LATENT TUBERCULOSIS INFECTION AND HUMAN LEUKOCYTE ANTIGEN POLYMORPHISMS IN PULMONARY TUBERCULOSIS PATIENTS AND THEIR HOUSEHOLD CONTACTS

SUSAN AKINYI ODERA

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DEPARTMENT OF MEDICAL MICROBIOLOGY AND IMMUNOLOGY

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UNIVERSITY OF NAIROBI

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DECLARATION

I affirm that this thesis is my original work and has not been presented for any award or degree at any other university. All referred published materials by others have been acknowledged.

Signature..... Date..12/12/2022.....

Susan Akinyi Odera,

Department of Medical Microbiology and Immunology,

Faculty of Health Sciences, University of Nairobi.

SUPERVISORS

The submission of this thesis has our approval as University Supervisors:

1. Prof Julius Oyugi

Signature..... Date..14/12/2022.....

Associate Professor
Department of Medical Microbiology and Immunology
Faculty of Health Sciences, University of Nairobi

2. Prof. Omu Anzala

Signature..... Date..14/12/2022.....

Professor
Department of Medical Microbiology and Immunology
Faculty of Health Sciences
University of Nairobi

3. Dr Marianne Mureithi

Signature: Date: 13th December 2022

Chairman and Senior Lecturer
Department of Medical Microbiology and Immunology
Faculty of Health, University of Nairobi

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DEFINITION OF TERMS

Household Contact: A person who shared the same enclosed living space and slept under the same roof with the PTB index case continuously for at least one week during the three months before the diagnosis.

Latent Tuberculosis Infection (LTBI): A state of persistent immune response to stimulation by *Mycobacterium tuberculosis* antigens with no evidence of clinically manifest active TB. There is no standard gold test for directly identifying *Mycobacterium tuberculosis* infection in humans. Most infected people have no signs or symptoms of TB but are at risk for active TB disease.

ACRONYMS AND ABBREVIATIONS

BCG …….. Bacillus Calmette- Guerin

HHC…….Household Contact

HIV ……Human Immunodeficiency Virus

HLA ……Human Leukocyte Antigen

IGRAs ……..Interferon Gamma Release Assays

INH …….……Isoniazid

MDR-TB ……Multi-drug resistant tuberculosis

MHC ……. Major Histocompatibility Complex

MIRU-VNTR …Mycobacterial Interspersed Repeat Units- Variable Nucleotide Tandem Repeats

MODS ……Microscopic Observed Drug Susceptibility

PTB ……Pulmonary Tuberculosis

RFLP ……..Restriction Fragment Length Polymorphism

RMP ……...Rifampicin

SNP ……Single Nucleotide Polymorphisms

TB ……..Tuberculosis

TST …….Tuberculin Skin Test

WHO ……World Health Organization

XDR-TB ……. Extensively Drug-Resistant Tuberculosis

ZN …….Ziehl- Nielsen

ABSTRACT

TITLE: Latent Tuberculosis and Human Leukocyte Antigen Polymorphisms in Pulmonary Tuberculosis Patients and their Household Contacts in Kenya

BACKGROUND: Household Contacts (HHCs) are the primary caregivers of Pulmonary Tuberculosis (PTB) patients at home and, subsequently, have a higher cumulative exposure to *Mycobacterium tuberculosis* through close physical contact and social interactions. Due to the mode of transmission of this pathogen, HHCs are more likely to be infected since the PTB patients are infectious before and during the early stages of their treatment. Due to underdiagnosis, latent tuberculosis infections (LTBI) are often missed, likely progressing to active TB. Sensitisation on LTBI dynamics and addressing challenges that HHCs face while caring for their PTB patients within the home setup could also help identify LTBI-positive cases for prompt initiation of preventive therapy. Host genetics have been identified as predictors for susceptibility to infectious diseases such as TB. For instance, the Human Leucocyte Antigen (HLA) class II alleles can influence the early immune response to TB by presenting antigenic peptides to CD4+ T cells for destruction. Polymorphisms in these genes could affect the efficiency of the body's immune response to TB infections, thereby determining the likelihood of progressing LTBI to active TB disease. In Kenya, the prevalence of LTBI diagnosed using IGRA among HHCs of PTB patients is lacking, presenting a knowledge gap. In addition, this population has not elucidated HLA class II allele polymorphism that influences susceptibility to TB and progression of LTBI to PTB.

OBJECTIVE: To determine the prevalence and risk factors of latent TB infection among household contacts of PTB patients and the association between frequency of HLA class II allele groups and outcome of exposure to *Mycobacterium tuberculosis*.

METHODS: A descriptive-analytic cross-sectional study of adult PTB patients and HHCs was conducted in outpatient clinics and isolation inpatient wards at Mbagathi County Hospital in Nairobi. The HHCs were recruited as they accompanied the patients to the outpatient clinics or during visiting hours at the inpatient section after the provision of consent. Sociodemographic data were collected using informal interviews and structured, pretested questionnaires, while clinical data were retrieved from patient files. Using a mixed-methods study design, we documented the challenges and experiences of PTB patients and their HHCs. Intravenous blood samples were drawn for Interferon Gamma Release Assay (IGRA) to determine Latent Tuberculosis Infection (LTBI) among HHCs and for extraction of DNA for typing of HLA-DQB1 and HLA-DRB1 allele groups via PCR sequence-specific primer amplification. Data analysis was done using R software version 4.2.0. The prevalence of LTBI was calculated using the Clopper Pearson method. Chi-square and Fisher's Exact tests were used in identifying potential factors associated with LTBI, while logistic regression was used for further comparative analyses. A linear regression model was used to investigate variation in the concentration of Interferon-gamma amongst those who tested positive for LTBI.

RESULTS: We evaluated 166 PTB and 175 HHCs. The overall prevalence of LTBI evaluated among the HHCs of PTB patients using the IGRA test was 55.7% (95% CI: 48-63.2). There wasn't enough statistical evidence to show an association between the Optical Density value measurements of IFN gamma concentrations and any assessed variables among the LTBI-positive individuals. Statistical evidence showed an association between several covariates and the risk of LTBI. These included the HHC HIV serostatus, relationship status (spousal or other) between the

household contact and the PTB patient, and the HIV serostatus of the index PTB patient. In the analysis, HHCs who were HIV seropositive had 98% fewer odds of testing positive for LTBI (OR: 0.02; CI: 0 - 0.3; p-value 0.006). The non-spouse relationship (OR: 0.09; CI: 0.01 – 0.69; p-value 0.021) and PTB patients positive for HIV (OR:0.41; CI: 0.19 - 0.87; p-value 0.02) were also significantly associated with reduced odds (91% and 59% less odds respectively) of testing positive for LTBI. On the contrary, HIV seropositive HHCs who were not spouses had over 63 times the odds of testing positive for LTBI (OR: 63.24; CI:2.44 – 1637.3; p-value 0.012). There was a significant interaction of terms where the non-spousal relationship seemed to modify the effect of the seropositive HHC. The huge confidence interval can be attributed to the smaller sample size falling in this category. The HLA-DQB1 and HLA-DRB1 allele groups were analysed in 54 participants: 17 PTB patients and 37 HHCs. In this group, 19 HHCs were LTBI positive while 18 were LTBI negative. The frequency of DRB3*1 was 0.17-fold lower [95% CI=0.03-0.83] among PTB patients compared to HHCs before adjusting for HIV status (p=0.048), while the frequency of the DRB5*2 allele was 23.5% higher among PTB patients compared to HHCs (p=0.013) before adjusting for HIV status. After adjusting HIV status, the frequency of DRB1*14 was 12-fold higher [95% CI=1.11-138.2] among PTB patients compared to HHCs (p=0.040). Evaluation of the experiences of the PTB patients and their HHCs revealed adequate access to PTB diagnosis and treatment. However, lack of knowledge on LTBI by the HHCs, psychosocial challenges and inadequate infection control measures at home are significant gaps that need to be addressed.

CONCLUSION: The HHCs of PTB patients in this population had a high prevalence of LTBI at 55.7%. Being a spouse of a PTB patient, prolonged co-habitation, and HIV serostatus were potential risk factors. The HIV serostatus of the PTB patient could impact infectiousness and, therefore, the risk of infection to close contacts. The HIV serostatus of the HHC could influence the performance of the IGRA test. The frequency of HLA-DRB5*2 and HLA-DRB1*14 alleles groups were higher among PTB patients, which suggested a possible association with the progression of LTBI to active PTB. The HLA-DRB3*1 allele had a higher frequency among LTBI negative HHCs, suggesting a potential protective role against *M. tuberculosis* infection.

RECOMMENDATIONS: The high prevalence of LTBI emphasises the need for TB control programs to focus more on sensitising household contacts of PTB patients on the importance and availability of screening for LTBI and preventive treatment to avoid reactivation. A proposed tool-the PTB HHC data card, can be evaluated for use in TB diagnostic centres to follow up on this high-risk group for implementation of TB preventive treatment. The TB prevention programs should also address the multifaceted challenges faced by caregivers of TB patients at home. We recommend further studies using higher resolution HLA typing kits to investigate the roles of HLA-DRB3*1, HLA-DRB5*2, HLA-DRB1*14 in TB immunopathogenesis as predictive HLA genetic biomarkers of the likely outcome of exposure to *Mycobacterium tuberculosis*.

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CHAPTER ONE

1 INTRODUCTION

Tuberculosis (TB) is a severe disease that impacts public health globally. From available data, about two billion individuals are latently infected; 8-10 million TB cases arise yearly. The World Health Organization (WHO) ranks TB among the top 10 leading causes of mortality worldwide. It is recognised as the primary cause of mortality globally as a single infectious agent, alongside HIV/AIDS and COVID-19. The disease has risen to epidemic proportions, particularly in sub-Saharan Africa (SSA), Kenya ranking among the top 30 countries with the highest TB burden (World Health Organization. Global Tuberculosis Programme, 2021). A consultative process by the WHO defined lists of high-burden countries between 2016 and 2020 in three levels: one list was for multidrug-resistant TB (MDR-TB), one for TB, and one for HIV-associated TB. Kenya is one of the 14 countries that appear on all three lists. These countries bear an enormous burden of the impact of TB. Global estimates project that about 63% of the incident TB cases in 2019 occurred in these countries (MacNeil, 2020).

Tuberculosis develops from infection with *Mycobacterium tuberculosis* (*Mtb*), an acid-fast bacillus. Due to the aerobic nature of the bacillus, it preferentially infects the lungs to cause pulmonary TB. However, it can also spread and infect other body systems, such as the urinary tract and the central nervous system. Shimeles et al discussed determinant factors associated with TB in adult populations in high-burden TB areas (Shimeles et al., 2019). These risk factors are likely predictors for tuberculosis and can be broadly categorised as environmental, socioeconomic and host/pathogen genetics. A distinctive feature of tuberculosis is the wide variation in the outcome of exposure to the pathogen, which ranges from a latent stage where individuals are exposed and infected but do not have any clinical symptoms to a more symptomatic infectious stage. Since only 10% of individuals infected with the bacillus in an HIV-1 negative population often progress to clinically evident active disease, many studies have postulated that exposure is significantly hinged on several host genetic factors that play a role in regulating disease expression (Duarte et al., 2018; Selvaraj, 2004; Wu et al., 2013).

Latent tuberculosis state can be identified when *Mycobacterium tuberculosis* antigens persistently stimulate the immune system without inducing the clinical symptoms of active TB. Most people who develop this state are asymptomatic but are more likely to develop active TB eventually (World Health Organization, 2018b). Although the actual burden of latent tuberculosis globally has not been established,

data shows that at least a third of humans have LTBI. Individuals who closely interact with PTB patients often have a significantly higher risk of getting infected due to prolonged exposure. Studies show that 10% of latent TB patients later progress to clinically symptomatic TB within the first five years of the initial infection (Kiazik S and Ball TB, 2017; Comstock et al., 1974), with clinical conditions associated with immune deficiency being the most common predictor for progressing to active TB (Blumberg & Ernst, 2016). People living with HIV and have LTBI (primary tuberculosis) are 26 times more likely to progress from latent to active TB (Kiazyk & Ball, 2017; Tilahun et al., 2019).

WHO has recommended a systematic screening approach for high-risk individuals and preventive therapy as control strategies for eliminating TB (Blumberg & Ernst, 2016). The preventative strategy postulates that TB elimination programs should incorporate screening for LTBI and prevent its progression to TB as part of primary care. The updated guidelines on the management of LTBI by the WHO (World Health Organization, 2018b) propose that children and both HIV-negative and positive adult HHCs of PTB patients who are living in a country with a high TB incidence should undergo a proper clinical evaluation to exclude active TB, and after that preventive therapy offered to those who test positive. Knowledge of LTBI and active TB dynamics in different populations at host and bacteria levels is crucial for controlling the disease.

Several studies on host genetics have investigated why some individuals are less prone to contracting TB than others. Infectious diseases such as TB exert much selective genetic pressure on victims because the genes that evoke immune responses are very diverse (Burgner et al., 2006). Many studies that have assessed the contributions of specific variations of specific DNA sequences (polymorphism) in the human genome and resistance or susceptibility to TB have elicited more questions than answers. Findings from some of these studies suggest that the outcome of exposure to *M. tuberculosis* and subsequent infection depends on bacteria, environmental, and host genetic factors, among several other factors (Coscolla & Gagneux, 2014).

The Human Leukocyte Antigen (HLA) has been studied extensively as a genetic risk factor for infectious disease transmission, auto-immune diseases and cancers (Kettaneh et al., 2006; Yim & Selvaraj, 2010Debebe et al., 2020). The nature and localisation of HLA polymorphism observed in populations would contribute significantly to disease susceptibility because different people express alleles and their specific extended haplotypes differently (Fernando *et al*., 2008; Pisanti *et al*., 2020). Understanding the

protective association of HLA in particular populations will be helpful for future studies in developing new epitope-based vaccines specific to phylogeographical lineages. The HLA class 2 genes, notably the HLA-DRB and HLA-DQB1 loci, have impacted the mycobacterial antigens presented to T helper cells for destruction (Bakir-Gungor & Sezerman, 2011).

This work investigates the prevalence of LTBI and socio-demographic dynamics among adult HHCs of PTB patients in a Kenyan setting. We will also highlight the experiences and challenges faced by the contacts of the PTB patients at the household level and propose a prototype PTB patient HHC Data card that can be customised for TB diagnostic centres. Furthermore, we discuss the findings of an exploratory analysis on the distribution of HLA-DQB and HLA-DRB allele groups among the study participants grouped as those with active disease (PTB patients), those who are LTBI positive (HHCs who are exposed and infected but asymptomatic) and those who are LTBI negative (HHCs who are exposed but do not have immunological evidence of infection). We explore which HLA alleles can be further investigated as markers for the acquisition and development of PTB in this population.

1.1 THESIS OVERVIEW

This thesis is divided into six chapters. The first chapter gives a general overview of *Mycobacterium tuberculosis* and this study's rationale and aims. The second chapter is an in-depth write-up on facts and emerging issues regarding the diagnosis and treatment of LTBI in various populations and the role of HLA in TB immunopathogenesis. The third chapter details the protocol used to measure the study's stated objectives. The findings and discussion of the results are presented in the fourth and fifth chapters. Finally, the study conclusions, limitations and recommendations are shown in the last chapter.

1.2 STUDY JUSTIFICATION

The HHCs are the TB patients' primary caregivers at home and, subsequently, have a high cumulative exposure to the infectious agent through close physical and social contact in the shared living space. Consequently, the HHCs are more likely to acquire *M. tb* infection. Identifying and treating individuals with LTBI significantly reduces active TB cases. However, the available data on the prevalence of LTBI and its associated factors among adults living with PTB patients in Kenya is relatively minimal. Increased sensitisation on the available testing and treatment options of LTBI among this population and highlighting the challenges they face while caring for the TB patient within the home setup will significantly reduce TB cases. Host genetics has been documented as an essential factor in susceptibility

to infectious diseases. The Human Leucocyte Antigen (HLA) class II alleles influence the early immune response to TB by presenting antigens to CD4+ T cells; hence polymorphisms in the genes can affect the immune response to infection regulation and progression to active disease. By identifying the polymorphisms within this population, this study will offer insight into alleles that can be explored as predictive biomarkers of infection outcomes among individuals exposed to the bacilli. Also, knowledge of the HLA alleles' contribution to the immunopathogenesis of TB within this population will provide valuable data for future studies on vaccine candidate development.

1.3 OBJECTIVE

To investigate Latent Tuberculosis Infection and Human Leukocyte Antigen polymorphisms in Pulmonary Tuberculosis patients and their household contacts in Kenya.

1.3.1 Study Objectives

1. To measure the prevalence of Latent Tuberculosis Infection among Household Contacts of Pulmonary Tuberculosis patients in Nairobi, Kenya.
2. To identify risk factors for Latent Tuberculosis Infection among the Household Contacts of Pulmonary Tuberculosis patients in Nairobi, Kenya.
3. To determine the distribution of Human Leukocyte Antigen Class II (HLA-DRB and HLA-DQB) allele groups in the study population.
4. To evaluate the association between Human Leukocyte Antigen Class II (HLA-DRB and -DQB) allele group frequencies and the outcome of exposure to *Mycobacterium tuberculosis.*

1.3.2 Secondary objectives

1. To document challenges experienced by Household Contacts of PTB patients in an urban setting.
2. To design a prototype PTB HHC Data card that can be customised in specific TB diagnostic centres to follow up on household contacts of PTB patients.

CHAPTER TWO

2 LITERATURE REVIEW

2.1 History of TB Disease

Tuberculosis has plagued humanity for several centuries. The earliest historical reference to TB disease is in Egypt's medical papyrus dated 1550 BC. In these records, spinal TB was reported in the Egyptian mummies. Paleopathological evidence in ancient skeletal remains presented at the time, together with others found from the paleomicrobiology of the tubercle bacillus, provided unprecedented information on the history of the bacillus (Barberis et al., 2017). Other early records of TB have also been found in India in a Rig Veda hymn dated 1550 BC. All the lineages of the *M.tb* complex are present in Africa, suggesting evolution from a common ancestor and dissemination to the rest of the world via the human migrations (Comas et al., 2013).

Hippocrates described TB in Book 1 of Epidemics as phthisis- a disease of the weakness of the lungs with cough and fever. The forms and stages of tuberculosis disease were later established by Gaspard Laurent and Rene Laennec in 1803 (Roguin, 2006). Laennec stated that the various lesions found in the phthisical lung were part of the same pathological process in different phases. In 1839, Schonlein opined that the word “tuberculosis” should be used as a reference for all the clinical presentations of TB’s pathological processes that involved the lungs. After being troubled with tuberculosis, Edward Livingstone, an American doctor, established a laboratory named after him that later became a modern hospital for treating tuberculosis.

