SERUM VITAMIN DLEVELS IN MALE BLOOD DONORS AND HUMAN IMMUNODEFICIENCY VIRUS AND TUBERCULOSIS COINFECTED MALE PATIENTS: A COMPARATIVE STUDY

BY

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A dissertation in partial fulfillment of the Masters in Medicine (Mimed) degree in the department of Human Pathology in the University of Nairobi

DECLARATION

I, CAROLINE W. NJERU, declare that this dissertation is my original work under the guidance of the supervisors named below and has not been submitted to the University of Nairobi or any institute of higher learning

Signature: _____ Date_____

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This proposal has been developed under our guidance and approval as University supervisors

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ACKNOWLEDGEMENT

I would like to thank my supervisors Prof. C.Kigondu and Dr.J.Kuria for their guidance in the development and writing of this dissertation.

My sincere gratitude to Prof A.Amayo for her input and advice .I thank my classmates for their immense support.

I appreciate the technical work done by Mr. Leonard Bosco and Mr.Kipmengich.

DEDICATION

I dedicate this work to my parents and Karimi.

LIST OF ABBREVIATIONS

1, 25(OH) D ..... 1, 25 dihydroxyvitamin D

25(OH) D ..... 25 hydroxyvitamin D

BMI ..... Basal Metabolic index

CCC ..... Comprehensive Care Clinic

CIMA ..... Chemiluminescent immunoassay

D_2 ..... Ergocalciferol

D_3 ..... Cholecalciferol

DNA .....Deoxyribonucleic acid

ECLIA .....Electro-chemiluminescent immunoassay

HBV .....Hepatitis B Virus

HCV ..... Hepatitis C virus

HIV/TB .....Human Immunodeficiency deficiency virus /Tuberculosis

GIT ..... Gastrointestinal tract

IU ..... International units

INH .....Isoniazid

KNH ..... Kenyatta National Hospital

LL-37 ..... Cathelicidin

MDR-TB .....Multiple drug resistant-Tuberculosis

MTB .....Mycobacterium Tuberculosis

NIH ..... National institute of Health

NIST .....National Institute of Technology

PTB .....Pulmonary Tuberculosis

RIA..... Radioimmunoassay

RR ..... Reference range

RMP ..... Rifampicin

SD .....Standard deviation

STATA .....Statistics and data

SVDD..... Severe Vitamin D Deficiency

TLR .....Toll like receptor

TTI .....Transfusion transmittable infection

UoN.....University of Nairobi

UV..... Ultraviolet light

VitD **.....**Vitamin D

VDBR .....Vitamin D Binding Protein

VDD.....Vitamin D Deficiency

VDR .....Vitamin D receptor

VDSP .....Vitamin D standardization program

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ABSTRACT

Introduction

Vitamin D plays a role in the immune function and itsdeficiency is associated with higher incidence of immune system disorders and faster progression of some infectious diseases. Tuberculosis is a major cause of death among people living with Human Immunodeficiency Virus/Acquired Immunodeficiency Syndrome. Evidence that vitamin D protects against tuberculosis has been supported by in vitro, epidemiological and some preliminary clinical studies.

Broad objective:

To compare vitamin D levels in healthy males and Human Immunodeficiency Virus and Tuberculosis co-infected males.

Specific objectives:

1. To compare vitamin D levels in male blood donors and malesco-infected with Human Immunodeficiency Virus patients and Tuberculosis.
2. To determine the prevalence of vitamin D deficiency in patients co-infected with Human Immunodeficiency virus and Tuberculosis.
3. To determine the reference interval for vitamin D in blood donors.

Methodology;

Study Design

This was a cross-sectional study, prospective descriptive study.

Study Area

Study area was Mbagathi District Hospital Tuberculosis clinic.

Study Population and Sample Size

Human immunodeficiency virus and tuberculosis co infected males and male blood donors were recruited to the study after giving their informed consent.

Sample size was 240.

Male blood donors were 120 and 120 male HIV/TB male patients.

Specimen Analysis

Vitamin D was run on the Cobas platform.

Data Analysis

Data was entered and stored using Microsoft excel 2013. Data was imported using STATA 13, coded, cleaned and analyzed.

Numeric (continuous/categorical) data were summarized using measures of central tendency and dispersion; summaries were presented in tables. Histograms were plotted to show distributions. Pearson correlation tests were used to evaluate the linear relationship between variables. Pearson correlation statistic and corresponding p-values was reported.

Two independent sample t-tests were used to compare the means of serum vitamin D between the two populations. The t statistics with corresponding p-values were reported.

Reference interval determination was done using male blood donors. The lower and upper reference limits of vitamin D levels will be obtained by X/±1.96 SD.

Ethical Consideration

Ethical approval was sought from the Kenyatta National Hospital Data /University of Nairobi ethical review committee before carrying out this research.

Results

The distribution of serum vitamin D was significantly different (K-sample test for equality of medians: chi-sq=86.38; p-value<0.001) between blood donors and HIV/TB patients. Among blood donors, serum vitamin D level ranged from 42.6nmol/L to 106.7 nmol/L. Median 68.7 and mean was 69.6(±)nmol/L. For HIV/TB patients' Vitamin D levels ranged between 33.9-89.8 median 44.0 and mean 44.3(±) nmol/L.

Out of 121 HIV/TB co-infected patients had 69 vitamin D deficiency (57.0%) and 49/121 had insufficiency (37.2%) based on the WHO reference interval.

Out of 121 patients, 75.2% (91) were on the intensive phase of treatment tuberculosis and 23.1% (28) in the continuation phase. Among the patients' majority Vitamin D deficiency was more frequent (68.5%) among patients in the intensive tuberculosis compared to those in the continuation phase. There was a significant association between tuberculosis phase and vitamin D status of the patient (chi-sq=27.67; p<0.001).

Based on the reference interval obtained in this study, 10/121 HIV/TB patients had deficient vitamin D. Prevalence of vitamin D deficiency among these patients was 8.3%. Achi-square test done to evaluate the association between HIV-TB co-infection and serum vitamin D status was significant (P value 0.002).

Conclusion

HIV/TB co-infected patients have a lower serum vitamin D levels as compared to blood donors (57% vs.5.7% as per WHO reference range). Prevalence of VDD is high among HIV/TB co-infected patients (57% as per WHO reference range). Using the reference interval determined in this study the prevalence of VDD was 8.3% in the HIV/TB co-infected patients. Serum vitamin D reference interval among blood donors was lower than WHO reference values. Co-infected patients in the intensive phase have lower Vitamin D than in those in the continuation phase (68.5% vs. 21.4%).

Recommendations

1. Patients with Human immunodeficiency virus co-infected with Tuberculosis should have their serum vitamin D measured.
2. Reference intervals obtained in this study should be used in the KNH laboratory.
3. Every laboratory is encouraged to establish reference intervals for serum Vitamin D.
4. Further study to establish serum vitamin D reference interval in females is recommended.

CHAPTER ONE

1.0 Introduction

Vitamin D is hormone of great physiological importance; it is essential micronutrient for the bone mineralization. In the immune system Vitamin D has been shown to activate macrophages and restricts intracellular Mycobacterium tuberculosis growth by up regulatingcathelicidins(1). Vitamin D has a potential effect on HIV (Human immunodeficiency Virus) for it influences the immune response to Tuberculosis, a common cause of morbidity and mortality in this group of patients. Deficiency has been associated with higher incidence of tuberculosis and faster progression of disease.

Tuberculosis (TB) now ranks alongside HIV as a leading cause of death .Worldwide 9.6 million people are estimated to have fallen ill with TB in 2014. Globally 12% of the 9.6million new TB cases were HIV positive.(2) Kenya reported 120,000 new tuberculosis cases in 2013(2).The emergence of drug resistant TB and HIV (Human Immunodeficiency Virus) co infection has challenged TB eradication programs worldwide.

Increased tuberculosis risk in vitamin D deficient individuals has been noted. High rates of Mycobacterium tuberculosis (MTB) infection and VDD have been reported in African immigrants in Australia, the United States, and Europe. The association between hypovitaminosis D and TB has been described in several case-control studies(3) .Investing in supplementation in poor settings such as sub Saharan Africa where HIV/TB co infection rates are high could be a cost-effective approach towards the eradication of TB morbidity and mortality.

CHAPTER TWO

2.0LITERATURE REVIEW

2.1 Vitamin D

Vitamin D (1,25dihydroxyvitamin D)is a fat-soluble vitamin that functions like a hormone. It is naturally found in fish-liver oils, fatty fish, mushrooms, egg yolks, and liver. The two major physiologically relevant forms are D2 (Ergocalciferol) and D3 (cholecalciferol). Ergocalciferol is synthesized by molds and yeasts by solar ultraviolet (UV) irradiation of ergosterol. Cutaneous synthesis of vitamin D occurs by the action of UV B wavelength 280-315nm on 7-dehydrocholesterol. Sun exposure between 10:00 am and 3:00 pm produces vitamin D in the skin that lasts twice as long in the blood compared with ingested vitamin D (3). Sun exposure that produces slight pinkness of the skin is equivalent to ingesting 10,000-25,000 IU (4). Increased skin pigmentation, aging, and the use of sunscreen decrease skin vitamin D production.

Vitamin D_2 and D_3 are biologically inactive and are transformed into the biologically active 1,25dihydroxyvitamin D. After being synthesized in the skin or absorbedfrom the gastrointestinal tract, most vitamin D is bound to vitamin D–binding protein (VDBP) and albumin and transported to the liver. In the liver, it undergoes two hydroxylations. It is hydroxylated by the enzyme 25-hydroxylase into 25-hydroxyvitamin D, the primary circulating form of vitamin D. From the liver, 25(OH) D is transported to the kidneys by carrier proteins mentioned above.

1, 25 dihydroxyvitamin D (1, 25(OH) D) is formed when 25(OH) D is hydroxylated by the enzyme 1α-hydroxylase, located in the mitochondria of proximal tubules of the kidney. Hepatic synthesis of 25(OH) D is loosely regulated, and blood levels reflect cutaneous and ingested levels of vitamin D .Activity of 1-alpha-hydroxylase is tightly regulated and is the major control point in production of the active hormone.

Synthesis of 1, 25(OH) 2 D is regulated by parathyroid hormone (PTH), serum calcium and phosphate. Increased levels of PTH and hypophosphatemia stimulate the enzyme 1α-hydroxylase which increases synthesis of 1, 25(OH) 2 D(4).

2.1.1 The Vitamin D Receptor and Mechanism of Action

The active form of vitamin D binds to intracellular receptors that then function as transcription factors to modulate gene expression. The vitamin D receptor(VDR) binds several forms of cholecalciferol. Its affinity for 1, 25(OH) 2 is roughly 1000 times that for 25OH, explaining their relative biological potencies.

The identification of VDRs in various cells has prompted the investigation of vitamin D in immunomodulation, cancer prevention and therapy, autoimmune disease and cardiovascular disease(5).

2.1.2 Physiological Effects of Vitamin D

Vitamin D is involved in mineral metabolism and bone growth.

In the small intestine, it facilitates absorption of calcium and stimulates absorption of phosphate and magnesium ions.

Vitamin D suppresses PTH production, decreases renal excretion of both calcium and phosphate.

2.2Vitamin D deficiency and Vitamin D reference range

2.2.1. Vitamin D Deficiency

Vitamin D deficiency in children is known as rickets and osteomalacia in adults. Genetic forms of rickets occur due to defects in the metabolism of vitamin D. Vitamin D–dependent rickets type I occurs because of a defect in the renal 1 alpha-hydroxylase. Vitamin D–dependent rickets type II occurs when a mutation exists in the VDR.

The daily maintenance dose of vitamin D varies by age, but most children and adults generally require 600-2000 IU of vitamin D daily. Higher dosesare recommended for deficient individuals.

Screening for vitamin D deficiency is recommended only in individuals who are at high risk for vitamin D deficiency such as patients who have; chronic kidney disease, osteoporosis, malabsorption syndrome and obese individuals.

Vitamin D deficiency can result from the following:
•Inadequate sunexposure.
•Vitamin D_2 malabsorption problems – Patients who have had small intestine resections, celiac sprue patients, short bowel syndrome sufferers, and cystic fibrosis.
•Exclusive breastfeeding - The American Academy of Pediatrics recommends supplementation starting at age 2 months for exclusively breast fed infants(6).
•Medications - phenobarbital, isoniazid, and rifampin can induce hepatic p450 enzymes to accelerate the catabolism of vitamin D (7). Efavirenz an antiretroviral drug is also associated with vitamin D deficiency.

Cutaneous vitamin D declines with advancing age, making elderly populations more dependent on dietary or supplemental vitamin D. Higher supplemental intakemay be required in elderly individuals to achieve optimal serum levels of D due to both reducedcutaneous and dietary absorption(8).

2.2.2 Vitamin D reference range

Currently controversy remains on recommended levels of vitamin D. Most laboratories agree that 75nMol/L (30ng/mL) is considered to be the optimal level, while individuals with lower concentrations are considered to be vitamin D insufficient or deficient. (9)

Many experts agree that Vitamin D reference ranges vary in different laboratories depending on what platform is used. Each laboratory is encouraged to establish its own reference interval for all analytes for the population they serve in addition to using a standard reference material. Although there is no formal definition of vitamin D deficiency, some groups including the National Institute of Health in the United States of America use the following values in adults:

	Cobas	WHO/HOLICK	NIST	IOM	RIA
Deficiency	<50	<50	<25	<24	<20
Insufficiency		52.5-72.5	<25-74	24-47.5	21-39
Optimal	>75	>75	75-250	50-125	40-195
Toxicity			>250	200	

Table 1: Serum 25-Hydroxyvitamin D [25(OH) D] Concentrations inHealth (10)in nanomoles per liter (nmol/L).

2.3Measurement of Vitamin D and Standardization

Different methods are used to measure vitamin D in blood.

1. Competitive protein binding,
2. Immunoassays which include enzyme immunoassays and radio immunoassays,
3. High performance liquid chromatography and
4. Liquid chromatographic separation followed by tandem mass spectrometric detection (LCMS/MS).

Liquid chromatography tandem mass spectrometry has the highest sensitivity and is considered the gold standard method. It is an expensive procedure and not routinely used. It is mostly used in research laboratories.

Steps towards vitamin D standardization were initiated by National Institute of Health, which created the Vitamin D Standardization Program(VDSP) in Nov 2010.The goal of the VDSP is to promote standardized laboratory measurement of 25(OH) to improve comparability of test results between laboratories and minimize variability in patient testing. Reference measurement procedures were developed by National Institute of Standards and Technology (NIST) and Ghent University. They are the “gold standard” laboratory procedures for measuring 25(OH) D.
The reference materials for 25(OH) D permit standardization of values in laboratories and improve method-related variability (11).

2.4Studies on Vitamin D

Vitamin D Reference Interval in Dark Skinned Individuals

Dark skin interferes with the cutaneous synthesis of vitamin D. Holick et aldemonstrated non-Hispanic black subjects require 6 times the amount of UV radiation necessary to produce a serum vitamin D concentration similar to that found in non-Hispanic white subjects(7). This is because melanin absorbs ultraviolet radiation thus the increased radiation needed. Decreased vitamin D production by dark-pigmented skin explains the higher prevalence of vitamin D insufficiency among darker-skinned adults. Dawson-Hughes and colleagues demonstrated that in Boston, 73% of elderly black subjects were vitamin D insufficient, compared with 35% of elderly non-Hispanic whites (7).

In a large survey of 1500 healthy African American and white women of reproductive(30-50years) carried out by the National Health and Nutrition Examination in 1988-1994 in 81 counties across the US, 40% of the African American women were vitamin Ddeficient (25[OH]D< 16ng/mL), compared with 4% of 1400 white women in that study(12).

Vitamin D has been shown to improve macrophage phagocytic capacity, cell-mediated immunity, and increase natural killer cell number suggesting an important role in response to infections (13).

In the presence of adequate 25(OH) D, VDR up regulation leads to cathelicidin induction, an antimicrobial peptide with direct action against intracellular pathogens such as MTB. Increased resistance to tuberculosis could potentially prolong survival in patients with HIVand slow HIV disease progression and preventing mortality(14).

Liu et al (2006) demonstrated MTB sensing by the Toll-like receptor 2/1 (TLR2/1) complex increases expression of VDRs in macrophages(15). Synthesis of 1, 25(OH) D promotes VDR-mediated transactivationof cathelicidin and killing of intracellular MTB. Cathelicidins have direct antimicrobial function in addition to anti-bacterial effects such as cationic membrane disruption.In vitro studies show macrophages are most efficient in producing cathelicidin (LL-

37) after infection with MTB, suggesting this antimicrobial peptide is important during mounting of the primary immune response towards MTB (7).

A further study by Liu (2006) demonstrated that transcriptional regulation of cathelicidin is be mediated by the active form of VitD. Stimulation of TLR receptors by microbial products results in increased production from the inactive form of the hormone to the active form(15).

Adams (2007) demonstrated TLR activation results in production of defensin-2(a antimicrobial peptide) and of cathelicidin: which are strongly up-regulated by 1, 25-hydroxyvitamin D(16).

Liu noted serum from donors with hypovitaminosis D had a low levels of LL-37 in macrophages compared to donors with normal vitamin D levels (15). Similar conclusions were made by Adams (16).

The role of VitD in the immune response to MTB is demonstrated in the production of the LL-37 and in promoting phagolysosome formation inmonocytes (17). Eun-Kyeong confirmed (2010) LL-37 plays an important role in innate immunity to mycobacterium and indirect immune modulation.(18).

Martineau (2011) observed that VDD was highly prevalent among black African adults living in Cape Town and was found to be associated with susceptibility to active TB in both the absence and the presence of HIV infection. The association was noted to be stronger in HIV-infected people. The study recommended testing for this group and supplementation for deficient individuals (19).

Gibney et al found a strong association between VDD and Latent Tuberculosis Infection (LTBI) in African immigrants in Melbourne (19). A further study, among sub-Saharan African immigrants in Melbourne, documented the frequency of both active and latent TB infection and the relationship with vitamin D deficiency (20).
A cohort follow-up study in Karachi, Pakistan, found thatdeficiency was associated with progression to active TB disease in healthy household contacts. Also noted wasa higher

susceptibility of women to the infection, which lead to little exposure to sunlight (21). A study among the Vietnamese populationfound hypovitaminosis Dstatus was an antecedent risk factor for TB(22).

A prospective cohort study conducted from 2009 to 2012 in Spain,Castellon to assess the relationship between serum baseline vitamin D status and the incidence of tuberculosis among contacts of PTB patients in Castellon, Spain. Mean vitamin D levels between the two populations was found to be 34.25nmol/L for cases and 64.25nmol/L for non-cases.Hypovitaminosis D showed a significant association with TB incidence. This result is in line with the hypothesis that vitamin D deficiency is associated with TB incidence(23).

Wilkinson et al (2000) in a case-control study in the Guajarati Indian population in NW London; Hypovitaminosis D was significantly associated with active TB disease. Undetectable vitamin levels carried the highest risk of contractingTB (24).

Iftikhar R et al in a case control study (2010-2012) in Kharian,found significant low Vitamin D levels in patients with TB as compared to controls. Deficiency was found to be more severe in females, individuals with low BMI, extra pulmonary and MDRTB(25).

In a meta analytical study published by Nnoahamthe association between low serum vitamin D and risk of tuberculosis was assessed. Findings from the meta-analysis were the probability of 70% that a healthy individual would have higher serum vitamin D level than an individual with TB if both were chosen at random from a population .Lower levels were associated with a higher risk of active TB. The study concluded that the potential role of vitamin D supplementation in people with tuberculosis and VDDassociated conditions like chronic kidney disease should be evaluated(26).

2 .4Study Rationale

Vitamin D testing has increased in the last few years worldwide and associations have been made with HIV, TBand HIV/TB co infection and several non-communicable diseases. Several publications have been made in the West documenting hypovitaminosis in apparently healthy population and in special groups. There is no consensus on what optimal vitamin D status is. There is need to establish a reference interval in the local population due to difference in skin color, geographicallatitude, age, gender and season. Each laboratory is encouraged to establish their own reference range for their local population. This study will establish distribution of vitamin D in the healthy population and HIV/TB co-infected individuals.

