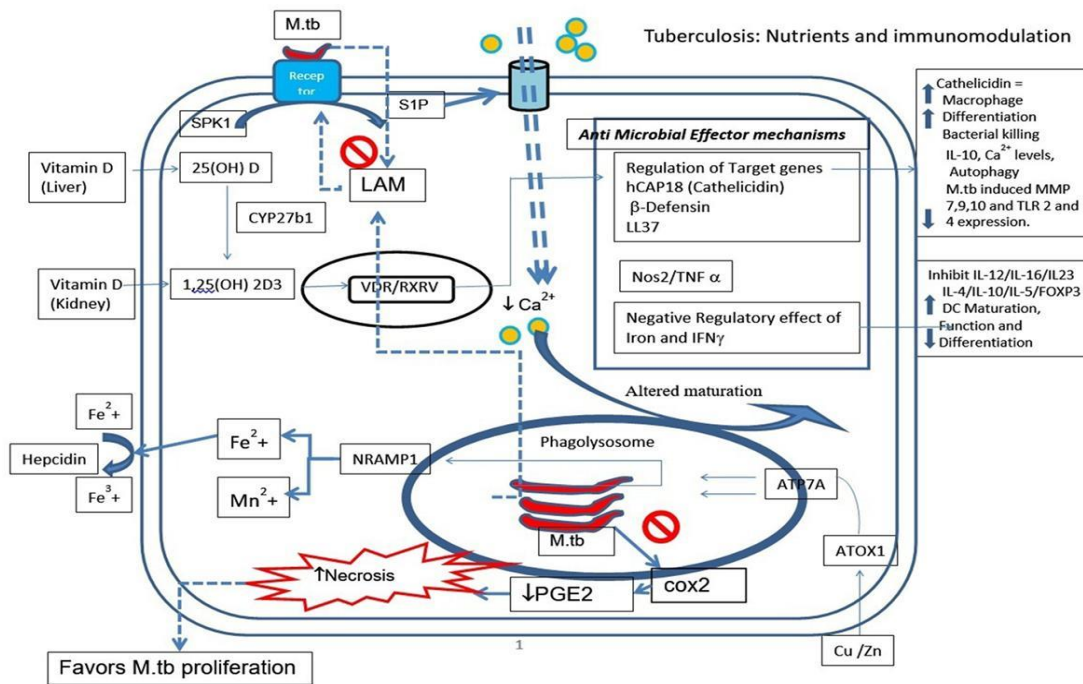
In healthy individuals, 25(OH) D3 is present in serum at concentrations in the range of 30-50 ng/mL. Individuals with 25(OH) D3 serum levels < 30 ng/mL are considered to have vitamin D insufficiency while levels < 20 ng/mL are proposed to define vitamin D deficiency.(Iftikhar, 2013).

Vitamin D as an immune modulator was triggered by the discovery of vitamin D receptors (VDRs) in almost all immune cells including activated CD4 and CD8 T cells, neutrophils and antigen presenting cells (APC). Importantly, VDR expression in some immune cells was demonstrated experimentally to be controlled by immune signals i.e. naïve Tcells displayed very low levels of VDRs compared to activated T cells, making them more sensitive to calcitriol. The ability of calcitriol to restrict the growth of virulent *mycobacteria tuberculosis* in humans was first demonstrated by (Crowle et al).



Monocytes and macrophages are crucial members of the innate immunity, which demonstrate a great ability to sense pathogen – associated molecular patterns (PAMPs) of various infectious agents by means of pattern recognition receptors e.g. Toll- like receptors (TLRs) (Gong, 2018). This provides the first line defense mechanisms against dangerous microbial invaders. Calcitriol has been shown to modulate the innate immunity by enhancing the chemo tactic and phagocytic responses of macrophages as well as production of an antimicrobial protein known as cathelicidin. It has also been demonstrated to exert pro differentiation on monocytes and monocyte derived cell lines as these cells acquire phenotypic features of macrophages when exposed to the hormone 1, 25(OH)<sub>2</sub> D<sub>3</sub>.(22). Important to note is that, TLRs activation of monocytes and macrophages results in up regulation of cathelicidin antimicrobial peptide (CAMPs) and killing of *mycobacterium tuberculosis*, through induction of autophagy within auto phagolysosomes.(Chung, 2018 ).

This hormone also inhibits TLR2 and TLR4 expression on monocytes resulting in a hypo responsiveness state to PAMPs. This inhibits excessive TLRs activation which subsequently prevents excessive inflammation. This step limits further progression of the infection through minimizing lung tissue damage (.Hunter, 2018) Tuberculosis disease progression relies on the extent of tissue damage. Vitamin D immunomodulatory mechanisms ensure the reduction of disease associated inflammation at least in the acute phases of the disease. This subsequently accelerates clinical improvement and eventual healing.



