

**THE PREVALENCE OF LATENT TUBERCULOSIS
INFECTION AMONG FINAL YEAR MEDICAL
STUDENTS OF THE UNIVERSITY OF NAIROBI,
KENYA**

BY;

GITAU JANE NJERI MBChB

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LIST OF ABBREVIATIONS

BCG	Bacille Calmette-Guerin
CD4	Cluster of differentiation 4
CFP-10	Culture Filtrate Antigen 10
ELISA	Enzyme-Linked Immunosorbent Assay
ESAT-6	Early Secretory Antigenic Target 6
HIV	Human Immunodeficiency Virus
HCWs	Health Care Workers
HBCs	High Burden Countries
IFN γ	Interferon Gamma
IGRA	Interferon Gamma Release assay
IU	International Units
KAVI	Kenya AIDS Vaccine Initiative
KNH	Kenyatta National Hospital
LTBI	Latent Tuberculosis Infection
MDR-TB	Multi-drug resistant TB
PPD	Purified Protein Derivative
QFT-G	Quantiferon TB Gold
QFT-GIT	Quantiferon TB Gold In Tube
REC	Research and Ethics Committee

TST	Tuberculin Skin Test
TB	Tuberculosis
TNF	Tumor Necrosis Factor
WHO	World Health Organization

ABSTRACT

Background: Health care workers (HCWs) are at higher than average risk of infection with mycobacterium tuberculosis (TB) and of developing tuberculosis disease. The risk includes infection with multi-drug resistant TB (MDR TB) strains. Little is known about the prevalence of latent tuberculosis infections (LTBI) in HCWs in Kenyatta National Hospital or among medical students who carry out clerkship in the medical wards.

Objective: The aim of the study was to determine the prevalence of latent TB Infection in fifth year medical students of the University of Nairobi using the interferon gamma release assay (IGRA)-Quantiferon Tb Gold (QFT-Gold) and to evaluate factors associated with the TB infection.

Design: A cross sectional study.

Methodology: The study was conducted between September and November 2013. A total of 181 final year medical students were interviewed using a standardized questionnaire and underwent QFT-Gold testing.

Results: The median age of the participants was 24 years (Range 22-31 years). Over half (51%) of the students were female. Majority (89.5%) had close contact with a person with TB. Many (72.9%) had been in boarding schools. The students had undergone a clerkship course in the medical wards for a mean duration of 19.2 weeks. The prevalence of TB infection using QFT-Gold was 30% (54/181). The prevalence of latent TB infection among final year medical students at was 24.9%. No variable was found to influence the prevalence of latent TB significantly.

Conclusion: A high prevalence of LTBI was found among final year medical students at the national referral hospital.

CHAPTER 1

INTRODUCTION

Tuberculosis (TB) is a contagious bacterial infection caused by mycobacterium tuberculosis that primarily involves the lungs, but may spread to other organs. TB occurs in every part of the World. According to the WHO Global Tuberculosis Report 2012, the global burden of TB remains enormous. In 2011, there were an estimated 8.7 million new cases of TB and 1.4 million people died from TB[1]. All countries are affected, but most cases occur in Asia (55%) and Africa (30%) where there are 22 high-burden countries (HBCs) that account for about 80% of the world's TB cases [2]. Kenya is one of them.

In Kenya, like the rest of the world, the interaction between TB and HIV infection has increased the burden of TB. The prevalence of TB in Kenya was 291 per 100,000 population in 2011 and new TB cases were estimated to be 288 per 100,000 population in the same year [1]. Over 9200 deaths were attributed to TB in the same year[1]. HCWs care for patients with open tuberculosis and hence are at risk of infection. Transmission of TB in health care settings to HCWs has been reported from virtually every country of the world, regardless of local TB incidence. The risk for TB among HCWs is consistently higher than the risk among the general population worldwide[3]. Galgalo et al carried out a case control study at Kenyatta National Hospital and found out that the annual incidence of TB from 2001 to 2005 in HCWs ranged from 645 to 1115 per 100000 population[4]. This incidence of TB was higher than that in the general population. Factors associated with TB disease identified in the same study were additional daily hours spent in rooms with patients, working in areas where TB patients received care, HIV infection and living in a slum or hospital-provided low-income housing[4].

The emergence of MDR TB complicates the picture further. MDR TB is defined as tuberculosis that is resistant to at least isoniazid and the two most powerful first-line treatment anti-TB drugs. A patient who develops active disease with a drug-resistant TB strain can transmit this form of TB to other individuals. Many health care workers are at risk of acquiring MDR-TB in health care settings[5].

LTBI definition and epidemiology

LTBI occurs when a patient is infected with mycobacterium tuberculosis, but does not have symptoms of active tuberculosis disease[6]. One third of the world's population (approximately 2 billion persons) is thought to be latently infected with TB[1]. Although persons with LTBI do not manifest overt symptoms of active TB and are not infectious, they are at increased risk for developing active disease and becoming infectious[7]. Five to ten percent of these will develop active disease during their lifetime. The greatest risk occurs within the first 2 years of infection [8].

Transmission and pathogenesis of M. Tuberculosis

TB is most commonly transmitted from a person with infectious pulmonary TB to others by droplet nuclei, which are aerosolized by coughing, sneezing, or speaking[5]. Majority of inhaled bacilli are trapped in the upper airways and expelled by ciliated mucosal cells. Only a fraction (usually <10%) reaches the alveoli [5]. Adhesion of mycobacteria to macrophages results in phagocytosis, which is enhanced by complement activation leading to opsonization of bacilli. After a phagosome forms, the survival of *M. tuberculosis* within it depends on reduced acidification. The bacterial cell-wall glycolipid (lipoarabinomannan) inhibits the intracellular increase of Ca^{2+} thus impairing phagosome-lysosome fusion, and the bacilli may survive within the phagosomes[5]. Replication begins and the macrophage eventually ruptures and releases its bacillary contents. Other uninfected phagocytic cells are then recruited to continue the infection cycle. The organisms grow for 2-12 weeks, until they reach 1000-10,000 in number, which is sufficient to elicit a cellular immune response. In the cellular immune response, activated CD4+ T lymphocytes differentiate into cytokine-producing T helper 1 (T_H1) or T helper 2 (T_H2) cells. T_H1 cells produce Interferon-gamma (INF-gamma) an activator of macrophages and monocytes and interleukin 2 (IL-2). The interplay of these various cytokines and their cross-regulation determine the host's response and containment of the infection[5].