In 1882 tuberculosis causing bacillus *Mycobacterium tuberculosis* was characterised by the German physician and microbiologist Robert Koch. The findings were published in the Berlin Society of Physiology on March 24th 1882 - a day now referred to as the “World Tuberculosis Day.” Two years in 1884, later he expounded the Koch’s postulates. In 1890 he announced the discovery of tuberculin, which later proved to be a valuable diagnostic tool. Koch was awared the Nobel prize in medicine in 1905. Over 13 years (1908-1921), a vaccine for tuberculosis was developed by two French bacteriologists, Albert Calmette and Camille-Guerin, from an attenuated bovine strain of TB and named Bacillus of Calmette and Guerin (BCG) (Fatima et al., 2020; Luca & Mihaescu, 2013). The history of tuberculosis is illustrated in **Figure 1**:

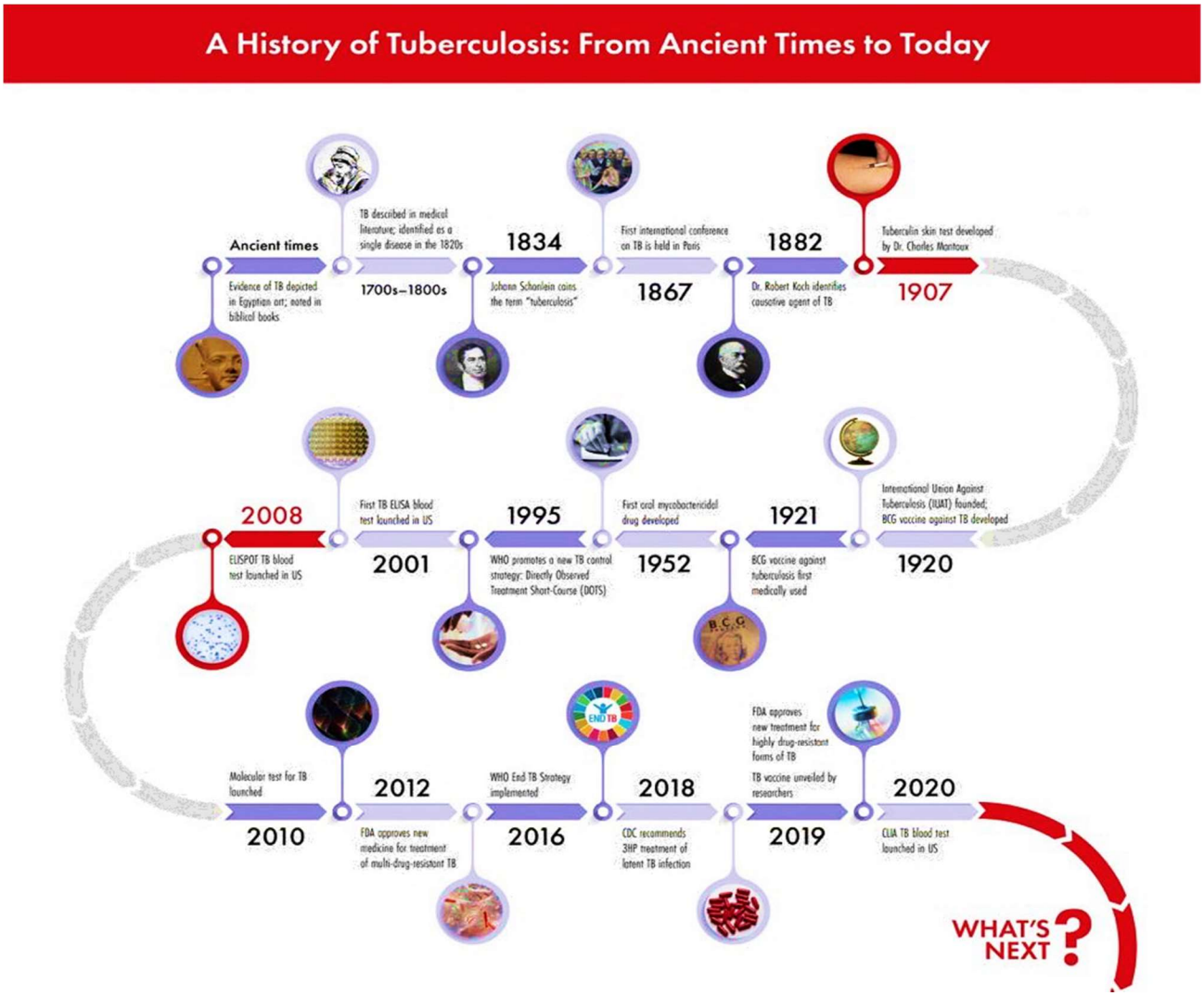

Figure 1. Adapted from History of Tuberculosis: from the ancient times to today (https://tspot.asia/education/educational-resources/infographics).

2.2 Epidemiology of Tuberculosis

Tuberculosis is a debilitating disease caused by a bacterium called *Mycobacterium tuberculosis.* It has been documented as a primary cause of mortality by a single infectious agent (worse than HIV/AIDS) (World Health Organization, 2018a). added After the emergence of Covid-19, it has been reported that TB is second only to COVID-19 as the cause of death from a single infectious agent WHO, 2021)

TB continues to have a sustained impact on medical health systems in low- and middle-income countries (LMIC) and has caused panic globally, particularly in sub-Saharan Africa, due to strained environment and socioeconomic conditions. The high prevalence of comorbidity with HIV has also had a heavy toll on strained resources. Tuberculosis has been declared a global emergency, but its incidence varies from

region to region. World Health Organization (WHO) reported that approximately 10.0 million people (between 8.9 and 11.0 million) contracted TB in 2019. In the same year, TB-related deaths were about 1.2 million (between 1.1 and 1.3 million people) among HIV-seronegative people. The number of dead TB related deaths in HIV-positive individuals stood at 208,000 (between 177 000 and 242 000).

The report also indicated that most people who contracted TB in 2019 were men (56%) aged ≥15 years). Female cases were 32%, and children aged <15 years were the least afflicted at around 12% (MacNeil, 2020). Studies that have analysed sex-specific factors and variations in susceptibility to infectious diseases postulate that a higher frequency of active TB among men could be linked to an increased risk for disease progression (McClelland et al., 2013).

Currently, the WHO ranks Kenya among the top 30 countries globally with a high TB burden all over the globe (World Health Organization. Global Tuberculosis Programme, 2021). The total notified cases of TB in Kenya in 2019 were 84, 345 of which 86% were pulmonary TB cases. There was 507 bacteriology confirmed cases of the MDR-RR TB and one case of the XDR-TB (World Health Organization. Global Tuberculosis Programme, 2021). The report also revealed that 82% of newly-enrolled people living with HIV were on preventive treatment. In comparison, only 39% of HHCs of laboratory-confirmed TB cases under-five years of age were on preventative treatments for LTBI.

2.3 Risk factors TB transmission

TB infection usually occurs after exposure to a patient with active pulmonary tuberculosis. Transmission occurs after inhalation of infectious droplet nuclei. TB development to an active disease involves both exogenous and endogenous risk factors, which influence the direction and pace of disease progression from exposure to infection and subsequently active disease (**Figure 2**).

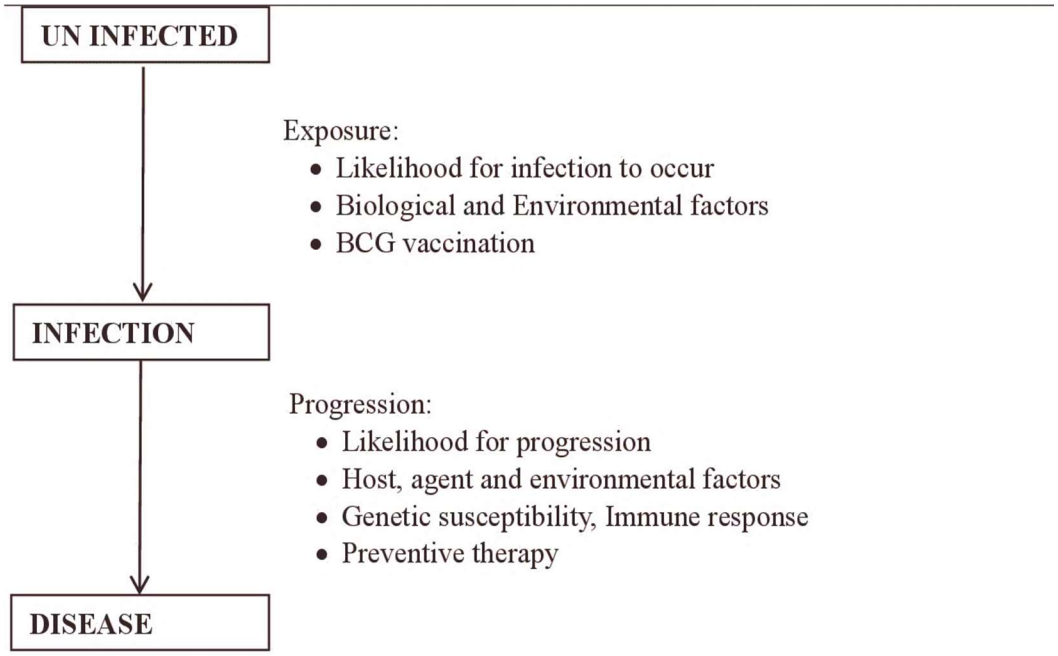

Figure 2. Flow chart on the course of progression of Tuberculosis disease. Adapted from Kakaire (2018).

Endogenous factors contribute to the observed variation in the progression from asymptomatic infection to active TB disease. Besides well-known risk factors for TB such as malnutrition, HIV positivity, and extremities of age, other commonly reported variables that influence infectivity at the population and individual level are indoor air pollution, alcohol consumption, immunosuppressive drug levels use and exposure to tobacco smoke. Higher cigarette smoking rates (20 sticks or more) per day increase the risk of progression from latent to TB active TB (Luca & Mihaescu, 2013; Silva et al., 2018).

Owing to the high burden of HIV in SSA countries, TB has been a prevalent co-infector of the HIV disease (World Health Organization, 2015). Co-morbidities predispose people to TB, including diabetes mellitus, Hodgkin's lymphoma, chronic respiratory diseases, end-stage renal disease, and malnutrition(Marais et al., 2013; Shield et al., 2013). Furthermore, studies have also found that host genetics can influence the outcome of exposure to infectious bacilli and subsequent progression to active TB disease (Abel et al., 2014a).

2.4 Microbiology of *Mycobacterium tuberculosis*

2.4.1 Biology and characteristics of *Mycobacterium tuberculosis*

Mycobacteria are non-motile slow-growing Acid Fast bacilli with a high concentration of G+C base pairs in their genome. They have a complex cell wall with high mycolic acid content that confers the acid-fast

staining characteristic that can be visualised using special techniques like Ziehl-Neelsen. The bacteria can be divided into non-tuberculous mycobacteria (NTM), *Mycobacterium leprae, and Mycobacterium tuberculosis* complex. *Mycobacterium leprae (*or Hansen's bacillus) causes leprosy- a chronic infectious disease. Infection requires close contact with a patient. Generally, the condition is curable, but late diagnosis can result in irreversible damage to nerve tracts. The NTM contain over 150 species and is mainly associated with opportunistic infections in immunocompromised individuals. The *M. avium* complex (MAC) comprises the *M. intracellulare* and *M. avium,* pathogenic NTM.

The primary causative agents of TB disease are the species of *Mycobacterium tuberculosis* complex (MTBC), which include: *Mycobacterium tuberculosis* (*Mtb*), the chief pathogen associated with TB in humans; *M. bovis (subs. bovis and caprae), M. africanum*, which infects humans in specific regions of Africa; *M. canetti*, a vaccine strain called *M. bovis* BCG (Bacille Calmette-Guerin), *M. pinnipedii*, which causes TB in different mammals; and *M. microti*, which only infects wolves (Delogu et al., 2013). The organisms have a genetic similarity but differ in their epidemiology, pathogenicity and host spectrum. Molecular studies on the genome and biology of the tubercle bacillus revealed novel properties (Cole et al., 1998) and emphasised the narrative of *Mtb* as a global human pathogen (Brosch et al., 2002).

Historical works have postulated the emergence of *Mtb* as a pathogenic human species about 70,000 years ago, with subsequent spread to other continents following human migrations (Brosch et al., 2002; Hershberg et al., 2008). History records suggest the first *Mtb* strains originated from the environmental mycobacteria (Supply et al., 2013), which evolved and persisted in low-density populations and caused disease reactivation after long latency periods (Blaser & Kirschner, 2007). After adopting the domestication of animals, transmission from humans to animals (zoonosis) was possible, thereby contributing to the emergence of. *M. bovis* is a pathogen of both wild and domesticated animals (Brosch et al., 2002). The impact of modern agriculture practices, the spread of civilisation and the consequences of rural to urban migration led to the selection of *Mtb* strains with enhanced pathogenicity, referred to as the modern *Mtb* strains (Comas et al., 2013; Wirth et al., 2008).

2.4.2 Genetic variation of *M. tuberculosis*

Clinical strains of *M.tb* have been divided into genetic groups based on Single Nucleotide Polymorphisms (SNPs) that occur at the $katG_{463}$ gene and $gryA_{95}$ gene (Filliol et al., 2006). These groups can be further classified into lineages. Based on available genome data, the *M.tb* complex can be divided into five

lineages (human-adapted); *M. tuberculosis sensu stricto* (Lineage 1-4 and Lineage 7) and two other human lineages representing *M. africanum* (Lineage 5-6) and at least nine animal adapted lineages (Brites et al., 2018). There is a paucity of data on the lineages circulating in Kenya. A 2014-2015 study by Mburu *et al.* (Mburu et al., 2018) on circuiting strains in Nairobi and Kiambu counties reported seven strains, out of which three strains- Indo oceanic, East Asia Beijing, and Euro America were common. Genetic variation of the pathogens contribute absence *of* presence to the differences in virulence profiles of bacterial strains and signals the movement of genes that encode virulence factors. The genomic diversity of the *Mtb* strain, for instance, influences TB pathogenesis. Genomic diversity affects the degree of virulence and susceptibility to antimicrobial agents and the host's immune response (Cui et al., 2019a).

There is increasing evidence of a high genetic variance within *M. tuberculosis,* and this variation can be used to predict disease outcomes (Möller et al., 2018). Clonal complexity in TB is well documented, and clinicians increasingly rely on the characterisation of strains to discriminate between re-activation and re-infection (McIvor et al., 2017). Genetic diversity in *M. tuberculosis* rarely involves the exchange of genetic material and instead takes the form of deletion, duplication and insertion events in addition to single nucleotide polymorphisms (SNPs). This has resulted in a clonal pattern of evolution, possibly acquiring differential pathogenic characteristics amongst lineages (Nicol & Wilkinson, 2008). A review of 100 published studies on the association between *M.tb c*omplex strain diversity and clinical phenotypes showed that virulence and immunogenicity of clinical strains of MTBC differ (Coscolla & Gagneux, 2010).

2.4.3 Significance of *M. tuberculosis* genetic variation

The onset and progression of disease after infection are influenced by the interplay between the genes of the human host and the pathogen's strains. The pathogenic potential of *M. tuberculosis* strain lineages are different (Coscolla & Gagneux, 2010). Several studies have shown that the pathogenic characteristics of different strain lineages arise because of interactions between human populations and particular lineages (Nicol & Wilkinson, 2008). Strain-specific virulence has been identified by grouping all strains affiliated with specific clinical outcomes and studying their in vitro characteristics. Studies have shown that most strains associated with extra-pulmonary manifestations of TB are more efficient in infecting macrophages than those responsible for pulmonary infection (García de Viedma et al., 2003). These findings show a varied potential for virulence and pathogenicity. They are of concern as a virulent bacillus will spread through a community more rapidly than strains of standard virulence (Kong et al., 2007).

Evidence of variation in strain virulence has also been investigated in studies evaluating the in-vitro characteristics of strains from households where several family members have active TB disease (Theus et al., 2006). The strains grew more rapidly in macrophages compared to non-transmitted control isolates. Implications of strain diversity have also been discussed extensively in work on Bacillus strains associated with significant outbreaks of tuberculosis in the UK and USA (Nicol & Wilkinson, 2008). The strains of *M. tuberculosis* lineages isolated in these outbreaks were shown to replicate significantly more rapidly in human macrophages in vitro than strains responsible for small clusters of infection. It has been hypothesised that strain diversity contributes to the global emergence and spread of Multi-Drug Resistant Tuberculosis (MDR-TB) and Extensively Drug-Resistant Tuberculosis (XDR-TB) (Borrell & Gagneux, 2011).

MDR-TB is tuberculosis that has built resistance to first-line (FLD) anti-TB drugs, i.e., rifampicin (RMP) and isoniazid (INH). Extensively Drug-Resistant Tuberculosis (XDR-TB) is tuberculosis resistant to at least rifampicin and isoniazid, any other member of the quinolone family and at least one anti-tuberculosis injectable second-line (SLD) drug. In 2010, Kenya had 112 confirmed cases of MDR-TB (World Health Organization. Global Tuberculosis Programme, 2021). The numbers increased in 2011, with laboratory-confirmed cases of MDR-TB reported as 160 (World Health Organization, 2015).

Studies have shown that the genetic background of different strains influences the acquisition of other drug-resistant traits and the overall fitness of the strains. Data has been presented showing a correlation with resistant strains in communities. (Gagneux et al., 2006; Nicol & Wilkinson, 2008). XDR-TB was first publicised following an outbreak of TB in South Africa in 2006. A rural hospital in the Kwa Zulu Natal region recorded 53 patients with XDR-TB; 52 of these patients died (Gandhi et al., 2006). The isolated strain showed a higher transmissibility and fatality rate than other resistant strains. Noting the significance of genetic diversity in *M. tuberculosis* is a reasonable basis for evaluating the implications of gene diversity in tuberculosis product development and management of patients (Gagneux et al., 2006; Nicol & Wilkinson, 2008).

2.4.4 The interplay of host and pathogen genetic markers in susceptibility to infection and risk of developing the disease

Exposure of humans to *M. tuberculosis* induces a highly variable response. Factors such as the virulence of the strains of the causative organism and the immune defences of the infected person are essential when

determining the risk of overt disease following exposure. Louis Pasteur termed these factors 'the seed and the soil' (Davies & Grange, 2001). Studies have assessed variation in susceptibility to infection and disease solely in the host or pathogen genome. A univariate view would suggest that those with the disease after infection either display low resistance or are infected with more virulent pathogen strains (Di Pietrantonio et al., 2011).

However, sometimes, the outcomes of people exposed to infectious agents depend on interactions between pathogen and human genotypes. *M. tuberculosis* has shown significant genomic variation in different countries and regions, which might indicate its adaptation to human populations (Chapman & Hill, 2012). Therefore, an alternate multivariate view would consider the possibility of a correlation between pathogen virulence and disease susceptibility (Di Pietrantonio et al., 2011; Gagneux et al., 2006). Specific *M. tuberculosis* genotypes may cause disease in certain hosts and not others, while some hosts may be susceptible to particular genotypes and resistant to others. Gagneux et al. state that this has far-reaching consequences in tuberculosis control and therapeutic product development. It would significantly impact intervention measures to determine the individual and combined effect of pathogen and host genetic variability on phenotype variance (Di Pietrantonio et al., 2011).

2.4.5 Molecular typing of *M. tuberculosis*

There are several molecular typing tools for studying the epidemiology of different strains of *M. tuberculosis*, such as Mycobacterial Interspersed Repeat Units- Variable Nucleotide Tandem Repeats typing (MIRU-VNTR), Restriction Fragment Length Polymorphism IS6110 (RFLP), and Spoligotyping are the genotyping techniques that have been used for *M. tuberculosis* (Glynn et al., 2010). The insertion sequence IS6110 can influence its genetic diversity by inserting genes disrupting its coding sequence. This method is useful for epidemiological investigations but not very useful for phylogenetic analysis, and it has been difficult to standardise the methodology for interpreting the fingerprints (Nicol & Wilkinson, 2008). Spoligotyping utilises a reverse-hybridization technique that assays the genetic diversity of the Direct Repeat locus of the *M. tuberculosis* (Driscoll, 2009). This technique evaluates the absence of presence of multiple spacer regions (unique ones) at a single locus, is not complex and can be reproduced. However, it may not help define the relationship between strains because the diversity of spoligotype results from single deletion events (Gori et al., 2005).

MIRU-VNTR genotyping of *M. tuberculosis* is close to real-time finger-printing, with strain identification based on large reference databases (Allix-Béguec et al., 2008; Weniger et al., 2010). The technique relies on analysing minisatellite elements that occur repetitively at different loci in chromosomes. These combine into a multidigit code that can be identified on reference databases. It is appropriate for phylogenetic analysis because numerous independent loci can be evaluated, although it is more labour intensive than the spoligotyping (Filliol et al., 2006).

2.4.6 Pathogenesis of *Mycobacterium tuberculosis*

Mycobacterium tuberculosis is a bacterial pathogen transmitted when a person inhales the bacilli from a TB-infected person. Infection with the pathogen has varied course and duration, from asymptomatic infection to clinically evident disease with pulmonary and extra-pulmonary involvement (**Figure 3**).