**Figure 2.2:** Summary of Immunomodulatory function of Vitamin D in TB infection (Nature Reviews immunology 2011)

## 2.4 Justification

Tuberculosis remains a major health problem in Kenya. Latest TB survey carried out between 2017 – 2018 reported a prevalence of 558 per 100,000 adult population. (Oketch, 2018) This figure translated to a mortality rate of 20 per 100,000 according to a WHO report of the same year. 83 % of the TB cases were HIV negative (WHO Global TB report, 2018). This suggests that interventions to control TB among people living with HIV has been successful and new strategies need to be developed to combat the large burden of TB existing among people not infected with HIV.

Despite WHO approach to treatment, combined with DOTs (Direct observational therapy) successful outcomes from the National Tuberculosis, Leprosy and Lung Disease Program (NTLD-program) has remained at 87% for the last 5 years. Factors attributed to this figure include both biological e.g. anemia, malnutrition and socioeconomic e.g. poverty, level of education, substance abuse just to mention a few. However more extensive studies are needed to provide concrete scientific evidence as to why these figures have remained constant despite numerous existing programs (Kenya TB survey, 2017)

High dose vitamin D was used widely during the pre-antibiotic era for the treatment of TB. Patients reported improved appetite and general wellbeing after taking fish oils. Despite this history, several studies have shown conflicting clinical outcomes when the standard anti-tuberculosis treatment was augmented with oral vitamin D (Daley, 2016). However, a study in Tanzania (Sudfeld, 2017) reported that low baseline serum vitamin D concentration in the black African population were associated with poor clinical outcomes.

Furthermore, some components of the anti-tuberculosis treatment used may pharmacologically lower serum concentration of useful vitamin D metabolites e.g. Rifampicin (Naik, Rajan and Manjrekar, 2017). It is therefore possible that low baseline vitamin D levels may further be compromised by drug therapy especially in the dark skinned African population.

However, there are other studies that support the use of vitamin D as an adjunctive therapy during Tuberculosis treatment. One study in particular from India reported that when TB patients with very low baseline vitamin D levels were given high doses of the same during treatment, their treatment outcomes was reduced from 6 months to 4 months. This could significantly improve patient compliance and in the long run save on treatment costs.

Documented studies for Kenya with regards to this matter are scarce. This study hopes to provide baseline data associating hypovitaminosis D as a risk factor in the progression of TB. This data may support and advocate for host directed adjuvant therapies, which translate to shorter TB treatment regimes in the future.

## **2.5 Research Questions**

Is vitamin D deficiency associated with increased risk of active pulmonary TB?

## **2.6 Null Hypothesis**

Vitamin D deficiency is not associated with an increased risk of active pulmonary TB

## **2.7 Objectives**

### **2.7.1 General objective**

To determine the prevalence of vitamin D deficiency in newly diagnosed TB patients.

### **2.7.2 Specific objectives**

- a) To compare serum vitamin D levels in newly infected TB patients and healthy TB negative individuals
- b) To compare vitamin D deficiency levels between female and male newly infected TB patients.

### 3.0 CHAPTER THREE: METHODOLOGY

#### 3.1 Study Site

The study was conducted in Kilifi County at The Malindi County Referral Hospital located along Casuarina road in Malindi Township (Latitude: -3.2165 Longitude: 40.1182). This site was selected due to its large catchment area with an active TB clinic and well-equipped laboratory services. The population of the area is estimated at 207,253 (KNBS, 2009) mostly of African descent.

#### 3.2 Study Design

This is a comparative descriptive study. Participants were recruited consecutively from the TB clinic after consenting. The clinic ran from Monday to Friday from 8am to 2pm. This was done daily between the months of November to March 2019 the hottest months with the maximum sunshine exposure in the year until the desired sample size was attained. The control group was recruited from the blood donors visiting the blood bank facilitated by the Kenya Red Cross Society Malindi Branch as part of an ongoing country wide blood donation drive. The serum samples were collected once the blood was screened.