Exposure to *M. tuberculosis* leads to active disease in only about 10% of the people. An effective immune response in remaining 90% of individuals stops *M. tuberculosis* multiplication. However, the pathogen is completely eradicated in about 10% people while others only succeed in containment of infection as some bacilli escape killing and remain in non-replicating

(dormant) state in old lesions[9]. The dormant bacilli can reactivate and cause active disease if a disruption of immune response occurs. The greatest risk for progression to active disease occurs within the first 2 years of infection. After the first 2 years following infection, the risk of developing active disease over an individual's lifetime is 5-10%. However, the risk of developing active disease is greatly increased (5%-15% every year and ~50% over a lifetime) by HIV co-infection[9].

Screening for TB infection: Interferon Gamma Release Assays(IGRA)

Tuberculin skin tests (TSTs) have been used worldwide for more than a century as an aid in diagnosing both LTBI and active tuberculosis. However, certain limitations are associated with the use of TSTs. A valid TST requires correct administration by the Mantoux method with intradermal injection of 0.1mL of tuberculin-purified protein derivative (PPD) into the dorsal surface of the forearm. Secondly, patients must return to a clinician for test reading, therefore intra reader bias and inaccuracies exist. Also, false-positive TSTs can result from contact with non-tuberculous mycobacteria or vaccination with Bacille Calmette-Guerin (BCG), because the TST test material contains antigens that are also in BCG and certain non-tuberculous mycobacteria[7].

In 2001, the United States food and drug administration (FDA) approved the first Interferon gamma release assay (IGRA) test. This test used an enzyme-linked immunosorbent assay (ELISA) to measure the amount of IFN-gamma released in response to PPD compared with controls. New IGRAs have since been developed with better specificity. In October 2007, QFT-Gold became the third IGRA approved by FDA as an aid for diagnosing M. tuberculosis infection. Control materials and antigens for QFT-Gold are contained in special tubes used to collect blood samples for the test. One tube contains test antigens that consist of a single mixture of 14 peptides representing the entire amino acid sequences of the early secretory antigenic target 6 (ESAT 6) and culture filtrate antigen 10 (CFP-10) and part of the sequence of TB7.7. The two accompanying tubes serve as negative and positive controls[7].

QFT-Gold Specificity and Sensitivity

Estimates of QFT-Gold sensitivity have varied widely in published studies. Studies that compared the sensitivity of QFT-Gold to that of TST in patients with culture-confirmed active tuberculosis have pooled QFT-Gold sensitivity of 83% [7]. In tested populations of persons unlikely to have *M. tuberculosis* infection, pooled QFT-Gold specificity was 99% and pooled TST specificity from these cohorts, when available, was 85% [7]. QFT-Gold therefore has similar sensitivity to TST but better specificity than the skin test.

Targeted populations for TB screening.

TSTs and IGRAs should be used as aids in diagnosing infection with *M. tuberculosis*. Screening is not for the general population. One should target those at high risk for TB infection such as HCWs. The other group is those at Risk of progression such as patients with HIV Infection (who have a 2 times risk of developing active TB), patients with abnormal chest x-ray compatible with past TB (risk of active TB is 10 times that of a normal chest x-ray), children less than 5 years and some medical conditions –diabetes mellitus, renal failure, lymphoma, leukemia, those on immunosuppressants such as tumor necrotic factor (TNF) inhibitors and transplant patients [7].

Medical Management after Testing

Diagnoses of *M. tuberculosis* infection and decisions about medical or public health management are not based on IGRA or TST results alone, but include consideration of epidemiologic and medical history as well as other clinical information.

Persons with a positive TST or IGRA result should be evaluated for the likelihood of *M. tuberculosis* infection, for risks for progression to active TB if infected, and for symptoms and signs of active TB. If risks, symptoms, or signs are present, additional evaluation and management is indicated. Neither an IGRA nor TST can distinguish LTBI from active TB [7].

Treatment of selected persons with LTBI aims at preventing active disease. This intervention is called preventive chemotherapy or chemoprophylaxis. A 6 to 12-month course of isoniazid reduces the risk of active TB in infected people by up to 90%. In the absence of reinfection, the protective effect is believed to be lifelong [5]. Candidates for treatment of LTBI defined as high-risk groups include HIV infected persons, persons receiving immunosuppressive therapy, close contacts with tuberculosis patients and persons with high risk medical conditions such as

diabetes mellitus, some hematologic and reticuloendothelial diseases and end-stage renal disease[5].The majority of HCWs may not have the risk factors for progression to disease that serve as the basis for the current recommendations for treatment of LTBI.

CHAPTER 2

2.1 LITERATURE REVIEW

TB Infection in Health Care workers and Medical Students

Transmission of tuberculosis (TB) in health care settings to health care workers (HCWs) has been reported from virtually every country of the world, regardless of local TB incidence. The risk for TB among HCWs is consistently higher than the risk among the general population worldwide[3].

Iacopo et al in a Metanalysis found stratified pooled estimates for LTBI in countries with high LTBI Incidence (>100/100,000 population) such as Kenya to be 8.4%; countries with intermediate LTBI incidence (50-100/100,000 population) was 6.9% and for countries with low LTBI incidence (<50/100,000 population) was 3.8%[3]. The reported incidence of LTBI among HCWs working in general health services was 69/100,000, whereas the incidence among staff at the TB services was 741/100,000, which was 10 times higher than among the general population[3].

Galgalo et al did a case control study in Kenya on tuberculosis risk among staff of Kenyatta National Hospital where 65 cases were staff diagnosed with TB and 316 controls were randomly selected staff without recent TB. The annual incidence of TB from 2001 to 2005 ranged from 645 to 1115 per 100,000 population. Factors associated with TB disease were additional daily hours spent in rooms with patients, working in areas where TB patients received care and HIV infection[4].

A cross-sectional study done in Kampala, Uganda from June 2001 to August 2001 reported a high prevalence of LTBI of 57% using a tuberculin skin test (TST \geq 10mm) in health care workers. The TST survey was conducted among 396 HCWs from three hospitals within Kampala[10].