2.5Broad Objective

To compare vitamin D levels in male blood donors and male Human immunodeficiency virus co-infected with tuberculosis

2.6Specific Objectives

1. To compare vitamin D level in male blood donors and male Human immunodeficiency viruspatients co-infected with Tuberculosis.
2. To determine the prevalence of vitamin D deficiency in males co-infected with Human immunodeficiency virus and tuberculosis.
3. To determine the reference interval for vitamin D in blood donors.

Secondary Objective

1. To correlate serum Vitamin D level with phase of tuberculosis treatment.

CHAPTER THREE

3.0 STUDY DESIGN AND METHODOLOGY

3.1Study Design

A cross-sectional study was conducted with the aim of comparing the distribution of serum vitamin D among male blood donors and male HIV patients co-infected with Tuberculosis.

3.2 Study Area.

The study was conducted in Mbagathi District Hospital, in Nairobi County, Kibra District which offers integrated health services to nearly 9800 HIV positive patients. Participants were recruited consecutively until the sample size was achieved. The clinic was visited on daily until the sample size was achieved. The Mbagathi District Hospital is located on Mbagathi Way, Nairobi. The catchment area includes the Kibra slum, one of the largest informal settlements in Africa. The Tuberculosis clinic is open weekdays, Monday to Friday.

3.3 Study Population and Sampling Method, Recruitment and Consenting

Participants were recruited consecutively until the sample size was achieved. The clinic was visited daily until the sample size was achieved.

The Mbagathi District Hospital is located on Mbagathi Way, Nairobi. The catchment area includes the Kibra slum, one of the largest informal settlements in Africa. The Tuberculosis clinic is open weekdays, Monday to Friday.

Blood donors were recruited into the study at different sites, depending on where Kenya National Blood Transfusion Service conducted their blood drive. Their serum was used once it has been screened and cleared for Syphilis, HIV hepatitis B and C.

3.4Inclusion and Exclusion Criteria

Inclusion criteria for blood donors:

- Over 18 years of age
- Males who will Consent to participate in the study
- Male gender

Exclusion criteria for blood donors

- Transfusion Transmittable Illnesspositive
- Female gender

Inclusion criteria for HIV positive patients with TB

- Consent
- confirmed HIV + and TB +
- Age above 18 years
- Male
- Newly diagnosed TB or on treatment for TB

Exclusion criteria for HIV positive patients with TB

- Patients on VitD supplementation
- Female patients

3.5 Sample Size

Vitamin D levels are assumed to follow a normal distribution in the general population. Therefore, the mean best summarizes this distribution. The aim of this study was to compare the distribution of serum vitamin D levels between male blood donors and male HIV patients co-infected with TB. The sample size was determined using the formula for comparison of means by Kelsey et al., (1996)(27).

$$n \geq (\frac{r+1}{r}) \frac{\sigma^2 (Z_\beta + Z_{\alpha/2})^2}{(d\text{ifference})^2}$$

Where:

n_1 = minimum sample size among exposed

$n_2 = r * n_1$ i.e. sample size among non-exposed

$n = n_1 + n_2$ i.e. Total sample size

r = is the ratio of non-exposed (blood donors) to exposed (HIV patients) (r=1)

σ = standard deviation in the population (σ =13, because vitamin D is measured in nmol/L)

$\mathbf{Z_\beta}$ =is the critical value for the desired power (Type II error β = 0.2, $\mathbf{Z_\beta}$ =0.84)

$\mathbf{Z_{\alpha/2}}$= is the critical value for standard normal distribution at α-level of significance (Type I error α= 0.05, $\mathbf{Z_{\alpha/2}}$=1.96)
Difference = expected effect size (the difference in means = 5)

Using this formula, the estimated minimum sample size for blood donors was105 and 105 for HIV male patients.

According to Tietz Fundamentals of Clinical Chemistryand Molecular Diagnostics for the estimation of a reference interval,40 values are sufficient, but at least 120 reference values are required to obtain reliable estimates (28).

Sample size was 240, 120 for male blood donors and 120 for patients co-infected with HIV/TB.

3.6Sample collection

HIV/TB CO-INFECTED PATIENTS

Four milliliters of blood were collected by the principal investigator or research assistant after consent is obtained in a red top serum separator tube.

BLOOD DONORS

For the blood donors sample collection was done during donation of blood after consent has been obtained. The sample was taken during cannulation of the donor.
The samples from the donors were assayed after screening for transfusion transmittable infections which was done at the National Blood Transfusion Unit.

3.7 Sample Transport

The blood was allowed to clot at room temperature. The sample was transported to theKenyattaNational Hospital Clinical Chemistry unit in a cool box where it was centrifuged 2000 rpm to separate serum from the cells.
The serum was stored at negative 20 degrees Celsius and batched.
Patient information wasentered into a questionnaire that did not bear the name of the patient.
Vitamin D was run on the Cobas 600 platform which is an electro-chemiluminscent microparticle immunoassay.

25(OH) D serum levels were measured for it is considered as the best indicator of vitamin D supply to the body. The circulating half-life of 25(OH) D is 2 weeks while that of 1, 25(OH) D is 4hrs.

3.8 Data Management and Analysis

Data was entered and stored using Microsoft excel 2013. Data was importedcoded, cleaned and analyzed using Statistics and data version 13(STATA).

Descriptive statistics was done to explore and summarize the data. Numeric data, were summarized using measures of central tendency (mean/median) and dispersion (standard deviation/inter-quartile range and; summaries were presented in tables. Histograms were plotted to show distributions.

Pearson correlation tests were used to evaluate the linear relationship between variables. Pearson correlation statistics and chi-square tests were done and corresponding p-values was reported. Two independent sample t-tests were used to compare the means of serum vitamin D between the two populations. The t statistics with corresponding p-values were reported. This study was conducted at alpha significance level of 0.05.

3.9 Vitamin D Reference Range Determination

Reference limit is a description of reference distribution that tells us about the variation of values in the selected set of reference individuals.

The lower and upper reference limits of vitamin D levels were obtained as.$\bar{X}\pm 1.96$ SD, where$\bar{X}$is the mean and SD is the standard deviation. All the values between and including the two reference limits are used to obtain the reference interval.

This reference interval can also be defined as the 95%central interval bounded by 2.5-97.5%.

The reference individual was the male blood donor.

4.0 Quality Assurance

Pre-analytical Errors

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

Analytical Errors

1. The manufacturers laid down standard operating procedures wereused to run all the tests.
2. VitaminD levels were interpreted based on the manufacturers insert and also after the reference interval was established.
3. QualityControl 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 is used.
5. If a control is out of its specified range, the associated test results were considered invalid and samples would be retested.
6. Calibration of the machines was done according to the manufacturer's' pamphlet procedures.

Post Analytical Errors

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

4.1 Ethical Consideration

Approval for study protocol was obtained from Kenyatta National Hospital/ University of Nairobi –Ethical and Research Committee (KNH/UON-ERC).Approval was also obtained from Mbagathi Level Five Hospital Ethical review Committee.

Informed consent was obtained from each study participant and samples collected according to standard procedure. Risks and benefits of the study were explained to the participants. Risks as pain during venipuncture and multiple pricks were explained to all participants.

4.2 Confidentiality

Patient information and results were kept confidential. A log book with patient details and study number werekept by the principal researcher. The questionnaire did not have patient's name. Thedata waskept under lock and key. Soft copy information waspassword protected. Results were communicated to patients' doctors and the national blood transfusion service to assist in patient management.

CHAPTER FOUR

RESULTS

4.0Demographic characteristics of patients and blood donors

A total of 121 HIV patients co-infected with TB and 141 blood donors were recruited for this study. Patients' age ranged between 20 and 67 years with a median of 44 and mean of 44.3(±)years whereas age donors ranged from 19 to 33 years with a median of 22 and meanof 22.7(±) years.

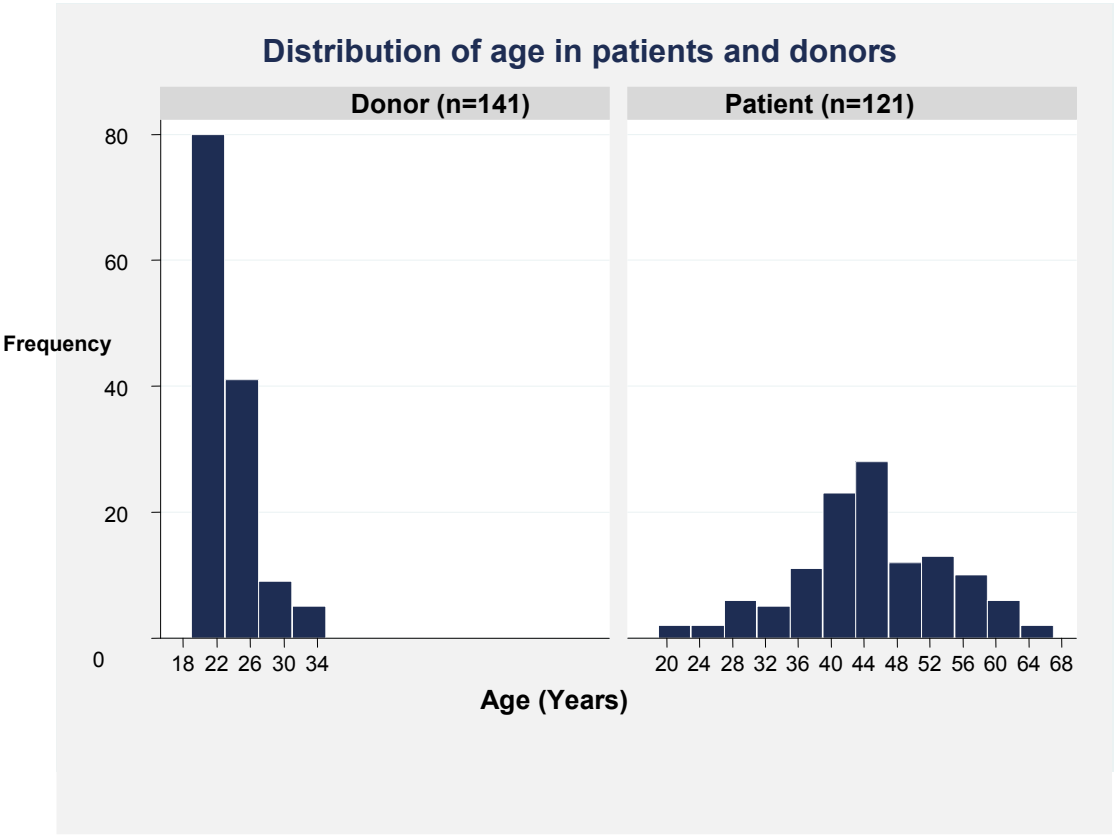

Figure 1: Distribution of age among HIV/TB patients and donors

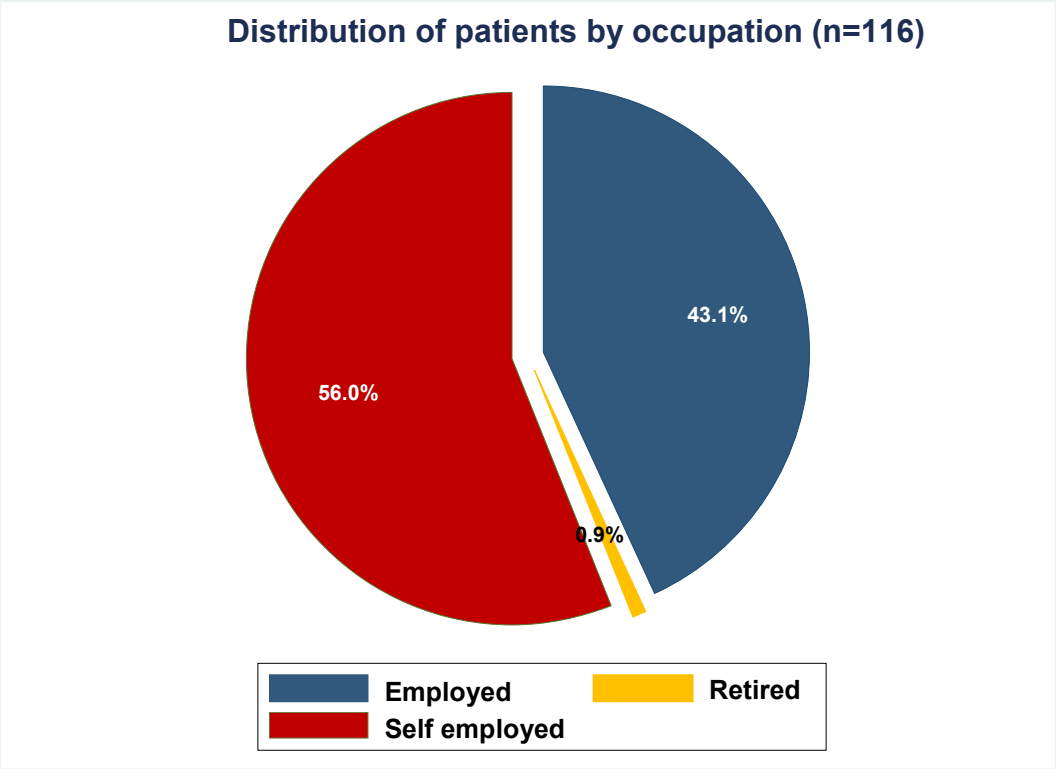

Figure 2: Distribution of co-infected patients by occupation

Self-employed co-infected HIV/TB patients were 56.0% (65/116), 43.1% (50/116) employed and 0.9% (1/116) retired. Five (5) patients did not respond.

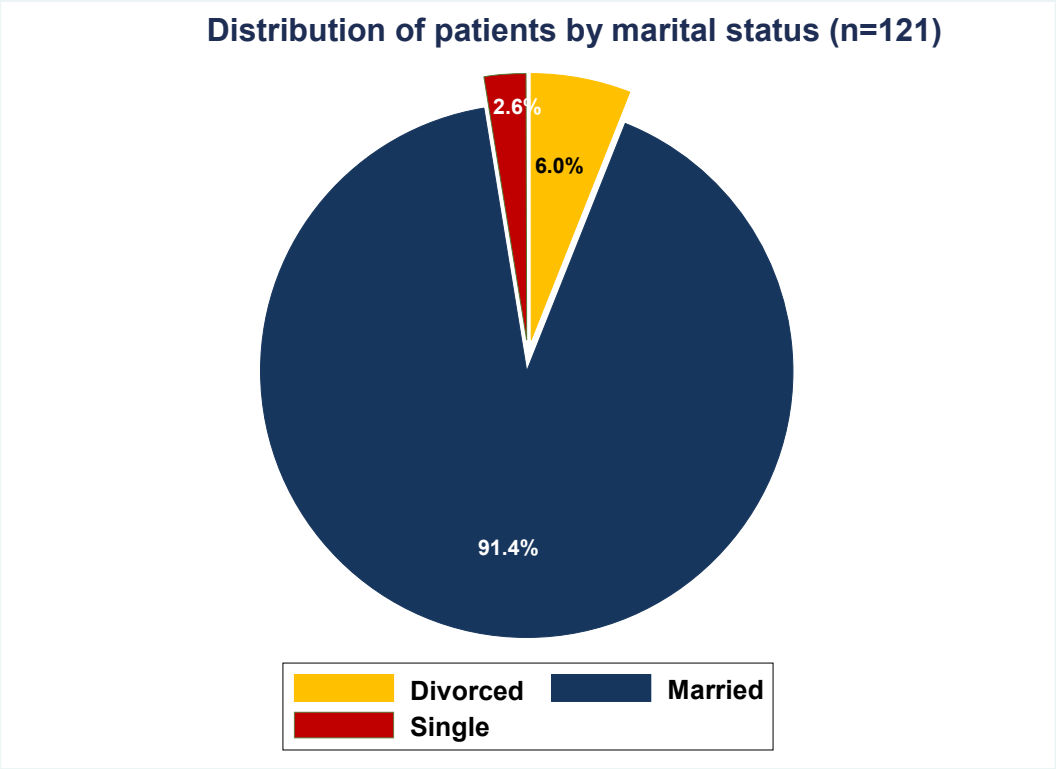

Figure 3: Distribution of patients by marital status

The majority of the patients were married (109/121; 90.1%) while 6% were divorced and 2.6% single.

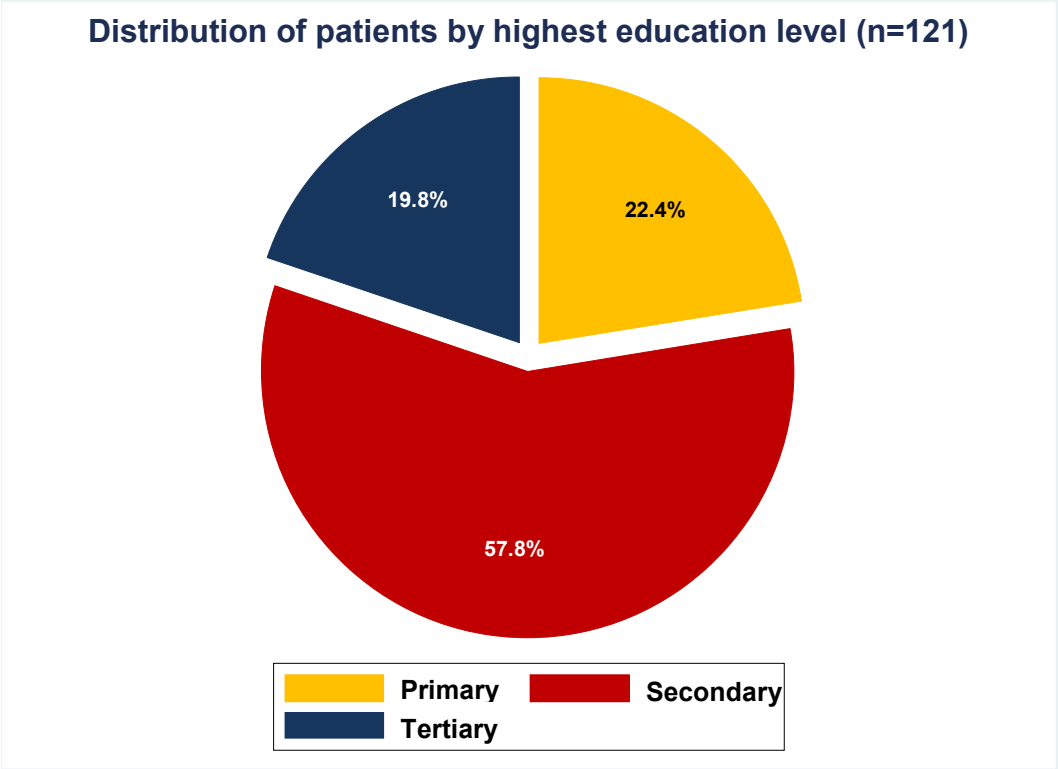

Figure 4: Distribution of HIV/TB patients by highest level of education

More than half of the HIV /TB co-infected patients (94/121; 57.9%) had attained secondary level education and above.

4.1 Distribution of Patients by Tuberculosis Phase

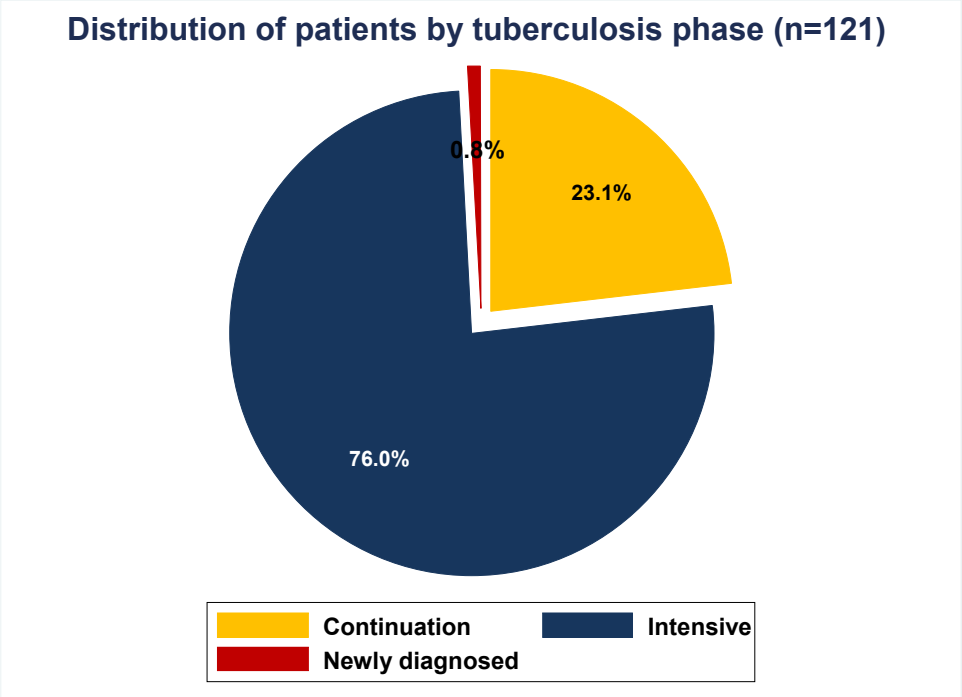

Figure 5: Distribution of Patients by Tuberculosis Phase

Out of 121 patients, 75.2% (91) were on the intensive phase of treatment tuberculosis and 23.1% (28) in the continuation phase. There was only one newly diagnosed tuberculosis case.