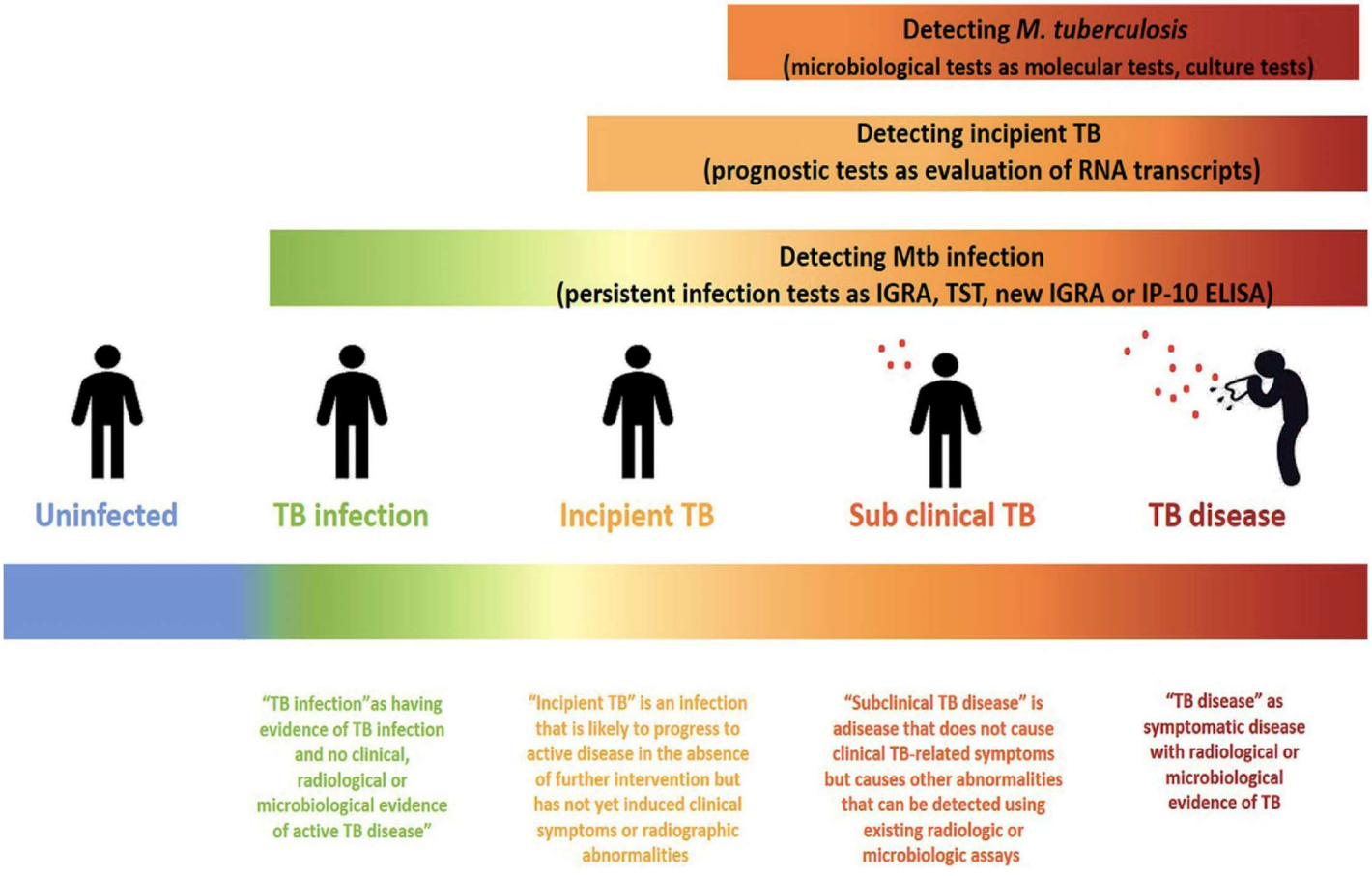

Figure 3. Diagram showing the spectrum of TB infection and disease. Adapted from Goletti et al. (2022)

The nuclei move from the upper to the lower respiratory tract and then to the lungs' alveoli (**Figure 4**). Tubercle bacilli are phagocytosed and inhibited by alveolar macrophages. Replication of the bacillus in the alveoli and the host's immune response produces characteristic symptoms, including productive cough, weight loss, night sweats, and cavities on chest X-ray.

Tuberculosis infections occur predominantly in the lung, but transmission to other body organs is also possible through hematogenous spread. Alive bacilli disseminate via the bloodstream or the lymphatic system to other organs and tissues such as the lung's apex, regional lymph nodes, bones, the brain, the kidney, et cetera.

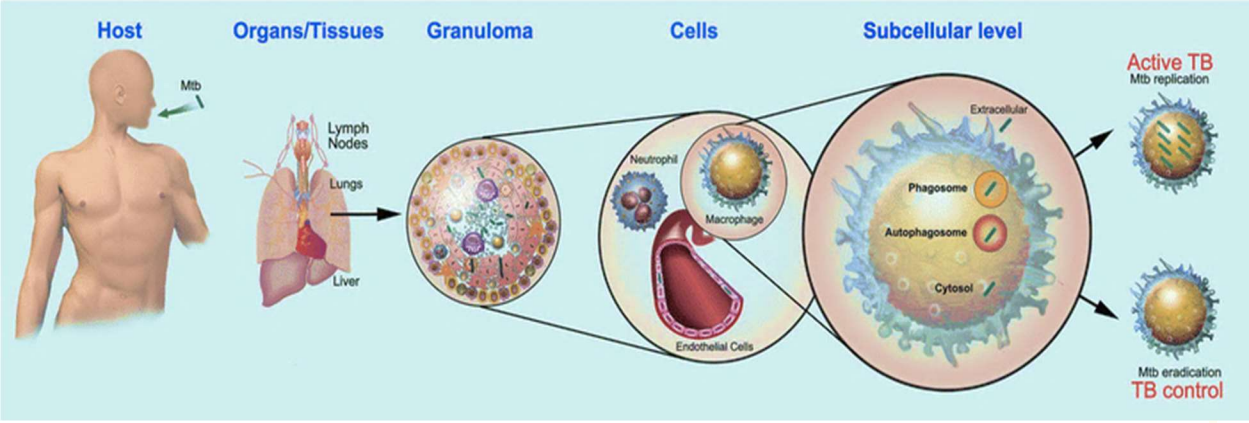

Figure 4. Host cells and environments for Mtb. Image adapted from Bussi & Gutierrez (2019).

2.4.6.1 Immunology of *Mycobacterium tuberculosis* infection

Following the inhalation of infectious droplet nuclei, there are three possible outcomes of exposure to *Mycobacterium tuberculosis* bacilli: First, the bacillus could be destroyed by the host's innate immune responses. Interestingly, this group of people do not have immunological evidence of *M.tuberculosis* infection despite having high exposure to active TB, as evidenced by negative Interferon Gamma (IFN-γ) Release Assays (IGRAs) and Tuberculin Skin Tests (TST) (Bifani et al., 2002; Dolla et al., 2019). Assessing such people's genetic and immunologic features can give an insight into protective immunity in TB (Dolla et al., 2019).

The second likely outcome of exposure is established infection and the development of active tuberculosis disease. The active disease occurs when the host's body cannot control the initial condition or develop a

protective immune response. Studies have shown that several biological, socio-economic and environmental factors contribute to the increased risk of developing TB (Comas et al., 2013). These studies have highlighted that risk factors for developing the disease following exposure and infection with the tubercle bacillus include patients with underlying conditions that contribute to immunosuppression such as HIV infection, use of immunosuppressive drugs, malnutrition, alcohol and intravenous drug abuse, people with diabetes, cancer patients, transplant patients, patients receiving corticosteroids among others (Shimeles et al., 2019).

The third likely outcome of exposure is the more significant majority of individuals with latent tuberculosis infection. These people with tuberculosis infection do not transmit the disease (are not contagious) and do not develop clinical symptoms (Flynn & Chan, 2001b). This population is an essential reservoir for reactivation of the disease from the latency (Dye et al., 1999; Selwyn et al., 1989). According to the WHO, one-fourth of the world population is latently infected, and 8-10 million cases of TB arise every year (World Health Organization, 2015).

2.4.6.2 Innate Immunity

In the early phase of infection, after inhalation of the droplet nuclei, mycobacteria are ingested by macrophages in the alveoli. Replication happens within the endosomes of the affected macrophages (Bussi & Gutierrez, 2019) and alveolar epithelial type II pneumocytes, as was described by (Bermudez & Goodman, 1996, JM Scordo et. al, 2016). The primary cellular mechanisms involved in innate immunity in the lungs include monocytes, macrophages, dendritic cells, and neutrophils, which readily engulf *M. tuberculosis* and either destroy or present them to other immune cells for destruction (Scordo et al., 2016; Sia & Rengarajan, 2019).

Biologically, the lysosomes and phagosomes fuse to form phagolysosomes. The bacterium survives inside the phagosome by inhibiting phagolysosome biogenesis and acidification processes and gaining access to the cytosol. This access to the cytosolic space is vital for specific immune responses. Cytokines recruit and activate macrophages, which engulf and wall off the bacteria and eventually destroy it (Cooper, 2009). Langhan's giant cell consists of activated macrophages that align around mycobacterial antigens and is a distinctive feature of tuberculous granulomas. Multiplication of infectious droplet nuclei in the alveoli results in the formation of a Ghon focus, and involvement of the regional lymph nodes produces the Ghon

complex. Manifestations of the disease covers a broad spectrum ranging from pulmonary infection to localised extra-pulmonary infection and disseminated disease (Nicol & Wilkinson, 2008).

In some individuals, the innate system has been shown to completely clear the infection after inhalation and before the adaptive immune system is activated (Cliff et al., 2015; O'Garra et al., 2013). Although there is a paucity of information about the mechanisms that modulate the sterilising immunity, studies show that macrophages' quick reactivation and a robust innate immune response are necessary for killing the bacilli immediately after exposure (Lerner et al., 2015). Granulocytes protect humans against *M. tuberculosis* infections(Weiss & Schaible, 2015). A chain series of events after exposure to and inhalation of the pathogen focus on containing the disease and preventing of dissemination of the pathogen. This includes the production of inflammatory cytokines and chemokines by alveolar macrophages to signal the presence of infection. This triggers the migration of neutrophils, monocytes and lymphocytes to the infection site to form the immune and physical barrier called the granuloma (Lin & Flynn, 2010).

2.4.6.3 Adaptive immunity

Studies indicate that adaptive Th1 immune responses are responsible for protection against TB (Bermudez & Goodman, 1996; Cooper, 2009; Flynn & Chan, 2001b; Sia & Rengarajan, 2019). Th1 is mediated by T cells after recognising infected or antigen containing Macrophages/ DCs (Berrington & Hawn, 2007; Flynn & Chan, 2001a). The production of IFN-γ contributes to the development of adaptive immunity by activating CD8+ and CD4+ T-cells (Ahmad, 2011; Sia & Rengarajan, 2019) and inhibiting bacterial replication. Bacterial clearance by macrophages is a crucial process that depends on the activation of macrophages by IFN-γ and cytokines secreted by CD8+ T cells, NK cells, and CD4+ T cells (Feng et al., 2006; Flynn & Chan, 2001a; Kaufmann, 2001; Wang et al., 2004).

Migration of monocytes that have been derived from dendritic cells and macrophages to infected sites happens whenever the diseased components of macrophages secrete pro-inflammatory cytokines such as IL-1, Tumour Necrosis Factor, and IL-6, and chemokines (Flynn & Chan, 2001a; Marino et al., 2004; Means et al., 1999). Granuloma occurs when cells migrate to the infected site to restrict further dissemination of bacteria (Co et al., 2004; Flynn & Chan, 2005). The adaptive immune response process is initiated when the migration of mature DCs infected with mycobacteria to local lymph nodes occurs and T cells are recognised (Flynn, 2004; Marino et al., 2004; Tascon et al., 2000; Tian et al., 2005).

Schwander and Dheda have described the sequence of events in immune-pathogenesis of pulmonary tuberculosis, 2011 (Schwander & Dheda, 2011), as illustrated in **Figure 5** below. The main events include exposure to the bacilli via inhalation from individuals with active TB, sterilising immunity or establishment of LTBI, and containment of the infection in latency or development of clinically detectable active disease.

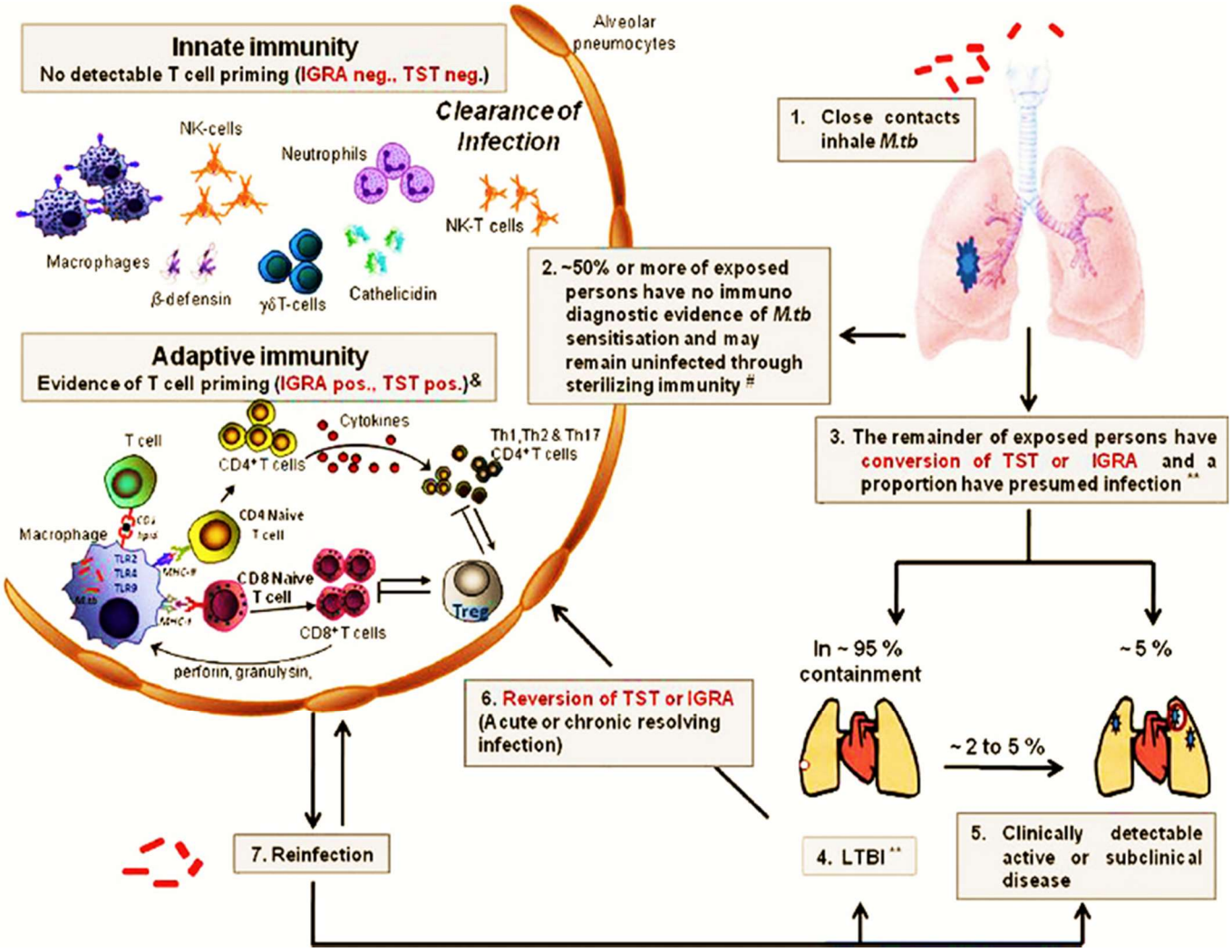

Figure 5. Image showing the immune-pathogenesis of pulmonary tuberculosis. Adapted from Schwander & Dheda (2011).

During *M. tb* infection, cellular and humoral mechanisms are involved and are responsible for the recruitment of inflammatory cells and control mycobacterial growth in the granuloma, as illustrated below in **Figure 6.**

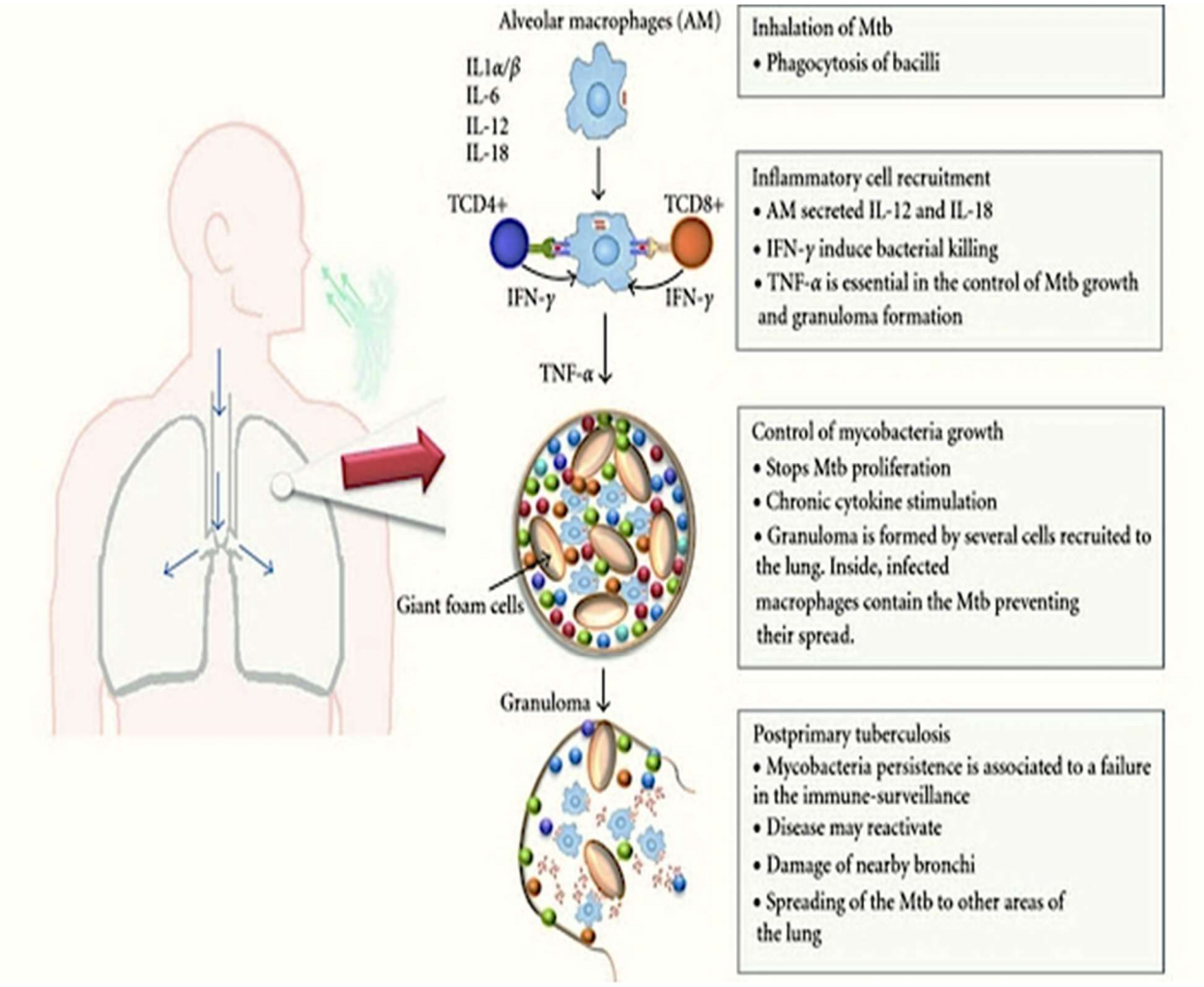

Figure 6. The inflammatory sequence in the lungs during Mtb infection (Adapted from (Zuñiga et al., 2012).

Though it is well established that T cells, predominantly $CD4^+$ T cells, can restrict the progression of TB, their contribution and limitations of the process are still being studied (Dawit et al., 2013). Studies involving animal models have been developed to identify characterize mechanisms of protective immunity to *M. tuberculosis* (Dharmadhikari & Nardell, 2008).

A tuberculosis infection primes adaptive immune responses. Antigen-presenting cells such as Dendritic cells (DCs) induce adaptive immunity by presenting *Mtb* antigens for destruction under the guidance of cytokines, costimulatory molecules, and the Human Leukocyte Antigen (HLA). The clinical symptoms of TB develop due to cytokine-induced cell-cell interactions after a person has an *M. tuberculosis* infection (Essone et al., 2019; Waruk et al., 2015). The CD4+ T lymphocytes recognise the mycobacterial antigens associated with HLA class II molecules presented by antigen-presenting cells such as macrophages. The

activated T cells release cytokines such as the macrophage migration inhibitory factor and Interferon-gamma (IFN-γ), which attract and activate even more macrophages to the site of the infection. Macrophages also release numerous cytokines, including tumour necrosis factor-alpha (TNF-α).