#### 3.3 Sample Size Determination

Sample size calculation was deduced using the below formula (Naing, Winn and Rusli, 2006). This being a preliminary study resource and time constraints were factors that affected the determinations of the absolute error (d). Expected proportion value (p) was estimated using systematic review paper on Vitamin D levels estimation (Martineau et al., 2016)

Sample size was calculated using the formula i.e.

$$\begin{aligned} \text{Sample size} &\equiv \frac{Z^2 \times p(1-p)}{d^2} \\ &= \frac{1.96^2 \times 0.85(1-0.85)}{0.05^2} \\ &= 196 \end{aligned}$$

Where Z -: standard normal variant (1.96)

p -: expected proportion in population as per previous studies (85%)

d -: absolute error /precision (5%)

### **3.4 Inclusion criteria for TB patients**

- Age : 18– 60 years consented individuals
- Newly diagnosed TB patients (positive on GeneXpert)
- Seronegative

#### **3.4.1 Inclusion criteria for TB Negative Healthy individuals/ Blood Donors**

- Blood donors

### **3.5 Exclusion criteria for TB patients**

- Co-existing malignancy/ metabolic syndrome
- Patients receiving corticosteroids, vitamin D supplementation, immunomodulators
- Patients with liver disease and renal failure
- Comorbidities especially Diabetes mellitus/ HIV infection.
- pregnancy

#### **3.5.1 Exclusion criteria for TB Negative Healthy individuals/ Blood donors**

- Acute bacterial infection
- Low iron

### **3.6 Laboratory Methods**

All study participants were accepted into the study following Ministry of Health/GOK guidelines for TB case definition i.e. positive GeneXpert. Informed consent was obtained from all the study participants. All blood samples were transported to the Malindi county referral

hospital laboratory using a cool box. All samples were processed within 8 hours of collection.

### **3.6.1 Specimen Collection and Transportation.**

Blood samples were collected in conformity with good laboratory practices following diagnosis and before commencement of anti-tuberculosis drugs. This was done by the principal investigator (PI). However, in the unlikely event that the PI was absent a qualified nurse assisted. Ten milliliters of venous blood were drawn and transferred to a standard sampling tubes without any anticoagulants (red top vacutainer).

The samples were subjected to centrifugation at 3000 revolutions per minute for 15 minutes at room temperature for serum separation. All serum samples were later stored in a freezer at -80 degrees Celsius awaiting vitamin D assay. Blood donor samples were collected during blood donation drives after consent.

Blood samples were carefully transferred into a cooler box. Triple packaging was employed using cotton wool, gauzes and cardboard. This ensured that the ice pack temperature was maintained throughout the journey. The cooler box was well labelled as infectious material and transported to the Kenyatta National Hospital biochemistry laboratory.

### **3.6.2 Vitamin D assay**

Serum vitamin D levels was done using an automated analyzer COBAS 601 platform of the cobas 6000 series from Roche diagnostics which is an electro-chemiluminescent assay (ECLIA)

Reagents for the assay were analyte specific calset (vit D) ready to use and predisposed in sealed reagent strips. The serum samples were mixed with the strips and placed in a machine. All of the assay steps are automatically done by the machine.

Final assay results were automatically calculated by the instrument after about 40 minutes, in accordance to the manufacturer's calibration curve then printed out. The results were expressed

in ng/mL.

### **3.6.3 Quality Assurance**

#### Pre-analytical Errors

1. Quality assurance was ensured by collecting the sample according to the laid down standard operating procedure in the correct vacutainers
2. The sample were transported in a cooler box.
3. Temperature of the refrigerators where samples and reagents were stored was checked twice daily during the week

#### Analytical Errors

1. The manufacturers laid down standard operating procedures were used to run all the tests.
2. Vitamin D levels were interpreted based on the manufacturers insert after reference ranges were established.
3. Quality Control was assured by running single samples of level one and two controls done for each set of assays.
4. Controls were run when a new bottle of reagent was used.
5. If a control was out of its specified range, the associated test result was considered invalid and the sample was retested.
6. Calibration of the machines was done according to the manufacturer's' pamphlet procedures.

#### Post Analytical Errors

Care was taken to avoid post transcriptional errors while transferring results from the assigned laboratory numbers to the data entry form.

### **3.6.4 Vitamin D Reference range**

Reference values of a particular analyte is usually related to a specific population. However, vitamin D<sub>3</sub> production in the skin is related to sun exposure. So, this source is highly variable depending on sun exposure and skin pigmentation. It is therefore impossible to establish a standard reference range for each case population.



For this reason, it is recommended that each lab establish its own expected values for the population it serves. However, this study adopted the WHO reference guidelines for vitamin D deficiency while interpreting the results.

**Table 3.1: WHO Reference guideline for Vitamin D deficiency**

<b>25 (OH) D in ng/dl</b>	<b>Interpretation</b>
< 20 ng/dl	Severe Deficiency
20 – 49 ng/dl	Deficiency
> 50 ng /dl	Optimum

### **3.7 Data Management**

#### **3.7.1 Data Collection**

Final assay was collected and recorded in a structured excel sheet, together with all their respective sociodemographic information, from the questioner that was provided to each study participant. This was later presented in tables and a box graph.