4.2 Distribution of Serum Vitamin D

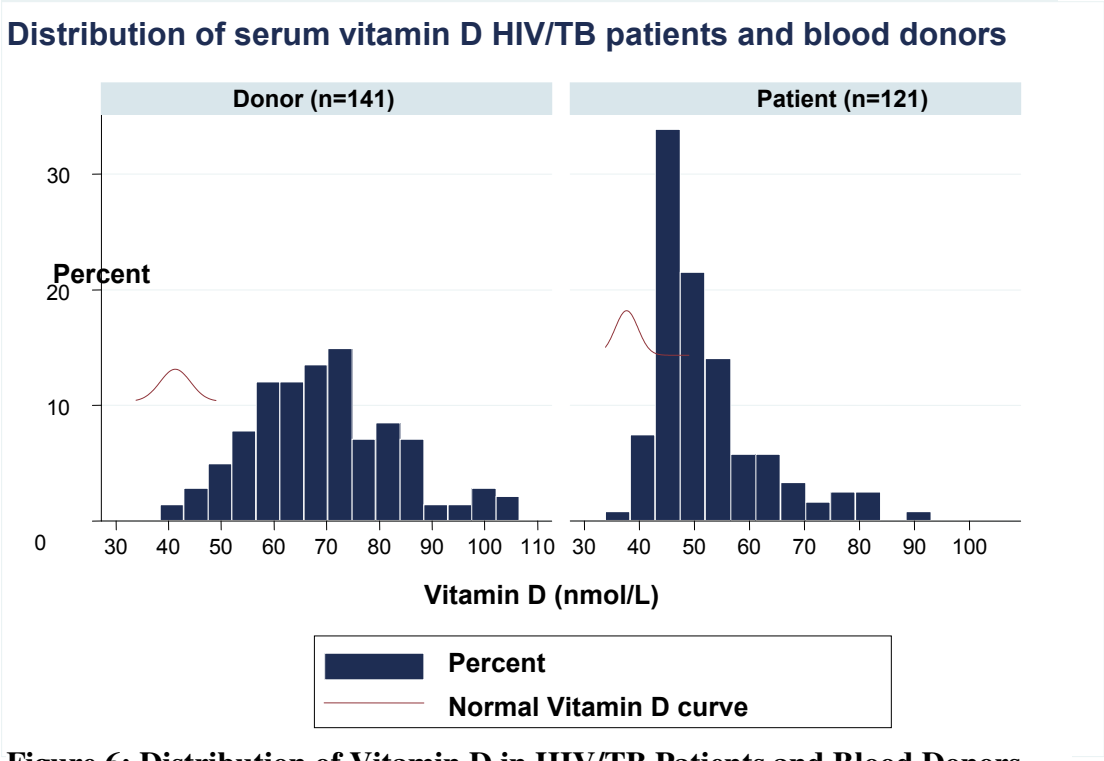

Figure 6: Distribution of Vitamin D in HIV/TB Patients and Blood Donors

Serum vitamin D level was normally distributed among blood donors and right skewed among patients. The Vitamin D range among donors was 38.9-106.7nmol/L with a mean and median 69.6(±) and 68.7nmol/L. Among HIV/TB co-infected patients the Vitamin D level range was 33.8-89.8nmol/L with a mean and median of 52.3(±) and 48.9nmol/L.

4.3 Comparison of Vitamin D levels in Blood donors and HIV/TB Co-infected Patients

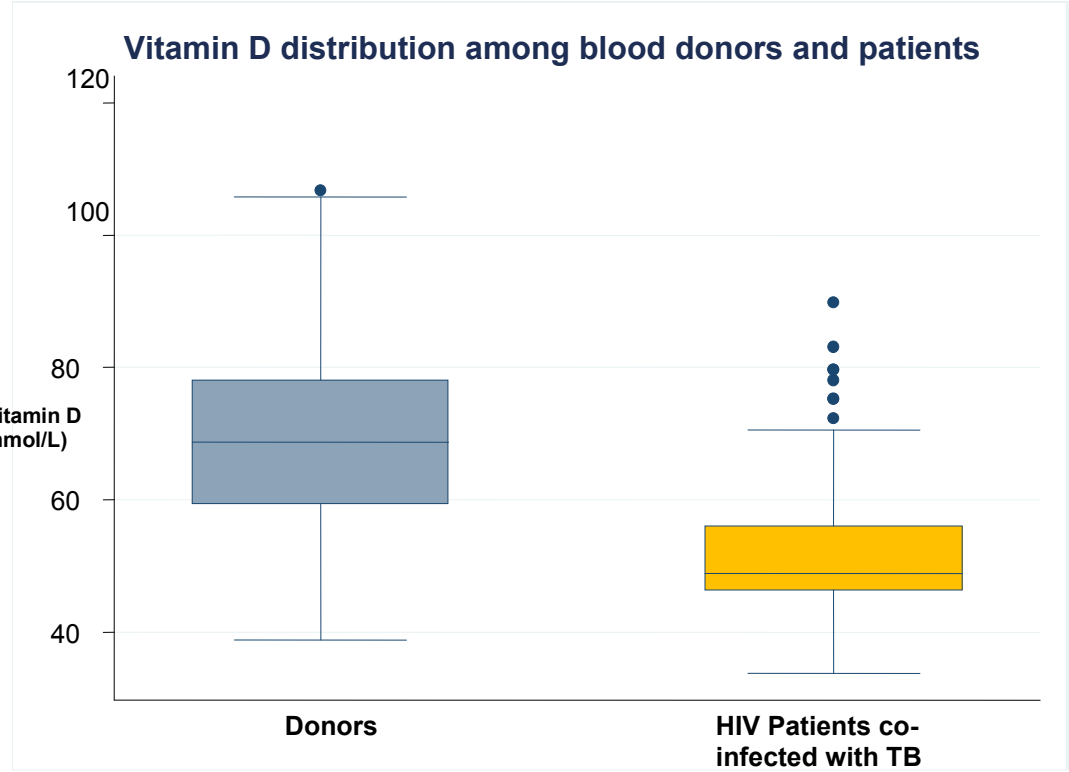

Figure 7: Serum Vitamin D Distribution among Blood Donors and HIV/TB Patients

The distribution of serum vitamin D was significantly different (K-sample test for equality of medians: chi-sq=86.38; p-value<0.001) between blood donors and HIV/TB patients.

4.4 Levels of Vitamin D vsTuberculosis Phase

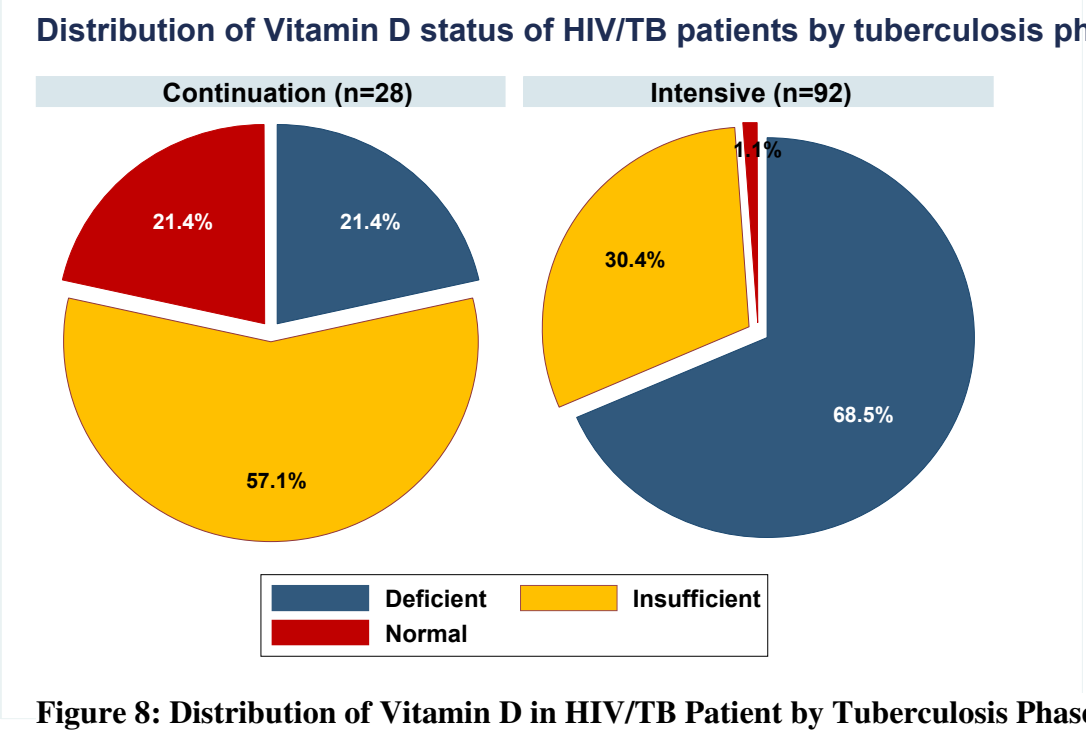

Figure 8: Distribution of Vitamin D in HIV/TB Patient by Tuberculosis Phase

Vitamin D deficiency was more frequent (68.5%) among patients in the intensive phase of treatment compared to those in the continuation phase. Majority of patients in the continuation phase had insufficient Vitamin D levels.

Table 2: Association between Tuberculosis Phase and Vitamin D Level

TB Phase\Vita min D level	Deficiency	Insufficiency	Normal	Chi-sq (df)	P-value
Continuation	**6**	16	6	27.67 (2)	**<0.001**
Intensive	**63**	28	1		

There was a significant association between tuberculosis phase and vitamin D status of the patient (chi-sq=27.67; p<0.001).

4.6 Comparison of Serum Vitamin D to WHO Reference Interval

The serum vitamin D level in patients and donors was compared to the WHO and Holick reference ranges: Normal (>75nmol/L), insufficient (50-74nmol/L) and deficient (<49mol/L).

Table 3: Distribution of Vitamin D in Donors (N=141) and Patients (N=121) Based on WHO Reference Interval

Reference range	Group	Frequency	Proportion
Normal	Donors	43	30.5
	Patients	7	5.8
Insufficient	Donors	90	63.8
	Patients	45	37.2
Deficient	Donors	8	5.7
	Patients	69	57.0

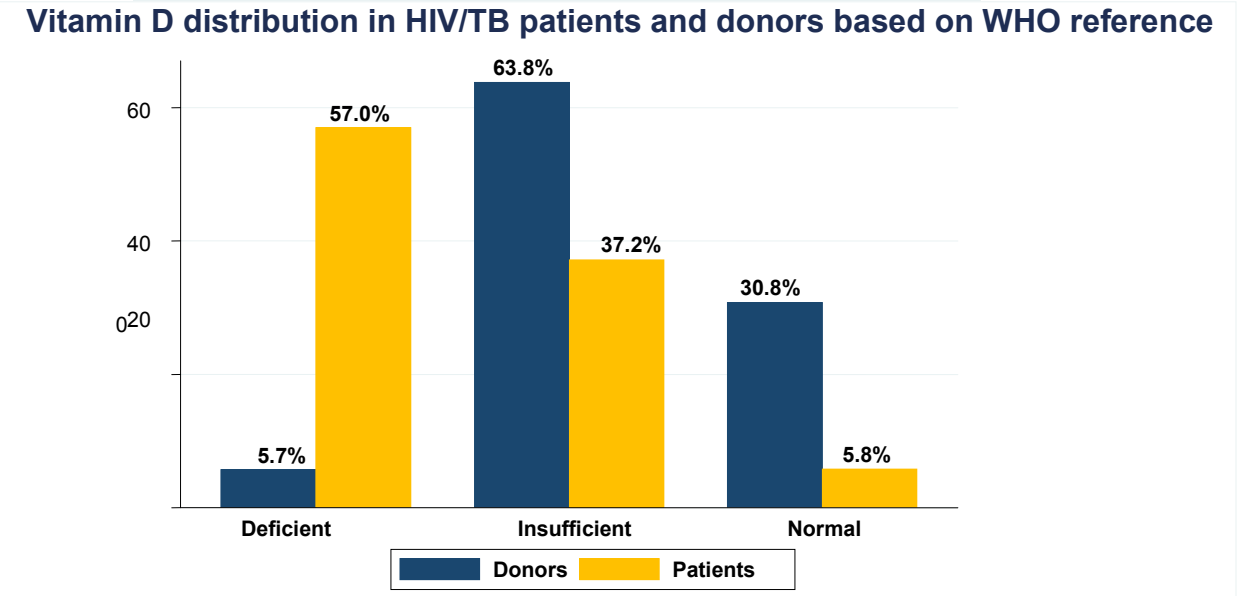

Figure 9: Distribution of Vitamin D in Patients and Donors Based on WHO Reference Interval

Among the patient's majority had vitamin D deficiency 69/121(57.0%) and insufficiency 45(37.2%).

Vitamin D deficiency was more frequent (68.5%) among patients in the intensive phase of treatment as compared to those with continuation phase. Only one had normal vitamin D

Majority of patients in the continuation phase tuberculosis patients had insufficient vitamin D levels.

Table 4: Association between Tuberculosis Phase and Vitamin D Level

TB Phase\Vita min D level	Deficiency	Insufficiency	Normal	Chi-sq (df)	P-value
Continuation	**6**	16	6	27.67 (2)	**<0.001**
Intensive	**63**	28	1		

There was a significant association between tuberculosis treatment phase and vitamin D status of the patient (chi-sq=27.67; p<0.001).

4.6Vitamin D Reference Interval for Blood Donors

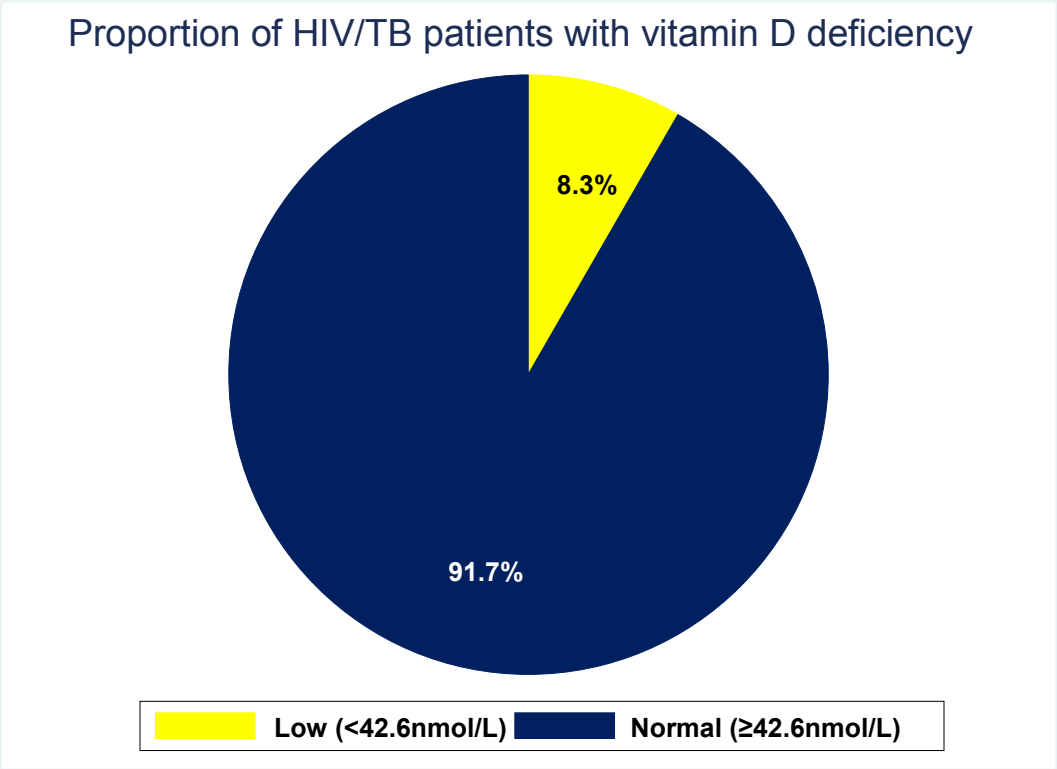

Figure 10: Prevalence of Vitamin D Deficiency Based on Reference Interval

Blood donors in this study were assumed to represent the healthy population so were used to establish population based reference intervals for serum Vitamin D. The cut-off for normal range for vitamin D level is ≥42.6nmol/L based on the lower limit of the 95% confidence interval of the mean serum vitamin D concentration (69.6±nmol/L; 95%CI [42.6; 96.7]) among donors. Insufficient level corresponds to serum vitamin D below 42.6nmol/L.

Among HIV patients co-infected with TB, 10/121 had insufficient vitamin D. Majority of the HIV/TB patients 91.7% had normal serum vitamin D. The prevalence of VDD was found to be 8.3% in HIV/TB co-infected patients using the reference interval obtained in this study.

A chi-square test done to evaluate the association between HIV-TB co-infection and serum vitamin D status was significant at α-level of significance 0.05 as shown in Table 6 below

Table 5: Association betweenHIV/TB Infection and Vitamin D Status (≥42.6nmol/L)

Group	Serum Vitamin D <42.6nmol/L	Serum Vitamin D ≥42.6nmol/L	Chi-square (df)	P-value
Donors	1	140	9.24(1)	0.002
HIV-TB patients	10	111		
Total	**11**	**251**		

The distribution of serum vitamin D was significantly different (K-sample test for equality of medians: chi-sq=86.38; p-value<0.001) between blood donors and HIV/TB patients.

CHAPTER FIVE

Discussion

There is no universal agreement on the optimal concentration of 25-OH D. Each laboratory is encouraged to establish their own reference intervals for their local population based on age, sex and special groups such as pregnancy and patients on renal replacement therapy. The question on what is an optimal vitamin D status remains a great topic of discussion. Some studies suggest that risk assessment on indicators of health such as bone and muscle function should be used to define optimal levels of this vitamin. Serum PTH levels and bone density should be assessed to truly assess for deficiency after low serum vitamin D level is realized. Many specialists consider the commonly used population based reference values too low. Health based reference values are recommended to replace population based referencevalues.Requiring patients to have levels greater than 80nmol/l implies that greater than 80% of the European population is VDD(29).

The World Health Organization (30) and Holick (31)defined vitamin D insufficiency as serum 25OHD below 50 nmol/L, a level that avoids skeletal and muscular problems.

Assay variability has been an alternate explanation to hypovitaminosis in different populations. Currently there are calibrators developed by the National Institute of Standards and Technology to provide traceability thus reducing assay variability(11).

In this study, serum Vitamin D level was normally distributed among blood donors and right skewed among patients. Among blood donor's serum Vitamin D level ranged from 38.9-106.7nmol/L mean was 69.6(±) nmol/L and median 68.7nmol/L. For HIV/TB patient's vitamin D levels ranged from 33.8-89.8nmol/L mean was 52.3(±) nmol/L and median 48.9nmol/L.