2.4.6.4 The Cytokine profiles in TB pathogenesis

Cytokines influence TB pathophysiology and development. The clinical features of TB are dependent on some cell-cell interactions moderated by cytokines that immune cells produce whilst responding to *M. tuberculosis* infection (Duarte et al., 2018). Macrophages get activated due to the synergistic action of IFN-γ and TNF-α. On the other hand, Interleukin 10 (IL-10), a macrophage deactivating cytokine that minimises inflammation, downregulates the production of IFN-γ and Th1-induced response to TB (Akgunes et al., 2011). Interferon-gamma-induced protein 10 (IP-10) production increases when a TB infection persists. Studies have shown that its expression, which is much higher than the IFN-gamma level, is not associated with variables such as age and sex (Aabye et al., 2013; Chowdhury et al., 2014; Ruhwald et al., 2012). It has also been investigated as a potential biomarker in the differentiation of active TB from latent TB (Aabye et al., 2013; Nonghanphithak et al., 2017; Tebruegge et al., 2015).

Recent investigations have evaluated the cytokines in TB patients to develop novel treatments and explore likely biomarkers used in diagnostic approaches (Dhanasekaran et al., 2013; John et al., 2012; Lalor et al., 2011; Mihret et al., 2013). One method involves comparing chemokine and cytokine levels of healthy HHCS and their PTBs. Mihret *et al*. have displayed that cytokines such as IL-4, IFN-γ, and IFN-γ inducible protein (IP-10), among others, are notably higher in active TB patients compared to treated TB patients or latently infected HHCs. These findings signalled the likelihood of further exploring the cytokine measurements as diagnostic markers or monitoring TB treatments' efficacy (Mihret et al., 2013; Nie et al., 2020).

2.4.6.5 Interferon-gamma (IFN-γ): Its role in TB infection

Interferons (IFNs) can be secreted by leucocytes (IFN-*α)* or fibroblasts (IFN-*β*) in response to viruses and also by Natural Killer cells and CD4+ or CD8+ lymphocytes (IFN-*γ*) in response to an inflammatory stimulus. IFN-γ helps the body to contain an active *M. tuberculosis* infection. This cytokine primes and augments the protective and effector functions of macrophages. Many robust anti-bacterial mechanisms and some effector molecules are modulated by IFN-γ stimulation (John et al., 2012; Lalor et al., 2011; Weniger et al., 2010). IFN-*γ* plays a critical role in the production of nitric oxide and oxygen free radicals

by inducing the transcription of the responsible genes in macrophages. These elements are effective antimicrobial molecules against *M. tuberculosis* (Cooper, 2009). Besides macrophage activation, IFN-*γ* also improves the antigen presentation process by inducing the expression of HLA class I and II molecules and CD4 T lymphocyte differentiation into a Th1 subpopulation (Fenton et al., 1997; Flesch et al., 1995; Oberholzer et al., 2000).

Several experimental and clinical studies have documented the importance of IFN-*γ* in *M. tuberculosis* pathogenesis. Flynn *et al*. in 1993 were able to demonstrate aggressive tuberculosis disease in mice whose IFN-*γ* genes were silenced (Flynn, 2004). Furthermore, a deficiency in the IFN-*γ* receptor gene has been shown to increase the susceptibility of individuals to mycobacterial infections (Newport et al., 1996), while a total lack of IFN-*γ* receptor has been shown to increase the gravity of disease and increase the risk of disseminated disease (Oberholzer et al., 2000). The importance of the IFN-*γ* receptor has been further emphasised in studies showing a higher susceptibility to infections by atypical mycobacteria in individuals with genetic mutations in the receptors (Jouanguy et al., 1996).

In vitro blood tests such as IFN-γ release assays (IGRAs) measure the release of IFN-γ from T-cells after being stimulated with *Mycobacterium tuberculosis* antigens (Redelman-Sidi & Sepkowitz, 2013). Currently, only two IGRAs have been approved for use globally. These are the T-SPOT.TB assay (Oxford Immunotec, Abingdon, UK) and the QuantiFERON-TB Gold In-Tube (QFT-GIT) assay (Cellestis Limited, Carnegie, Australia). Various studies show that these immunological assays have comparable sensitivity to the TST assay and even higher specificity for LTBI diagnosis in an immunocompetent person (Jouanguy et al., 1996; Newport et al., 1996).

Redelman-Sidi and Sepkowitz 2013 evaluated the use of the available IGRA tests and noted several advantages in their use over the TST in specific populations, including immunocompromised adults, for example, HIV-infected persons and cancer patients; individuals who have immune-mediated inflammatory disorders; those undergoing hemodialysis; and candidates for solid-organ transplant and management using TNF-α inhibitors. Pai *et al*., 2008(Pai et al., 2008) suggested the need for more studies to investigate the usefulness of IGRAs in immunocompromised patients due to the inability of immunocompromised individuals to mount an adequate immune response.. However, recent improvements have been implemented on the IGRA assays to increase their efficacy for use on immunocompromised individuals. New generation IGRA tests have more and shorter M. tb antigen peptides that also evaluate the response from CD8+ cells, giving a better evaluation of the individuals

immune response. Other advantages of the IGRA over TST include: previous exposure to BCG vaccination does not affect the test because the antigens used are neither found in the vaccine nor in nontuberculous mycobacteria, potential risks of adverse events are reduced because they are ex vivo tests, and because they require one patient visit, they have an operational advantage.

2.4.6.6 Burden and Risk factors of Latent *M. tuberculosis* infection

Latent tuberculosis is a non-communicable asymptomatic condition where an individual has an *M. tuberculosis* infection that does not progress to active disease. Although latent TB-infected individuals are not infectious and do not show any clinical manifestations of disease they are significant reservoirs for developing secondary TB. Certain geographic, epidemiologic factors (indoor air pollution, tobacco smoke, malnutrition, homelessness or congested living conditions, incarceration, excessive alcohol use) and host genetic factors can regulate disease expression. Latent TB-infected individuals are not infectious (are asymptomatic) but are prone to disease reactivation and infecting their close contacts (Bloom et al., 2017; Druszczyńska et al., 2012; Mack et al., 2009). In people with LTBI, the average lifetime risk of progression to active tuberculosis is between 5% and 20%. HIV-negative individuals bear the most significant risk of reactivation in the first five years of the infection (World Health Organization, 2015). Determinants of chances of developing TB have been seen to change with age with projections showing that infants and under-fives are at high risk of *M. tb* infection (Holmberg et al., 2019).

Global estimates of patients who have LTBI stand at about 2 billion. Of these, around 200 million cases are more likely to advance to the clinically evident TB disease (Houben & Dodd, 2016a; World Health Organization, 2018c). LTBI burden is seen to vary highly around the globe. In developing countries, LTBI prevalence has reached 60% in Ethiopia and 50% in Kenya (Cohen et al., 2019; Fox et al., 2013). Published data reviewing 203 studies of LTBI among HHCs of PTB found the prevalence of LTBI to be 51.5% (95% CI of 47.1–55.8%) in LMIC areas compared to 28.1% (95% CI of 24.2–32.4%) in high-income areas (Fox et al., 2013)(Cohen et al., 2019). People living with HIV are in high-risk fields(Corbett et al., 2003) with global statistics showing that more than 30% have LTBI coinfection, ranging from 14%, 46%, and 50% in Europe, Southeast Asia, and sub-Saharan Africa (Corbett et al., 2003, 2006).

Knowledge of reactivated or latent TB dynamics at the bacterial and host levels is critical for disease control. Key health-care interventions that have been shown to prevent new *M. tb* infections and progression of latent infections to active TB are the provision of Bacille Calmette-Guérin (BCG) vaccines

at birth and treatment of LTBI. Interventions for preventing activation of latent infections and treatment of active TB are in place, but uptake, apart from BCG vaccination, is relatively low in high-risk settings (World Health Organization, 2015). In high-burden countries, management approaches for LTBI should include a focused and systematic screening of vulnerable groups such as HHCs of TB patients, creating individualised treatment plans, and managing toxicity to boost adherence to the treatment (Sharma et al., 2012).

2.4.6.7 Latent TB Infection Diagnosis

The available tests for detecting TB infection are indirect tests that investigate immune responses suggestive of present or past exposure to the TB bacilli. On the other hand, investigation of active disease can be explored through direct tests that include microscopy, culture and molecular tests that show the presence of the actual bacilli or its genomic material.

LTBI diagnosis can be made using immunological tests that challenge the acquired immune responses of patients using attenuated mycobacterial antigens. These tests ideally identify individuals with previous sensitisation to mycobacterial antigens, but they cannot differentiate between latent and active TB infections. The tests can be conducted in vitro using the Interferon-gamma release assays (IGRAs) or in vivo using the Tuberculin Skin Test (TST) (Buonsenso et al., 2020; Veronique, 2015). Both assays require supplies and well-trained personnel.

Tuberculin Skin Test is based on measurements of the wheal response that occurs after intradermal injection of 0.1ml of tuberculin purified protein derivative (PPD) into the inner surface of the forearm. The PPD is a mixture of antigens found in *Mycobacterium tuberculosis*, the BCG vaccine, and non-tuberculous mycobacteria.

IGRAs evaluate the adaptive immune response's memory against *Mycobacterium tuberculosis* antigens located in the specific region of difference 1 (RD1)- the early secretory antigenic target 6 (ESAT 6) and the culture filtrate protein 10 (CFP 10) as well as an additional single peptide from the TB7.7 which is encoded in RD11. Two IGRA tests that are commercially available are the QuantiFERON®-TB Gold in-tube assay (QFT) (Cellestis, Victoria, Australia), which utilises ELISA to measure the amount of IFN-γ response (illustrated in **Figure 7**) and T-SPOT.TB Assay (ELISPOT) (Oxford Immunotec, Oxford, UK) identifies the T cells that produce IFN-γ.

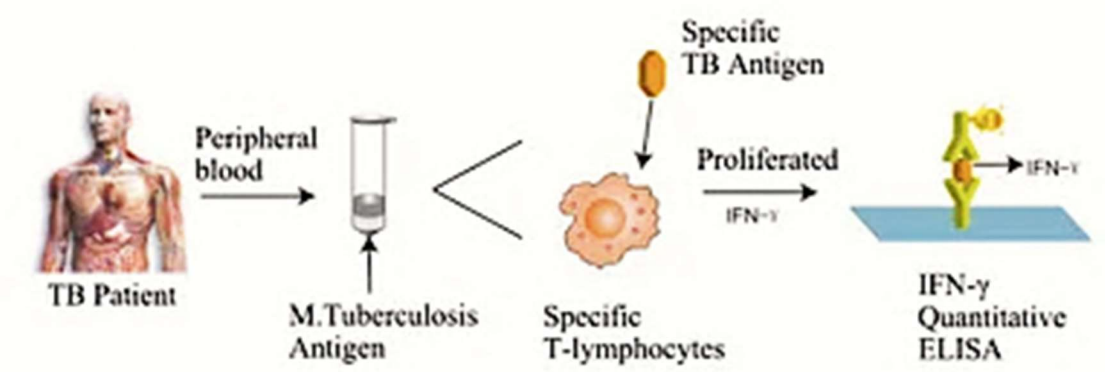

Figure 7. Interferon-Gamma Release Assays (IGRA) image Adapted from Invitro-test.com.

A significant advantage of the IGRA assays is the increased specificity compared to TST because it evaluates the body's response to those *Mycobacterium tuberculosis* antigens ESAT 6 and CFP 10 that are not encoded for in other forms of mycobacteria (Cheallaigh et al., 2013). Both proteins are immunodominant antigens due to their high immunogenicity. Therefore, individuals who have had BCG vaccinations and are not infected with *Mycobacterium tuberculosis* should not have any immunologic reaction to the test antigens. In addition, individuals who have non-tuberculous mycobacterial infections would not have their results influenced by cross-reactivity. The IGRA tests have internal quality control measures because the setup includes positive (mitogen tube) and negative (no antigen tube) controls that are useful when interpreting test results.

Furthermore, they require one patient visit and have a quick turnaround time (approximately 24-48 hours). However, IGRA tests are more expensive than the TST assay, require complex laboratory infrastructure, stable electricity supply, refrigeration and need a blood specimen to be drawn from the patient. Comparatively, though not expensive, the TST assay requires two patient visits, results are available in 48-72 hours, and BCG has been reported to cause false-positive reactions, especially in younger individuals. Furthermore, in immunosuppressed individuals, the sensitivity of TST is compromised. The test's specificity is also compromised by the likelihood of cross-reaction with antigens of non-tuberculous mycobacteria. A summary of the differences between the LTBI diagnosis tests has been displayed in **Table 1**.

Table 1. Comparison of Latent TB diagnostic tests

	Tuberculin Skin Test	QuantiFERON-TB Gold In-Tube	T-SPOT.TB
Intended use	latent TB diagnosis	latent TB diagnosis	latent TB diagnosis
Studies	in-vivo	ex-vivo	ex-vivo
Technique	skin prick test	ELISA	ELISPOT
Antigen(s) used	PPD	ESAT-6, CFP-10, TB 7.7(p4)	ESAT-6, CFP-10
Results reported as	skin induration in mm	IFN-gamma concentration	spot-forming number
Result interpretation	subjective	objective	objective
Result availability	48–72 hours	24 hours	24 hours
Patients visit required	two	one	one
Influence by prior BCG vaccination	yes	no	no
Cross-reactions with non-TB bacteria	yes	rare (*M. fortuitum*)*	no
Side effects	yes (rare)	no	no
Booster effect	yes (possible)	no	no

Table Adapted from Dyrhol-Riise et al. (2010)

Both TST and the first-generation IGRA tests would be adversely affected by low CD4+ count. At the time of this study, the most advanced and available IGRA test was the three tube (Gold-In-Tube) test with a mitogen test tube as a positive control, negative control test tube without any antigens and an antigen test tube containing the peptides. The current generation IGRA test (Gold plus) has been optimised with more tuberculosis-specific antigens that elicit strong CD4+ and CD8+ T cell responses. This offers the advantage of providing a more precise assessment of a patient's immune response to TB infection after infection with *M. tb*. The improved IGRA test has four tubes consisting of the Mitogen- which is the positive control for confirming baseline immune status; the Nil or negative control, which adjusts for Interferon-gamma; the TB1 antigen tube, which detects T cell (CD4 +) immune responses; and a TB2 antigen tube, which is optimised for T cell responses (CD8+ and CD4+). This assay can detect low CD4+ T cell counts more accurately than other analytic tests by inducing a stronger, more robust, and better IFN-gamma release. This new generation of IGRA tests has advantages over TST, including the single patient visit. They are also unaffected by the BCG vaccine, and environmental non-tuberculous mycobacteria. The assays use positive and negative controls in multiple screening situations, which is better compared to TST which can have a booster effect in multiple screening.

In 2018 WHO released new guidelines on LTBI that expanded the number of tests that can be used for diagnosis, the different high-risk groups that should be considered in screening programs and the available

treatment options (World Health Organization, 2018b). Based on these guidelines, IGRAs and TST could be used for LTBI testing globally, having acknowledged the higher benefit of preventive treatment in HIV-positive persons who are also LTBI positive and the shortage of TST kits globally as a barrier to scale-up of programs for preventing LTBI. A Kenyan LTBI 2020 policy document states that LTBI diagnosis can be conducted using IGRA or TST depending on the cost and availability of kits.

2.5 Host Genetic factors and Susceptibility to Infectious Disease

Several studies have highlighted factors involved in susceptibility or resistance to infectious diseases (Burgner et al., 2006). In their research, Burgner *et al*. suggest that infectious diseases exert an intense genetic pressure (selective) on organisms and that diverse genes evoke immune responses (Burgner et al., 2006). However, several questions remain unanswered on the functional consequences that gene translations, transversions, or deletions have on the resistance and susceptibility to TB. These polymorphisms explain why some individuals can resist infection better than their peers (Coscolla & Gagneux, 2010) Coscolla & Gagneux, 2014), hence reports of variation in susceptibility to exogenous pathogens (Klebanov, 2018). These reports of associations between genetic variations and exposure to or resistance to infectious diseases such as malaria, human immunodeficiency virus (HIV) infection, and Creutzfeldt-Jacob disease (CJD) have been highlighted in the literature. For example, in studies on malaria, the presence of α- and ß-thalassemia have been shown to confer a protective effect against *Plasmodium falciparum* malaria, even though the mechanism of this association is not well understood (Flint et al., 1986). According to published data on the differences in susceptibility of different ethnic groups, the Fulani ethnic group from West Africa is less susceptible to contracting clinical malaria than the Fulani from Burkina Faso (Modiano et al., 1996).

Although our knowledge of the immunogenetics of some infectious diseases is still scanty; HLA and non-HLA genes implicated in the process (Dheenadhayalan et al., 2001), with genomic studies suggesting strong associations between susceptibility to infectious diseases and polymorphisms in human leukocyte antigen (HLA) genes (Klebanov, 2018). Global studies have indicated that the occurrence of TB varies among different subsets of populations with additional attributes such as race, family, and ethnicity, influencing TB susceptibility (Hayward S et al.,; Azad et al., 2012). Familial risks of TB infections have been documented in reports by Naranbhai. (Naranbhai, 2016). In twin studies, evidence on the influence of genetic variation in susceptibility to TB disease has reported that monozygotic twins are twice more

likely to contract TB than dizygotic twins (Abel et al., 2014b; Casanova & Abel, 2002; Hayward et al., 2018a; World Health Organization, 2018b).

The main setback with immunogenomics is the candidate gene selection approach because of the diversity of the composition of genes inferring resistance to infections. However, microarray technology for instance, has made the identification of novel genes more feasible (Bryant et al., 2004) and this has led to discoveries of important essential genes affecting response to human infections, using animal models such as mice or drosophila (Lazzaro et al., 2004; Reiling et al., 2002; Wasserman, 2004). Studies that have evaluated family-based approaches are also diverse. In some reports, investigators have linked large numbers of families with some common infectious diseases (Bellamy et al., 2000; Bucheton et al., 2003; Jepson et al., 1997; Marquet et al., 1999; Mira et al., 2003; Siddiqui et al., 2001; Tosh et al., 2002). Some genetic linkage studies have concluded that susceptibility phenotypes result from mutations rather than polymorphisms (Levin et al., 1995; Newport et al., 1996).

Complex segregation analysis, twin studies, and familial clustering data have also suggested a correlation between gene composition and tuberculosis, leprosy, and mycobacterial disease susceptibility. In some studies, segregation analyses have identified genes that could be implicated in susceptibility to infectious disease (Abel & Demenais, 1988; Comstock, 1978; Fine, 1981). Several studies have identified significant infectious disease susceptibility loci, as shown in **Figure 8** below.

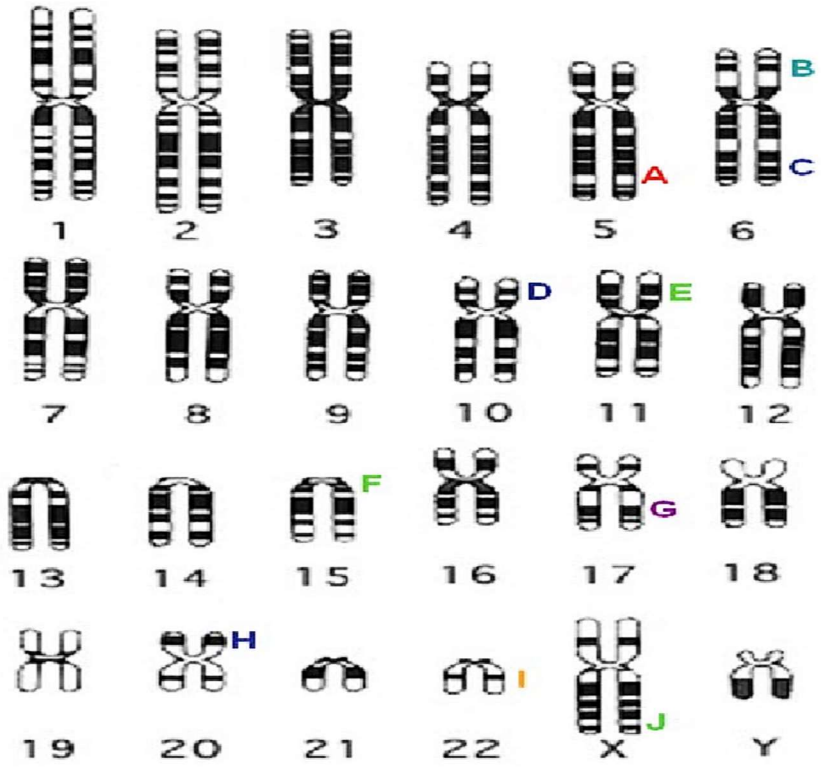

Figure 8. Diagrammatic representation of susceptibility loci location of some infectious diseases investigated via genome-wide linkage analysis. A disease is represented by a text colour: red- the burden of schistosomiasis parasite; aqua- represents malaria; leprosy is represented by blue; tuberculosis is represented by green; purple, tuberculosis and leprosy combined are represented by colour purple; Kala-Azar is represented by orange (Frodsham & Hill, 2004).