Da Ho Jung et al investigated the prevalence of latent tuberculosis infection (LTBI) among medical students in South Korea. Students, who were in second- or third-year classes before clerkship course, were enrolled for three consecutive years in the study. A standard questionnaire

was given to each participant, and tuberculin skin test (TST), Quantiferon-TB GOLD In-Tube (QFT-GIT) assay, and chest radiography were performed. Of the 153 subjects, 23 (15.0%) tested positive for the TST, and 8 (5.2%) tested positive for the QFT-GIT. Of the four students who had a history of contact with TB patients, only one subject tested positive for both tests, and the other three students tested negative for both tests[11].

Zimbabwe is ranked as one of the high-burden TB countries. Elizabeth Cobert et al compared institutional rates and community rates of tuberculin skin test (TST) conversion in Harare, Zimbabwe. Nursing students experienced 19.3 TST conversions (increase in induration diameter, ≥ 10 mm) per 100 person-years, and polytechnic school students experienced 6.0 conversions per 100 person-years. The rate of difference was 13.2 conversions per 100 person-years. Nursing students reportedly nursed 20,868 in patients with tuberculosis during 315 person-years of training.

Both groups had high TST conversion rates, but the extremely high rates among nursing students implied high occupational exposure to TB. Intense exposure to inpatients with tuberculosis was reported during training[12].

LuuThi Lien et al conducted a cross-sectional study to determine the prevalence of TB infection and its risk factors among HCWs in Hanoi, Viet Nam. The Prevalence of TB infection estimated by either IGRA or TST was high among HCWs in the hospital. IGRA seemed to estimate TB infection more accurately than any other criteria using TST. A total of 300 HCWs including all staff members in a municipal TB referral hospital received an interferon-gamma release assay (IGRA), Quantiferon-TB Gold In-Tube, followed by tuberculin skin test (TST) and a questionnaire-based interview. The prevalence of TB infection estimated by IGRA, was 47.3%, TST was 66.3%. Working in TB hospital was associated with twofold increase in odds of TB infection estimated by IGRA. Increased age, low educational level and the high body mass index also demonstrated high odds ratios of IGRA positivity[13].

In India, Pai et al conducted a study to estimate latent tuberculosis infection prevalence in health care workers using the tuberculin skin test (TST) and a whole-blood interferon gamma (IFN-gamma) assay. It was a cross-sectional comparison study of 726 health care workers aged 18 to

61 years with no history of active tuberculosis conducted from January to May 2004, at a rural medical school in India. A total of 493 (68%) of the health care workers had direct contact with patients with tuberculosis and 514 (71%) had BCG vaccine scars. A large proportion of the health care workers were latently infected; 360 (50%) were positive by either TST or IFN-gamma assay, and 226 (31%) were positive by both tests. The prevalence estimates of TST and IFN-gamma assay positivity were comparable (41% and 40% respectively). Increasing age and years in the health profession were significant risk factors for both IFN-gamma assay and TST positivity. BCG vaccination had little impact on TST and IFN-gamma assay results[14].

In Germany Anja et al conducted a multi center study to determine the prevalence and putative risk factors of LTBI. They tested 2028 employees in the healthcare sector with the Quantiferon-Gold In-tube (QFT-GIT) test between December 2005 and May 2009.

A positive IGRA was found in 9.9% of the healthcare workers (HCWs). Nurses and physicians showed similar prevalence rates (9.7% to 9.6%). By occupation, the highest prevalence was found in administration staff and ancillary nursing staff (17.4% and 16.7%). None of the individuals in the trainee group showed a positive IGRA result. In the different workplaces the observed prevalence was 14.7% in administration, 12.0% in geriatric care, 14.2% in technicians (radiology, laboratory and pathology), 6.5% in admission ward staff and 8.3% in the staff of pulmonary/infectious disease wards. Putative risk factors for LTBI were age (>55 years), being foreign-born, TB in the individual's own history and previous positive TST results. There was no statistically significant association with gender, BCG vaccination, workplace or profession[15].

It is therefore clear from these studies that HCWs are at an increased risk of TB infection and factors associated with the infection include older age, years in the health profession, coming from areas with high prevalence and incidence of TB, working in TB clinics/hospitals amongst many others.

2.2 PROBLEM STATEMENT

HCWs are at higher than average risk for infection with mycobacterium tuberculosis and of developing TB disease[3]. Little work has been done on the prevalence of TB infection in HCWs in Kenyan health Institutions especially using the newer screening techniques. Additionally

students have not been screened for TB infection despite being exposed to open TB cases nursed in the medical wards and at the clinics. The risk of acquiring TB Infection while undergoing clerkship courses at Kenyatta National Hospital is not known. My study proposes to establish the prevalence of TB infection in outgoing 5th year medical students who have been exposed to patients during their clerkship courses in the wards where open TB cases were nursed. This would reflect on the ongoing transmission of M. tuberculosis in the medical wards at Kenyatta National Hospital.

2.3 JUSTIFICATION/SIGNIFICANCE

Accurate determination of the prevalence of LTBI is important for the design and evaluation of TB control strategies at the hospital. Screening HCWs for infection with *M. tuberculosis* is an essential administrative measure for the control of transmission of *M. tuberculosis* in health-care settings. Screening of medical students will provide important information as the first step in assessing TB infection control measures at Kenyatta National Hospital. The University of Nairobi needs information on the risk of acquiring TB infection while students are undergoing clerkship courses at Kenyatta National Hospital, so as to enable the university to contribute to infection control measures at the Hospital. This could be of benefit to all HCWs.

My study will serve as a baseline on prevalence of LTBI in health institutions in Kenya.

2.4 RESEARCH QUESTION

What is the prevalence of latent TB Infection in Medical Students at Kenyatta National Hospital using the interferon gamma release assay (IGRA)-[Quantiferon-TB Gold (QFT-Gold)]?

2.5 RESEARCH OBJECTIVE

Primary objective

The primary objective was to determine the prevalence of latent TB infection in 5th year medical students at Kenyatta National Hospital using QFT-Gold.

Secondary objective

The secondary objective was to identify factors associated with Latent TB Infection in 5th year medical students such as gender, history of contact with open TB case, history of co-morbid condition and history of medical ward rotation.