The distribution of serum vitamin D was significantly different (K-sample test for equality of medians chi-sq 86.38; p-value <0.001) between blood donors and HIV/TB patients. This corresponds to the p values obtained in a Meta analytical study where the medians of TB patients were compared to matched healthy controls. A significant difference in medians between the healthy controls and TB patients were found,and after testing for the equality of medians p values were found to be less than 0.05. This meta analytical studied concluded that the

probability that a healthy individual would have higher serum vitamin D level than an individual with tuberculosis if both were chosen at random from a population was 70%(26).

Using the reference range suggested by Holick and WHO (30) blood donors in this study who had hypovitaminosis D were 63.8% (90/141) and sufficient levels were found in only 30.5%(43/141). This is similar to a study done in Hawaii where 93 subjects (63 male and 30 female) were recruited to the study. Mean age of the Hawaiian participants was 24(mean age of donors was 22). Applying a cut point of 75nmol, 51% (47 of 93) of these subjects had low vitamin D status. The highest serum 25 (OH) D concentration observed was 155 nmol/L.

Kagotho et al(32)found among 258 male and femaleblood donors at Aga Khan University Hospital in Nairobi 17.4% were VDD.She noted that males were less likely to have VDD as compared to females and explained this could be due to differences in clothing styles between two.

This study confirms previous studiesthat patients presenting with active TB have significantly lower mean concentrations of serum 25-OH where majority had vitamin D deficiency 57.0% (69/121) and insufficiency 37.2% (45/121) using WHO reference values.
In a case control study done in Guinea Bissau (33)comparing TB patients with healthy controls hypovitaminosis D was found to be in 46% (167/362) of the TB patients and in 39% (193/494) of the healthy controls. Hypovitaminosis was defined as 25(OH) <50nmol/ L which is similar to the WHO definition of VDD. Hypovitaminosis D was highly prevalent among TB patients and healthy controls; Hypovitaminosis D was more frequent among the TB patient which is concordance to this study at 57%. Hypovitaminosis D in the blood donors was 8% vs.39% in the healthy control subjects in Bissau study which was a disparity. Perhaps this could be due to the controls used in the Bissau study were contacts of the patients thus if it was a nutritional deficit the contacts would also be deficient.

In a study done by Kibirige et al(34)where HIV/TB co-infected patients had VDD, VDI, SVDD and very SVDD 44.2%, 23.5%, 13.5% and 4.2% resp. which is in concordance in with findings in this study where most co-infected patients were deficient 57%(69/121)and insufficient

37.2%(45/121).No patients in this studywere found to have severe and very severe deficiency. This perhaps could be due to the patients who were recruited to the Mulago study who were all in-patients thus more ill corresponding to lower serum vitamin D levels due to perhaps the acute phase response to infection.

In a 22-multicenter study done in Canada and United States of America, a markedly high prevalence of vitamin D insufficiency was seen in the cohort of patients with active pulmonary tuberculosis, where 86% of the study subjects with measured concentrations of serum 25(OH)D <75 nmol/L. These findings echo the results in this study where among the patients' the majority had vitamin D deficiency (57.0%) and insufficiency (37.2%).

Most patients were in the intensive phase (92/141) of treatment versus the continuation phase (28/121). Vitamin D deficiency was more frequent 68.5% (68/92) among patients in the intensive phase of treatment for tuberculosis as compared to the continuation phase where 57.1% (16/28)had insufficient levels. These findings correspond to a study done in Malawi (35) where patients' serum vitamin D levels were followed up from diagnosis of Tuberculosis to treatment. Trends in serum 25 (OH) D concentrations over time were assessed for 133 patients who reached a final outcome. Median serum 25(OH) D rose to 62 nmol/L by week 8 of treatment and 64 nmol/L by end of treatment. This occurred despite daily administration of RMP and INH to all patients and increased use of ART by HIV-infected participants.

Vitamin D plays an important role in activation of 1 α-hydroxylase to convert 25(OH) D to its active form [1, 25 (OH) 2D] that leads to expression of cathelicidin, a microbicidal peptide which has been shown to kill intracellular *M.Tuberculosis*. Serum levels >75 nmol/L provide an adequate substrate for activation of 1 α-hydroxylase to convert 25(OH) D to its active form [1, 25 (OH) 2D] that leads to expression of cathelicidin, a microbicidal peptide for *Mycobacterium tuberculosis*. Serum levels <50nmol/L are said to impair the macrophage-initiated innate immune response thus causing individuals to be more susceptible to infection.(14).

The significance of an association between vitamin D deficiency and tuberculosis is 2-fold. First, already low vitamin D levels in tuberculosis patients may fall further on commencement of treatment. Isoniazid (INH) inhibits both hydroxylation steps of Vitamin D cutaneous synthesized into 25(OH) and 1,25(OH)D. Rifampicin induces alternative enzyme activity to degrade

25(OH)D into a waste product. Combined rifampicin and isoniazid treatment may reduce serum concentrations of useful vitamin D metabolites by 23–34%. In addition, efavirenz an antiretroviral drug is also known to lower vitamin D levels. It induces the cytochrome P450 pathway which causes 1,25OH and 25OH to be inactivated to their inactive metabolites. Also, efavirenz has been said to induce the cytochrome CYPR21 which hydroxylates D3 and D2 which are needed for Vitamin D activation (36).

The reference interval for serum Vitamin D level in this study was 42.6-96.7nmol/L based on X±1.96SD.Mean was 69.6(±)nmol/L. Based on this reference 10/121 patients had insufficient vitamin D and the prevalence of VDD was 8.3%. A chi-square test done to evaluate the association between HIV-TB co-infection and serum vitamin D status was significant (P 0.002).

A reference study (37) was done between November and July in Northern Germany conducted with samples from apparently healthy individuals of Caucasian heritage where the age range was 20-77 years. The number of males in the study was 201, the mean serum vitamin D was 48.5nmol/L and reference interval determined to be 12.3-107nmol/L using percentiles (2.5-97.5%). The lower limits of the reference limits in the Germany study were much lower compared to the blood donors in this study (42.6-96.7nmol/L). A reason could be that the study period in Germany was winter time thus less sun exposure. Another reason could be the study participants age was 20-77years.Cutaneous synthesis of Vitamin D is known to decrease with age.

The reference interval determined in this study(42.6-96.7nmol/L) was lower than the WHO reference interval for vitamin D. Vitamin D deficiency according to WHO are serum Vitamin D levels less <50nmol/L,insufficiency as 52.5-72.5 nmol/L and optimal levels as >75nmol/L.

Conclusion.

1. HIV/TB co-infected patients have a lower serum vitamin D levels as compared to blood donors (57% vs.5.7% as per WHO reference range).
2. Prevalence of VDD is high among HIV/TB co-infected patients (57% as per WHO reference range).
3. Using the reference interval determined in this study the prevalence of VDD was 8.3% in the HIV/TB co-infected patients.
4. Serum vitamin D reference interval among blood donors was lower than the manufacturers insert reference values.
5. HIV/TB co-infected patients in the intensive phase have lower Vitamin D than in continuation (68.5% vs. 21.4%)

Study Limitations

1. This study was not a matched case-control design, which impedes strong conclusions when comparing TB patients and the random population sample.

Recommendations

1. Patients with Human immunodeficiency virus co-infected with Tuberculosis should have their serum vitamin D measured.
2. Reference intervals obtained in this study should be used in the KNH laboratory
3. Every laboratory is encouraged to establish reference intervals for serum Vitamin D.
4. Further study to establish serum vitamin D reference interval in females is recommended.
5. Vitamin D supplementation is recommended based on clinical correlation and laboratory investigation.

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APPENDICES

1. Appendix 1- Consent information and consent form
2. Appendix 2-Questionnaire for participants
3. Standard operating procedure for blood collection
4. Appendix 4-Laboratory procedure for vitamin D.

APPENDIX 1

CONSENT INFORMATION AND CONSENT FORM

Informed consent form male patients and male blood donors. The title of the research is serum vitamin D and calcium levels in male blood donors and HIV/TB co infected male patients.

Name of Principal Investigator-Dr.Caroline Njeru

Name of Organization-University of Nairobi

This Informed Consent Form has two parts:

- Information Sheet (to share information about the research with you)
- Certificate of Consent (for signatures if you agree to take part)

You will be given a copy of the full Informed Consent Form

PART I: Information Sheet

Introduction and purpose of study

I am doing a study on serum vitamin D, to enable a reference range to be established and also to improve treatment modalities in patients with Tuberculosis. I am a postgraduate student at the University of Nairobi doing masters in human pathology.

You do not have to decide today whether or not you will participate in the research. You can talk to anyone you feel comfortable with before deciding to participate. If you have questions later, you can ask me or the nurses.

Vitamin D levels will be assayed in blood. One blood sample of 4mls will be taken. Your participation in this research is entirely voluntary. Whether you choose to participate or not, all services you receive at this clinic will continue. Nothing will change. If you decide to participate, the results from the tests shall be communicated to the physicians in this clinic and treatment modalities initiated if needed.

If you decide not to participate later after your blood sample has been drawn, the sample shall not be run. Services you receive in this clinic will continue normally.

Number of participants

240 male patients will be involved in this study.

Period of Participation

Only one sample will be taken from the participant. No repeat samples will be taken.

SPECIMEN COLLECTION

One blood sample will be taken from your arm using a syringe and needle.4ml of blood will be drawn only once tea spoonful is the equivalent of 4ml.The procedure will last less than 5minutes.The blood will be tested at the University of Nairobi clinical chemistry unit. The sample will be stored for the period of the study and the sample shall be destroyed after the study is concluded. The sample shall not be used for any other study other than this study.

Confidentiality

Patient information will be stored securely and will not be available to any other researcher. A unique number will be given to each sample to enable tracking of samples. No names will be used on samples.

Results will be communicated back to the clinic or patient. If any pathological values are noted the patient will be notified for corrective action to be taken.

Risks

Side effects of the blood collection are-some pain during the collection, temporary swelling may occur at the area of collection.

Benefits

The participants will get to know their serum vitamin D, calcium and albumin levels.

Who to Contact

In case of any questions, please contact

Dr.C.Njeru

0726 247 829

Dr.Kuria

0721 570 812

This proposal has been reviewed and approved by 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.

PART II: Certificate of Consent

I have read the foregoing information, or it has been read to me. I have had the opportunity to ask questions about it and any questions that I have asked been answered to my satisfaction. I consent voluntarily to participate as a participant in this research.

Print Name of Participant_____

Signature of Participant _____

Date _____
Day/month/year

I have witnessed the accurate reading of the consent form to the potential participant, and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely.

Print name of witness_____

Signature of witness _____

Date _____
Day/month/year

Statement by the researcher/person taking consent

I have accurately read out the information sheet to the potential participant, and to the best of my ability made sure that the participant understands it.I confirm that the participant was given an opportunity to ask questions about the study, and all the questions asked by the participant have been answered correctly and to the best of my ability. I confirm that the individual has not been coerced into giving consent, and the consent has been given freely and voluntarily.

A copy of this Informed Consent Form has been provided to the participant.

Print Name of Researcher/person taking the consent_____

Signature of Researcher /person taking the consent_____

Date _____

Day/month/year

Thumb print of participant

_____

Who to Contact

In case of any questions, please contact

Dr.C.Njeru

0726 247 829

Dr.Kuria

0721 570 812

This proposal has been reviewed and approved by 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 2

QUESTIONNAIRE SERUM VITAMIN D LEVELS IN MALE BLOOD DONORS AND HIV/TB CO INFECTED MALE PATIENTS

DATE …../……/…….

STUDY NUMBER ……

SOCIAL DEMOGRAPHIC CHARACTERISTICS

Age……..Years

Occupation……..

Education

1. None
2. Primary
3. Secondary
4. Tertiary
5. Others

Marital status

1. Single
2. Married
3. Divorced
4. Widowed

Medical History

1. When were you diagnosed with HIV?.....weeks…..Months……years

2. How long have you had HIV for ……? Months/……..Years

3. How long have you had TB for ……..Months/……..Weeks

Do you have any other medical conditions?

1.
2.
3.
4.

Medication

HAART

1.
2.
3.
4.

TB Phase of treatment

A. Intensive phase

B. Continuation phase

DRUGS

1.
2.
3.
4.
5.
6.
7.

MWISHO 2

TAARIFA KUHUSU IDHINI NAFOMU YA IDHINI

Taarifa ya idhini iliyoko katika fomu ya idhini kwa wagonjwa wanaume na wanaume wanaotoa damu.Kichwa cha Utafiti huu ni viwango vya Chembechembe za damu (serum) vitamini D, katika damu ya wanaume wanaotoa damu na wale wanaoshi na virusi vya ukimwi na Ungonjwa wa kifua kikuu (TB).

Jina la mtafiti Mkuu-Daktari.Caroline Njeru

Jina la shirika –Chuo kikuu cha Nairobi

Hii fomu ya taarifa ya idhini ipo na sehemu mbili:

- Karatasi ya taarifa (Kuhusu kukupa taarifa kuhusu utafiti huu)
- Cheti cha idhini (ya sahihi iwapo utakubali kushiriki)

Utapokea nakala ya fomu ya idhini.

Sehemu ya kwanza : Taarifa

Utangulizi na lengo la utafiti

Ninafanya utafiti kuhusu viwango vya chembechembe za serum vitamin D, kusaidia kutambua njia mbalimbali za kuboresha matibabu kwa wagonjwa walio na ugonjwa wa kifua kikuu. Mimi ni mwanafunzi wa uzamili(postgraduate) katika chuo kikuu cha Nairobi nikisomea shahada ya masters ya pathologia kwa binadamu(human pathology) .

Sio lazima uamue kushiriki leo au kama hautashiriki katika utafiti huu. Unaweza kumuongelesha yeyote ambaye unaona anafaa kabla ya kuamua kushiriki. Iwapo una maswali baadaye, unaweza kuniiliza mimi au wauguzi.

Viwango vya vitamin D vitatambuliwa katika damu. Kiwango cha 4mls cha damu kitachukuliwa. Kushiriki kwako katika utafiti huu ni kwa kujitolea.Endapo utakubali kushiriki au la, huduma zote utapokea katika kituo hiki zitaendelea.Hakuna chochote kitabadilika.Ukiamua kushiriki , matokeo utayapokea kutoka kwa daktari wa kliniki hii na matibabu kuanzishwa kama kuna hitaji.

Ukiamua kutoshiriki baadaye baada ya damu yako kuchukuliwa, damu yako haitatumiwa .Huduma unazopokea katika kituo hiki zitaendelea kwa kawaida

Nambari ya wahusika

Wanaume mia mbili na arubaini watashiriki katika utafiti huu.

Sampuli moja pekee itachukuliwa kwa mshiriki. Hakuna Kuchukua sampuli nyingine.

Damu kutolewa

Sampuli moja ya damu itatolewa kutumia sindano kutoka kwa mkono wako .Kiwango cha damu cha 4ml kitatolewa .Kijiko kimoja cha chai kinalingana na 4ml.Kazi hii itachukua muda wa chini ya dakika tano.

Damu itapelekwa chuo kikuu cha Nairobi kitengo cha kliniki ya chemia.(chemist).Sampuli itawekwa kwa muda ambao utafiti unaendelea na kuharibiwa baada ya utafiti kumalizika.Sampuli hiyo haitatumika kwa utafiti mwingine.

Usiri

Taarifa kuhusu mgonjwa itawekwa vyema na haitatumiwa na mtafiti mwingine. Nambari ya kipekee itapeanwa hurahisisha kutambua sampuli za damu.Hakuna kutumia majina kwa sampuli hizo.

Matokeo yatawasilishwa kwa kliniki au mgonjwa. Endapo kuna shida Fulani zitapatika katika damu ,mgonjwa ataelezwa na hatua dhibiti kuchukuliwa.

Hatari

Madhara kutokana na kutoa damu ni kama kuhisi uchungu kidogo wakati wa damu kutolewa , kuvimba kwa muda kunaweza kutokea katika sehemu ambayo damu imetolewa.

Manufaa

Mshiriki ataweza kufahamu chembechembe za Serum vitamin D, Calcium na viwango vya albumin.

Wa kuwasiliana naye

Kwa maswali yeyote, tafadhali wasiliana na

Daktari.C.Njeru

0726 247 829

Dr.Kuria

0721 570 812

Pendekezo hili limedhibitishwa na kuangaliwa na Hospitali kuu Ya Kenyatta/Chuo kikuu cha Nairobi –kamati ya maadili na utafiti (KNH/UON-ERC), ambayo ni kamati yenye kazi ya kuhakikisha kuwa wanaohusika katika utafiti wamezuiwa kutokana na madhara.Nambari ya simu (254-020) 2726300 Ext 44355

Sehemu ya pili: Cheti cha Idhini

Nimesoma habari hii, au nimesomewa.Nimeweza kupata muda wa kuuliza maswali kuihusu na maswali ambayo nimeuliza yamejibiwa vyema.Ninakubali Kushiriki katika utafiti huu.

Jina la mshiriki_____

Sahihi ya mshiriki _____

Tarehe _____
siku/mwezi/mwaka

Nimeshuhudia kusomwa kwa fomu ya idhini kwa mshiriki wa utafiti huu, na mshiriki amepata nafasi ya kuuliza maswali.Nadhibitisha mshiriki amepeana idhini kwa hiari yake.

Jina la shahidi_____

Sahihi ya shahidi _____

Tarehe_____
Siku/Mwezi/mwaka

Kutoka kwa mtafiti/anayechukua idhini

Nimesoma vyema taarifa kwa mshiriki mtarajiwa .Nimehakikisha kwa uwezo wangu kuwa mshiriki ameweza kuielewa. Nadhibitisha kuwa mshiriki amepewa nafasi ya kuuliza maswali kuhusu utafiti , na maswali yamejibiwa kulingana na uwezo wangu.Nadhibitisha mhusika hajachanganywa kwa kupeana idhini, na amepeana idhini bure na kwa kujitolea

Nakala hii ya idhini imepeanwa kwa mshiriki.

Jina la mtafiiti/Jina la anayechukua idhini_____

Sahihi ya mtafiti /anayechukua idhini_____

Tarehe_____

Siku/mwezi/mwaka

Alama ya kidole ya mshiriki

_____

Wa kuwasiliana naye

Kwa maswali yeyote, tafadhali wasiliana na

Daktari.C.Njeru

0726 247 829

Dr.Kuria

0721 570 812

Pendekezo hili limedhibitishwa na kuangaliwa na Hospitali kuu Ya Kenyatta/Chuo kikuu cha Nairobi –kamati ya maadili na utafiti (KNH/UON-ERC), ambayo ni kamati yenye kazi ya kuhakikisha kuwa wanaohusika katika utafiti wamezuiwa kutokana na madhara.Nambari ya simu (254-020) 2726300 Ext 44355

Mwisho 2

Hojajaji ya viwango vya Serum Vitamini D katika damu ya wanaume ambao hutoa damu na wanaoshi na virusi vya ukimwi /Ungonjwa wa kifua kikuu (TB) wagonjwa wanaume walioshirikiana maambukizi .

ufupisho ……/……./…….

Tarehe …../……/…….

Nambari ya utafiti ……

Kuhusu mshiriki

miaka……..mwaka

Kazi……..

Masomo

1. Hakuna
2. shule ya msingi
3. Shule ya upili
4. Chuo kikuu
5. Zingine

Hali ya ndoa

1. Pekee
2. Ameowa/ameolewa
3. Amepewa talaka
4. Amefiwa mume au mke

Historia ya matibabu

1. Uligundua unaugua virusi vya ukimwi lini?

2. Umeishi na virusi vya ukimwi kwa muda wa …… Miezi/……..Miaka

3. Umeishi na ugonjwa wakifua kikuu kwa muda gani ……..Miezi/……..Wiki/

Una shida ingine yeyote ya kiafya?

1.
2.
3.
4.

Matibabu

HAART

1.
2.
3.
4.

Sehemu ya matibabu ya kifua kikuu

A. Sehemu ya matibabuya kuanzia(Intensive Phase)

B. Sehemu ya kuendelea(Continuation phase)

Dawa

1.
2.
3.
4.
5.
6.
7.

APPENDIX 3

STANDARD OPERATING PROCEDURE FOR BLOOD EXTRACTION

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 centre (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.