HLA Molecular component

The HLA complex refers to the human version of the major histocompatibility complex (MHC), a gene family in many other species. The HLA is located on the 3500kb segment of the human MHC (Bodmer, 1987) on chromosome 6 (**Figure 9**) and (**Figure 10**).

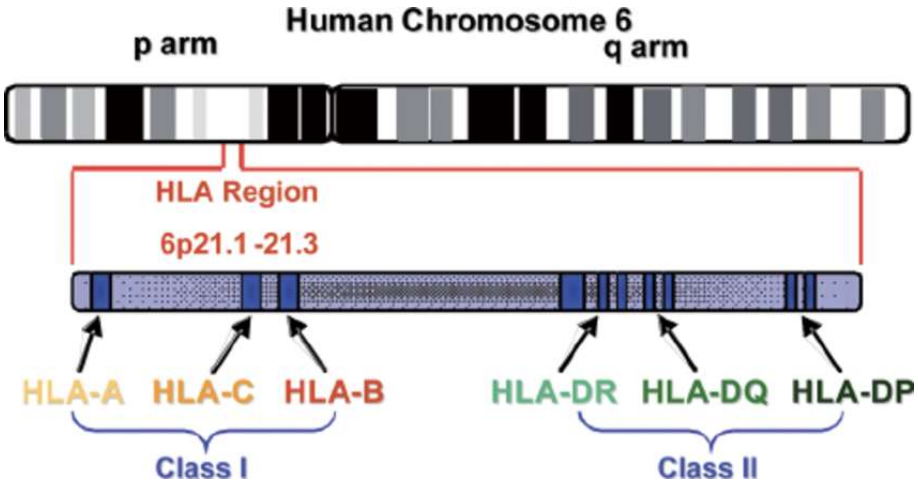

Figure 9. Showing the gene map of HLA. Gene map of the human leukocyte antigen (HLA) region. The HLA region is located on the short arm of chromosome 6, at 6p21.31 (McCarty et al., 2010).

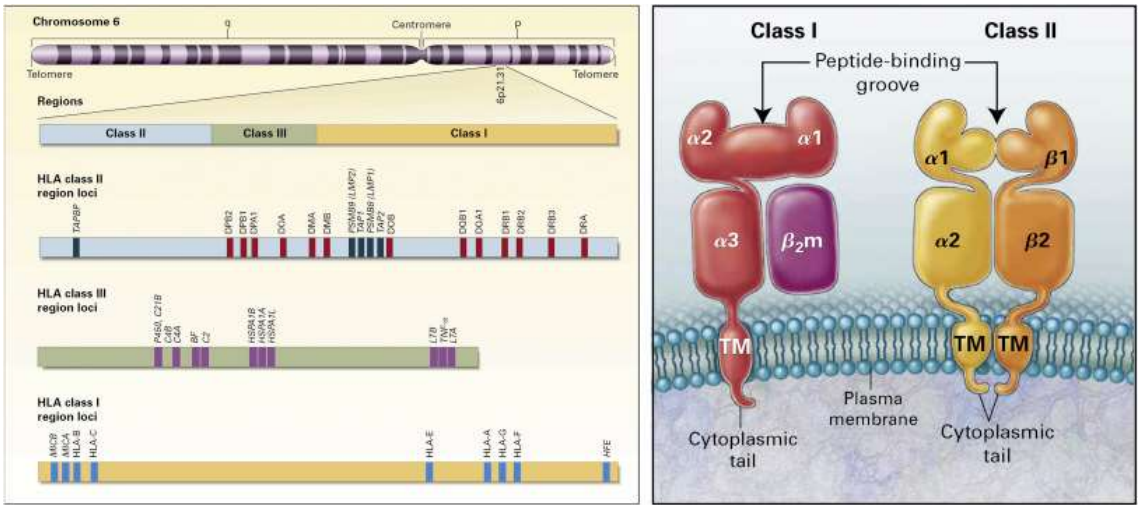

Figure 10. Adapted image showing the Genetic organization of the human leukocyte antigen (HLA) complex and structure of HLA class I and II molecules (adapted from Klein & Sato (2000).

Around 6810 HLA alleles have been described in studies published in the IMGT/ HLA Database (http://www.ebi.ac.uk/imgt/hla/stats.html) and in HLA nomenclature (Helmberg, 2012). In HLA Nomenclature, as illustrated in **Figure 11** below, all alleles receive a four-digit name after the HLA prefix and locus: the first field represents the allele group, e.g. HLA-DRB1*13; the second represents a specific HLA allele, e.g. HLA-DRB1*13:01; the third represents an allele that differs by synonymous DNA substitution in the coding region, e.g. HLA-DRB1*13:01:02 and the fourth represents an allele which has a mutation outside the coding part.

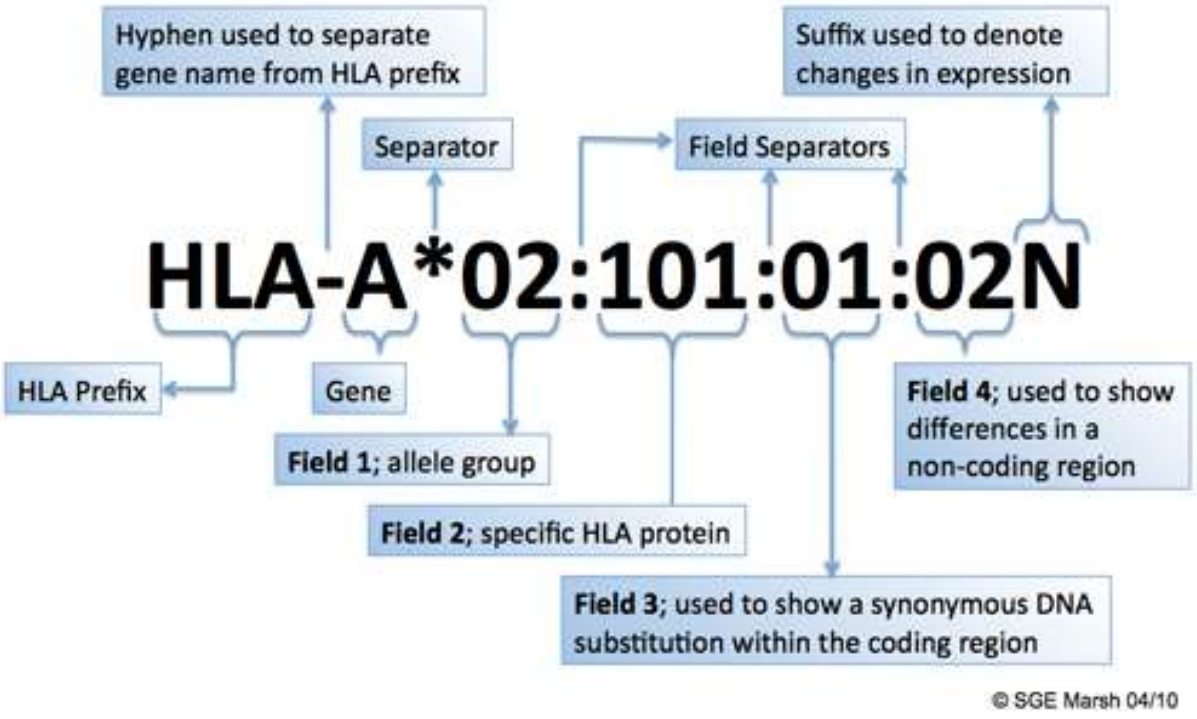

Figure 11. Illustration of the section details used in the nomenclature system for identifying HLA alleles. Adapted from hla.alleles.org HLA nomenclature: how an allele is named. Downloaded on 7th July 2022.

The HLA apparatus is of a high polymorphic nature. Varying frequencies of its alleles and their subtypes have been described, most of which have differences in the composition of their extended haplotypes (Choo, 2007). Because of the diversity of this genetic complex, the immune response is often very efficient (Sadki et al., 2012).

It has three sub-regions: HLA classes I, II and III. The class I A, B, C and classes II DRB1, DQA1, DQB1, and DPB1 loci are the most polymorphic HLA genes.

Molecular typing methods have been used to evaluate these gene loci and distinct allelic sequences described for most of these variants (Jagielski et al., 2014). The highly polymorphic nature of the HLA complex system makes it a suitable genetic biomarker for identifying different diseases (Allison, 1954). However, case-control studies that could identify and describe the specific haplotypes of HLA alleles that influence the infection process have proved difficult, mainly due to linkage disequilibrium and high polymorphism (Berlin et al., 1997; Sato et al., 2002).

The membrane-distal domain (shown in **Figure 12** below), which forms the binding region for MHC class II peptides, comprises β1 and α1 regions. The β2 area has pleomorphic peptide binding cleft regions instead of Class I molecules (McCarty et al., 2010).

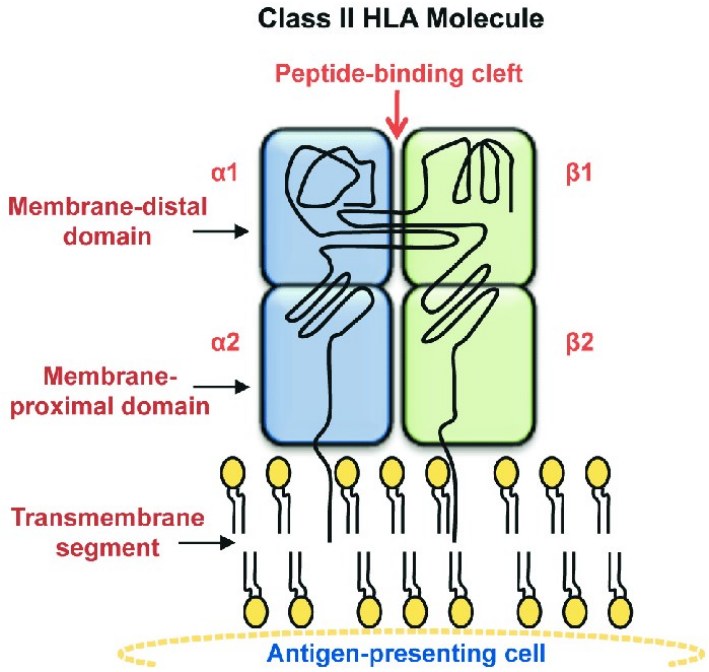

Figure 12. Diagram showing the structure of an HLA class II molecule

The position of the HLA-I gene is at the telomere of chromosomes in three sections - A, B, and C genes. The $CD8^{+}$T cells recognise HLA-I molecules combined with endogenous antigens and are displayed on the cell surface. Similarly, the $CD4^{+}$ T cells recognise exogenous antigens bound to the HLA-II molecules and displayed on the cell surface. HLA-III genes are located between HLA II and HLA I genes and are not often involved in antigen presentation. A minority of these genes modulate immune regulation via complement. Overall, the regulation of immune response and antigen presentation are modulated by HLA-I and HLA-II genes and, therefore, directly impact the susceptibility of individuals to different infectious diseases (Yim & Selvaraj, 2010). HLA class II genes also modulate protein synthesis, which is presented on the surface of immune system cells.

2.5.1 HLA in Infectious Diseases

The human major histocompatibility complex- the HLA encodes the genetic components of antigen-presenting molecules and plays many additional roles, key among them cellular cooperation functions and recognition of the T lymphocyte's substratum. The HLA is a critical genetic risk factor for transmissible

diseases such as leprosy, tuberculosis, hepatitis B, HIV, and hepatitis C. The primary role is to ensure that antigen-presenting cells generate and present peptides to regulate immune responses against immunological abnormalities during infections.

Some common West African HLA genes (HLA-B*53) have been reported to confer protection against severe forms of malaria (A. V. Hill et al., 1991). The DQB1*0301 confers protection against the hepatitis C virus, while expression of the DRB1*1302 allele has been shown to confer protection against the hepatitis B virus (A. V. Hill et al., 1991). Several mechanisms have been suggested to explain the association with disease, including antigenic cross-reactivity between pathogens and HLA antigens (Cunningham, 2019).

A study on Kenyan sex workers found a strong and positive correlation between HLA class II DRB1*01 expression and HIV seroconversion by restricting the activity of CD4+ cells (MacDonald et al., 2000). Expression of both class I and II HLA genes has been shown to impact the resistance and susceptibility of individuals to HIV (Singh & Spector, 2009). Immunocompromised individuals because of AIDs have been shown to have a higher risk of contracting opportunistic pathogens. Beginning in the early 1980s, for instance, the AIDS epidemic accelerated the transmission of TB and mortality due to TB.

These and other studies have pointed out possible associations between TB disease and HLA and non-HLA genes, including transporter associated with antigen processing (TAP), TNF α and β, mannose-binding lectin (MBL), the vitamin D receptor (VDR) gene, and Interleukin-1 receptor antagonist (IL-1RA) (Goldfeld et al., 1998; Sharma et al., 2003). The kind of genetic polymorphisms observed in these genes can serve as genetic markers of predisposition to develop active TB and failure to respond to therapy (Alcaïs et al., 2005; Azad et al., 2012), some of which have been investigated using different study designs including candidate gene approaches, case-control studies, genome-wide linkage studies, and family-based studies (Harishankar et al., 2018). In a systemic review of candidate gene studies, Daly AK and Day CP 2001 indicated that there had been pitfalls both in the design and interpretation of candidate gene associations, but this can be addressed by informative data on genetic polymorphisms and an increased repository of data on access to systems for studying specific gene defects that can influence the pathogenicity or prognosis of diseases (Daly & Day, 2001).

Genome-wide association studies: When hosts and parasites coevolve, these interactions between the host genes and between pathogen and host genes make the repetition of genome-wide association studies

complex across different host populations (A. MacPherson et al., 2018). Understanding HLA's importance in diseases has shown differences in ethnic groups. One such study by Modiano et al. in 1996 in West Africa showed a lower risk of clinical malaria among West African Fulani people than the residents of Burkina Faso (Modiano et al., 1996). Luoni *et al*., 2001 and Tangteerawatana *et al*., 2009 showed an association between some HLA cytokine gene polymorphisms and lower susceptibility to clinical malaria in this population (Luoni et al., 2001; Tangteerawatana et al., 2009).

Both class I and II HLA alleles have been shown to influence the susceptibility of Africans to malaria (A. V. Hill et al., 1991; Thursz et al., 1995). Variability in these alleles also affects the strains' diversity causing clinical malaria (Gilbert et al., 1998; Young et al., 2005). Because linkage genes have not been revealed, there is a need for more studies.

Therefore, the HLA loci seem to play a crucial role in initiating and regulating immune responses. Many other studies have been conducted to understand better associations between gene polymorphisms and progression or susceptibility to some diseases, some of which are summarised in **Table 2**.

Table 2. Presentation of HLA loci that have been investigated as markers of susceptibility and resistance to various diseases

HLA allele	Disease	Effect
HLA-B53	Malaria	Resistance
HLA-B35	H1V-1 progression	Susceptibility
HLA-B27	HIV-1 progression	Resistance
HLA-B57	HIV-1 progression	Resistance
HLA-DR.B1*1302	Malaria	Resistance
HLA-DPB1	Tuberculosis	Resistance
HLA-DQB1	Tuberculosis	Susceptibility
HLA-DQB1	Leprosy	Resistance
HLA-DR2	Tuberculosis	Susceptibility
HLA-DR2	Leprosy	Susceptibility
HLA-DRB1*1302	HBV persistence	Resistance
HLA-DR2	HBV persistence	Resistance
HLA-DR7	HBV persistence	Susceptibility
HLA-DRB 1*11	HCV persistence	Resistance

Adapted from Frodsham & Hill (2004)

The pathogenesis of TB is very complex. The outcome of exposure to *Mycobacterium tuberculosis* ranges from absolute clearance by sterilising innate or adaptive immunity and latent infection to active disease. Phagocytosis by alveolar macrophages influences the initial stages of host-pathogen interactions, which can often affect the outcome of exposure to the pathogen. The course of progression from infection to active disease is also highly variable. Several studies have indicated that the interplay of host genetic and environmental factors determines the eventual outcome (Duarte et al., 2011). For nearly five decades, many authors have demonstrated strong and statistically significant associations between the pathogenesis of different immunological disorders and MHC specificities, especially for infectious diseases such as TB (P. C. Hill et al., 2006; Yee et al., 2003). Due to the critical role that the HLA plays in the modulation of immune responses, studies have examined the correlations between TB and these antigens in different populations and had varied results (Wilkinson, 1999). The molecules influence people's susceptibility to TB by affecting the ability of macrophages, B cells, and Langerhans cells to present *Mycobacterium tuberculosis* antigens for destruction by T helper cells and CD4 cells (Shiina et al., 2009).

Not everybody exposed to *Mycobacterium tuberculosis* gets infected, and not all infected progress to active TB or show symptoms and signs of the disease. The human immune response to tuberculosis is multi-factorial. The genetic makeup of hosts and other host factors not only affect susceptibility to infections and health outcomes such as the severity of infections. Studies have shown that polymorphisms in some genes are associated with TB and clinical phenotype in different ethnic groups (Wu et al., 2013). Associations between TB pathology and HLA gene polymorphisms have been demonstrated in case-control studies by Kattaneh *et al*. and Yim and Selvaraj (Kettaneh et al., 2006; Yim & Selvaraj, 2010).

Coinfection of TB and HIV-1 endangers the health of millions of people worldwide. Little information exists on how the HLA profile of individuals is associated with *M. tuberculosis* co-infection, predominantly susceptibility to HIV 1. Evaluation of HLA alleles in PTB/HIV positive patients in Mexico indicated a higher prevalence of a subtype of HLA-DR antigens, suggesting that genetic predisposition to this marker seemed to fast-track the development of AIDS in patients with a coinfection (Dong et al., 2013). These effects could be modified by HIV-1 co-infection (Louie et al., 2004). The factors that influence the variability in the susceptibility of different individuals to PTB/HIV co-infection are yet to be identified.

The HLA class II alleles play an essential role in the early immune response to TB by presenting antigenic peptides to CD4+ T cells. HLA molecules are among the most polymorphic human gene products known. Polymorphisms affecting antigen processing and presentation, and hence the profile of cytokines secreted, can influence the efficiency of the immune response to infection and can play a significant role in the host response (Chang et al., 2008). This diversity would also limit the global use of a vaccine candidate if the analysis were based on pathogen strains and allele frequencies of HLA molecules in only specific populations. The distribution and frequency of HLA alleles within a particular population would be valuable baseline data for vaccine design studies and formulation of population-specific intervention strategies, especially in countries with a high TB burden.

2.5.2 HLA Typing Techniques

Tissue typing laboratories need to use flexible HLA typing techniques to type required loci accurately at the best resolution. Many techniques for HLA typing, essential among them Sequence-specific oligonucleotide probe (SSOP), serology, Sequence-specific primer (SSP), Genomic DNA sequencing,

Reference strand–mediated conformational analysis (RSCA), Microarrays, and RNA SBT, have been developed and adopted by clinical laboratories all over the world.

2.5.2.1 Serology

Serological techniques identify HLA antigens using a battery of antisera and viable lymphocyte preparations. It is thought that most laboratories have ceased using serological methods for HLA typing together because of their high cost and inefficiency compared to genomic DNA analysis. Genomic DNA typing is the most preferred because of its reproducibility, accuracy, higher resolution, and greater flexibility (Middleton, 1999). Genomic DNA typing methods also use synthetic oligonucleotides, which are easily standardised to provide reproducible results and confirm homozygosity; failing to reflect expressed HLAs is a rare occasion (Elsner & Blasczyk, 2004; Smith et al., 2005).