CHAPTER 3

MATERIALS AND METHODS

3.1 STUDY DESIGN:

The study was cross-sectional. It was carried out from September to November 2013 among final year undergraduate medical students at Kenyatta National Hospital, a referral and teaching Hospital in Nairobi, Kenya.

3.2 STUDY LOCATION:

Kenyatta National Hospital (KNH) is a state owned national referral and teaching Hospital, situated in Nairobi, Kenya. Within the KNH complex are College of Health Sciences (University of Nairobi); the Kenya Medical Training College; Kenya Medical Research Institute and Public Health Laboratory Service (Ministry of Health). The hospital offers a wide range of diagnostic services such as laboratories, radiology/imaging and endoscopy among other specialized services. Kenyatta National Hospital has a capacity of 1800 beds and over 6000 staff members. At any given day the hospital hosts in its wards between 2500 and 3000 patients[16]. The internal medicine department runs 7 general medicine wards and one specialized respiratory medicine ward where TB patients are taken care of. The University of Nairobi, school of medicine collaborates with KNH to provide areas of teaching to its students. In this regard the students are required to undergo clerkship courses in the different medical wards over 3rd and 5th years under guidance and supervision of the various consultants. The study participants were from University of Nairobi-School of Medicine.

3.3 STUDY SUBJECTS

Study population

The target population was medical students enrolled in University of Nairobi -School of Medicine in their final year of study.

Inclusion criteria

Those included in the study were adult participants (age 18 and above) who consented.

Exclusion criteria

Any student on treatment or who had been on treatment for tuberculosis was excluded

3.4 SAMPLE SIZE

The sample size calculated was exceeded as **182 students** volunteered.

The minimal sample size was calculated using Fisher's formula was 168:

$$n = \frac{z^2(p(1-p))}{e^2}$$

n = the sample size

z = the statistic associated with the level of significance we wish to test our result at

p = an estimate of the proportion of LTBI in participants (40% in Indian population)

e = the proportion of error we are prepared to accept

$$n = \frac{3.8416 \times [0.4 \times (1-0.4)]}{0.05 \times 0.05} = 368$$

However the total study population was 310 final year students. Therefore the sample size exceeded the total population. If the target population is less than 10,000 then the final estimate is calculated using the formula:

$$n_f = \frac{n}{1 + n/N}$$

where n_f = desired sample size where population < 10,000

N = total study population

$$\begin{aligned} n_f &= \frac{368}{1 + 368/310} \\ &= 168 \end{aligned}$$

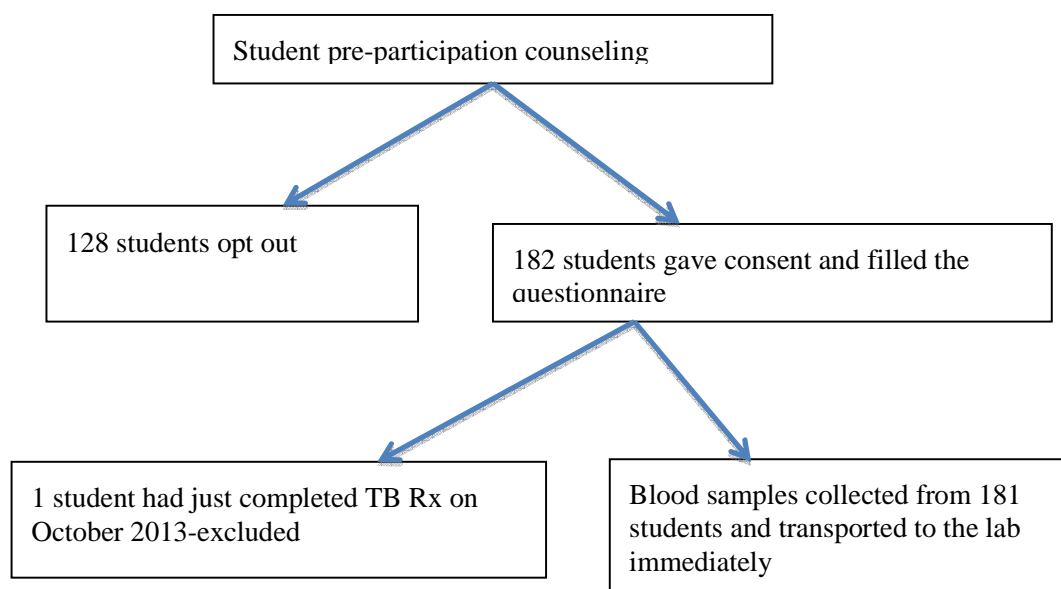
3.4 SAMPLING TECHNIQUE

Consecutive sampling was done. A class list containing the names of the total 310 students was obtained from the department of medicine. All willing students were counseled and allowed to sign up for participation. This was done until a total of 182 participants volunteered.

3.5 RECRUITMENT AND CONSENTING PROCEDURE

Final year students were recruited at their Kenyatta campus classroom. Explanation to the students as to the nature of the study was given in a classroom set up. There was pre-participation counseling on the definition of latent TB, and its relevance. Willing and eligible students were asked to sign a consent form. The consent form clearly outlined the purpose of the test, the risks involved and the benefits. It spelt out that participation was voluntary and students could refuse to take part in the study. Figure 1 summarizes the recruitment and consenting procedure.

Figure 1: A flow chart of the recruitment and consenting process



3.6 DATA COLLECTION

Once consent was given, the students were asked to fill a self-administered questionnaire detailing their contacts, age, sex and previous exposure to the medical wards with PTB patients, previous treatment for PTB, any significant medical illness such as HIV infection, diabetes, and current use of immunosuppressant drugs. Phlebotomy by qualified clinical officers (research

assistants) was then done on consenting participants who met the inclusion criteria. Venous blood sample of 3ml volume was obtained aseptically from the antecubital fossa of each consenting student. The students did not undergo any clinical examination.

The blood was put directly into three 1-mL heparin-containing tubes. One tube contained only heparin as negative control, another contained the T-cell mitogen phytohemagglutinin as positive control, and the third tube had overlapping TB peptides. Blood vials were transported to the Kenya Aids Vaccine Initiative (KAVI) Lab immediately at room temperature for incubation and further analysis.