SERUM VITAMIN DLEVELS IN MALE BLOOD DONORS AND HUMAN IMMUNODEFICIENCY VIRUS AND TUBERCULOSIS COINFECTED MALE PATIENTS: A COMPARATIVE STUDY

BY

CAROLINE W.NJERU

A dissertation in partial fulfillment of the Masters in Medicine (Mimed) degree in the department of Human Pathology in the University of Nairobi

DECLARATION

I, CAROLINE W. NJERU, declare that this dissertation is my original work under the guidance of the supervisors named below and has not been submitted to the University of Nairobi or any institute of higher learning

Signature: _____ Date_____

SUPERVISOR APPROPVAL

This proposal has been developed under our guidance and approval as University supervisors

1. Prof. Christine Kigondu
   Associate Professor, Unit of Clinical Chemistry
   Department of Human Pathology,
   College of Health Sciences,
   University of Nairobi.

   Signature_____ Date _____

2. Dr. Julius Kuria

   MbCHB, Mimed Path (Pathology) UON

   Department of Clinical Chemistry

   College of Health Sciences

   University of Nairobi.

   Signature_____ Date _____

ACKNOWLEDGEMENT

I would like to thank my supervisors Prof. C.Kigondu and Dr.J.Kuria for their guidance in the development and writing of this dissertation.

My sincere gratitude to Prof A.Amayo for her input and advice .I thank my classmates for their immense support.

I appreciate the technical work done by Mr. Leonard Bosco and Mr.Kipmengich.

DEDICATION

I dedicate this work to my parents and Karimi.

LIST OF ABBREVIATIONS

1, 25(OH) D ..... 1, 25 dihydroxyvitamin D

25(OH) D ..... 25 hydroxyvitamin D

BMI ..... Basal Metabolic index

CCC ..... Comprehensive Care Clinic

CIMA ..... Chemiluminescent immunoassay

D_2 ..... Ergocalciferol

D_3 ..... Cholecalciferol

DNA .....Deoxyribonucleic acid

ECLIA .....Electro-chemiluminescent immunoassay

HBV .....Hepatitis B Virus

HCV ..... Hepatitis C virus

HIV/TB .....Human Immunodeficiency deficiency virus /Tuberculosis

GIT ..... Gastrointestinal tract

IU ..... International units

INH .....Isoniazid

KNH ..... Kenyatta National Hospital

LL-37 ..... Cathelicidin

MDR-TB .....Multiple drug resistant-Tuberculosis

MTB .....Mycobacterium Tuberculosis

NIH ..... National institute of Health

NIST .....National Institute of Technology

PTB .....Pulmonary Tuberculosis

RIA..... Radioimmunoassay

RR ..... Reference range

RMP ..... Rifampicin

SD .....Standard deviation

STATA .....Statistics and data

SVDD..... Severe Vitamin D Deficiency

TLR .....Toll like receptor

TTI .....Transfusion transmittable infection

UoN.....University of Nairobi

UV..... Ultraviolet light

VitD **.....**Vitamin D

VDBR .....Vitamin D Binding Protein

VDD.....Vitamin D Deficiency

VDR .....Vitamin D receptor

VDSP .....Vitamin D standardization program

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ABSTRACT

Introduction

Vitamin D plays a role in the immune function and itsdeficiency is associated with higher incidence of immune system disorders and faster progression of some infectious diseases. Tuberculosis is a major cause of death among people living with Human Immunodeficiency Virus/Acquired Immunodeficiency Syndrome. Evidence that vitamin D protects against tuberculosis has been supported by in vitro, epidemiological and some preliminary clinical studies.

Broad objective:

To compare vitamin D levels in healthy males and Human Immunodeficiency Virus and Tuberculosis co-infected males.

Specific objectives:

1. To compare vitamin D levels in male blood donors and malesco-infected with Human Immunodeficiency Virus patients and Tuberculosis.
2. To determine the prevalence of vitamin D deficiency in patients co-infected with Human Immunodeficiency virus and Tuberculosis.
3. To determine the reference interval for vitamin D in blood donors.

Methodology;

Study Design

This was a cross-sectional study, prospective descriptive study.

Study Area

Study area was Mbagathi District Hospital Tuberculosis clinic.

Study Population and Sample Size

Human immunodeficiency virus and tuberculosis co infected males and male blood donors were recruited to the study after giving their informed consent.

Sample size was 240.

Male blood donors were 120 and 120 male HIV/TB male patients.

Specimen Analysis

Vitamin D was run on the Cobas platform.

Data Analysis

Data was entered and stored using Microsoft excel 2013. Data was imported using STATA 13, coded, cleaned and analyzed.

Numeric (continuous/categorical) data were summarized using measures of central tendency and dispersion; summaries were presented in tables. Histograms were plotted to show distributions. Pearson correlation tests were used to evaluate the linear relationship between variables. Pearson correlation statistic and corresponding p-values was reported.

Two independent sample t-tests were used to compare the means of serum vitamin D between the two populations. The t statistics with corresponding p-values were reported.

Reference interval determination was done using male blood donors. The lower and upper reference limits of vitamin D levels will be obtained by X/±1.96 SD.

Ethical Consideration

Ethical approval was sought from the Kenyatta National Hospital Data /University of Nairobi ethical review committee before carrying out this research.

Results

The distribution of serum vitamin D was significantly different (K-sample test for equality of medians: chi-sq=86.38; p-value<0.001) between blood donors and HIV/TB patients. Among blood donors, serum vitamin D level ranged from 42.6nmol/L to 106.7 nmol/L. Median 68.7 and mean was 69.6(±)nmol/L. For HIV/TB patients' Vitamin D levels ranged between 33.9-89.8 median 44.0 and mean 44.3(±) nmol/L.

Out of 121 HIV/TB co-infected patients had 69 vitamin D deficiency (57.0%) and 49/121 had insufficiency (37.2%) based on the WHO reference interval.

Out of 121 patients, 75.2% (91) were on the intensive phase of treatment tuberculosis and 23.1% (28) in the continuation phase. Among the patients' majority Vitamin D deficiency was more frequent (68.5%) among patients in the intensive tuberculosis compared to those in the continuation phase. There was a significant association between tuberculosis phase and vitamin D status of the patient (chi-sq=27.67; p<0.001).

Based on the reference interval obtained in this study, 10/121 HIV/TB patients had deficient vitamin D. Prevalence of vitamin D deficiency among these patients was 8.3%. Achi-square test done to evaluate the association between HIV-TB co-infection and serum vitamin D status was significant (P value 0.002).

Conclusion

HIV/TB co-infected patients have a lower serum vitamin D levels as compared to blood donors (57% vs.5.7% as per WHO reference range). Prevalence of VDD is high among HIV/TB co-infected patients (57% as per WHO reference range). Using the reference interval determined in this study the prevalence of VDD was 8.3% in the HIV/TB co-infected patients. Serum vitamin D reference interval among blood donors was lower than WHO reference values. Co-infected patients in the intensive phase have lower Vitamin D than in those in the continuation phase (68.5% vs. 21.4%).

Recommendations

1. Patients with Human immunodeficiency virus co-infected with Tuberculosis should have their serum vitamin D measured.
2. Reference intervals obtained in this study should be used in the KNH laboratory.
3. Every laboratory is encouraged to establish reference intervals for serum Vitamin D.
4. Further study to establish serum vitamin D reference interval in females is recommended.

CHAPTER ONE

1.0 Introduction

Vitamin D is hormone of great physiological importance; it is essential micronutrient for the bone mineralization. In the immune system Vitamin D has been shown to activate macrophages and restricts intracellular Mycobacterium tuberculosis growth by up regulatingcathelicidins(1). Vitamin D has a potential effect on HIV (Human immunodeficiency Virus) for it influences the immune response to Tuberculosis, a common cause of morbidity and mortality in this group of patients. Deficiency has been associated with higher incidence of tuberculosis and faster progression of disease.

Tuberculosis (TB) now ranks alongside HIV as a leading cause of death .Worldwide 9.6 million people are estimated to have fallen ill with TB in 2014. Globally 12% of the 9.6million new TB cases were HIV positive.(2) Kenya reported 120,000 new tuberculosis cases in 2013(2).The emergence of drug resistant TB and HIV (Human Immunodeficiency Virus) co infection has challenged TB eradication programs worldwide.

Increased tuberculosis risk in vitamin D deficient individuals has been noted. High rates of Mycobacterium tuberculosis (MTB) infection and VDD have been reported in African immigrants in Australia, the United States, and Europe. The association between hypovitaminosis D and TB has been described in several case-control studies(3) .Investing in supplementation in poor settings such as sub Saharan Africa where HIV/TB co infection rates are high could be a cost-effective approach towards the eradication of TB morbidity and mortality.

CHAPTER TWO

2.0LITERATURE REVIEW

2.1 Vitamin D

Vitamin D (1,25dihydroxyvitamin D)is a fat-soluble vitamin that functions like a hormone. It is naturally found in fish-liver oils, fatty fish, mushrooms, egg yolks, and liver. The two major physiologically relevant forms are D2 (Ergocalciferol) and D3 (cholecalciferol). Ergocalciferol is synthesized by molds and yeasts by solar ultraviolet (UV) irradiation of ergosterol. Cutaneous synthesis of vitamin D occurs by the action of UV B wavelength 280-315nm on 7-dehydrocholesterol. Sun exposure between 10:00 am and 3:00 pm produces vitamin D in the skin that lasts twice as long in the blood compared with ingested vitamin D (3). Sun exposure that produces slight pinkness of the skin is equivalent to ingesting 10,000-25,000 IU (4). Increased skin pigmentation, aging, and the use of sunscreen decrease skin vitamin D production.

Vitamin D_2 and D_3 are biologically inactive and are transformed into the biologically active 1,25dihydroxyvitamin D. After being synthesized in the skin or absorbedfrom the gastrointestinal tract, most vitamin D is bound to vitamin D–binding protein (VDBP) and albumin and transported to the liver. In the liver, it undergoes two hydroxylations. It is hydroxylated by the enzyme 25-hydroxylase into 25-hydroxyvitamin D, the primary circulating form of vitamin D. From the liver, 25(OH) D is transported to the kidneys by carrier proteins mentioned above.

1, 25 dihydroxyvitamin D (1, 25(OH) D) is formed when 25(OH) D is hydroxylated by the enzyme 1α-hydroxylase, located in the mitochondria of proximal tubules of the kidney. Hepatic synthesis of 25(OH) D is loosely regulated, and blood levels reflect cutaneous and ingested levels of vitamin D .Activity of 1-alpha-hydroxylase is tightly regulated and is the major control point in production of the active hormone.

Synthesis of 1, 25(OH) 2 D is regulated by parathyroid hormone (PTH), serum calcium and phosphate. Increased levels of PTH and hypophosphatemia stimulate the enzyme 1α-hydroxylase which increases synthesis of 1, 25(OH) 2 D(4).

2.1.1 The Vitamin D Receptor and Mechanism of Action

The active form of vitamin D binds to intracellular receptors that then function as transcription factors to modulate gene expression. The vitamin D receptor(VDR) binds several forms of cholecalciferol. Its affinity for 1, 25(OH) 2 is roughly 1000 times that for 25OH, explaining their relative biological potencies.

The identification of VDRs in various cells has prompted the investigation of vitamin D in immunomodulation, cancer prevention and therapy, autoimmune disease and cardiovascular disease(5).

2.1.2 Physiological Effects of Vitamin D

Vitamin D is involved in mineral metabolism and bone growth.

In the small intestine, it facilitates absorption of calcium and stimulates absorption of phosphate and magnesium ions.

Vitamin D suppresses PTH production, decreases renal excretion of both calcium and phosphate.

2.2Vitamin D deficiency and Vitamin D reference range

2.2.1. Vitamin D Deficiency

Vitamin D deficiency in children is known as rickets and osteomalacia in adults. Genetic forms of rickets occur due to defects in the metabolism of vitamin D. Vitamin D–dependent rickets type I occurs because of a defect in the renal 1 alpha-hydroxylase. Vitamin D–dependent rickets type II occurs when a mutation exists in the VDR.

The daily maintenance dose of vitamin D varies by age, but most children and adults generally require 600-2000 IU of vitamin D daily. Higher dosesare recommended for deficient individuals.

Screening for vitamin D deficiency is recommended only in individuals who are at high risk for vitamin D deficiency such as patients who have; chronic kidney disease, osteoporosis, malabsorption syndrome and obese individuals.

Vitamin D deficiency can result from the following:
•Inadequate sunexposure.
•Vitamin D_2 malabsorption problems – Patients who have had small intestine resections, celiac sprue patients, short bowel syndrome sufferers, and cystic fibrosis.
•Exclusive breastfeeding - The American Academy of Pediatrics recommends supplementation starting at age 2 months for exclusively breast fed infants(6).
•Medications - phenobarbital, isoniazid, and rifampin can induce hepatic p450 enzymes to accelerate the catabolism of vitamin D (7). Efavirenz an antiretroviral drug is also associated with vitamin D deficiency.

Cutaneous vitamin D declines with advancing age, making elderly populations more dependent on dietary or supplemental vitamin D. Higher supplemental intakemay be required in elderly individuals to achieve optimal serum levels of D due to both reducedcutaneous and dietary absorption(8).

2.2.2 Vitamin D reference range

Currently controversy remains on recommended levels of vitamin D. Most laboratories agree that 75nMol/L (30ng/mL) is considered to be the optimal level, while individuals with lower concentrations are considered to be vitamin D insufficient or deficient. (9)

Many experts agree that Vitamin D reference ranges vary in different laboratories depending on what platform is used. Each laboratory is encouraged to establish its own reference interval for all analytes for the population they serve in addition to using a standard reference material. Although there is no formal definition of vitamin D deficiency, some groups including the National Institute of Health in the United States of America use the following values in adults:

	Cobas	WHO/HOLICK	NIST	IOM	RIA
Deficiency	<50	<50	<25	<24	<20
Insufficiency		52.5-72.5	<25-74	24-47.5	21-39
Optimal	>75	>75	75-250	50-125	40-195
Toxicity			>250	200	

Table 1: Serum 25-Hydroxyvitamin D [25(OH) D] Concentrations inHealth (10)in nanomoles per liter (nmol/L).

2.3Measurement of Vitamin D and Standardization

Different methods are used to measure vitamin D in blood.

1. Competitive protein binding,
2. Immunoassays which include enzyme immunoassays and radio immunoassays,
3. High performance liquid chromatography and
4. Liquid chromatographic separation followed by tandem mass spectrometric detection (LCMS/MS).

Liquid chromatography tandem mass spectrometry has the highest sensitivity and is considered the gold standard method. It is an expensive procedure and not routinely used. It is mostly used in research laboratories.

Steps towards vitamin D standardization were initiated by National Institute of Health, which created the Vitamin D Standardization Program(VDSP) in Nov 2010.The goal of the VDSP is to promote standardized laboratory measurement of 25(OH) to improve comparability of test results between laboratories and minimize variability in patient testing. Reference measurement procedures were developed by National Institute of Standards and Technology (NIST) and Ghent University. They are the “gold standard” laboratory procedures for measuring 25(OH) D.
The reference materials for 25(OH) D permit standardization of values in laboratories and improve method-related variability (11).

2.4Studies on Vitamin D

Vitamin D Reference Interval in Dark Skinned Individuals

Dark skin interferes with the cutaneous synthesis of vitamin D. Holick et aldemonstrated non-Hispanic black subjects require 6 times the amount of UV radiation necessary to produce a serum vitamin D concentration similar to that found in non-Hispanic white subjects(7). This is because melanin absorbs ultraviolet radiation thus the increased radiation needed. Decreased vitamin D production by dark-pigmented skin explains the higher prevalence of vitamin D insufficiency among darker-skinned adults. Dawson-Hughes and colleagues demonstrated that in Boston, 73% of elderly black subjects were vitamin D insufficient, compared with 35% of elderly non-Hispanic whites (7).

In a large survey of 1500 healthy African American and white women of reproductive(30-50years) carried out by the National Health and Nutrition Examination in 1988-1994 in 81 counties across the US, 40% of the African American women were vitamin Ddeficient (25[OH]D< 16ng/mL), compared with 4% of 1400 white women in that study(12).

Vitamin D has been shown to improve macrophage phagocytic capacity, cell-mediated immunity, and increase natural killer cell number suggesting an important role in response to infections (13).

In the presence of adequate 25(OH) D, VDR up regulation leads to cathelicidin induction, an antimicrobial peptide with direct action against intracellular pathogens such as MTB. Increased resistance to tuberculosis could potentially prolong survival in patients with HIVand slow HIV disease progression and preventing mortality(14).

Liu et al (2006) demonstrated MTB sensing by the Toll-like receptor 2/1 (TLR2/1) complex increases expression of VDRs in macrophages(15). Synthesis of 1, 25(OH) D promotes VDR-mediated transactivationof cathelicidin and killing of intracellular MTB. Cathelicidins have direct antimicrobial function in addition to anti-bacterial effects such as cationic membrane disruption.In vitro studies show macrophages are most efficient in producing cathelicidin (LL-

37) after infection with MTB, suggesting this antimicrobial peptide is important during mounting of the primary immune response towards MTB (7).

A further study by Liu (2006) demonstrated that transcriptional regulation of cathelicidin is be mediated by the active form of VitD. Stimulation of TLR receptors by microbial products results in increased production from the inactive form of the hormone to the active form(15).

Adams (2007) demonstrated TLR activation results in production of defensin-2(a antimicrobial peptide) and of cathelicidin: which are strongly up-regulated by 1, 25-hydroxyvitamin D(16).

Liu noted serum from donors with hypovitaminosis D had a low levels of LL-37 in macrophages compared to donors with normal vitamin D levels (15). Similar conclusions were made by Adams (16).

The role of VitD in the immune response to MTB is demonstrated in the production of the LL-37 and in promoting phagolysosome formation inmonocytes (17). Eun-Kyeong confirmed (2010) LL-37 plays an important role in innate immunity to mycobacterium and indirect immune modulation.(18).

Martineau (2011) observed that VDD was highly prevalent among black African adults living in Cape Town and was found to be associated with susceptibility to active TB in both the absence and the presence of HIV infection. The association was noted to be stronger in HIV-infected people. The study recommended testing for this group and supplementation for deficient individuals (19).

Gibney et al found a strong association between VDD and Latent Tuberculosis Infection (LTBI) in African immigrants in Melbourne (19). A further study, among sub-Saharan African immigrants in Melbourne, documented the frequency of both active and latent TB infection and the relationship with vitamin D deficiency (20).
A cohort follow-up study in Karachi, Pakistan, found thatdeficiency was associated with progression to active TB disease in healthy household contacts. Also noted wasa higher

susceptibility of women to the infection, which lead to little exposure to sunlight (21). A study among the Vietnamese populationfound hypovitaminosis Dstatus was an antecedent risk factor for TB(22).

A prospective cohort study conducted from 2009 to 2012 in Spain,Castellon to assess the relationship between serum baseline vitamin D status and the incidence of tuberculosis among contacts of PTB patients in Castellon, Spain. Mean vitamin D levels between the two populations was found to be 34.25nmol/L for cases and 64.25nmol/L for non-cases.Hypovitaminosis D showed a significant association with TB incidence. This result is in line with the hypothesis that vitamin D deficiency is associated with TB incidence(23).

Wilkinson et al (2000) in a case-control study in the Guajarati Indian population in NW London; Hypovitaminosis D was significantly associated with active TB disease. Undetectable vitamin levels carried the highest risk of contractingTB (24).

Iftikhar R et al in a case control study (2010-2012) in Kharian,found significant low Vitamin D levels in patients with TB as compared to controls. Deficiency was found to be more severe in females, individuals with low BMI, extra pulmonary and MDRTB(25).

In a meta analytical study published by Nnoahamthe association between low serum vitamin D and risk of tuberculosis was assessed. Findings from the meta-analysis were the probability of 70% that a healthy individual would have higher serum vitamin D level than an individual with TB if both were chosen at random from a population .Lower levels were associated with a higher risk of active TB. The study concluded that the potential role of vitamin D supplementation in people with tuberculosis and VDDassociated conditions like chronic kidney disease should be evaluated(26).

2 .4Study Rationale

Vitamin D testing has increased in the last few years worldwide and associations have been made with HIV, TBand HIV/TB co infection and several non-communicable diseases. Several publications have been made in the West documenting hypovitaminosis in apparently healthy population and in special groups. There is no consensus on what optimal vitamin D status is. There is need to establish a reference interval in the local population due to difference in skin color, geographicallatitude, age, gender and season. Each laboratory is encouraged to establish their own reference range for their local population. This study will establish distribution of vitamin D in the healthy population and HIV/TB co-infected individuals.