2.5.2.2 Sequence-specific primer (SSP) Typing

The SSP method is a PCR-based HLA typing technique from DNA using sequence-specific primers (Bunce et al., 1995). The technique is based on DNA amplification using group- or allele-specific primers and detecting an amplified product of the correct size by gel electrophoresis. The size is determined by running an agarose gel that separates the PCR products according to their size. The assignment of alleles merely consists of determining whether amplification has occurred, visualization and detection of the appropriate-sized amplicon by agarose gel electrophoresis. The typing process can be completed within 2.5 hours. Specially designed software can then be used to quickly and accurately interpret the results.

2.5.2.3 Sequence-Specific Oligonucleotide Probe Typing

The SSOP is considered to be the first PCR-based method for typing HLA. Initially, SSOP was referred to as a forward “dot-blot”, conducted first by using group-specific or locus primers to amplify genomic DNA, which is then analysed using hybridised oligonucleotide probes to detect single nucleotide differences in HLA genes (Saiki et al., 1986). Synthetic oligonucleotides hybridise PCR products. The PCR products have to be immobilised on a nitrocellulose or nylon membrane (Saiki et al., 1986).

2.5.2.4 Sequence-Based Typing

SBT is a comprehensive laboratory technique used to type HLA genes. Like some of the most common analytic methods, SBT involves amplification of the locus of interest using PCR. The PCR amplicons are the sequences (Sanger) used to determine the nucleotide sequences of amplified genes. Because SBT is a high-resolution technique, it makes the identification of unrelated yet genetically compatible hematopoietic stem cell recipient and donor pairs and new alleles. When conducted at a high throughput level, SBT is permitted and is recommended for routine HLA typing in the laboratories (Cecka et al., 2014).

2.5.2.5 Oligonucleotide primer design and PCR amplification

A key element of the DNA-based HLA typing procedure is in the design of the primers used for PCR amplification. The sensitivity and specificity of the HLA typing procedure is markedly enhanced by PCR amplification of the target sequence corresponding to the region in the HLA gene encoding the polymorphism (Cecka et al., 2014).

A summary of the stregnths and weaknesses of the different HLA typing techniques have been presented in **Figure 13** below.

Method	Summary	Advantages	Disadvantages
Serology	Serologically testing expressed HLA antigens on the surface of lymphocytes by using monoclonal antibodies	Quicker and cheaper than molecular methods	Does not provide direct information about sequence variation in alleles. Incapable of detecting some differences in DR molecule
PCR - RFLP	PCR amplified DNA digested with restriction enzyme to generate specific restriction pattern and alleles are identified according to pattern	Distinguishes polymorphisms associated with DR3, DR5 and DR6 haplotypes. Higher specificity than serological methods	Lacks accuracy in precise allelic typing, especially DR4 haplotypes. Long procedure and extensive handling of samples
PCR - SSO	Labeled sequence specific probes are hybridized to PCR amplified DNA and then detected	Most specific technique. High resolution typing within 10 hours. Easy handling of several samples in one run. Does not require controls at each step	Sequences of alleles must be known. Hybridization temperature is critical, could lead to false negative hybridization. Lacks accuracy in precise allelic typing
PCR – SSP	PCR amplified DNA using Sequence specific primers (SSP). Primers are designed with specificity-dependent nucleotide on the 3' end	Faster than PCR – RFLP and PCR – SSO. As accurate as PCR – SSO. Cheaper than other Methods	Unequivocal typing of the eight DB1806 alleles have been observed
PCR - SBT	DNA amplified by PCR using primers specific for the site of interest. PCR products are purified and then sequenced	More reliable and specific than other methods. New alleles can be detected quite easily	Apparatus needed is expensive

Figure 13. Gene map of the human leukocyte antigen (HLA) region (adapted from Transplant Diagnostics Human Leukocyte Antigen landscape accessed from Dolcera.com website 8/12/2022 Dolcera (2022)

CHAPTER THREE

3 MATERIALS AND METHODS

This chapter provides a detailed report on the investigations carried out in the study, including a description of the study site, study design, and the recruitment process of study participants. A detailed description of the study participants' sociodemographic, exposure data and clinical database has been included. These were collected and documented through structured questionnaires and informal interviews. The collection of blood specimens for investigation of LTBI using the IGRA test and the laboratory procedures for extraction and quantification of DNA from blood specimens have also been described. Finally, the Interferon Gamma Release Assay and the polymerase chain reaction sequence-specific primer genotyping of the HLA class II (-DRB and -DQB) alleles have also been elaborated.

3.1 Study Site

This study was conducted at Mbagathi County Hospital in Nairobi, Kenya. The hospital is a public health facility under the County Government of Nairobi's Department of Health Services. It provides a broad range of services, including an outpatient clinic and in-patient wards for PTB patients. Most of the study participants were from Kibra slums, one of the largest informal settlements in sub-Saharan Africa. Other participants were residents of various estates within Nairobi. The hospital was a suitable study site with its known history of being a centre of excellence in the management of TB patients. It is a public facility and therefore serves a big population with varied clientele.

3.2 Study Design

This was a mixed-methods study that included a descriptive cross-sectional survey of LTBI among HHCs of the PTB patients, a cross-sectional analytical study of HLA class II alleles of LTBI positive HHCs, LTBI negative HHCs, and PTB patients and an analysis of qualitative data on the experiences of HHCs and the PTB patients.

A study questionnaire collected demographic, behaviuoral and medical characteristics of the study participants.

3.3 Study Population and Recruitment

3.3.1 Pulmonary Tuberculosis (PTB) patients

All recruited PTB patients had been diagnosed at Mbagathi District Hospital. They were confirmed to have TB by sputum smear microscopy and GeneXpert test with complementary information from chest X-ray examined by a medical specialist. The PTB patients were recruited at the TB outpatient clinic and from the in-patient TB wards. Sociodemographic and clinical characteristics were obtained using the study questionnaire.

3.3.1.1 Inclusion criteria

- Adult patients: 18 years and above.
- Confirmed diagnosis of PTB at Mbagathi Hospital by sputum smear microscopy, GeneXpert, and chest X-ray.
- Provided informed consent to participate in the study.

3.3.1.2 Exclusion criteria

- PTB patients younger than 18 years.
- Failure to provide consent.

3.3.2 Household contacts (HHC)

Household contacts were defined as persons who had been living with, shared meals and slept under the same roof with a PTB patient. The household contacts should have lived continuously with the patient for at least one week in the month prior to initiation of treatment. Adult HHCs were recruited as they accompanied their patients to the out-patient TB clinic or during the hospital visiting hours in the wards. We identified the HHCs with latent TB using an Interferon-gamma release assay (IGRA) test.

3.3.2.1 Inclusion criteria

- Showed no signs/symptoms of TB
- The individuals did not have any known chronic underlying illness
- Were not pregnant
- Were not on immunosuppressive drugs
- Did not have any known history of TB disease

- Did not have prior preventive therapy for LTBI
- Provided informed consent to participate in the study

3.3.2.2 Exclusion criteria

- HHCs younger than 18 years
  Failure to provide consent
- Household contacts who started living with the patient after initiation of TB treatment

3.3.3 Sample size determination

A 2013 prospective cross-sectional study evaluated the prevalence of latent tuberculosis in a BCG vaccinated population in Taiwan using the IGRA test. Interferon-gamma release assay results showed the majority of LTBI to be 14.5% in the high-risk population of health care workers studied. This finding was used to calculate the sample size (N) for this study using Fisher's formula (1981) for cross-sectional studies at a 95% confidence interval (CI). The desired precision was set at 5%.

Statistical formula: $N = \frac{Z^2 xp(1-p)}{d^2}$

Parameters:

N: Required sample size

P: Prevalence of LTBI (14.5 % from a study by (Hung et al., 2015)

Z^2: Normal variate for alpha at 95% confidence interval (1.96)

d: Desired precision or absolute error (5%)

$$N = \frac{1.96^2 x 0.145(1-0.145)}{0.05^2} = 190$$

The required sample size was 190 HHCs. PTB patients were recruited as index cases of the HHCs. One hundred and ninety (190) HHCs were approached and interviewed, but we could only document all required sociodemographic factors and obtain blood samples for IGRA analysis of 175 HHC of 166 PTB. One HHC had indeterminate IGRA results (positive control failure) and was eliminated from analyses. Sixty (60) HLA test kits were available to analyse the HLA-DRB and HLA-DQB alleles. Forty (40) test kits were allotted to randomly selected HHCs (stratified as latent TB positive and latent TB negative), while 20 kits were allotted to randomly selected PTB patients. Typing on 6 DNA blood extract samples

(3 PTB and 3 HHC DNA samples) was unsuccessful despite repeated tests. These were eliminated from the analysis. HLA-DRB and HLA-DQB alleles were identified in 17 PTB samples and 37 HHC.

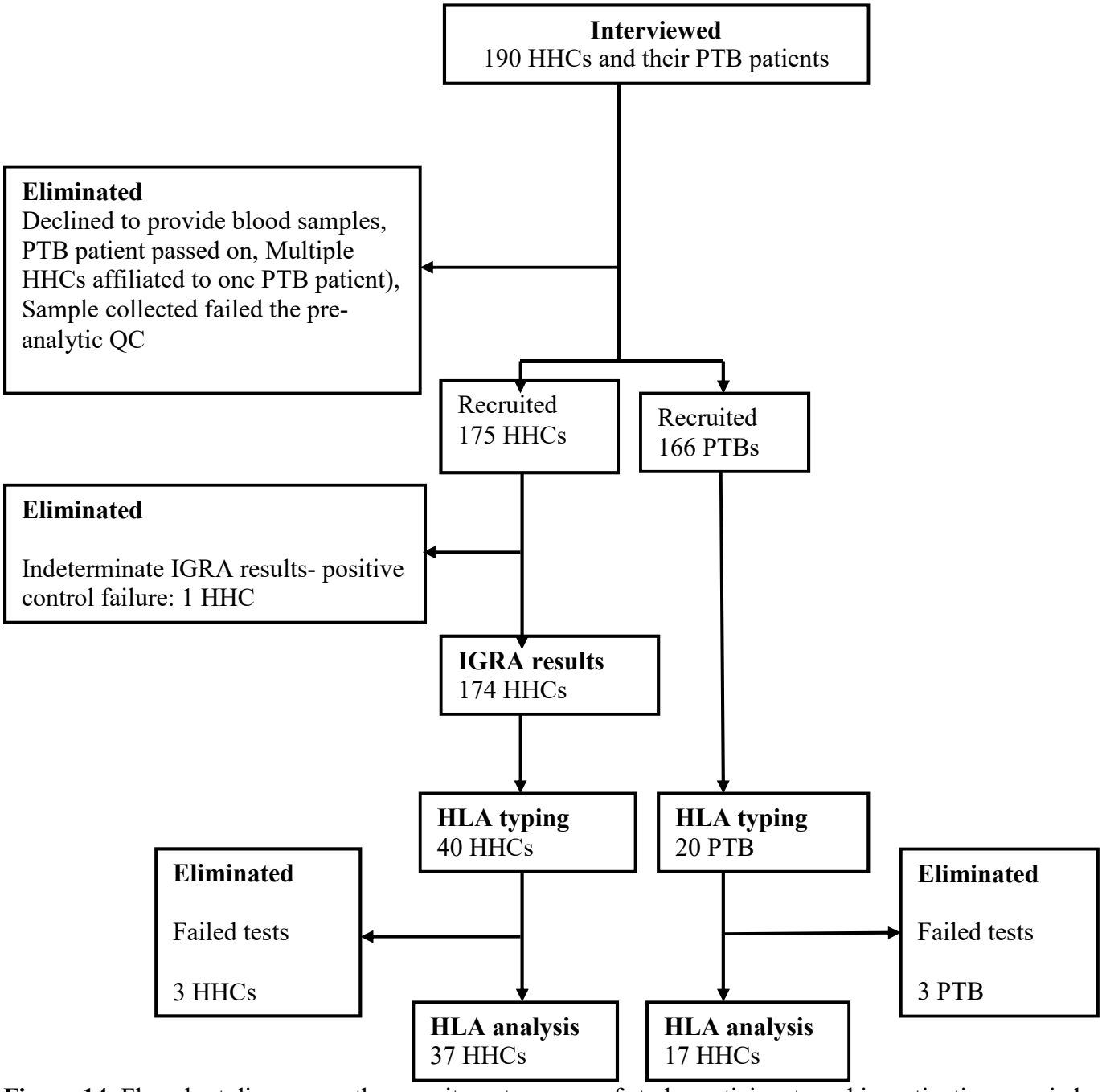

Figure 14. Flowchart diagram on the recruitment process of study participants and investigations carried out

3.4 Specimen Collection and Storage

A blood specimen was obtained to identify the presence or absence of LTBI from the recruited HHCs using the IGRA test and for HLA genotyping. For the IGRA test, 5 mL of blood were collected from the HHCs through venous puncture directly into the three quanti-FERON tubes (Nil, Antigen, and Mitogen tubes) provided in the QuantiFERON®-TB Gold In-Tube kit (QFT-GIT), (Qiagen, Germany). The blood samples were transported immediately to the Biozeq KAVI-ICR laboratory at the University of Nairobi for processing.

For the HLA typing assay, 5ml of blood was collected in EDTA blood collection tubes from the PTB patients and the HHCs. The samples were centrifuged and Buffy coats were aliquoted into 2 ml screw-cap tubes and stored at -20^{o}C till needed for DNA extraction.

3.5 Interferon-gamma release assay

3.5.1 The Interferon-gamma release assay (IGRA) using QuantiFERON Gold In-Tube test

In this study, the determination of LTBI was performed using IGRA. The IGRA test identifies a memory of an adaptive immune response against mycobacterial antigens and is more sensitive than TST (Cheallaigh et al., 2013). It is a quantitative and qualitative test for cell-mediated responses to peptide antigens that simulate mycobacterial proteins. The test used, the QuantiFERON®-TB Gold In-Tube test (QFT GIT) (Qiagen, Germany), was performed according to the manufacturer's instructions. Blood was collected by venipuncture from each HHC into three blood collection tubes: one tube containing a cocktail of peptide antigens (ESAT-6, CFP-10, and TB7.7) that simulate the *M. tuberculosis* proteins; a second tube containing a mitogen, and the third tube- a nil tube, with no antigen.

The ESAT-6, CFP-10, and TB7.7 proteins are absent from all BCG strains and nontuberculous mycobacteria except for *M. kansasii, M. szulgai, and M. marinum*. Individuals infected with the Mtb complex organisms usually have sensitised lymphocytes recognising these antigens. Recognising these antigens involves the generation and secretion of the Interferon-gamma cytokine. The detection and subsequent quantification of this cytokine form the basis of the test. Immunosuppressive medical treatments would reduce the IFN gamma responses to conditions that impair the immune response, such as infections. Individuals infected with the exception mentioned above of 3 Mycobacterium species may also give a positive result since these species have genes that code for the ESAT-6, CFP-10, and TB7.7

proteins. The Mitogen-stimulated plasma sample was an IFN-γ positive control for each specimen tested and quality control for correct blood handling and incubation.

Whole blood was incubated for 16 hours at 37ºC and transferred to 4ºC until processing, but for no longer than 48 hours. Tubes were centrifuged at 2000g for 15 min, and then supernatants were stored at -80ºC until the QFT ELISA could be conducted. Supernatants and IFN-γ standards (50 ml) plus conjugate (50 ml) were incubated for 2 hours, washed six times, and incubated for 30 min with substrate solution: Tetramethylbenzidine. After 30 min, 50 ml stop solution was added, and the wells and the plates were read at 450 nm with a 650 nm reference filter.

Concentrations of IFN-γ were calculated based on the standard curve, and test outcomes (positive, negative, or indeterminate) were determined using a mathematical algorithm from the manufacturer. Samples of participants with indeterminate QFT results were rerun, and positive or negative outcomes on the second run were interpreted as their final results. Individuals with indeterminate on the second run were scored as QFT indeterminate. A test was positive if the ELISA value for interferon-gamma (IFN-γ) was above the Nil IFN-γ value (> 0.35 IU/ml). Optical densities (ODs) were read using a spectrophotometer machine. These ODs were introduced into software provided to Qiagen by Cellestis (Cellestis Limited, Australia), downloadable from their website: https://www.quantiferon.com.

The QFT-GIT used in the analysis differs from the previous versions of the Quantiferon test in that it includes an additional TB antigen, TB7.7, and the blood specimen is drawn directly into tubes coated with TB antigens, ensuring immediate T-cell stimulation. The assay, therefore, gives more positive results and fewer indeterminate results than previous versions of the Quantiferon assay (Cheallaigh et al., 2013).

3.6 DNA extraction and quantification

3.6.1 Extraction of DNA from blood

Deoxyribonucleic acid (DNA) was extracted from buffy coat samples using the QIAmp DNA mini kit (Qiagen, Strasse 1, Hilden Germany), following the manufacturer's instructions. Briefly, 200 µl of the buffy coat samples were lysed with proteinase K, and DNA molecules adsorb onto silica-gel membranes in the presence of high chaotropic salt concentration. The resulting DNA was washed and eluted in 200 µl of buffer AE.

3.6.2 Quantification of extracted DNA from blood

The extracted DNA was quantified by standard UV spectro- photometric analysis. The A260/A280 ratio for all DNA extracts was between 1.6 – 2.0 by UV spectrophotometry for optimal band visualisation during electrophoresis.

3.7 HLA-DQB and HLA-DRB genotyping

The Olerup SSP® HLA qualitative in vitro diagnostic kit was used for DNA typing of HLA-DQB1 and -DRB1 alleles. The PCR method used the HLA SSP (sequence-specific primer) technique to identify the alleles from DNA. Genotyping was performed using the -DR and -DQ low-resolution kit (yields a DNA-based typing result at the level of the first field according to HLA nomenclature- refer to **Figure 15**) following the manufacturer's (Olerup SSP®, AB Franzengatan 5, SE- 112 51 Stockholm, Sweden) instructions.

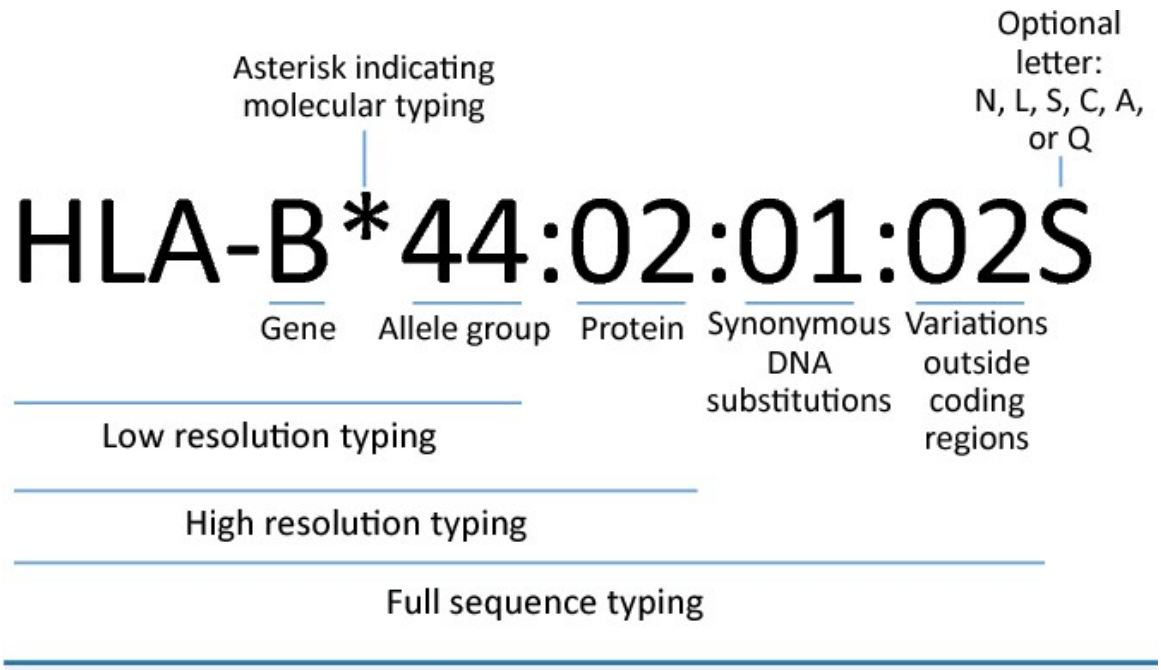

Figure 15. An illustration of the scope of the different fields identified in low resolution, high resolution and complete sequence typing of HLA. Adapted from Sypek et al. (2018)

Unlike most PCR-based tests where the PCR technique is used as an amplification step of target DNA, the PCR sequence-specific primer PCR-SSP technique discriminates different allele groups during the polymerase chain reaction process. This simplified the interpretation of results as either positive or negative.