3.6 LABORATORY METHODS AND PROCEDURE

As recommended by the manufacturer, blood samples collected, were incubated at 37°C for 24 hours. After 24 hours of incubation, the tubes were centrifuged and plasma harvested ready for analysis by enzyme-linked immunosorbent assay (ELISA). Plasma was frozen at -80°C until all samples were collected. This allowed for batching of samples to enable efficiency during analysis by ELISA. This process took 3 weeks. (Plasma in centrifuged tubes is stable for 28 days at 2-8°C and indefinitely stable at -80°C).

After thawing, the amount of IFN-gamma was then quantified using the enzyme-linked immunosorbent assay. Thawing was only done once. The result was reported as quantification of IFN-gamma in international units (IU) per mL. An individual is considered positive for *M. tuberculosis* infection if the IFN-gamma response to TB antigens is above the test cut-off (after subtracting the background IFN-gamma response in the negative control)[8]. As recommended by the manufacturer the cut-off value for a positive test is IFN-gamma of at least 0.35 IU/mL[9].

3.7 QUALITY ASSURANCE

To ensure quality control, blood collection tubes were stored in a temperature-controlled room as per manufacturer's instructions. Five research assistants (registered clinical officers) were recruited and trained on communication skills, phlebotomy, medical ethics including elements of informed consent and disclosure of medical information, biohazard and medical waste management. The recommended procedure for specimen collection, proper labeling and storage was followed strictly. Administration of the questionnaires and the collection of blood samples

was overseen by the principal investigator. The KAVI laboratory runs internal and external quality controls whereby the internal controls are done every 2 weeks and the external controls done every 6 months by Qualify limited. KAVI laboratory is ISO certified. Well-trained and qualified Lab technicians carried out the QFT-Gold lab procedure. All the assays met quality control standards.

3.8 DATA ANALYSIS

Data from the questionnaire was cleaned, verified and coded before being entered into a pre-designed Microsoft excel database. Statistical analysis was performed using SPSS version 17.0. The study population was described by summarizing categorical data such as sex, into relative frequencies and continuous data such as age and duration of patient exposure into means or medians. Prevalence of latent TB infection was calculated and presented as a proportion with 95% confidence interval. Factors associated with TB infection were explored using Student's t test to compare means such as age and Chi square test for categorical variables such as sex, history of previous medical ward exposure, co-morbid conditions and history of contact with open TB. Independent predictors of latent TB infection were determined using logistic regression analysis. Odds ratios were calculated and presented as estimates of relative risks of disease. All statistical tests were performed at 5% level of significance (95% confidence interval).

3.9 ETHICAL CONSIDERATIONS

Approval to carry out the study was sort from the Kenyatta National Hospital/University of Nairobi Research and Ethics Board (KNH/UON-REC). Pre-counseling was done before student participation detailing the relevance and implication of the test. Participants were enrolled into the study only after giving informed consent. Those who gave consent were not exposed to unnecessary risk. Freedom to withdraw from the study was observed without prejudice. Strict confidentiality was maintained and all data obtained was securely stored and used for purposes of the study only. All those who tested positive were counseled on its implication and need for evaluation due to the risk of progression to active TB infection. Referral letters for review and treatment at the university health services were given to the students who had positive test results. An alternative option of review by a physician of their choice at the students' own cost was made available to them. The need for treatment was to be determined on an individual basis.

Treatment option for those with LTBI and at a risk of progression was offered at the university clinic (Isoniazid for 9 months) at no cost.

CHAPTER 4

RESULTS

4.1 Participants Description

Three hundred and ten final year medical students were eligible to participate in the study. Of the eligible students 181 (58%) participated in the study. The median age was 24 years (Range 22-31 years). Over half (51%) of the students were female.

Table 1: Socio-Demographic Characteristics Of Study Participants (N= 181) Final Year Students From Faculty Of Medicine, University Of Nairobi.

Variable	Frequency (%)
Age	
Mean (SD)	24.4 (1.2)
Median (IQR)	24 (24-25)
Min-Max	22-31
Sex	
Male	88 (48.6)
Female	93 (51.4)

One hundred and thirty two students (72%) had been residents of boarding schools prior to joining the university. Boarding schools are high-risk congregate settings for TB aerosol spread. During their medical clerkship years, 162 students (89%) had close contact with persons suspected to have had pulmonary TB with most of them having attended to patients with active pulmonary TB.

The average time spent in medical wards was 19.2 (± 2.1) weeks, and 3.5 (± 2.5) weeks in casualty where patients with active pulmonary TB are seen daily. (Table 2)

Table 2: Tuberculosis Screening: Characteristics Of Study Participants (N= 181) Final Year Students From School Of Medicine, University Of Nairobi

Variable	Frequency (%)
Close contact to person suspected to have TB	
Yes	162 (89.5)
No	19 (10.5)
Resident of high risk congregate settings	
Yes	132 (72.9)
No	49 (27.1)
Duration in weeks of rotation, mean (SD)	
Medical wards	19.2 (2.1)
Surgical wards	19.2 (3.2)
Casualty	3.5 (2.5)
Paediatric wards	11.2 (1.1)

Of the participants interviewed, 157 (86.7%) reported no symptoms associated with active TB. Ten students (5.5%) had a cough for three weeks or more. Other symptoms such as chest pain, unexplained weight loss, night sweats, fever and anorexia, were reported variably by the rest of the participants. (Table 3)

Table 3: Clinical Characteristics Of Study Participants (N= 181) Final Year Students From School Of Medicine, University Of Nairobi

Variable	Frequency (%)
Asymptomatic	157(86.7)
Symptomatic	24 (13.3)
Cough (3 weeks or more)	10 (5.5)
Hemoptysis	0 (0)
Chest pain	5 (2.8)
Unexplained weight loss	3 (1.7)
Night sweats	8 (4.4)
Fever	4 (2.2)
Anorexia	2 (1.1)
≥ 2 symptoms	5(2.7)
Presence of Co-morbidity	
HIV infection:	0 (0)
Receiving immunosuppressive therapy	2 (1.1)
Chronic infection	0 (0)
Cigarette smokers	3 (1.7)
Alcohol users	19 (10.5)

Only 2 of the participants reported use of high dose steroids previously. No one reported to be HIV positive or to abuse alcohol/drugs. Three participants admitted that they were smokers.