2.5Broad Objective

To compare vitamin D levels in male blood donors and male Human immunodeficiency virus co-infected with tuberculosis

2.6Specific Objectives

1. To compare vitamin D level in male blood donors and male Human immunodeficiency viruspatients co-infected with Tuberculosis.
2. To determine the prevalence of vitamin D deficiency in males co-infected with Human immunodeficiency virus and tuberculosis.
3. To determine the reference interval for vitamin D in blood donors.

Secondary Objective

1. To correlate serum Vitamin D level with phase of tuberculosis treatment.

CHAPTER THREE

3.0 STUDY DESIGN AND METHODOLOGY

3.1Study Design

A cross-sectional study was conducted with the aim of comparing the distribution of serum vitamin D among male blood donors and male HIV patients co-infected with Tuberculosis.

3.2 Study Area.

The study was conducted in Mbagathi District Hospital, in Nairobi County, Kibra District which offers integrated health services to nearly 9800 HIV positive patients. Participants were recruited consecutively until the sample size was achieved. The clinic was visited on daily until the sample size was achieved. The Mbagathi District Hospital is located on Mbagathi Way, Nairobi. The catchment area includes the Kibra slum, one of the largest informal settlements in Africa. The Tuberculosis clinic is open weekdays, Monday to Friday.

3.3 Study Population and Sampling Method, Recruitment and Consenting

Participants were recruited consecutively until the sample size was achieved. The clinic was visited daily until the sample size was achieved.

The Mbagathi District Hospital is located on Mbagathi Way, Nairobi. The catchment area includes the Kibra slum, one of the largest informal settlements in Africa. The Tuberculosis clinic is open weekdays, Monday to Friday.

Blood donors were recruited into the study at different sites, depending on where Kenya National Blood Transfusion Service conducted their blood drive. Their serum was used once it has been screened and cleared for Syphilis, HIV hepatitis B and C.

3.4Inclusion and Exclusion Criteria

Inclusion criteria for blood donors:

- Over 18 years of age
- Males who will Consent to participate in the study
- Male gender

Exclusion criteria for blood donors

- Transfusion Transmittable Illnesspositive
- Female gender

Inclusion criteria for HIV positive patients with TB

- Consent
- confirmed HIV + and TB +
- Age above 18 years
- Male
- Newly diagnosed TB or on treatment for TB

Exclusion criteria for HIV positive patients with TB

- Patients on VitD supplementation
- Female patients

3.5 Sample Size

Vitamin D levels are assumed to follow a normal distribution in the general population. Therefore, the mean best summarizes this distribution. The aim of this study was to compare the distribution of serum vitamin D levels between male blood donors and male HIV patients co-infected with TB. The sample size was determined using the formula for comparison of means by Kelsey et al., (1996)(27).

$$n \geq (\frac{r+1}{r}) \frac{\sigma^2 (Z_\beta + Z_{\alpha/2})^2}{(d\text{ifference})^2}$$

Where:

n_1 = minimum sample size among exposed

$n_2 = r * n_1$ i.e. sample size among non-exposed

$n = n_1 + n_2$ i.e. Total sample size

r = is the ratio of non-exposed (blood donors) to exposed (HIV patients) (r=1)

σ = standard deviation in the population (σ =13, because vitamin D is measured in nmol/L)

$\mathbf{Z_\beta}$ =is the critical value for the desired power (Type II error β = 0.2, $\mathbf{Z_\beta}$ =0.84)

$\mathbf{Z_{\alpha/2}}$= is the critical value for standard normal distribution at α-level of significance (Type I error α= 0.05, $\mathbf{Z_{\alpha/2}}$=1.96)
Difference = expected effect size (the difference in means = 5)

Using this formula, the estimated minimum sample size for blood donors was105 and 105 for HIV male patients.

According to Tietz Fundamentals of Clinical Chemistryand Molecular Diagnostics for the estimation of a reference interval,40 values are sufficient, but at least 120 reference values are required to obtain reliable estimates (28).

Sample size was 240, 120 for male blood donors and 120 for patients co-infected with HIV/TB.

3.6Sample collection

HIV/TB CO-INFECTED PATIENTS

Four milliliters of blood were collected by the principal investigator or research assistant after consent is obtained in a red top serum separator tube.

BLOOD DONORS

For the blood donors sample collection was done during donation of blood after consent has been obtained. The sample was taken during cannulation of the donor.
The samples from the donors were assayed after screening for transfusion transmittable infections which was done at the National Blood Transfusion Unit.

3.7 Sample Transport

The blood was allowed to clot at room temperature. The sample was transported to theKenyattaNational Hospital Clinical Chemistry unit in a cool box where it was centrifuged 2000 rpm to separate serum from the cells.
The serum was stored at negative 20 degrees Celsius and batched.
Patient information wasentered into a questionnaire that did not bear the name of the patient.
Vitamin D was run on the Cobas 600 platform which is an electro-chemiluminscent microparticle immunoassay.

25(OH) D serum levels were measured for it is considered as the best indicator of vitamin D supply to the body. The circulating half-life of 25(OH) D is 2 weeks while that of 1, 25(OH) D is 4hrs.

3.8 Data Management and Analysis

Data was entered and stored using Microsoft excel 2013. Data was importedcoded, cleaned and analyzed using Statistics and data version 13(STATA).

Descriptive statistics was done to explore and summarize the data. Numeric data, were summarized using measures of central tendency (mean/median) and dispersion (standard deviation/inter-quartile range and; summaries were presented in tables. Histograms were plotted to show distributions.

Pearson correlation tests were used to evaluate the linear relationship between variables. Pearson correlation statistics and chi-square tests were done and corresponding p-values was reported. Two independent sample t-tests were used to compare the means of serum vitamin D between the two populations. The t statistics with corresponding p-values were reported. This study was conducted at alpha significance level of 0.05.

3.9 Vitamin D Reference Range Determination

Reference limit is a description of reference distribution that tells us about the variation of values in the selected set of reference individuals.

The lower and upper reference limits of vitamin D levels were obtained as.$\bar{X}\pm 1.96$ SD, where$\bar{X}$is the mean and SD is the standard deviation. All the values between and including the two reference limits are used to obtain the reference interval.

This reference interval can also be defined as the 95%central interval bounded by 2.5-97.5%.

The reference individual was the male blood donor.

4.0 Quality Assurance

Pre-analytical Errors

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

Analytical Errors

1. The manufacturers laid down standard operating procedures wereused to run all the tests.
2. VitaminD levels were interpreted based on the manufacturers insert and also after the reference interval was established.
3. QualityControl 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 is used.
5. If a control is out of its specified range, the associated test results were considered invalid and samples would be retested.
6. Calibration of the machines was done according to the manufacturer's' pamphlet procedures.

Post Analytical Errors

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

4.1 Ethical Consideration

Approval for study protocol was obtained from Kenyatta National Hospital/ University of Nairobi –Ethical and Research Committee (KNH/UON-ERC).Approval was also obtained from Mbagathi Level Five Hospital Ethical review Committee.

Informed consent was obtained from each study participant and samples collected according to standard procedure. Risks and benefits of the study were explained to the participants. Risks as pain during venipuncture and multiple pricks were explained to all participants.

4.2 Confidentiality

Patient information and results were kept confidential. A log book with patient details and study number werekept by the principal researcher. The questionnaire did not have patient's name. Thedata waskept under lock and key. Soft copy information waspassword protected. Results were communicated to patients' doctors and the national blood transfusion service to assist in patient management.

CHAPTER FOUR

RESULTS

4.0Demographic characteristics of patients and blood donors

A total of 121 HIV patients co-infected with TB and 141 blood donors were recruited for this study. Patients' age ranged between 20 and 67 years with a median of 44 and mean of 44.3(±)years whereas age donors ranged from 19 to 33 years with a median of 22 and meanof 22.7(±) years.

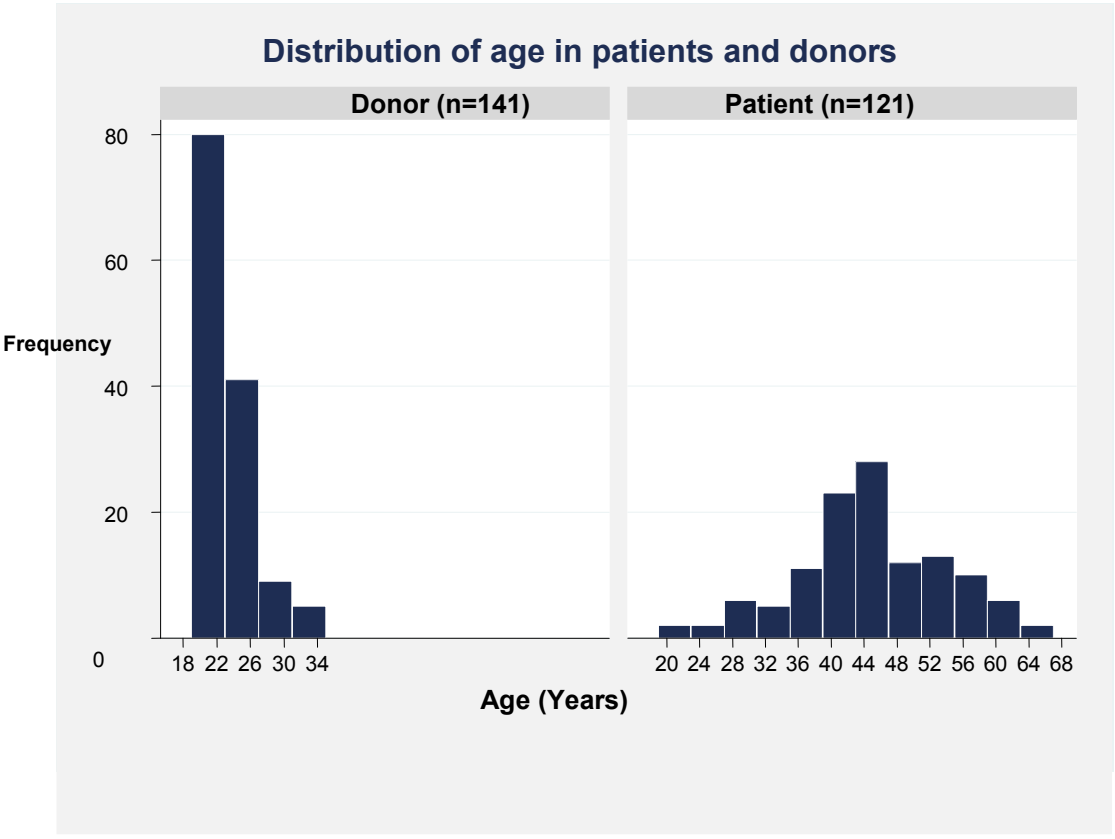

Figure 1: Distribution of age among HIV/TB patients and donors

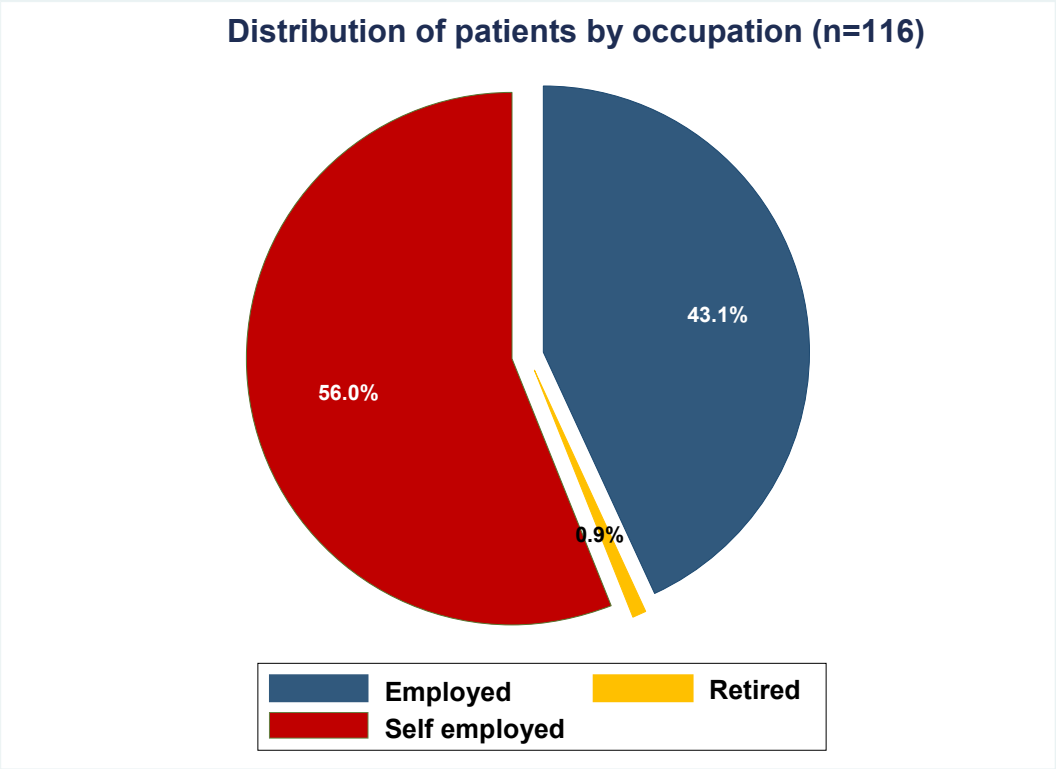

Figure 2: Distribution of co-infected patients by occupation

Self-employed co-infected HIV/TB patients were 56.0% (65/116), 43.1% (50/116) employed and 0.9% (1/116) retired. Five (5) patients did not respond.

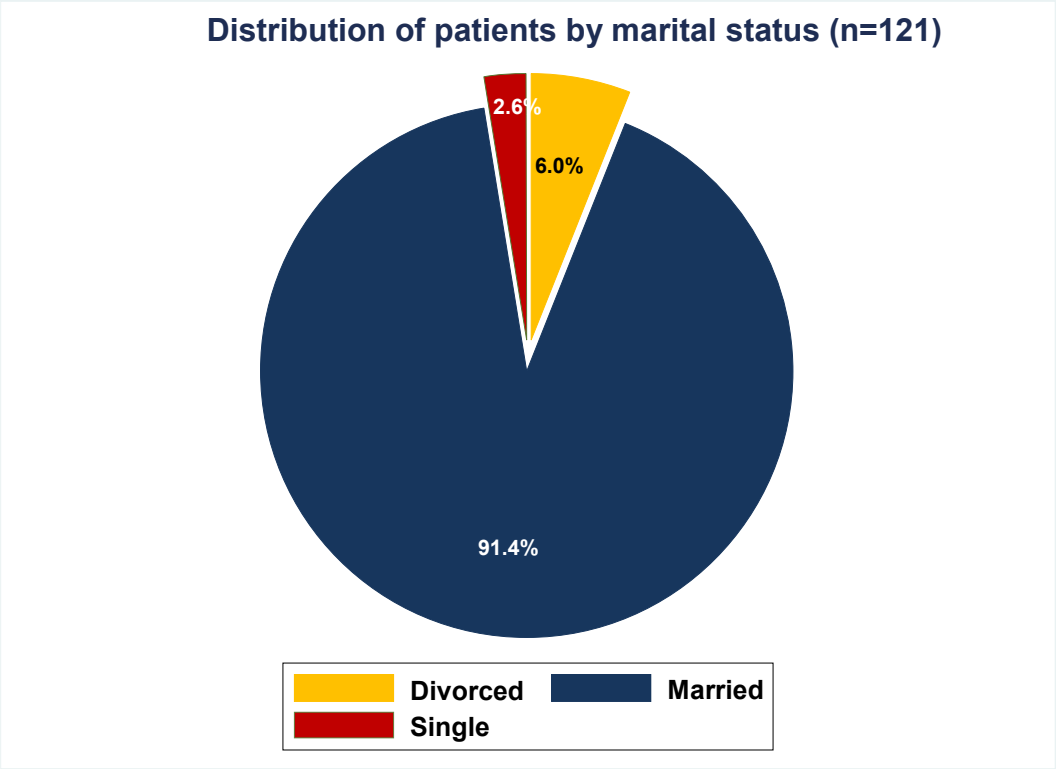

Figure 3: Distribution of patients by marital status

The majority of the patients were married (109/121; 90.1%) while 6% were divorced and 2.6% single.

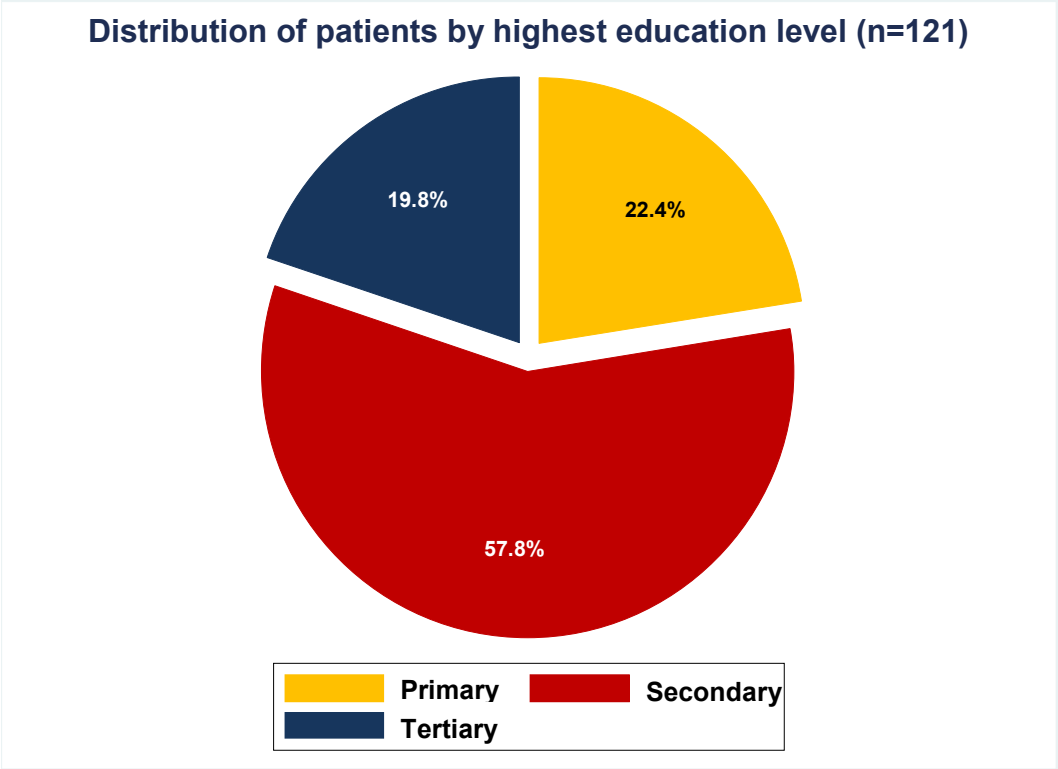

Figure 4: Distribution of HIV/TB patients by highest level of education

More than half of the HIV /TB co-infected patients (94/121; 57.9%) had attained secondary level education and above.