#### **3.7.2 Data Analysis**

Data was cleaned to get rid of inconsistencies i.e. missing values, duplication and out of range values. This data was analyzed using STATA version 15.1.

Uni variant data was presented in percentages, while median and mean were used as measures of central tendency. Wilcoxon Mann Whitney test was used as measures of association. All statistical tests were performed at 5% level of significance.

#### **3.7.3 Data Confidentiality and Storage**

Study identity numbers were used for each specimen for anonymity. Filled consent forms were kept under lock and key after the study. The data was stored and backed up in electronic

computer devices with only access to the researcher by use of a password. Data collected will be kept for a minimum period of 5 years.

### **3.8 Ethical Consideration**

#### **3.8.1 Informed Consent**

Informed consent was sought from all participants voluntarily once the study was approved by the Kenyatta National Hospital/ University of Nairobi Ethical and Research Committee (KNH/UON-ERC) – study ref no KNH-ERC/A/266 and the Kilifi county ethical approval committee ( see annex)

Every participant was required to read through the consent form either in English or Kiswahili. For those unable to read, this was done for them by the principal investigator. Afterwards all the benefits and potential harms of the study were carefully highlighted for emphasis. All participants were required to sign a consent form before being enrolled. This was witnessed by the study principle investigator.

### **3.8.2 Laboratory Approval**

Permission was sought from the head of Kenyatta National hospital Biochemistry laboratory – study ref KNH/DLM/60 as the sites for specimen processing and analysis. Once the approval certificate was issued the samples were presented to the laboratory for processing.

## 4.0 CHAPTER FOUR: RESULTS

**Table 4.1: Sociodemographic and clinical characteristics of study population**

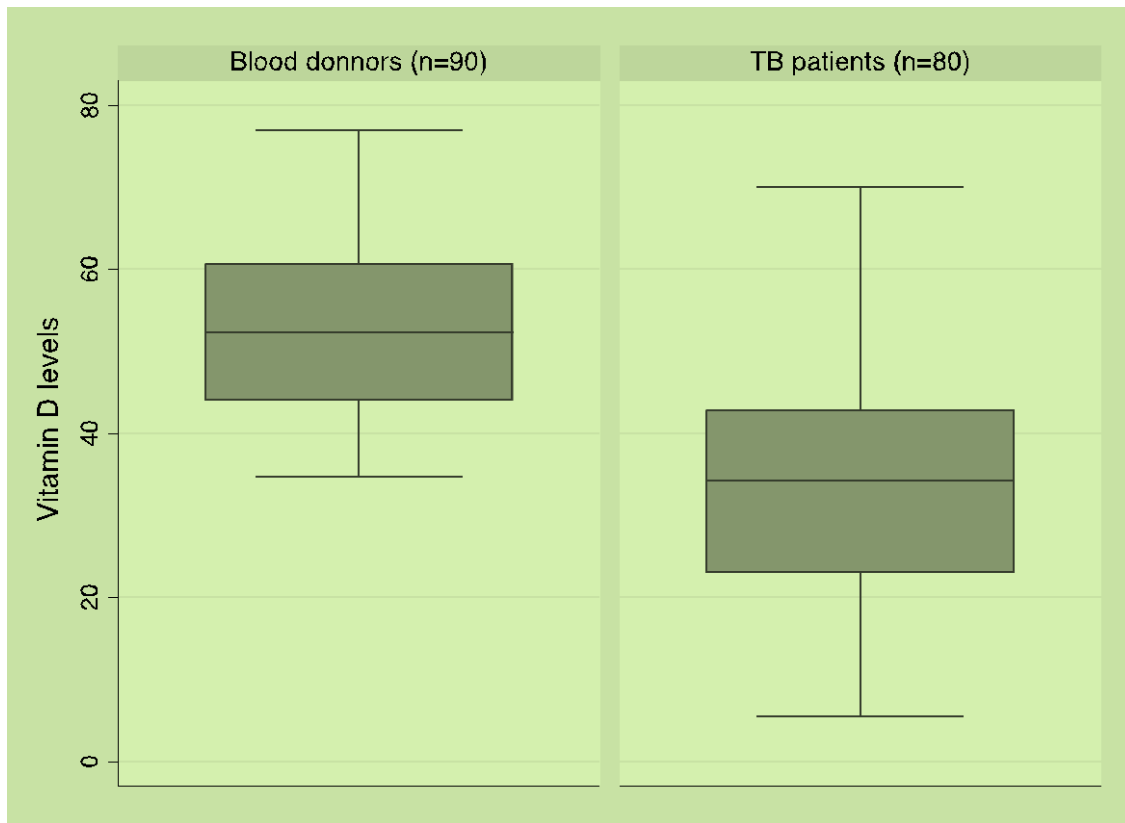
Variables	n	n%	Median (p25 - p75)	Mean (SD)
Age	170		32 (23 - 40)	
18 – 35		62.3		
>35 – 49		26.5		
>=50		11.2		
Sex	170			
Male		67		
Female		33		
BMI	80			20.7 (4.7)
< 18.5		36.3		
18.5 - < 25		51.3		
>= 25		12.4		
Hemoglobin (g/dl)	80			9.7 (1.8)
Anaemia*		77.5		
Non anemic		22.5		
Education	80			
Primary		7.5		
Secondary		27.5		
Tertiary		28.8		
Others		13.7		
Non		22.5		
Marital Status	79			
Single		19.0		
Married		70.9		
Single		10.1		
Other				
Residence	80			
Rural		30		
Urban		70		
Occupation	80			
white Collar		23.8		
Blue Collar		42.5		
Pink Collar		12.5		
None		21.2		
vitamin D levels (ng/dl)	170			44.1 (16)
< 20		8.2		
20 – 49		57.1		
> 50		34.7		
* Anaemia = < 11 in male, < 10 in females.				