Interferon gamma assay results

Valid INF-gamma assay results were available for 180 (99%) of the 181 participants. The median INF-gamma assay level was 0.06 (Range -4.53 – 10.0). A cut off level of 0.35IU/ml was used to determine if participants were either positive or negative.

With this cut-off point, 54 (29.8%) had a positive assay.

One participant had an indeterminate response due to an insufficient sample collection.

Table 4: Interferon gamma assay results

Variable	Frequency (%)
Positive results	54 (29.8)
Negative results	126 (69.6)
Indeterminate results	1 (0.0)

4.2Prevalence Of Latent Tb

Latent TB is TB infection without any symptoms of TB. From the study out of the 54 participants who had a positive result, 45 had no symptoms (Table 5). Therefore the prevalence of Latent TB infection in final year medical students at Kenyatta National Hospital using the INF gamma release assay was **24.9 %**.

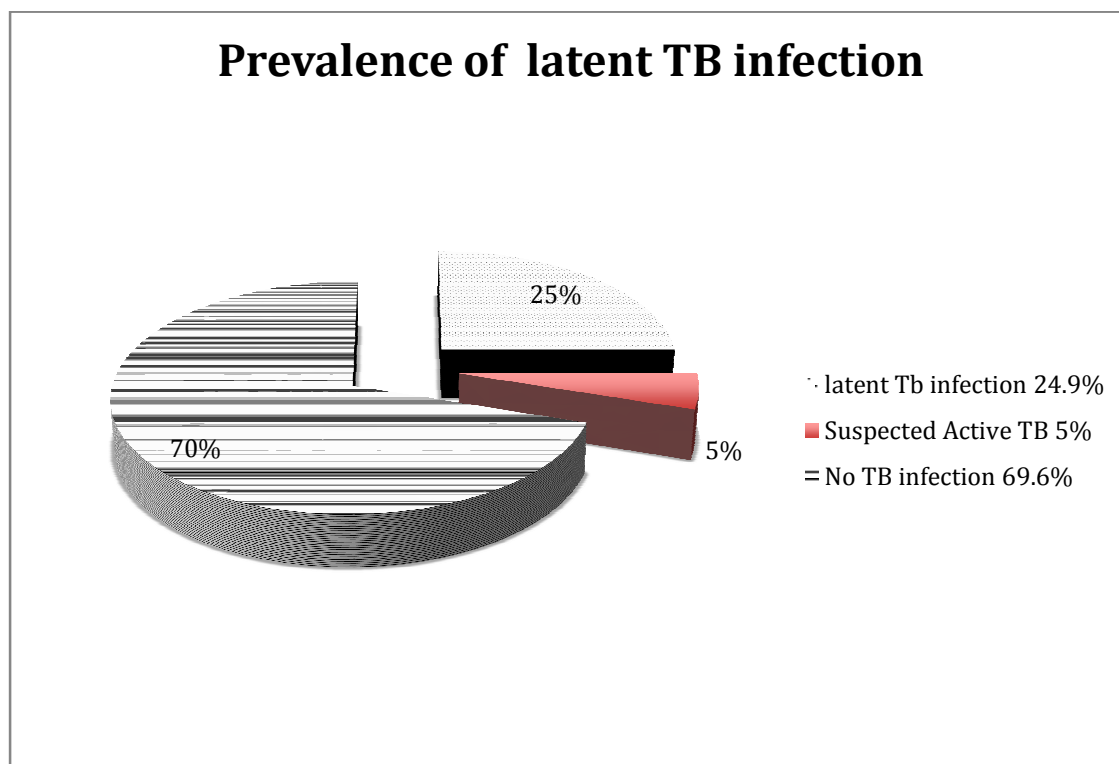


Figure 2: A Pie Chart On The Participants TB Infection Status.

Table 5: Tb Infection

Variable	Frequency (%)	95% CI
Latent TB (IGRA positive and asymptomatic)	45 (24.9)	18.8-31.5
Suspected Active TB (IGRA positive + symptomatic)	9 (5.0)	2.2-8.8

4.3 Factors Associated With Latent Tb Infection

Factors associated with Latent TB Infection in 5th year medical students were explored. They included age, sex, previous ward exposure, co-morbid conditions and history of contact with open TB.

No variable was found to influence the prevalence of latent TB significantly.

There was no difference in ages between the group with latent TB and the group without. There was no statistically significant difference in the prevalence of latent TB between male and female.

The likelihood of having latent TB was 1.9 times higher in persons who had contact with PTB than those without contact. This however was not statistically significant.

The odds of having latent TB in persons that had been residents of a high risk congregatesetting such as a boarding school was equal to those with no such history. This association was not statistically significant.

Receiving immunosuppressive therapy as a factor could not be analyzed as only 2 students reported use of steroids-one for asthma and the other for multiple sclerosis. None of these 2 students tested positive for TB infection.

Use of alcohol did not influence LTB infection significantly

Medical rotation duration (whether the required 20 weeks or greater) was not associated with latent TB significantly. This trend was also seen in the surgical rotation. It seemed rotating in casualty was protective. However no statistical significance was found between any ward rotation and latent TB.

Table 6 summarizes the factor association with the diagnosis of latent TB infection that was explored in this study.

Table 6: Factors Associated With Latent TB Diagnosis

Variable	Latent TB (n=45)	Normal (n=126)	OR (95% CI)	P value
Mean age (SD)	24.4 (1.2)	24.3 (1.0)	-	0.633
Sex				
Male	19 (22.6%)	65 (77.4%)	0.7 (0.3-1.4)	0.281
Female	26 (29.9%)	61 (70.1%)	1.0	
Close contact to person with active/suspected TB				
Yes	42 (27.5%)	111 (72.5%)	1.9 (0.5-6.9)	0.407
No	3 (16.7%)	15 (83.3%)	1.0	
Resident of high risk congregate settings				
Yes	33 (26.4%)	92 (73.6%)	1.0 (0.5-2.2)	0.967
No	12 (26.1%)	34 (73.9%)	1.0	
Receiving immunotherapy				
Yes	0 (0.0%)	2 (100.0%)	-	1.000
No	45 (26.6%)	124 (73.4%)		
Alcohol use				
Yes	5 (27.8)	13 (72.2)	1.1 (0.4-3.2)	1.000
No	40 (26.1)	113 (73.9)	1.0	
Duration of rotation in weeks				
Medical ward				
≤20	41 (26.3)	115(73.3)	1.0	1.000
>20	4 (26.7)	11 (73.3)	1.0 (0.3-3.4)	
Surgical ward				
≤20	39 (26.2)	110 (73.8)	1.0	0.913
>20	6 (27.3)	16 (72.7)	1.1 (0.4-2.9)	
Casualty round				
Yes	40 (25.8)	115 (74.2)	0.8 (0.3-2.3)	0.766
No	5 (31.3)	11 (68.8)	1.0	

CHAPTER 5

5.1 DISCUSSION

Health care students in clinical training are at risk of being exposed to mycobacterium tuberculosis in hospital settings. Our study is the first to evaluate the prevalence of latent TB and factors associated with it in medical students in a referral teaching hospital in Nairobi, Kenya.