4.1 Distribution of Patients by Tuberculosis Phase

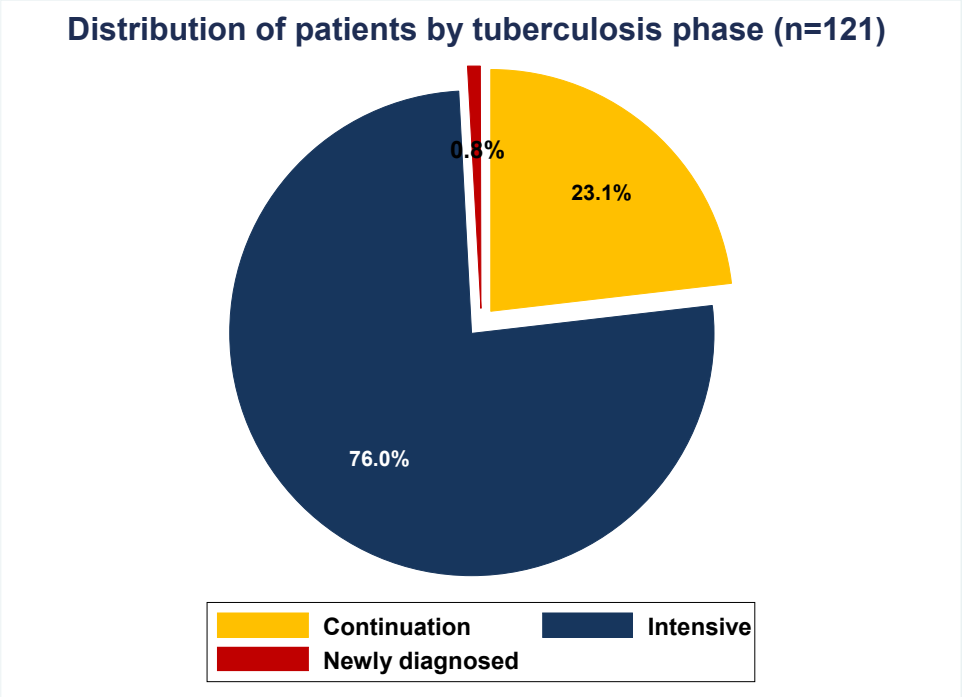

Figure 5: Distribution of Patients by Tuberculosis Phase

Out of 121 patients, 75.2% (91) were on the intensive phase of treatment tuberculosis and 23.1% (28) in the continuation phase. There was only one newly diagnosed tuberculosis case.

4.2 Distribution of Serum Vitamin D

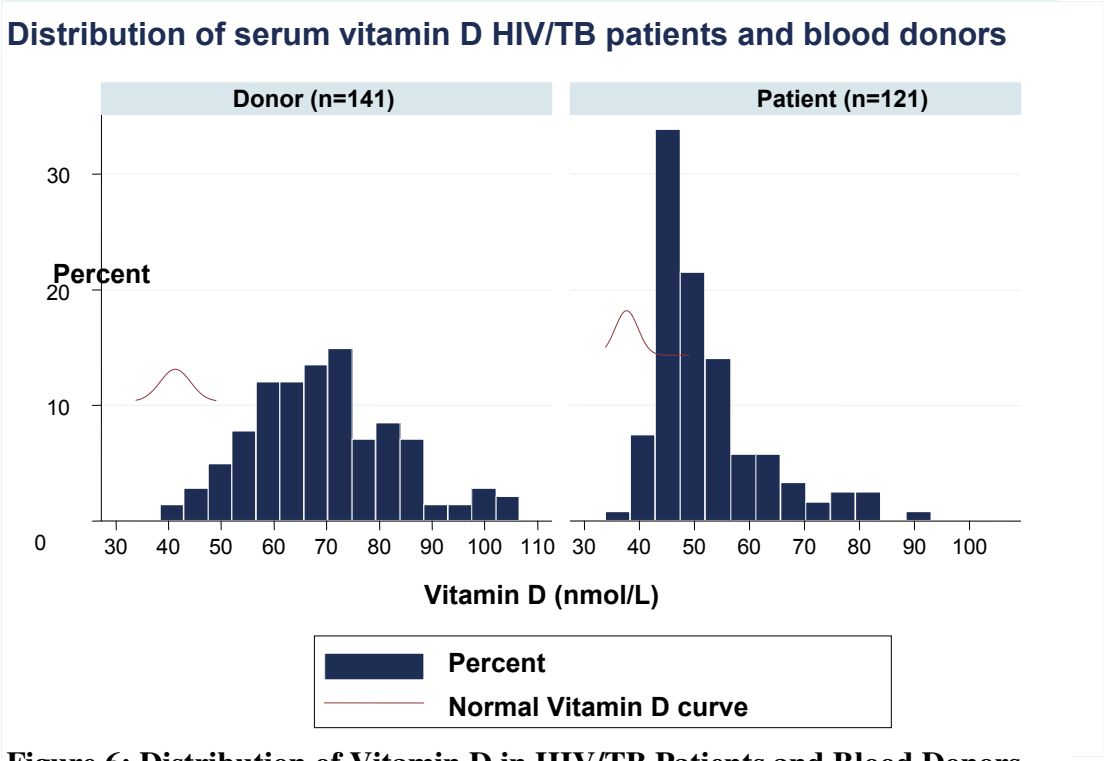

Figure 6: Distribution of Vitamin D in HIV/TB Patients and Blood Donors

Serum vitamin D level was normally distributed among blood donors and right skewed among patients. The Vitamin D range among donors was 38.9-106.7nmol/L with a mean and median 69.6(±) and 68.7nmol/L. Among HIV/TB co-infected patients the Vitamin D level range was 33.8-89.8nmol/L with a mean and median of 52.3(±) and 48.9nmol/L.

4.3 Comparison of Vitamin D levels in Blood donors and HIV/TB Co-infected Patients

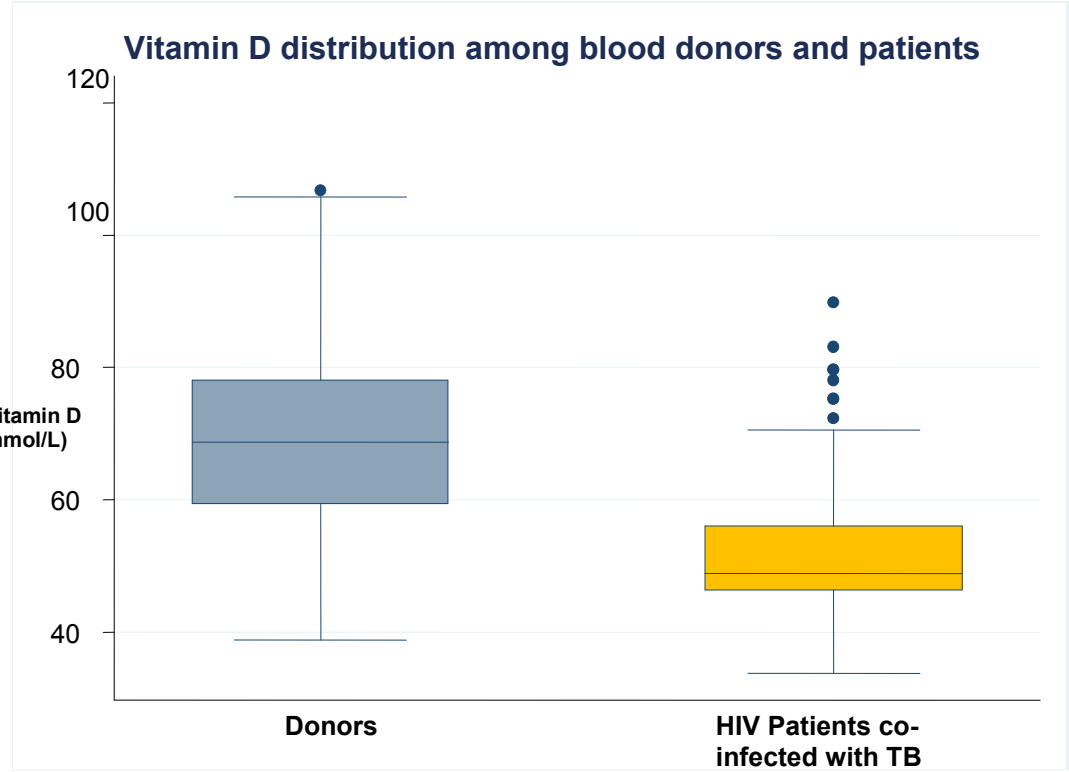

Figure 7: Serum Vitamin D Distribution among Blood Donors and HIV/TB Patients

The distribution of serum vitamin D was significantly different (K-sample test for equality of medians: chi-sq=86.38; p-value<0.001) between blood donors and HIV/TB patients.

4.4 Levels of Vitamin D vsTuberculosis Phase

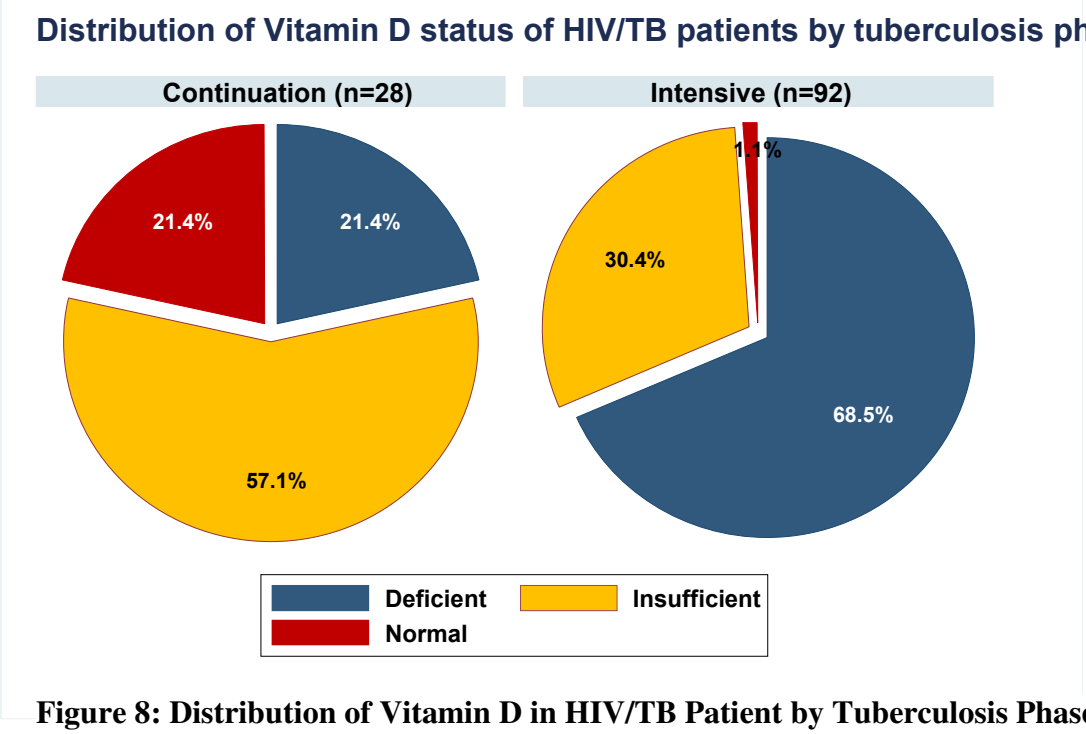

Figure 8: Distribution of Vitamin D in HIV/TB Patient by Tuberculosis Phase

Vitamin D deficiency was more frequent (68.5%) among patients in the intensive phase of treatment compared to those in the continuation phase. Majority of patients in the continuation phase had insufficient Vitamin D levels.

Table 2: Association between Tuberculosis Phase and Vitamin D Level

TB Phase\Vita min D level	Deficiency	Insufficiency	Normal	Chi-sq (df)	P-value
Continuation	**6**	16	6	27.67 (2)	**<0.001**
Intensive	**63**	28	1		

There was a significant association between tuberculosis phase and vitamin D status of the patient (chi-sq=27.67; p<0.001).

4.6 Comparison of Serum Vitamin D to WHO Reference Interval

The serum vitamin D level in patients and donors was compared to the WHO and Holick reference ranges: Normal (>75nmol/L), insufficient (50-74nmol/L) and deficient (<49mol/L).

Table 3: Distribution of Vitamin D in Donors (N=141) and Patients (N=121) Based on WHO Reference Interval

Reference range	Group	Frequency	Proportion
Normal	Donors	43	30.5
	Patients	7	5.8
Insufficient	Donors	90	63.8
	Patients	45	37.2
Deficient	Donors	8	5.7
	Patients	69	57.0

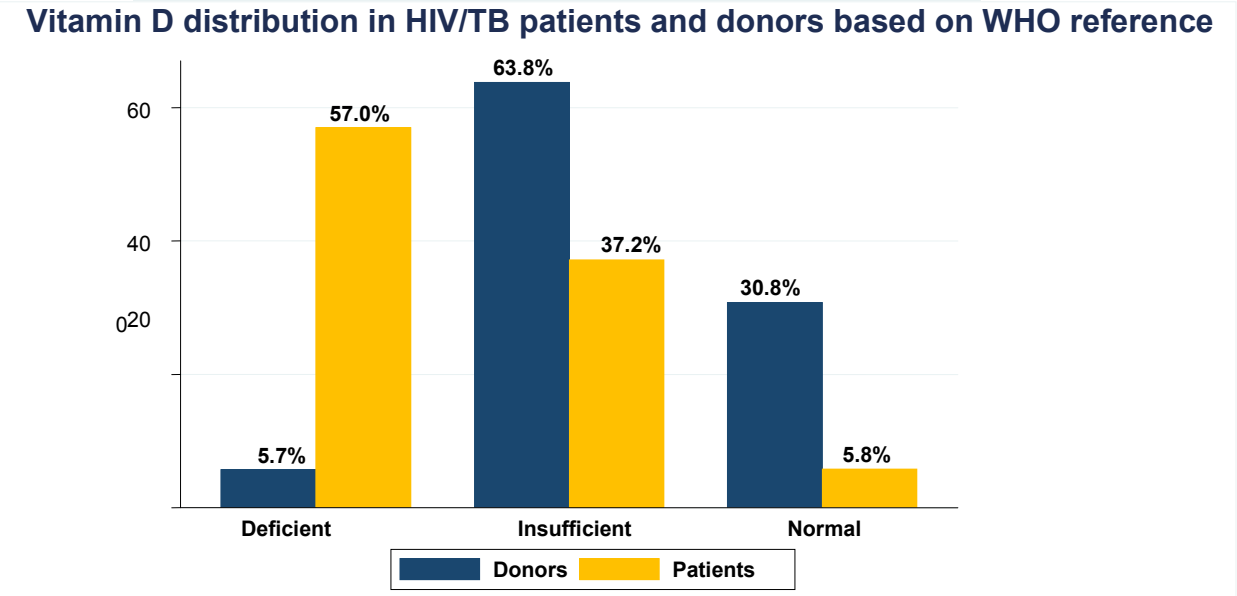

Figure 9: Distribution of Vitamin D in Patients and Donors Based on WHO Reference Interval

Among the patient's majority had vitamin D deficiency 69/121(57.0%) and insufficiency 45(37.2%).

Vitamin D deficiency was more frequent (68.5%) among patients in the intensive phase of treatment as compared to those with continuation phase. Only one had normal vitamin D

Majority of patients in the continuation phase tuberculosis patients had insufficient vitamin D levels.

Table 4: Association between Tuberculosis Phase and Vitamin D Level

TB Phase\Vita min D level	Deficiency	Insufficiency	Normal	Chi-sq (df)	P-value
Continuation	**6**	16	6	27.67 (2)	**<0.001**
Intensive	**63**	28	1		

There was a significant association between tuberculosis treatment phase and vitamin D status of the patient (chi-sq=27.67; p<0.001).

4.6Vitamin D Reference Interval for Blood Donors

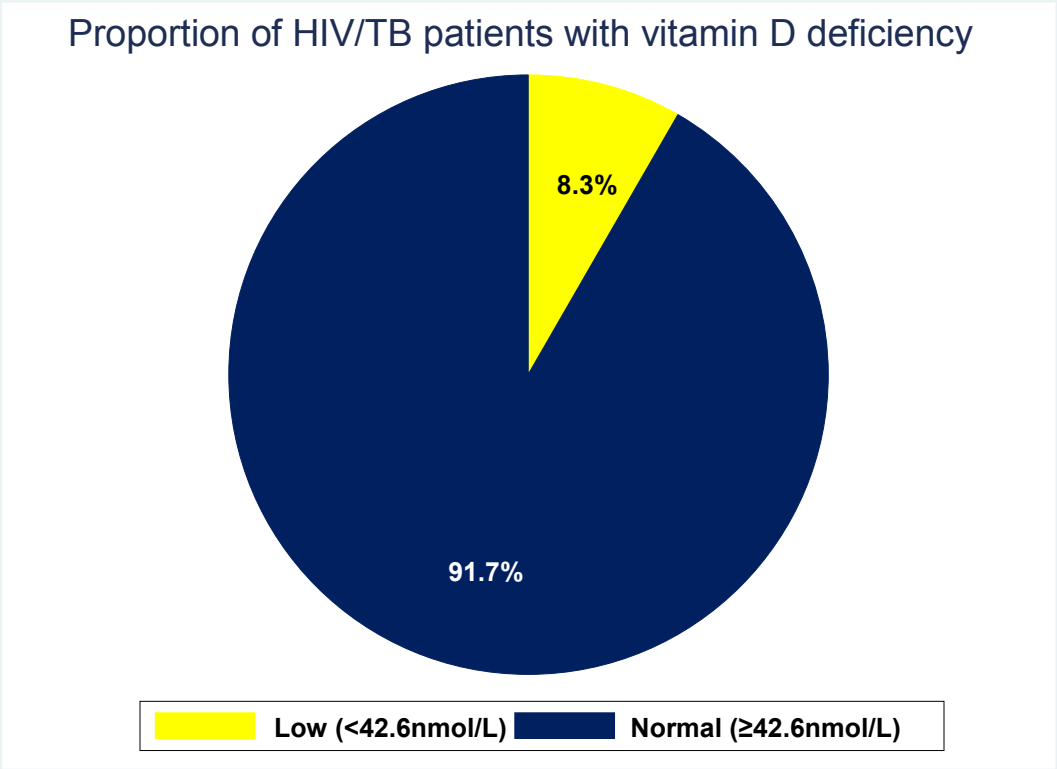

Figure 10: Prevalence of Vitamin D Deficiency Based on Reference Interval

Blood donors in this study were assumed to represent the healthy population so were used to establish population based reference intervals for serum Vitamin D. The cut-off for normal range for vitamin D level is ≥42.6nmol/L based on the lower limit of the 95% confidence interval of the mean serum vitamin D concentration (69.6±nmol/L; 95%CI [42.6; 96.7]) among donors. Insufficient level corresponds to serum vitamin D below 42.6nmol/L.

Among HIV patients co-infected with TB, 10/121 had insufficient vitamin D. Majority of the HIV/TB patients 91.7% had normal serum vitamin D. The prevalence of VDD was found to be 8.3% in HIV/TB co-infected patients using the reference interval obtained in this study.

A chi-square test done to evaluate the association between HIV-TB co-infection and serum vitamin D status was significant at α-level of significance 0.05 as shown in Table 6 below

Table 5: Association betweenHIV/TB Infection and Vitamin D Status (≥42.6nmol/L)

Group	Serum Vitamin D <42.6nmol/L	Serum Vitamin D ≥42.6nmol/L	Chi-square (df)	P-value
Donors	1	140	9.24(1)	0.002
HIV-TB patients	10	111		
Total	**11**	**251**		

The distribution of serum vitamin D was significantly different (K-sample test for equality of medians: chi-sq=86.38; p-value<0.001) between blood donors and HIV/TB patients.

CHAPTER FIVE

Discussion

There is no universal agreement on the optimal concentration of 25-OH D. Each laboratory is encouraged to establish their own reference intervals for their local population based on age, sex and special groups such as pregnancy and patients on renal replacement therapy. The question on what is an optimal vitamin D status remains a great topic of discussion. Some studies suggest that risk assessment on indicators of health such as bone and muscle function should be used to define optimal levels of this vitamin. Serum PTH levels and bone density should be assessed to truly assess for deficiency after low serum vitamin D level is realized. Many specialists consider the commonly used population based reference values too low. Health based reference values are recommended to replace population based referencevalues.Requiring patients to have levels greater than 80nmol/l implies that greater than 80% of the European population is VDD(29).

The World Health Organization (30) and Holick (31)defined vitamin D insufficiency as serum 25OHD below 50 nmol/L, a level that avoids skeletal and muscular problems.

Assay variability has been an alternate explanation to hypovitaminosis in different populations. Currently there are calibrators developed by the National Institute of Standards and Technology to provide traceability thus reducing assay variability(11).

In this study, serum Vitamin D level was normally distributed among blood donors and right skewed among patients. Among blood donor's serum Vitamin D level ranged from 38.9-106.7nmol/L mean was 69.6(±) nmol/L and median 68.7nmol/L. For HIV/TB patient's vitamin D levels ranged from 33.8-89.8nmol/L mean was 52.3(±) nmol/L and median 48.9nmol/L.