In the SSP-PCR method, the primer pairs are designed to be matched with single alleles, depending on the typing resolution required. A positive result is obtained where complete matching of the primer and amplification occurs, while a negative result is due to a lack of amplification because of mismatched primer pairs. Interpretation of the results would be guided by the presence or the absence of PCR products from the amplification process. For quality control, an internal positive control primer pair that matches conserved regions of the human growth hormone gene is included in each PCR reaction. The amplification products (amplicons) generated by the specific HLA primer pairs are shorter than the amplicons of the internal positive control primer pair but larger than unincorporated primers.

The PCR Master Mix was prepared by adding PCR water dNTPs, $MgCl_2$ and Taq-Polymerase. The PCR Master Mix was then aliquoted into 96-well plates containing primer mixes. Extracted DNA (75ng) was added to each well, and PCR amplification was carried out.

This method allowed simultaneous genotyping of the two loci on the same plate (multiplex). Gel-electrophoresis was done using the Qiaxcel automated machine, which uses a particular commercial cartridge. PCR products were displayed on the screen after running for 25 minutes on the Qiaxcel machine. After PCR amplification, the Gel Picture was uploaded into the Olerup SSP Analysis Software, where positive bands were marked in the Input Data section and analysed (**Figure 16**). Genotypes were attributed using the Olerup SSP HLA allele typing software.

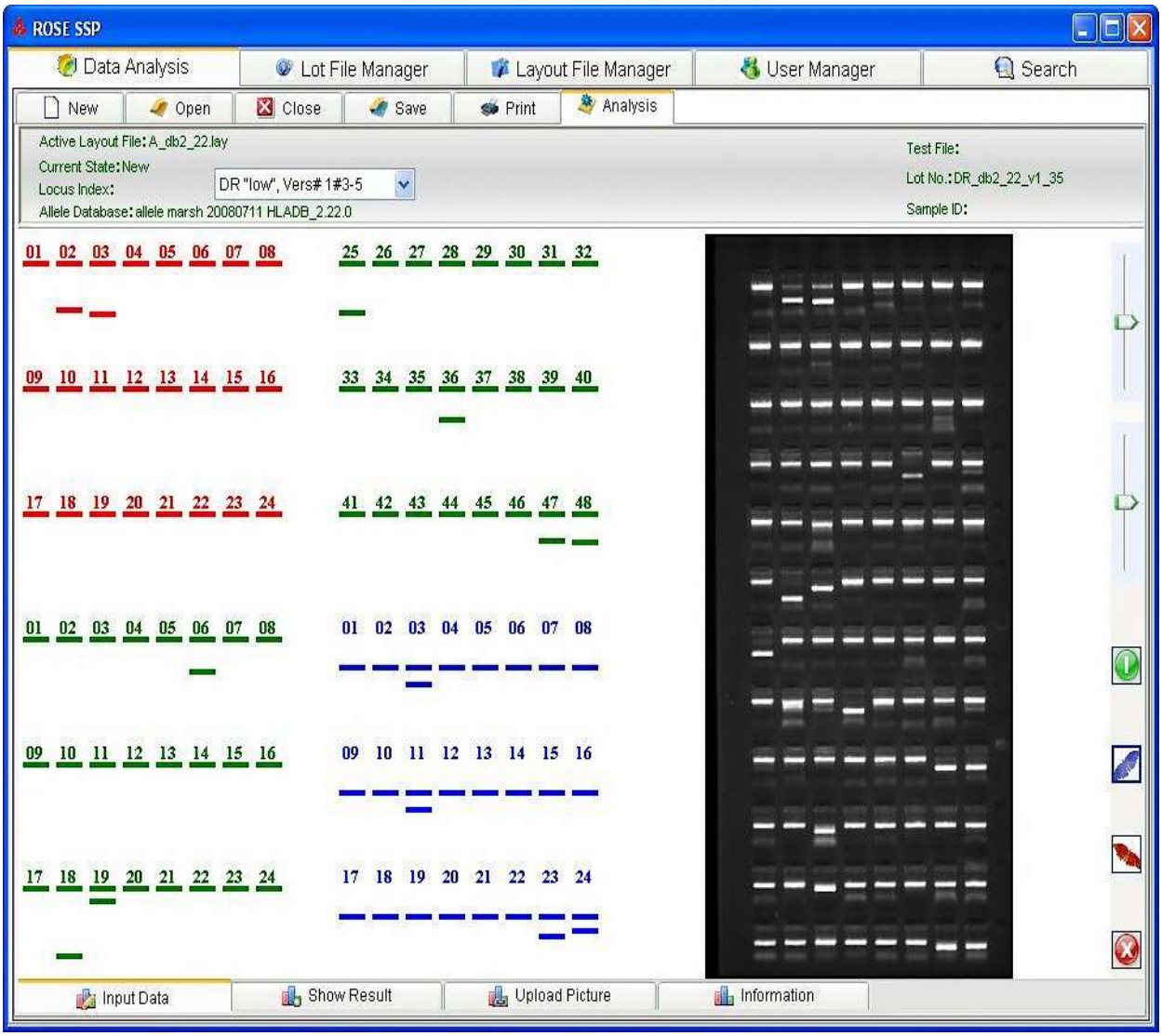

Figure 16. Snapshot of a screen display of uploaded PCR products and Analysis with Olerup software. The generated PCR products (amplicons) are displayed as a gel picture (extreme right), then uploaded for analysis using the Olerup SSP HLA allele typing software to identify the genotype.

3.8 Study participant interviews

The study sought to illuminate the experiences of the PTB patients and their Household contacts, some of whom happen to be their home caregivers. Sociodemographic data and individual perspectives on the experiences of the patients and the HHCs were obtained using structured questionnaires (**Appendix 3** and **4**) and through informal interviews. Clinical data were obtained from the patient files.

The patient questionnaires obtained data that included age, sex, ethnic background, marital status, the highest level of formal education attained, occupation and history of smoking and alcohol use. The HHC questionnaire included similar questions to the patient questionnaire and other questions that obtained data

on the duration of association with the patient as a member of the same household and their relationship to the patient.

Unstructured questions and conversations sought to establish the experiences of the PTB patients in seeking and obtaining medical care. We also had similar informal face-to-face interviews with the HHCs, documenting their challenges living with and caring for a PTB patient at home.

3.9 Data Analysis procedure

3.9.1 Statistical analysis methods of Latent TB infection prevalence and associated Risk factors

The prevalence of latent tuberculosis infection (LTBI) among HHCs of PTB patients was calculated using the Clopper Pearson method.

- Descriptive analysis:

Data analysis was done using R software version 4.2.0. A dichotomous table highlighting all variables assessed during the study was developed, and data were summarised using counts and proportions for categorical variables. While evaluating risk factors, the LTBI status (either positive or negative) was the dependent variable. Demographic, behavioural, and medical factors such as gender, age group, relation to PTB patient (grouped as spouse and others: brother, sister, son, daughter, cousin, aunt, uncle, friend), duration of contact with the PTB patient, educational level, occupation, smoking status, alcohol use, and HIV status were evaluated as independent variables.

Continuous variables such as age were grouped into mutually exclusive categories. The Chi-square test for independence was then applied to check for the association between the grouping variables and the outcome of the Latent TB test. Fisher's exact test was used as an alternative in variables with a number less than six in at least one of the counts. The summarised data was presented in tables and charts such as bar graphs and dot plots. Logistic regression was then used for the comparative analyses.

-Logistic regression

All variables with p-values less than 0.2 and those deemed necessary from the literature review were passed for modelling. First, simple binary logistic regression models were independently fitted for all variables to assess for association with the outcome of LTBI. After that, the variables were collectively included in a saturated multiple binary logistic regression model, adjusting for the potential confounders. A backward stepwise regression was run on the saturated model to retain variables explaining the highest variation in the outcome of LTBI using the stepaic function. The final selected model was compared to the saturated model using Akaike Information Criterion (AIC) value, and the model with a lower AIC

value was only passed after meeting the assumptions for logistic models including tests for multicollinearity, influential values and variance inflation factors. Interaction of terms were also tested for in the stepwise regression with an upper limit set for a number equal to the independent variables and a lower limit set at one. The significance level was set at 0.05 for this study, with coefficients accompanying their corresponding 95% confidence intervals and p-values. The performance of the final selected model was assessed using a confusion matrix comparing the predicted values of LTBI outcome versus the actual value from the collection.

-Linear regression

Here, all variables used in the binary logistic regression were fitted to explain variation in the concentration of Interferon-gamma amongst those who tested positive for LTBI. The variable capturing optical density (OD) values for interferon-gamma was set as the dependent variable to assess which explanatory variables were significantly associated with the level of immune response mounted following exposure to the *Mycobacterium tuberculosis* antigens. Similar to the logistic regression, simple models were first independently fitted, and the covariates were then collectively fitted to form a saturated linear regression model. A backward stepwise regression included a test for interaction of variable terms was applied to the saturated model and the final selected model compared to the saturated model using their respective AIC values. The better performing model was subjected to model assumptions including linearity, normality of residuals, homogeneity of residuals variance and independence of residuals error terms.

The final selected model had 60% performance in explaining the variation in the outcome variable, as shown in the confusion matrix below.

	Predicted negative	Predicted positive
Observed negative	64	13
Observed positive	56	41

Model efficiency = (True positive + True negative) / (True positive + True negative + False positive + False negative); = (64+41) / (64+41+56+13) * 100 = **60.34%**

Qualitative data on challenges faced by household contacts were extracted from the filled out questionnaires and from recorded excerpts from the informal interviews. These were grouped in to 4 main themes and discussed.

3.9.2 Statistical analysis methods of HLA-DRB and -DQB distribution and association with outcome of exposure to *Mycobacterium tuberculosis*

The HLA -DRB and -DQB allele distribution was summarised using frequency distributions and percentages. The distribution of alleles groups between PTB patients and HHCs; PTB patients, LTBI positive HHCs, and LTBI negative HHCs; and between males and females were evaluated using the Chi-square test at a 95% confidence interval. Fisher's test was used when an expected frequency in any cell of two-by-two tables was less than five. Logistic regression was also used to adjust for the HIV status of participants while conducting comparative analyses. For univariable and inferential comparative analysis, odds ratios (OR) and adjusted odds ratios (AOR) were interpreted as the measures of effect size, with a p-Value <0.05 considered statistically significant. The results of the study were presented in tables and bar graphs.

3.10 Ethical considerations

The Kenyatta National Hospital/the University of Nairobi Ethics and Research Committee (KNH/UoN ERC) reviewed and approved the study, Reference No. P649/12/2012. Mbagathi District Hospital/Nairobi County granted permission to conduct the study. Only consenting participants were enrolled, and a study questionnaire was administered, after which biological specimens were collected. Before samples were collected, the participants gave written informed consent (Appendix 2a and 2b).

CHAPTER FOUR

4 RESULTS

4.1 Introduction

This chapter reports the findings of this study as per the stipulated objectives. The data is presented in tables, figures and charts, beginning with a description of the socio-demographic and clinical characteristics of the study participants.

The findings of the first objective on the prevalence of LTBI are presented by reporting the proportion of HHCs that tested positive according to the cut-off value for the IGRA test. Further analysis was conducted on the interferon-gamma concentrations measured across select characteristics of the HHCs.

The findings of the second objective on the risk factors for LTBI in the study population are then presented in tables analysing the various variables (socio-demographic, behavioural, and medical characteristics) of the study participants.

The results of the third and fourth objectives show the distribution of HLA-DQB and HLA-DRB allele groups across the study participants and an analysis of the association between the frequency of specific HLA allele groups and the outcome of exposure to *Mycobacterium tuberculosis* among PTB patients, LTBI positive HHCs and LTBI negative HHCs are then presented in graphs, dot-plots and tables.

Finally, the results of the secondary objectives on the challenges faced by the contacts of the PTB patients at the household level and a proposed data card for follow-up of HHC of PTB patients are presented.

4.2 Demographics of Study participants

4.2.1 Socio-demographic and clinical characteristics of PTB patients

The socio-demographic and clinical characteristics of PTB patients are presented in **Table 3**. Most were females (51.8%) ages 30-39 years (33.1%) with secondary education (39.2%). Most were unemployed (63.9%), single (44.6%), non-smokers (69.9%) and HIV positivity was 65.1%.

Table 3. Socio-demographic characteristics of PTB patients, N = 166

		Frequency	Percent
Sex	Male	80	48.2
	Female	86	51.8
Age group	18-29	41	24.7
	30-39	55	33.1
	40-49	37	22.3
	50+	33	19.9
Educational Level	None	15	9.0
	Primary	65	39.2
	Secondary	65	39.2
	College	21	12.7
Occupation	Professional	46	27.7
	House wife	14	8.4
	Unemployed	106	63.9
Marital status	Single	74	44.6
	Married	68	41.0
	Separated	11	6.6
	Widowed	12	7.2
	Divorced	1	0.6
Smoking status	Smoker	21	12.7
	Previous smoker	29	17.5
	Non smoker	116	69.9
Alcohol use		43	25.9
HIV Serostatus	Positive	108	65.1
	Negative	58	34.9

4.2.2 Socio-demographic characteristics of HHCs

Of the one hundred and seventy-five HHCs, 29.7% (52/175) were male, and 70.3% (123/175) were female. Approximately 37% (66/175) were 30-39 years old, while 39.4% (69/175) had lived with the TB patients for over 20 weeks. About 38.9% (68/175) had secondary education, 42.9% (75/175) were unemployed,

while 65.7% (115/175) lived in a one-room structure with the TB patient. Cigarette smoking, alcohol consumption, and HIV seropositivity constituted 5.1% (5/175), 16.6% (29/175), and 9.1% (16/175) of the HHCs, respectively (**Table 4**).

Table 4. Socio-demographic characteristics of Household Contacts N = 175

		Frequency	**Percent**
Gender	Male	52	29.7
	Female	123	70.3
Age group	18-29	53	30.3

	30-39	66	37.7
	40-49	29	16.6
	50+	27	15.4
Relation to patient	Spouse	20	11.4
	Others	155	88.6
Duration of association	1-4 Weeks	58	33.1
	4.1-8 Weeks	32	18.3
	8.1-12 weeks	10	5.7
	16.1-20 Weeks	6	3.4
	>20.1 Weeks	69	39.4
Educational Level	None	6	3.4
	Primary	55	31.4
	Secondary	68	38.9
	College	46	26.3
Occupation	Professional	69	39.4
	House wife	31	17.7
	Unemployed	75	42.9
Room sharing	Yes	115	65.7
	No	60	34.3
Patients in household	1	170	97.1
	>1	5	2.9
Smoking status	Smoker	9	5.1
	Previous smoker	4	2.3
	Non smoker	162	92.6
Alcohol use	Yes	29	16.6
	No	146	83.4
HIV sero-status	Positive	16	9.1
	Negative	159	90.9

4.2.3 Comparison of demographic characteristics of PTB patients and Household contacts

Pulmonary tuberculosis patients (PTB) were more likely to be male [OR=2.20 (1.39-3.44) p=0.001], single [OR=2.49 (1.54-3.99), p=0.001] or separated [OR=6.04 (1.75-20.65), p=0.004] compared to married, be active smokers [OR=3.26, (1.48-7.59), p=0.004] or previous smokers [OR=10.13 (3.77- 27.2),

p=0.001], consumed alcohol [OR=1.76 (1.03-2.99), p=0.046) and were HIV positive (OR=18.50 (10.22-33.7), p=0.001. These have been presented in **Table 5** below.

Table 5. Comparison of select demographic characteristics of PTB patients and Household contacts

Variable		PTB (N=166)	HHCs (N=175)	p- value
Gender	Male	80 (48.2)	52 (29.7)	**0.001**
	Female	86 (41.1)	123 (58.9)	
Marital status	Single	74 (44.6)	49 (28.0)	**0.001**
	Married	68 (41.0)	112 (64.0)	
	Separated	11 (6.6)	3 (1.7)	**0.004**
	Widowed	12 (7.2)	9 (5.1)	0.101
	Divorced	1 (0.6)	2 (1.1)	1.000
Smoking	Smoker	21 (12.7)	9 (5.1)	**0.004**
	Previous	29 (17.5)	4 (2.3)	**0.001**
	Non smoker	116 (69.9)	162 (92.6)	
Alcohol use		43 (25.9)	29 (16.6)	**0.046**
HIV	Positive	108 (65.1)	16 (9.1)	**0.001**
	Negative	58 (34.9)	159 (90.9)	

This comparison showed that in our study population, the odds of being a pulmonary tuberculosis case were higher among males, single or separated individuals, smokers, individuals who were consuming alcohol, and individuals who were HIV positive.

4.3 Results of Research Objective 1: Prevalence of Latent Tuberculosis Infection among the Household contacts of PTB patients

4.3.1 Prevalence of LTBI

Of the175 HHCs evaluated, 174 had QFT-GIT assay results for the diagnosis of LTBI. One sample had indeterminate results and was eliminated from the analysis. The overall prevalence of LTBI during the

study period among the household members living with PTB patients visiting Mbagathi hospital was 55.7% (95% CI: 48-63.2), calculated using the Clopper-Pearson method. The majority of those who tested positive [46.4% (45/97)] had an interferon-gamma (IFN-γ) measurement of >0.35 and 4.9 IU/ml; while 27.8% (27/97) had a value greater than 9.0 IU/ml **(Figure 17)**.

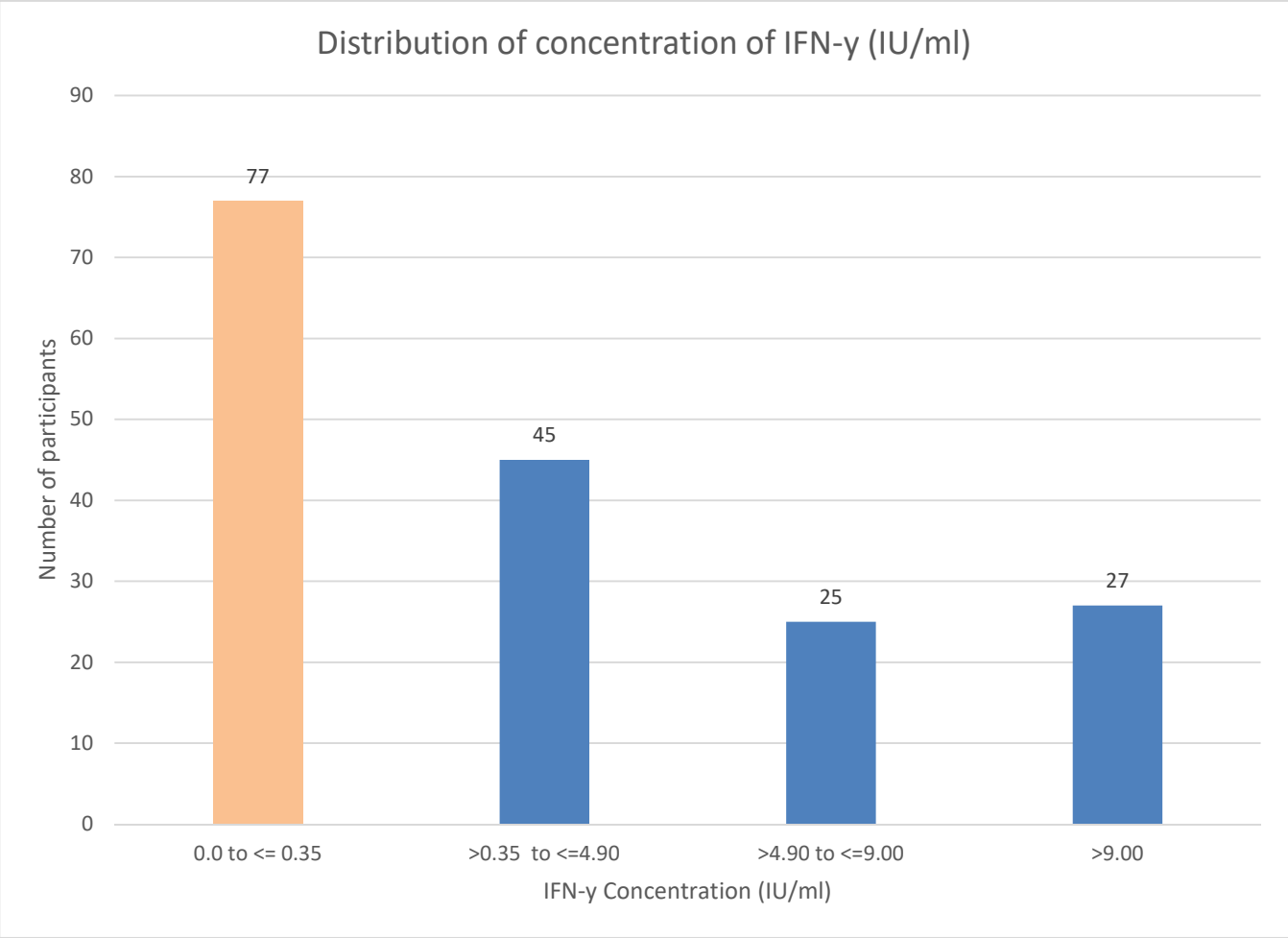

Figure 17. The distribution QFT GIT ELISA test results showed that most participants (97/174) were LTBI positive while 77 tested negative. Most of those diagnosed with LTBI had IFN IU/ml test values for the QFT GIT test of between >0.35 and 4.90.