In this study, the prevalence of LTBI in final year medical students with a mean age of 24.4 years was investigated using the interferon gamma release assay (QFT-Gold) test. We observed a high LTBI prevalence of about 25%. IGRA positivity however was 30%. This finding is important because the dormant bacilli can reactivate and cause active disease in 10% of individuals with latent TB infection [9]. It therefore means that 5 of the study students with LTBI are at an even higher risk of developing TB later on in their lives. One student had been excluded from the study already due to having had active TB Infection and been on TB medication. This information is vital to the school administration and the hospital Management as it may reflect on infection control practices at the hospital.

In comparison to other regions, our study prevalence falls within the range of studies performed in high TB incidence countries, which have reported LTBI prevalence figures ranging from 9.2% to 72% among healthcare students [17,18,19,20]. In these studies however TST was used for the diagnosis of LTBI.

In studies where testing of LTBI was done with IGRA the prevalence of LTBI in medical school students varied: 0.5% in Italian undergraduate medical students [21]

5.2% in South Korean medical students [11], 15.2% in South African Medical Students [22] and 43.9% in Ethiopian medical and paramedical male students [23].

In the Ethiopian study performed in December 2008 to February 2009 at the Faculty of Medicine, Addis Ababa University, 43.9% of 107 healthy medical and paramedical male students with mean age of 20.9 yearshad a positive QFT-GIT test Result [23].

Our study had a lower prevalence than Ethiopian students. This may be explained by the fact that Ethiopia is a country of high incidence TB: 247 per 100,000 population [2]. Secondly, the study population was different from ours. It included nursing students and laboratory medicine students. It is supposed that nurses spend more time with patients than physicians. Our study

population only included medical students who spend less time with patients than nursing students.

Our students had a higher LTBI prevalence than South Africa students. Van et al found a LTBI prevalence of 15.2% among 79 low-exposed medical students and 69.2% amongst 120 highly exposed HCWs in a hospital in Johannesburg [22]. Our study population included final year medical students with high patient exposure whilst the South African study was of low exposed first year medical students. The difference may also be explained by South Africa's infection control practices, which may be a contributing factor since South Africa is a high TB burden country with a prevalence of 857/100,000 population [2].

Looking outside the African continent, South Korea's Da Ho Jung et al carried out a study from March 2010 to March 2012 among 153 medical students in 2nd and 3rd classes before clerkship course found that only 8 (5.2%) tested positive for the QFT-GIT [11]. This prevalence level is low as compared to our study population. It may be attributed to the fact that these were pre-clerkship students and the country's incidence of TB is 80.7/100,000 population [11] which is lower than Kenya's: 288 per 100,000 population [1]. Our study population had a higher prevalence than Japan's student population. The Japan study evaluated TB risk at the time of admission in university campus and medical schools in Japan. 969 students (585 Japanese students and 384 international students) were screened for TB using the IGRAs at the time of admission. Eight Japanese students (0.9%) were positive for IGRAs, and 30 international students (7.8%) were positive for IGRAs [24]. These students were tested upon admission and had no ward exposure.

When student prevalence figures are compared to health care workers, it is clear that the health workers have a higher prevalence of LTBI. Kampala, Uganda reports a high prevalence of LTBI of 57% using a tuberculin skin test (TST \geq 10mm) in 396 health care workers from 3 hospitals within Kampala [10]. This was informed by a cross-sectional study conducted between June and August 2001 [10]. The mean age of this population was 31 ± 9 years [10]. This population was older and may have had more contact with TB patients than our study population of students. In India, Pai et al evaluated 726 health care workers aged 18 to 61 years with no history of active tuberculosis from January to May 2004, at a rural medical school in India. A total of 493 (68%) of the health care workers had direct contact with patients with tuberculosis. The prevalence estimates of TST and IFN-gamma assay positivity were comparable (41% and 40%

respectively)[14].It can be assumed that health care workers have more exposure to patients than students hence the prevalence value was way higher than students in Kenya.

Generally from the studies reviewed here, HCWs in the high incidence countries, have a higher prevalence than students.

This underscores that precaution need to be taken to avoid the exposure of students to known infectious TB cases during the training activities. Proper use of individual protection devices and measures at the hospital should be encouraged.

Factors associated with Latent TB Infection in the final year medical students in our study were investigated. They included age, gender, previous ward exposure, co-morbid conditions and history of contact with open TB. No variable was found to be to influence the prevalence of latent TB significantly. This compared well with other studies.

In Ethiopia no statistically significant differences in QFT-GIT assay positivity was found when analyzed for any variables (year of study, nursing versus lab students, religion and tribe) [22]. However, the odds of having a positive QFT-Gold assay results was nearly 2 times higher as age increased by one year [22].Their study population included students from 1st year to 3rd year. Our study population only involved one class with a median age of 24 years.

In Italy, a cross-sectional study, performed between January and May 2012, on 733 undergraduate students attending the Medical, Nursing, Pediatric Nursing and Midwifery Schools of the University of Genoa, were tested for LTBI. No statistically significant association between TST positivity and age and gender was observed [21]. The main independent variable associated with TST positivity was to be born in a country with a high TB incidence (i.e., >20 cases per 100,000 population) [21].

Likewise, in the South Korean study of 2nd and 3rd year medical students, no variables (sex, History of previous TB, History of contact with TB patients) were found to influence positivity of Latent Tb testing [11]. This was a similar finding to our study.