Table 4.1 demonstrates the sociodemographic and clinical characteristics of the study population. There was a total of 170 participants comprising of Tb infected cases (80) and blood donors (90). More than half of the population belonged to the youth group with a median age of 32 years. There were more than twice the number of men than females especially contributed by the blood donor group. One third of the TB infected cases were underweight with a BMI of less than 18.5 while interestingly enough 12.4% of them were either overweight or obese. It was alarming to note that more than 77% of the TB patients were anemic with least recorded HB being 6.0g/dl. A quarter of the cases were uneducated, majority (42.5 %) worked in the informal sector .Seventy percent of the Tb patients resided in the urban areas of Malindi. Three quarters of them were married, with a 10 % divorce rate among them. Vitamin D deficiency was at 8.2 %, while more than half (57.1 %) recorded insufficiency levels. 34.7 % of the total study population had sufficient vitamin D levels. Overall the mean vitamin D levels in the total study population was 44.1g/dl.

**Table 4.2: Comparison of vitamin D levels according to Age and Sex among TB patients and Blood donors**

<b>Variables</b>	<b>TB cases(n=80)</b>		<b>Blood Donors (n=90)</b>	
	<b>median (p2 -p75)</b>	<b>p value</b>	<b>median(p25-p75)</b>	<b>p value</b>
Age		0.871		0.4427
18 – 35	35.57 (3.19-42.76)		50.45 (43.94 -59.09)	
>35 – 49	30.9 (22.27-42.41)		55.43 (46.87 - 65.95)	
>= 50	30.39 (24.05-43.25)		56.03 (44.09 - 67.95)	
Sex		0.665		0.116
male	32.27 (20.59 - 43)		51.42 (44.54-60.73)	
female	35.57 (23.37- 42.41)		53.81 (42.9-62.98)	

Table 4.2 compares vitamin D levels in TB cases with healthy blood donors. Vitamin D levels in TB cases decrease with increase in age however male TB patients recorded slightly lower vitamin D levels than females. In healthy donors there was an increase of vitamin D levels as the ages advanced, however female healthy donors recorded higher levels of vitamin D compared to their male counter parts. Generally, both age and sex did not demonstrate any statistical significance in both TB cases and healthy blood donors with regards to vitamin D levels.



**Figure 4.1:** Graph showing comparison of vitamin D levels among TB cases and Blood donors.

Figure 4.1 shows lower levels of vitamin D in TB cases (median 37) compared to healthy blood donors (median 53). More than half of the newly diagnosed TB cases recorded insufficient levels of vitamin D, while about 11% recorded deficiency levels of vitamin D.