The distribution of serum vitamin D was significantly different (K-sample test for equality of medians chi-sq 86.38; p-value <0.001) between blood donors and HIV/TB patients. This corresponds to the p values obtained in a Meta analytical study where the medians of TB patients were compared to matched healthy controls. A significant difference in medians between the healthy controls and TB patients were found,and after testing for the equality of medians p values were found to be less than 0.05. This meta analytical studied concluded that the

probability that a healthy individual would have higher serum vitamin D level than an individual with tuberculosis if both were chosen at random from a population was 70%(26).

Using the reference range suggested by Holick and WHO (30) blood donors in this study who had hypovitaminosis D were 63.8% (90/141) and sufficient levels were found in only 30.5%(43/141). This is similar to a study done in Hawaii where 93 subjects (63 male and 30 female) were recruited to the study. Mean age of the Hawaiian participants was 24(mean age of donors was 22). Applying a cut point of 75nmol, 51% (47 of 93) of these subjects had low vitamin D status. The highest serum 25 (OH) D concentration observed was 155 nmol/L.

Kagotho et al(32)found among 258 male and femaleblood donors at Aga Khan University Hospital in Nairobi 17.4% were VDD.She noted that males were less likely to have VDD as compared to females and explained this could be due to differences in clothing styles between two.

This study confirms previous studiesthat patients presenting with active TB have significantly lower mean concentrations of serum 25-OH where majority had vitamin D deficiency 57.0% (69/121) and insufficiency 37.2% (45/121) using WHO reference values.
In a case control study done in Guinea Bissau (33)comparing TB patients with healthy controls hypovitaminosis D was found to be in 46% (167/362) of the TB patients and in 39% (193/494) of the healthy controls. Hypovitaminosis was defined as 25(OH) <50nmol/ L which is similar to the WHO definition of VDD. Hypovitaminosis D was highly prevalent among TB patients and healthy controls; Hypovitaminosis D was more frequent among the TB patient which is concordance to this study at 57%. Hypovitaminosis D in the blood donors was 8% vs.39% in the healthy control subjects in Bissau study which was a disparity. Perhaps this could be due to the controls used in the Bissau study were contacts of the patients thus if it was a nutritional deficit the contacts would also be deficient.

In a study done by Kibirige et al(34)where HIV/TB co-infected patients had VDD, VDI, SVDD and very SVDD 44.2%, 23.5%, 13.5% and 4.2% resp. which is in concordance in with findings in this study where most co-infected patients were deficient 57%(69/121)and insufficient

37.2%(45/121).No patients in this studywere found to have severe and very severe deficiency. This perhaps could be due to the patients who were recruited to the Mulago study who were all in-patients thus more ill corresponding to lower serum vitamin D levels due to perhaps the acute phase response to infection.

In a 22-multicenter study done in Canada and United States of America, a markedly high prevalence of vitamin D insufficiency was seen in the cohort of patients with active pulmonary tuberculosis, where 86% of the study subjects with measured concentrations of serum 25(OH)D <75 nmol/L. These findings echo the results in this study where among the patients' the majority had vitamin D deficiency (57.0%) and insufficiency (37.2%).

Most patients were in the intensive phase (92/141) of treatment versus the continuation phase (28/121). Vitamin D deficiency was more frequent 68.5% (68/92) among patients in the intensive phase of treatment for tuberculosis as compared to the continuation phase where 57.1% (16/28)had insufficient levels. These findings correspond to a study done in Malawi (35) where patients' serum vitamin D levels were followed up from diagnosis of Tuberculosis to treatment. Trends in serum 25 (OH) D concentrations over time were assessed for 133 patients who reached a final outcome. Median serum 25(OH) D rose to 62 nmol/L by week 8 of treatment and 64 nmol/L by end of treatment. This occurred despite daily administration of RMP and INH to all patients and increased use of ART by HIV-infected participants.

Vitamin D plays an important role in activation of 1 α-hydroxylase to convert 25(OH) D to its active form [1, 25 (OH) 2D] that leads to expression of cathelicidin, a microbicidal peptide which has been shown to kill intracellular *M.Tuberculosis*. Serum levels >75 nmol/L provide an adequate substrate for activation of 1 α-hydroxylase to convert 25(OH) D to its active form [1, 25 (OH) 2D] that leads to expression of cathelicidin, a microbicidal peptide for *Mycobacterium tuberculosis*. Serum levels <50nmol/L are said to impair the macrophage-initiated innate immune response thus causing individuals to be more susceptible to infection.(14).

The significance of an association between vitamin D deficiency and tuberculosis is 2-fold. First, already low vitamin D levels in tuberculosis patients may fall further on commencement of treatment. Isoniazid (INH) inhibits both hydroxylation steps of Vitamin D cutaneous synthesized into 25(OH) and 1,25(OH)D. Rifampicin induces alternative enzyme activity to degrade

25(OH)D into a waste product. Combined rifampicin and isoniazid treatment may reduce serum concentrations of useful vitamin D metabolites by 23–34%. In addition, efavirenz an antiretroviral drug is also known to lower vitamin D levels. It induces the cytochrome P450 pathway which causes 1,25OH and 25OH to be inactivated to their inactive metabolites. Also, efavirenz has been said to induce the cytochrome CYPR21 which hydroxylates D3 and D2 which are needed for Vitamin D activation (36).

The reference interval for serum Vitamin D level in this study was 42.6-96.7nmol/L based on X±1.96SD.Mean was 69.6(±)nmol/L. Based on this reference 10/121 patients had insufficient vitamin D and the prevalence of VDD was 8.3%. A chi-square test done to evaluate the association between HIV-TB co-infection and serum vitamin D status was significant (P 0.002).

A reference study (37) was done between November and July in Northern Germany conducted with samples from apparently healthy individuals of Caucasian heritage where the age range was 20-77 years. The number of males in the study was 201, the mean serum vitamin D was 48.5nmol/L and reference interval determined to be 12.3-107nmol/L using percentiles (2.5-97.5%). The lower limits of the reference limits in the Germany study were much lower compared to the blood donors in this study (42.6-96.7nmol/L). A reason could be that the study period in Germany was winter time thus less sun exposure. Another reason could be the study participants age was 20-77years.Cutaneous synthesis of Vitamin D is known to decrease with age.

The reference interval determined in this study(42.6-96.7nmol/L) was lower than the WHO reference interval for vitamin D. Vitamin D deficiency according to WHO are serum Vitamin D levels less <50nmol/L,insufficiency as 52.5-72.5 nmol/L and optimal levels as >75nmol/L.

Conclusion.

1. HIV/TB co-infected patients have a lower serum vitamin D levels as compared to blood donors (57% vs.5.7% as per WHO reference range).
2. Prevalence of VDD is high among HIV/TB co-infected patients (57% as per WHO reference range).
3. Using the reference interval determined in this study the prevalence of VDD was 8.3% in the HIV/TB co-infected patients.
4. Serum vitamin D reference interval among blood donors was lower than the manufacturers insert reference values.
5. HIV/TB co-infected patients in the intensive phase have lower Vitamin D than in continuation (68.5% vs. 21.4%)

Study Limitations

1. This study was not a matched case-control design, which impedes strong conclusions when comparing TB patients and the random population sample.

Recommendations

1. Patients with Human immunodeficiency virus co-infected with Tuberculosis should have their serum vitamin D measured.
2. Reference intervals obtained in this study should be used in the KNH laboratory
3. Every laboratory is encouraged to establish reference intervals for serum Vitamin D.
4. Further study to establish serum vitamin D reference interval in females is recommended.
5. Vitamin D supplementation is recommended based on clinical correlation and laboratory investigation.

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APPENDICES

1. Appendix 1- Consent information and consent form
2. Appendix 2-Questionnaire for participants
3. Standard operating procedure for blood collection
4. Appendix 4-Laboratory procedure for vitamin D.

APPENDIX 1

CONSENT INFORMATION AND CONSENT FORM

Informed consent form male patients and male blood donors. The title of the research is serum vitamin D and calcium levels in male blood donors and HIV/TB co infected male patients.

Name of Principal Investigator-Dr.Caroline Njeru

Name of Organization-University of Nairobi

This Informed Consent Form has two parts:

- Information Sheet (to share information about the research with you)
- Certificate of Consent (for signatures if you agree to take part)

You will be given a copy of the full Informed Consent Form

PART I: Information Sheet

Introduction and purpose of study

I am doing a study on serum vitamin D, to enable a reference range to be established and also to improve treatment modalities in patients with Tuberculosis. I am a postgraduate student at the University of Nairobi doing masters in human pathology.

You do not have to decide today whether or not you will participate in the research. You can talk to anyone you feel comfortable with before deciding to participate. If you have questions later, you can ask me or the nurses.

Vitamin D levels will be assayed in blood. One blood sample of 4mls will be taken. Your participation in this research is entirely voluntary. Whether you choose to participate or not, all services you receive at this clinic will continue. Nothing will change. If you decide to participate, the results from the tests shall be communicated to the physicians in this clinic and treatment modalities initiated if needed.

If you decide not to participate later after your blood sample has been drawn, the sample shall not be run. Services you receive in this clinic will continue normally.

Number of participants

240 male patients will be involved in this study.

Period of Participation

Only one sample will be taken from the participant. No repeat samples will be taken.

SPECIMEN COLLECTION

One blood sample will be taken from your arm using a syringe and needle.4ml of blood will be drawn only once tea spoonful is the equivalent of 4ml.The procedure will last less than 5minutes.The blood will be tested at the University of Nairobi clinical chemistry unit. The sample will be stored for the period of the study and the sample shall be destroyed after the study is concluded. The sample shall not be used for any other study other than this study.

Confidentiality

Patient information will be stored securely and will not be available to any other researcher. A unique number will be given to each sample to enable tracking of samples. No names will be used on samples.

Results will be communicated back to the clinic or patient. If any pathological values are noted the patient will be notified for corrective action to be taken.

Risks

Side effects of the blood collection are-some pain during the collection, temporary swelling may occur at the area of collection.

Benefits

The participants will get to know their serum vitamin D, calcium and albumin levels.

Who to Contact

In case of any questions, please contact

Dr.C.Njeru

0726 247 829

Dr.Kuria

0721 570 812

This proposal has been reviewed and approved by 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.

PART II: Certificate of Consent

I have read the foregoing information, or it has been read to me. I have had the opportunity to ask questions about it and any questions that I have asked been answered to my satisfaction. I consent voluntarily to participate as a participant in this research.

Print Name of Participant_____

Signature of Participant _____

Date _____
Day/month/year

I have witnessed the accurate reading of the consent form to the potential participant, and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely.

Print name of witness_____

Signature of witness _____

Date _____
Day/month/year

Statement by the researcher/person taking consent

I have accurately read out the information sheet to the potential participant, and to the best of my ability made sure that the participant understands it.I confirm that the participant was given an opportunity to ask questions about the study, and all the questions asked by the participant have been answered correctly and to the best of my ability. I confirm that the individual has not been coerced into giving consent, and the consent has been given freely and voluntarily.

A copy of this Informed Consent Form has been provided to the participant.

Print Name of Researcher/person taking the consent_____

Signature of Researcher /person taking the consent_____

Date _____

Day/month/year

Thumb print of participant

_____

Who to Contact

In case of any questions, please contact

Dr.C.Njeru

0726 247 829

Dr.Kuria

0721 570 812

This proposal has been reviewed and approved by 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 2

QUESTIONNAIRE SERUM VITAMIN D LEVELS IN MALE BLOOD DONORS AND HIV/TB CO INFECTED MALE PATIENTS

DATE …../……/…….

STUDY NUMBER ……

SOCIAL DEMOGRAPHIC CHARACTERISTICS

Age……..Years

Occupation……..

Education

1. None
2. Primary
3. Secondary
4. Tertiary
5. Others

Marital status

1. Single
2. Married
3. Divorced
4. Widowed

Medical History

1. When were you diagnosed with HIV?.....weeks…..Months……years

2. How long have you had HIV for ……? Months/……..Years

3. How long have you had TB for ……..Months/……..Weeks

Do you have any other medical conditions?

1.
2.
3.
4.

Medication

HAART

1.
2.
3.
4.

TB Phase of treatment

A. Intensive phase

B. Continuation phase

DRUGS

1.
2.
3.
4.
5.
6.
7.

MWISHO 2

TAARIFA KUHUSU IDHINI NAFOMU YA IDHINI

Taarifa ya idhini iliyoko katika fomu ya idhini kwa wagonjwa wanaume na wanaume wanaotoa damu.Kichwa cha Utafiti huu ni viwango vya Chembechembe za damu (serum) vitamini D, katika damu ya wanaume wanaotoa damu na wale wanaoshi na virusi vya ukimwi na Ungonjwa wa kifua kikuu (TB).

Jina la mtafiti Mkuu-Daktari.Caroline Njeru

Jina la shirika –Chuo kikuu cha Nairobi

Hii fomu ya taarifa ya idhini ipo na sehemu mbili:

- Karatasi ya taarifa (Kuhusu kukupa taarifa kuhusu utafiti huu)
- Cheti cha idhini (ya sahihi iwapo utakubali kushiriki)

Utapokea nakala ya fomu ya idhini.

Sehemu ya kwanza : Taarifa

Utangulizi na lengo la utafiti

Ninafanya utafiti kuhusu viwango vya chembechembe za serum vitamin D, kusaidia kutambua njia mbalimbali za kuboresha matibabu kwa wagonjwa walio na ugonjwa wa kifua kikuu. Mimi ni mwanafunzi wa uzamili(postgraduate) katika chuo kikuu cha Nairobi nikisomea shahada ya masters ya pathologia kwa binadamu(human pathology) .

Sio lazima uamue kushiriki leo au kama hautashiriki katika utafiti huu. Unaweza kumuongelesha yeyote ambaye unaona anafaa kabla ya kuamua kushiriki. Iwapo una maswali baadaye, unaweza kuniiliza mimi au wauguzi.

Viwango vya vitamin D vitatambuliwa katika damu. Kiwango cha 4mls cha damu kitachukuliwa. Kushiriki kwako katika utafiti huu ni kwa kujitolea.Endapo utakubali kushiriki au la, huduma zote utapokea katika kituo hiki zitaendelea.Hakuna chochote kitabadilika.Ukiamua kushiriki , matokeo utayapokea kutoka kwa daktari wa kliniki hii na matibabu kuanzishwa kama kuna hitaji.

Ukiamua kutoshiriki baadaye baada ya damu yako kuchukuliwa, damu yako haitatumiwa .Huduma unazopokea katika kituo hiki zitaendelea kwa kawaida

Nambari ya wahusika

Wanaume mia mbili na arubaini watashiriki katika utafiti huu.

Sampuli moja pekee itachukuliwa kwa mshiriki. Hakuna Kuchukua sampuli nyingine.

Damu kutolewa

Sampuli moja ya damu itatolewa kutumia sindano kutoka kwa mkono wako .Kiwango cha damu cha 4ml kitatolewa .Kijiko kimoja cha chai kinalingana na 4ml.Kazi hii itachukua muda wa chini ya dakika tano.

Damu itapelekwa chuo kikuu cha Nairobi kitengo cha kliniki ya chemia.(chemist).Sampuli itawekwa kwa muda ambao utafiti unaendelea na kuharibiwa baada ya utafiti kumalizika.Sampuli hiyo haitatumika kwa utafiti mwingine.

Usiri

Taarifa kuhusu mgonjwa itawekwa vyema na haitatumiwa na mtafiti mwingine. Nambari ya kipekee itapeanwa hurahisisha kutambua sampuli za damu.Hakuna kutumia majina kwa sampuli hizo.

Matokeo yatawasilishwa kwa kliniki au mgonjwa. Endapo kuna shida Fulani zitapatika katika damu ,mgonjwa ataelezwa na hatua dhibiti kuchukuliwa.

Hatari

Madhara kutokana na kutoa damu ni kama kuhisi uchungu kidogo wakati wa damu kutolewa , kuvimba kwa muda kunaweza kutokea katika sehemu ambayo damu imetolewa.

Manufaa

Mshiriki ataweza kufahamu chembechembe za Serum vitamin D, Calcium na viwango vya albumin.

Wa kuwasiliana naye

Kwa maswali yeyote, tafadhali wasiliana na

Daktari.C.Njeru

0726 247 829

Dr.Kuria

0721 570 812

Pendekezo hili limedhibitishwa na kuangaliwa na Hospitali kuu Ya Kenyatta/Chuo kikuu cha Nairobi –kamati ya maadili na utafiti (KNH/UON-ERC), ambayo ni kamati yenye kazi ya kuhakikisha kuwa wanaohusika katika utafiti wamezuiwa kutokana na madhara.Nambari ya simu (254-020) 2726300 Ext 44355

Sehemu ya pili: Cheti cha Idhini

Nimesoma habari hii, au nimesomewa.Nimeweza kupata muda wa kuuliza maswali kuihusu na maswali ambayo nimeuliza yamejibiwa vyema.Ninakubali Kushiriki katika utafiti huu.

Jina la mshiriki_____

Sahihi ya mshiriki _____

Tarehe _____
siku/mwezi/mwaka

Nimeshuhudia kusomwa kwa fomu ya idhini kwa mshiriki wa utafiti huu, na mshiriki amepata nafasi ya kuuliza maswali.Nadhibitisha mshiriki amepeana idhini kwa hiari yake.

Jina la shahidi_____

Sahihi ya shahidi _____

Tarehe_____
Siku/Mwezi/mwaka

Kutoka kwa mtafiti/anayechukua idhini

Nimesoma vyema taarifa kwa mshiriki mtarajiwa .Nimehakikisha kwa uwezo wangu kuwa mshiriki ameweza kuielewa. Nadhibitisha kuwa mshiriki amepewa nafasi ya kuuliza maswali kuhusu utafiti , na maswali yamejibiwa kulingana na uwezo wangu.Nadhibitisha mhusika hajachanganywa kwa kupeana idhini, na amepeana idhini bure na kwa kujitolea

Nakala hii ya idhini imepeanwa kwa mshiriki.

Jina la mtafiiti/Jina la anayechukua idhini_____

Sahihi ya mtafiti /anayechukua idhini_____

Tarehe_____

Siku/mwezi/mwaka

Alama ya kidole ya mshiriki

_____

Wa kuwasiliana naye

Kwa maswali yeyote, tafadhali wasiliana na

Daktari.C.Njeru

0726 247 829

Dr.Kuria

0721 570 812

Pendekezo hili limedhibitishwa na kuangaliwa na Hospitali kuu Ya Kenyatta/Chuo kikuu cha Nairobi –kamati ya maadili na utafiti (KNH/UON-ERC), ambayo ni kamati yenye kazi ya kuhakikisha kuwa wanaohusika katika utafiti wamezuiwa kutokana na madhara.Nambari ya simu (254-020) 2726300 Ext 44355

Mwisho 2

Hojajaji ya viwango vya Serum Vitamini D katika damu ya wanaume ambao hutoa damu na wanaoshi na virusi vya ukimwi /Ungonjwa wa kifua kikuu (TB) wagonjwa wanaume walioshirikiana maambukizi .

ufupisho ……/……./…….

Tarehe …../……/…….

Nambari ya utafiti ……

Kuhusu mshiriki

miaka……..mwaka

Kazi……..

Masomo

1. Hakuna
2. shule ya msingi
3. Shule ya upili
4. Chuo kikuu
5. Zingine

Hali ya ndoa

1. Pekee
2. Ameowa/ameolewa
3. Amepewa talaka
4. Amefiwa mume au mke

Historia ya matibabu

1. Uligundua unaugua virusi vya ukimwi lini?

2. Umeishi na virusi vya ukimwi kwa muda wa …… Miezi/……..Miaka

3. Umeishi na ugonjwa wakifua kikuu kwa muda gani ……..Miezi/……..Wiki/

Una shida ingine yeyote ya kiafya?

1.
2.
3.
4.

Matibabu

HAART

1.
2.
3.
4.

Sehemu ya matibabu ya kifua kikuu

A. Sehemu ya matibabuya kuanzia(Intensive Phase)

B. Sehemu ya kuendelea(Continuation phase)

Dawa

1.
2.
3.
4.
5.
6.
7.

APPENDIX 3

STANDARD OPERATING PROCEDURE FOR BLOOD EXTRACTION

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 centre (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.