The fact that no factors influenced LTBI could mean that our sample size was not powered to achieve our secondary objective. Out of a class of 310, 182 students volunteered. This left out 128 students, majority of whom opted out due to fear of needles. It is possible that inclusion of the whole study population would have brought out a significant association.

Our study had several limitations. This was a cross-sectional study hence it was not possible to attribute the TB infection to hospital wards and its environment. TB infection could have been acquired from previous exposure such as residences (e.g. slum dwelling). The risk of TB infection could also not be determined due to the study design.

Recall Bias could not be eliminated. It was difficult to ascertain the specific duration students spent in different rotations at the hospital and contact time with patients suspected to have TB. We could also not compare pre clinical and clinical students as all study participants were from one class.

The effect of alcohol or cigarette smoking to IGRA positivity could not be assessed as very few student admitted to this.

Selection Bias of the study population was seen as only willing students who consented were tested. One out of two students opted not to have TB test done for fear of needles.

5.2 CONCLUSION AND RECOMMENDATIONS

The prevalence of latent TB infection in final year medical students at Kenyatta National Hospital was high at 25%. These 45 students should be followed up closely to investigate for active TB in future as studies show that 10% develop active TB in their lifetime.

The school administration and the hospital management could use this information in guiding policy in infection control practices. The principle investigator will disseminate the outcome of this study at the appropriate venue to University of Nairobi, Kenyatta National Hospital Annual Scientific Conference. The findings will also be availed to the Board of Undergraduate Studies.

Further studies could be done on the incoming medical students and HCWs of Kenyatta National Hospital as these two groups would provide for good comparison.

APPENDIX I

QUESTIONNAIRE

Part 1: Demographics

ID code _____

Age (yrs) _____

Sex _____

Year of study _____

Phone contacts _____ email address _____

Part II: Tuberculosis (TB) Screening

Please answer the following questions by circling the correct response:

1. Have you ever had close contact with persons known or suspected to have active TB disease?
A) YES
B) NO

2. Have you been a resident of high-risk congregate settings (e.g., correctional facilities, boarding school)?
A) YES
B) NO

3. Have you been a volunteer or health-care worker who served clients who have active TB disease?
A) YES
B) NO

4. Indicate the duration of time spent rotating in the different medical wards during your clerkship years.

- a) Medical wards _____ weeks
- b) Surgical wards _____ weeks
- c) Casualty _____ weeks
- d) Pediatric wards _____ weeks

Part III. Clinical Assessment

Please answer the following questions

1. Do you have any of the following signs or symptoms? Tick the appropriate response.

- A) Cough (lasting for 3 weeks or longer) with or without sputum production
- B) Coughing up blood (hemoptysis)
- C) Chest pain
- D) Unexplained weight loss
- E) Night sweats
- F) Fever
- G) Anorexia

2. Have you been on treatment for TB recently (within the past 2 years)

- A) YES
- B) NO

3. Do you have any of the following conditions?

(Tick the appropriate response)

- A) Infected with HIV
- B) Receiving immunosuppressive therapy such as tumor necrosis factor-alpha (TNF) antagonists, systemic corticosteroids equivalent to/greater than 15 mg of prednisone per day, or immunosuppressive drug therapy following organ transplantation

- C) Diagnosed with diabetes mellitus, chronic renal failure, leukemia, or cancer
- D) Cigarette smokers and persons who abuse drugs and/or alcohol

4. Interferon Gamma Release Assay (IGRA)-[To be filled by lab technician]

Date Obtained: ____/____/____ DMY

Result: negative_____ positive_____ indeterminate_____

Part IV. Management of Positive TST or IGRA

All students with a positive TST or IGRA with no signs and symptoms of active disease have latent TB and will be referred to a clinician for further evaluation and management.

THE END

Adapted from a sample form prepared originally by ACHA's Tuberculosis Guidelines Task Force Revised by Emerging Public Health Threats and Emergency Response Coalition

American College Health Association 1362 Mellon Road, Suite 180 Hanover, MD 21076 (410) 859-1500 www.acha.org

APPENDIX II

INFORMED CONSENT EXPLANATION

Title of the study:

The prevalence of Latent TB infection in Final year Medical students at Kenyatta National Hospital

Principal Investigator: Jane Gitau

Introduction:

We are interested in finding out the magnitude of TB infection and other risk factors of TB infection among students. We will require 3mls of blood for Interferon Gamma Release assay, a test that measures your exposure to TB infection. The test cannot detect if an individual has active disease.

Procedure to be followed:

Upon accepting to participate in this study you will be requested to sign a consent form after which you will answer questions relating to your socio-demographics, present and past illnesses and current treatments. We shall then draw 3mls of venous blood for testing. We shall give you the results and accord you appropriate advice and referral for follow up.

Participation of this study is voluntary and you can choose to decline or withdraw from the study without any penalty.

Risk:

Mild discomfort may be experienced during withdrawal of blood sample for the lab tests. You may develop a bruise or swelling where the needle will be inserted

Benefits:

The mentioned procedure will be done free of charge. A copy of the results will be availed to you at the Kenya Aids Vaccine Initiative Lab for collection.

Confidentiality:

Strict confidentiality will be maintained and all data obtained will be securely stored and used for purposes of this study only.

Who can participate in this study: a person above 18 years and is a medical student in 5th year of study.

Participation: Your participation in this study is voluntary. Refusal to participate will not be penalized. You may discontinue participation at any time without any penalty.

If during the course of this you have any questions concerning the nature of the research you should contact:

Dr Jane Gitau, P.O. Box 116-202 Nairobi, Kenya. Telephone +254722282955

Permission to conduct this study has been sort from **Kenyatta National Hospital/ University of Nairobi Ethics Review Committee (KNH/UoN-ERC).**

Contact Information:

P.O Box 20723-00202 Nairobi

Email: uonbi_erc@uonbi.ac.ke

TEL 726300-9

APPENDIX III:

CONSENT FORM:

I.....

Do hereby give consent/permission to Dr Jane Gitau to include me in this study on ‘The Prevalence of latent TB Infection in Final year Medical Students at Kenyatta National Hospital’.

I have read and understood the contents of this form. I am also aware I can withdraw from this study without losing any benefits.

VOLUNTEER

NAME.....SIGNED.....

WITNESS.....

DATE.....

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