**Table 4.3: Comparison of vitamin D levels in TB cases with other sociodemographic and clinical variables**

Variables	n	median (p25 - p75)	p value
BMI	80		0.679
< 18.5		36.67(25.35-44.11)	
18.5-<25		32.87(22.27-42.41)	
>= 25		33.55(22.27-44.78)	
Hemoglobin g/dl	80		
Anaemia*		35.17(34.67-35.66)	
Normal		33.3(22.79-42.88)	
Education	80		0.2621
Primary		23.16(20.59-25.35)	
Secondary		33.95(23.19-39.02)	
Tertiary		34.67(16.75-39.32)	
others		39.02(29.18-44.78)	
None		33.9(23.37-45.1)	
Marital Status	79		0.6393
Single		36.03(23.19-42.76)	
Married		33.3(23.65-43.24)	
Divorced		27.46(21.63-36.3)	
Residence	80		0.979
Rural		31.15(24.00-42.88)	
Urban		35.23(22.48-43.26)	
Occupation	80		0.6963
white collar		29.63(12.92-55.8)	
Blue Collar		36.19(22.27-43)	
Pink Collar		32.27(22.17-35.71)	
None		36.03(27.82-41.56)	

Table 4.3. Compares vitamin D status with other variables apart from age and sex. These were all gathered from the questioner given to all the TB cases. Neither of the variables demonstrated any p values that were statistically significant. Higher levels of vitamin D were recorded among TB patients who were underweight. There were also higher levels of vitamin D among the anemic TB cases ( median 37.17).Lower levels of vitamin D was noted among the divorced TB patients compared to the single and married ones. However, it was interesting to note the slightly higher levels of vitamin D levels in TB patients who worked in the informal sector compared to the ones who were in the formal sector. Urban patients had slightly higher levels of vitamin D compared to the TB patients from the rural area. Majority of the TB cases recorded insufficient levels of vitamin D.



## 5.0 CHAPTER FIVE: DISCUSSION.

In this study we aimed to establish whether hypovitaminosis D was a risk factor in the acquisition of acute pulmonary TB and also whether sex of the patient played a role as well. We presented 80 newly diagnosed treatment naïve TB patients and tested for serum vitamin D levels. Overall serum vitamin D levels were below the normal levels in 89 % of the TB cases. 78% recorded deficient vitamin D levels while 11% of them had severely deficient vitamin D levels of below 20ng/dl. 18 % of the TB cases diagnosed were rifampicin resistant according to gene expert results. These figures slightly correlate to a similar study published by (Bengele, 2018) in the DRC that recorded 95% insufficient and deficient vitamin D levels in their TB patients.

However, there was no difference in the median vitamin D levels between male (median 32.27) and female (35.57) TB patients. When we focused on the age of the TB cases, a socioeconomical stratification was used to categorize age into 3 groups; youth, adult and old age. A trend was observed in that the median vitamin D levels significantly dropped between the ages of 18 years to about 40 years old but then slightly differed after the ages of 49 years and above. These results were compared to a study that was done in the US between the years of 2011 and 2014 by (Herrick, 2015 ) looking at whether age and sex played a role in the average vitamin D levels. Although a similar picture was seen when it came to gender in that no difference was observed, it differed in the age trends in that the older cases observed a higher level of vitamin D level in the US study compared to this study. This can be explained in that in the US, senior citizens are mostly cared for in homes where their nutritional wellbeing are carefully monitored whereas in Kenya majority of our senior citizens retire to our village homes with very little or no nutritional care accorded to them at all.

Body mass index results didn't seem to influence the vitamin D levels in the TB cases studied. There was no trend that was observed in the results. Higher levels of vitamin D (median 36.67) were observed in the underweight category compared to the obese group. Loss of weight being one of the cardinal signs of TB infections these results were somehow conflicting. Since this

was a cross sectional study it was difficult to ascertain whether the individual BMI levels recorded at the time the TB cases presented at the clinic had changed or remained constant before and after the acquisition of the TB infection. Similar results were observed in several other studies trying to establish causality between BMI and vitamin D levels. These results were not conclusive and rather conflicting. However these results conflicted with similar studies closer to home particularly one done in Ethiopia by (Workineh, 2017) which showed that lower BMI was associated with vitamin D deficiency, however as eluded earlier one isn't sure whether the BMI levels had changed over time before the onset of the disease, since most of the studies were cross sectional studies.

Severe anemia was reported in 97.5 % among the TB cases. Anaemia in this study was defined as hemoglobin levels lower than 11 g/dl in males and 10 g/dl in females. (Karita, 2009) established that the leading cause of anemia in the Kenyan coast as multifactorial with 77% presenting as Iron deficiency (IDA). However, we were unable to categorize the type of anemia our TB cases presented. Tuberculosis infection is known to induce a systemic inflammatory state affecting iron hemostasis. Iron is also an important element required in the many enzymatic systems including those required for vitamin D activation. Studies linking vitamin D deficiency and anemia has presented conflicting data in the different populations (Aziz – soleiman, 2018). Our study didn't not establish any correlation between anemia and vitamin D status in the TB cases we studied. This being a cross sectional study the researcher was unable to know whether the reason for the anemia was due to other factors other than Tuberculosis infection and whether or not that had an effect on the vitamin D levels.

Several socio demographic data i.e. marital status, residency, education level and occupation were also taken from the TB cases and we tried to establish whether they influenced their vitamin D levels. This study did not establish any correlation between an individual's marital status, residence address, education level and/or occupation to their level of vitamin D. However, TB patients who held blue collar jobs demonstrated higher levels of vitamin D (median 36.19) compared to the other occupations especially those in white collar jobs. This is because of the longer amounts of time they spent outside in the sun earning a living compared to their counterparts who spent most of their time in offices. Majority of the TB cases lived in

the urban areas (70%) and recorded higher levels of vitamin D compared to the rural folks. This is attributed to the difference in their socioeconomic levels in that most of the one in urban centers were more educated and held jobs while those in the rural areas were mostly old, retired or unemployed poor people. This could have maybe influenced their eating habits in terms of vitamin D rich foods and also health seeking behavior. However urban living involves shared living which can encourage easy spread of TB.

During this study a lot of challenges were encountered during the recruitment phase, this was mainly due to poor health seeking behaviors among the population in the Kilifi county region. Also, disruption in the supply of gene expert cassettes by the Ministry of Health disrupted the recruitment process during the time of the study. Lack of diagnostic tools to measure vitamin D levels was also a challenge in that it was only available in big private institutions and the National referral centers. Seasonal variations played a role towards the beginning of the year since majority of the rural folks were busy preparing their lands for the seasonal rains. Proper matching was not achieved mainly due to cultural reasons in that majority of the blood donors were male because women in the coastal region are discouraged culturally to donate blood.

## **5.1 CONCLUSION**

The prevalence of vitamin D insufficiency and deficiency was higher among the TB cases (78% deficient and 11% severely deficient). Slightly lower levels of vitamin D were recorded among men compared to women. Majority of the TB cases were already anemic before commencement of treatment (77.5%). An alarming 18 % of the newly diagnosed TB cases were rifampicin resistant before commencement of treatment.

## **5.2 STUDY LIMITATION**

This study was not a matched case control design, thus impeding strong conclusions when we compared the TB patients to the random population sample. There was a big gap in the laboratory diagnostics aspect in the county referral hospital.

### **5.3 RECOMMENDATIONS**

Further well-matched studies to be conducted locally in different counties to investigate the relationship between vitamin D deficiency and tuberculosis. This study strongly recommend the use of Gene expert especially in the diagnosis of new TB cases. Routine hemoglobin testing in all TB cases before treatment should be strictly adhered.

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## **APPENDICES**

### **Appendix I: Informed Consent Document**

#### **VOLUNTEER INFORMATION SHEET**

**Study Title:** Prevalence of Vitamin D deficiency among TB patient at a Kenyan Health Facility.

#### **Introduction:**

You are being invited to take part in a research study. Before you decide, it is important to understand why the research is being done and what it will involve. Please take time to read (or have it read to you) and ask the doctor or nurse to explain anything you do not understand. Take time to decide whether or not you wish to take part. You will sign two copies of this document. One copy is for you to keep and one will be kept in the clinic's files. If you do not want to keep a copy, we will keep it for you.

Your participation is voluntary; you can choose to take part in this study or not. If you decide not to take part, your legal rights and the medical care otherwise available to you will not be affected. The study is taking place at Malindi general Hospital TB clinic. The principal investigator in charge of this study is Dr. Andrew Karani.

#### **What is the purpose of this study?**

To gather data that will be helpful in future studies aimed at TB prevention and treatment.

#### **How are study participants selected?**

Men and women who have been recently diagnosed with TB will be asked if they would like to volunteer. Also, we will be asking blood donors who fit the inclusion criteria to do the same. Volunteers will be required to provide 10mls of venous blood prior to start of anti TB medication.

#### **Why have I been chosen?**

You have been chosen because you have been newly diagnosed with TB and have been scheduled to start your anti TB medication. Blood donors have been selected to represent the normal healthy population



**What are the study procedures?**

- The PI/nurse will explain the study and answer your questions.
- You will be asked to answer questions to make sure you understand the study.
- You will sign the informed consent form if you want to join the study.
- You will be asked for permission to obtain information from your medical record such as bio data, weight, height and home address.
- You will be asked to donate 10mls of blood. No extra blood sample will be drawn for this study.

**How long will the study last?**

You will not be required to attend any separate study visit.

**Storage of Specimen**

After the laboratory tests are done, if you agree, the remaining samples will be discarded. Only your study number will be used to label the tubes holding your samples.

**Risks and Discomforts**

There are no additional risks involved. If you provide a blood sample, blood will be collected by inserting a needle into one of your veins. This can cause temporary mild pain or discomfort (common), local bruising (rare), infection and fainting (very rare).

**Benefits**

There are no benefits to you for taking part in the study. However, in the event that your blood vitamin D levels are severely diminished we shall facilitate your referral to a physician for appropriate care and management. By contributing your blood sample, you will be contributing to our understanding of TB infection and how to prevent and or improve on treatment.

**Injuries**

We do not expect you to be harmed from taking part in this study. The blood draw may cause some discomfort, bleeding, or bruising at the site of vein puncturing and in rare cases, lightheadedness and fainting. The volume of blood collected is highly unlikely to cause harm to your health.

**Reimbursement**

Participants will be reimbursed for their time and transport a total sum of sh.300

**Will my taking part in this study be kept confidential?**

Your participation in the study, all personal information collected about you and all research test results will be confidential. All information and samples collected from you will be identified by a study number and not your name. Only you and the clinic staff know your study number. Your identity will not be revealed in any publication or presentation.

**Contact Numbers**

If you have any questions about the study, your participation in the study or a medical problem, please call: Dr. Andrew Karani (Principal Investigator).Tel 0722225812

If you have a question about your rights as a study volunteer, please contact Dr Marianne Mureithi supervisor department of Medical Microbiology University of Nairobi. Tel 0703704711.

The Kenyatta National Hospital/ University of Nairobi Ethical and Research Committee (KNH/UON-ERC), which is a committee whose task it is to make sure that research participants are protected from harm. The KNH/UON-ERC telephone number is (254-020) 2726300 Ext 44355.

## Appendix II: Consent Form

I, (printed name of volunteer) \_\_\_\_\_ of (address) \_\_\_\_\_

1. I agree to take part in the research project entitled: : Prevalence of vitamin D deficiency among TB patients at a Kenyan Health Facility.

Yes  No

2. I agree to allow the study staff to obtain information needed for this study from my me.

Yes  No

3. I agree to donate 10mls blood sample to be used in the study.

Yes  No  N/A

4. I agree that my samples may be stored temporarily and later discarded.

Yes  No

5. I wish to take a copy of the signed and dated informed consent form with me.

Yes  No

\_\_\_\_\_

Volunteer Name

\_\_\_\_\_

Volunteer Signature

\_\_\_\_\_

Date

Impartial Witness (*If volunteer was not able to read and understand this Informed Consent*)

I affirm that the Informed Consent Document has been read to the volunteer and he/she understands the study, had his/her questions answered, and I have witnessed the volunteer's consent to study participation.

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Impartial Witness signature

---

Date

**Person obtaining consent:**

I have fully explained the nature and purpose of the above-described study and the risks and benefits that are involved in its performance. I have answered all questions to the best of my ability.

---

Name of Person Obtaining Consent

---

Signature of Person Obtaining Consent

---

Date

**Emergency Contacts**

**Dr Andrew Karani (principle investigator) - 0722225812**

Dr Marianne Mureithi (Study supervisor) - 0703704711.

The KNH/UON-ERC - (254-020) 2726300 Ext 44355.

### **Appendix III: Standard Operating Procedure for Blood Extraction- MOH/ Malindi Hospital**

1. Obtain informed consent from participant
2. Explain the procedure clearly to participant giving time for any questions, ensuring the participant is comfortable about the procedure
3. Ensure all equipment is ready to hand in a tray next to the participant.
4. Identify a good-sized vein, usually in the antecubital fossae or on the dorsum (back) of the hand.
5. Apply a tourniquet proximal to the site of venipuncture to ensure engorgement of vein with blood.
6. Prepare a 10ml syringe with either a green or blue needle depending upon the size of the vein
7. Clean the site of venipuncture with an alcohol swab.
8. Insert needle into vein looking for blood flashback in the bevel of the syringe
9. Gently withdraw approximately 4mls of blood into the syringe
10. Once enough blood has been withdrawn, undo the tourniquet with the needle still in place.
11. Take cotton swab and place over site of needle insertion (Venipuncture) and gently remove the needle.
12. Apply direct pressure with the cotton swab over the puncture site to stem any bleeding. This should be carried out for 2mins, after which the swab should be removed to ensure bleeding has stopped. If not affix the swab with gauze tape.
13. Transfer blood from syringe into a red top tube, either by directly puncturing the top of the tube in the center (rubber black area) or remove the tube top and gently inject blood into the empty tube prior to replacing the cap.
14. Carefully label the tubes with patient study number and date and time blood sample was taken.