The median OD for the LTBI-negative group was 0.01 IU/ml. The median for the LTBI-positive group was 5.57 IU/ml. There was a significant difference in the median values of IFN gamma measurements between the LTBI negative and LTBI positive groups (p-value <0.0001). (**Figure 18**).

Distribution of Interferon-gamma between LTBI positive and LTBI negative HHCs

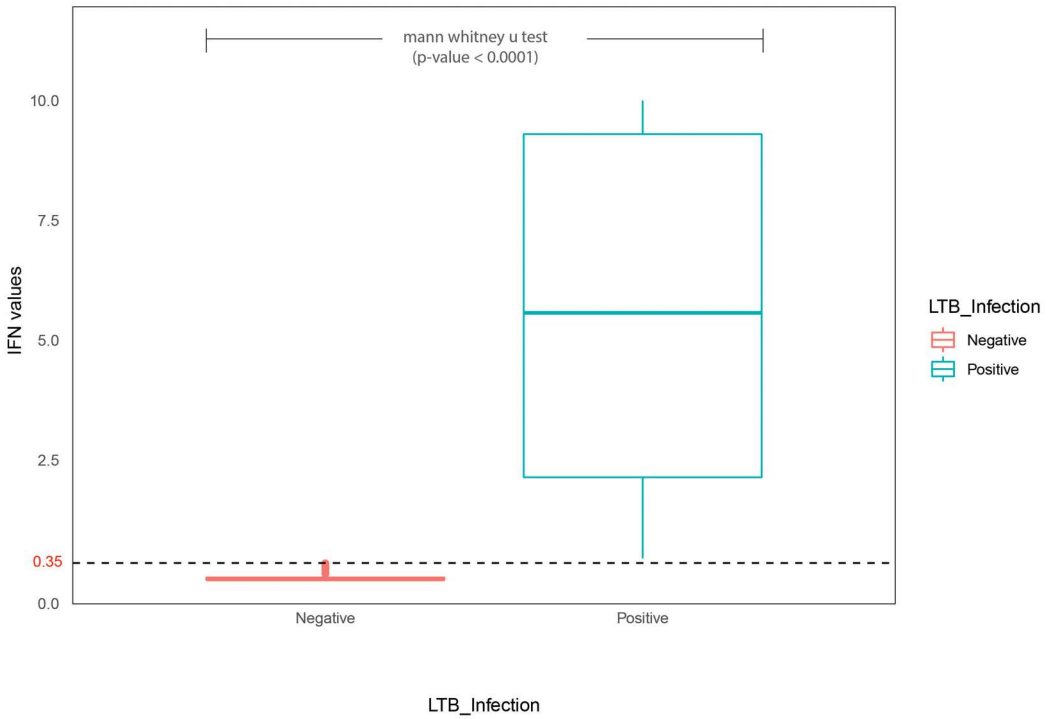

Figure 18. The distribution of IFN gamma concentrations between the LTBI-negative and LTBI-positive household contacts.

4.3.2 Association between IFN-γ measured in LTBI positive HHCs and select demographic characteristics.

We evaluated the IFN-γ level concentrations measured across the LTBI positive HHCs stratified by select demographic characteristics. There wasn't enough statistical evidence to show an association between the measurements of IFN gamma concentrations and any assessed variables among the LTBI-positive individuals. This is presented in **Table 6**.

Table 6. A comparison of the risk factors coefficients between the univariate and multivariate linear regression of IFN-γ levels in LTBI-positive Household contacts

Risk factor	Number	Crude OR	LCI	UCI	P-value	Adjusted OR	LCI	UCI	P-value
HHC HIV status: Negative	91	-	-	-	-	-	-	-	-
Positive	6	0.15	-2.74	3.04	0.917	-0.08	-3.13	2.97	0.96
Duration of contact: 1- 4 weeks	30	-	-	-	-	-	-	-	
Above 4 to 8 weeks	18	-0.55	-2.62	1.52	0.599	-0.72	-2.86	1.42	0.505
Above 8 to 12 weeks	8	0.3	-2.46	3.06	0.832	-0.69	-3.54	2.15	0.629
Above 12 to 16 weeks	3	-1.43	-5.63	2.77	0.501	-0.62	-4.92	3.68	0.776
More than 20 weeks	38	0.01	-1.68	1.7	0.991	-0.47	-2.22	1.28	0.597
Relationship: Spouse	14	-	-	-	-	-	-	-	-
Others	83	-1.71	-3.66	0.24	0.086	-1.54	-3.62	0.54	0.144
Patient HIV status: Negative	32	-	-	-	-	-	-	-	-
Positive	65	1.24	-0.21	2.7	0.094	1.23	-0.29	2.75	0.112
Age Group: 18-29	26	-	-	-	-	-	-	-	-
30-39	42	0.79	-0.88	2.47	0.348	0.8	-0.92	2.52	0.360
40-49	17	-0.08	-2.18	2.01	0.936	0.07	2.15	2.28	0.953
50-59	11	-1.9	-4.31	0.51	0.121	-1.72	-4.25	0.81	0.18
60-69	1	-4.2	-11.04	2.64	0.226	-5.14	-12.3	2.05	0.159
Room sharing: Yes	68	-	-	-	-	-	-	-	-
No	29	0.05	-1.47	1.57	0.948	0.56	-1.12	2.23	0.509

4.4 Research Objective 2: Risk factors for LTBI infection among HHCs of PTB

4.4.1 Potential risk factors for LTBI infection

While evaluating potential risk factors, the LTBI status (either positive or negative) was the dependent variable. Demographic, behavioural and medical factors such as gender, age group, relation to PTB patients, duration of association with PTB patients, educational level, occupation, smoking status, alcohol use, and HIV status were considered independent variables. Potential risk factors for LTBI were evaluated using the Chi-square test, where the Index PTB patient's HIV status was statistically significant. The findings are presented below in **Table** 7.

Table 7. Summary statistics of potential risk factors for Latent TB Infection among Household

Variable	LTBI+ (n = 97)	LTBI- (n = 77)	p-value
HHC HIV status			0.2012
Positive	6(6.2)	10(13)	
Negative	91(93.8)	67(87)	
Duration of contact(weeks)			0.6251
1 to 4	30(30.9)	27(35.1)	
Above 4 to 8	18(18.6	14(18.1)	
Above 8 to 12	8(8.25)	2(2.6)	
Above 12 to 16	3(3.1)	3(3.9)	
More than 20 weeks	38(39.2)	31(40.3)	
Relationship with patient			0.2607
Spouse	14(14.4)	6(7.8)	
Other	83(85.7)	71(92.2)	
Marital status			0.5232
Single	26(26.8)	23(29.9)	
Married	65(67)	46(59.7)	
Separated	2(2.1)	1(1.3)	
Divorced	0(0)	2(2.6)	
Widowed	4(4.1)	5(6.5)	
Patient's HIV status			0.0254
Positive	65(67)	64(83.1)	
Negative	32(33)	13(16.9)	
Sex			0.72
Male	30(30.9)	21(27.3)	
Female	67(69.1)	56(72.7)	
Age (mean)	36	36.4	0.7239

Age group			0.2927
18-29	26(26.8)	27(35.1)	
30-39	42(43.3)	24(31.2)	
40-49	17(17.5)	11(14.3)	
50-59	11(11.3)	11(14.3)	
60-69	1(1)	3(3.9)	
70-79	0(0)	1(1.3)	
Education			0.6561
None	4(4.1)	2(2.6)	
Primary	31(32)	23(29.9)	
Secondary	40(41.2)	28(36.4)	
Tertiary	22(22.7)	24(31.2)	
Occupation			0.8939
Unemployed	42(43.2)	32(41.6)	
House wife	18(18.6)	13(16.9)	
Professional	37(38.1)	32(41.6)	
Number of Rooms in House			0.5899
1	50(60.8)	46(59.7)	
2	29(29.9)	18(23.4)	
3	7(7.2)	7(9.1)	
4	5(5.2)	1(1.3)	
5	4(4.1)	3(3.9)	
6	2(2.1)	1(1.3)	
7	0(0)	1(1.3)	
Room sharing with patient at night			0.2048
Yes	68(70.1)	46(59.7)	
No	28(29.9)	31(40.3)	
Number of PTB patients in House			0.7929
1	95(97.9)	74(96.1)	
2	2(2.1)	3(3.9)	
Smoking status			1
Current Smoker	5(2.9)	4(2.3)	
Previous Smoker	2(1.1)	2(1.1)	
Never Smoked	90(51.7)	71(40.8)	
Alcohol			0.5382
Yes	14(8)	14(8)	
No	83(47.7)	63(36.2)	
Access to Information			0.7747
Yes	86(49.7)	67(38.7)	

No	10(5.8)	10(5.8)	
Medication			1
Yes	80(60)	64(36.8)	
No	17(9.8)	13(7.5)	
Nutrition			0.4178
Yes	87(50)	65(37.4)	
No	10(5.7)	12(6.9)	
Counselling			1
Yes	89(51.1)	71(40.8)	
No	8(4.6)	6(3.4)	
Support			1
Yes	91(52.3)	73(42)	
No	6(3.4)	4(2.3)	

Other*: brother, sister, son, daughter, aunt, uncle, cousin, friend, mother, father

4.4.2 Comparative analysis of potential risk factors for Latent TB infection using bivariate and multivariate logistic regression.

The data collected in the present study had enough statistical evidence to show an association between several covariates. These included the HHC HIV serostatus, relationship status (spousal or other) between the household contact and PTB patient, and the HIV serostatus of the index PTB patient. In the analysis, HHCs who were HIV seropositive had 98% fewer odds of testing positive for LBTI (OR: 0.02; CI: 0 - 0.3; p-value 0.006). The non-spouse relationship (OR: 0.09; CI: 0.01 – 0.69; p-value 0.021) and PTB patients positive for HIV (OR:0.41; CI: 0.19 - 0.87; p-value 0.02) were also significantly associated with reduced odds (91% and 59% less odds respectively) of testing positive for LTBI. On the contrary, HIV seropositive HHCs who were not spouses had over 63 times the odds of testing positive for LBTI (OR: 63.24; CI:2.44 – 1637.3; p-value 0.012). There was a significant interaction of terms where the non-spousal relationship seemed to modify the effect of the seropositive HHC. The huge confidence interval can be attributed to the smaller sample size falling in this category. These results have been presented in **Table 8** below.

Table 8. A comparison of the odds ratios for the risk factors covariates between the univariate and multivariate logistic regression

Risk factor	LTBI-ve	*LTBI+ve*	Crude OR	LCI	UCI	P-value	Adjusted OR	LCI	UCI	P-value
HHC HIV status: HIV-ve	67(87%)	91(93.8%)	-	-	-	-	-	-	-	-
HIV +ve	10(13%)	6(6.2%)	0.44	0.15	1.28	0.131	0.02	0	0.3	0.006
Duration of contact: 1 week	27(35.1%)	30(30.9%)	-	-	-	-	-	-	-	
2 weeks	14(18.2%)	18(18.6%)	1.16	0.48	2.76	0.743	1.33	0.52	3.41	0.554
3 weeks	2(2.6%)	8(8.2%)	3.6	0.7	18.45	0.124	3.31	0.58	18.74	0.177
5 weeks	3(3.9%)	3(3.1%)	0.9	0.17	4.84	0.902	0.91	0.16	5.29	0.915
6 weeks	31(40.3%)	38(39.2%)	1.1	0.55	2.23	0.784	1.39	0.65	2.97	0.402
Relationship: 5	6(7.8%)	14(14.4%)	-	-	-	-	-	-	-	-
6	71(92.2%)	83(85.6%)	0.5	0.18	1.37	0.179	0.09	0.01	0.69	0.021
Patient HIV status: Negative	13(16.9%)	32(33%)	-	-	-	-	-	-	-	-
Positive	64(83.1%)	65(67%)	0.41	0.2	0.86	0.81	0.41	0.19	0.87	0.02
Age Group: 1	27(35.1%)	26(26.8%)	-	-	-	-	-	-	-	-
2	24(32.1%)	42(43.3)	1.82	0.87	3.79	0.112	1.67	0.77	3.36	0.193
3	11(14.3%)	17(17.5)	1.6	0.63	4.07	0.319	1.71	0.64	4.6	0.286
4	11(14.3%)	11(11.3%)	1.04	0.38	2.81	0.941	0.93	0.31	2.75	0.893
5	3(3.9%)	1(1%)	0.35	0.03	3.54	0.371	0.48	0.04	5.24	0.55
6	1(1.3%)	0(0%)	0	0	Infinity	0.987	0	0	Infinity	0.987
Room sharing: 1	46(59.7%)	68(70.1%)	-	-	-	-	-	-	-	-
2	31(40.3%)	29(29.9%)	0.63	0.34	1.19	0.154	0.59	0.29	1.19	0.14
HHC HIV positive: Relation 6	5(6.5%)	5(5,2%)					63.24	2.44	1637.30	0.012

Relationship code: 5* spouse, 6*other: sibling,parent,child,aunt,uncle
Age group code: 1*18-29, 2*30-39, 3*40-49, 4*50-59, 5*60-69, 6* 70-79
Room sharing with patient at night code: 1*Yes, 2*No

4.5 Research Objective 3: Distribution of HLA -DRB and -DQB allele groups in the study population

We analysed DNA extracts of 60 randomly selected study participants and identified allele groups in HLA-DQB and -DRB loci in 54 samples comprising 17 PTB patients and 37 HHCs. The DNA extracts from the unyielding samples were re-submitted to the processing protocol, but the typing was unsuccessful. The PCR products from the successful amplification processes were analysed through gel electrophoresis using a particular cartridge in the Qiaxcel automated machine. The products were then displayed on the screen, and the gel picture was uploaded into the Olerup SSP Analysis Software.

4.5.1 Demographic characteristics of sub-group of study participants typed for HLA-DRB and -DQB allele groups.

Deoxyribonucleic Acid (DNA) extracted from blood samples of PTB patients and HHCs were randomly selected for the HLA typing. The demographic characteristics of this sub-set of study participants are presented in **Table 9**. The mean age of HHCs (36.3±11.2 years)was higher than that of PTB (32.9±9.8 years). Most participants were female in the PTB patients and HHCs groups, at 58.8% and 56.8%. More PTB patients had a history of smoking (5.9%) compared to HHCs (2.7%), while alcohol consumption was higher among HHCs (16.2%) than PTB patients (5.9%). The gender differences, history of smoking and alcohol consumption were also not statistically significant. We observed that 41.2% of the PTB patients were HIV positive compared to 100% of HHCs who were all HIV negative ($p<0.01$) (**Table 9**). The demographic characteristics were comparable except for HIV status where none of the HHCs typed for HLA was HIV positive.

Table 9. Demographic characteristics of sub-group of participants selected for HLA typing

Parameters		**PTB (17)**	**HHC (37)**	**p value**
Age (years)	Mean±SD	32.9±9.8	36.3±11.2	0.26
Gender	Male	7 (41.2)	16 (43.2)	1.00
	Female	10 (58.8)	21 (56.8)	1.00
Cigarette smoking		1 (5.9)	1 (2.7)	0.53
Alcohol consumption		1 (5.9)	6 (16.2)	0.41
HIV status	+ve	7 (41.2)	0 (0.0)	<0.01
	-ve	10 (58.8)	37 (100)	<0.01

4.5.2 The distribution of HLA-DRB and HLA-DQB allele groups across the study participants was stratified as LTBI negative HHCs, LTBI positive HHCs and PTB patients.

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From 54 samples analysed via SSP-PCR genotyping, HLA-DRB genotypes were identified in 47 samples with 18 allelic groups. HLA-DQB genotypes were identified in 36 samples with six allelic groups. The DNA extracts from unyielding samples were submitted to the sequencing protocol, but the typing was unsuccessful. The distribution of the allele group frequencies within the LTBI negative HHCs, LTBI positive HHCs and the PTB patients has been presented in a bar graph in **Figure 19** and a dot plot in **Figure 20** below.

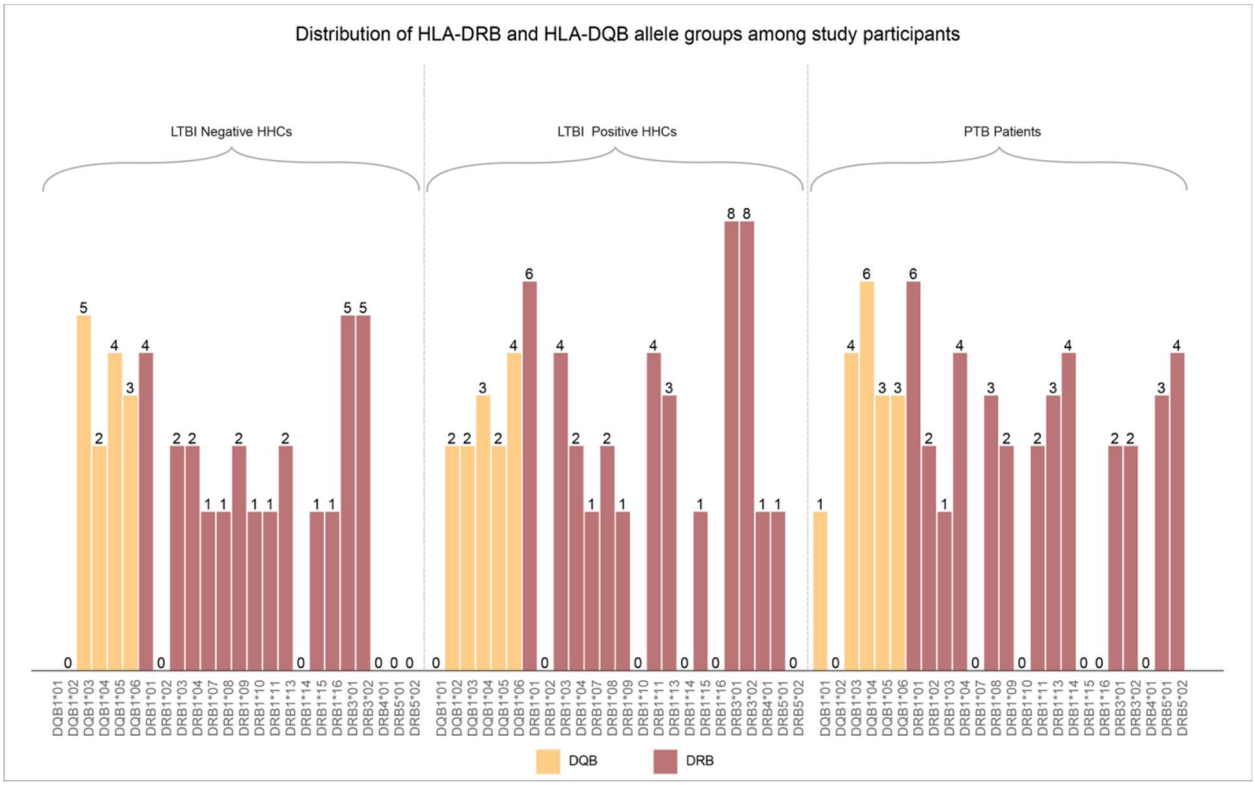

Figure 19. Illustration of frequency counts of allele groups across the study participants stratified as PTB patients, LTBI-positive HHCs and LTBI-negative HHCs.

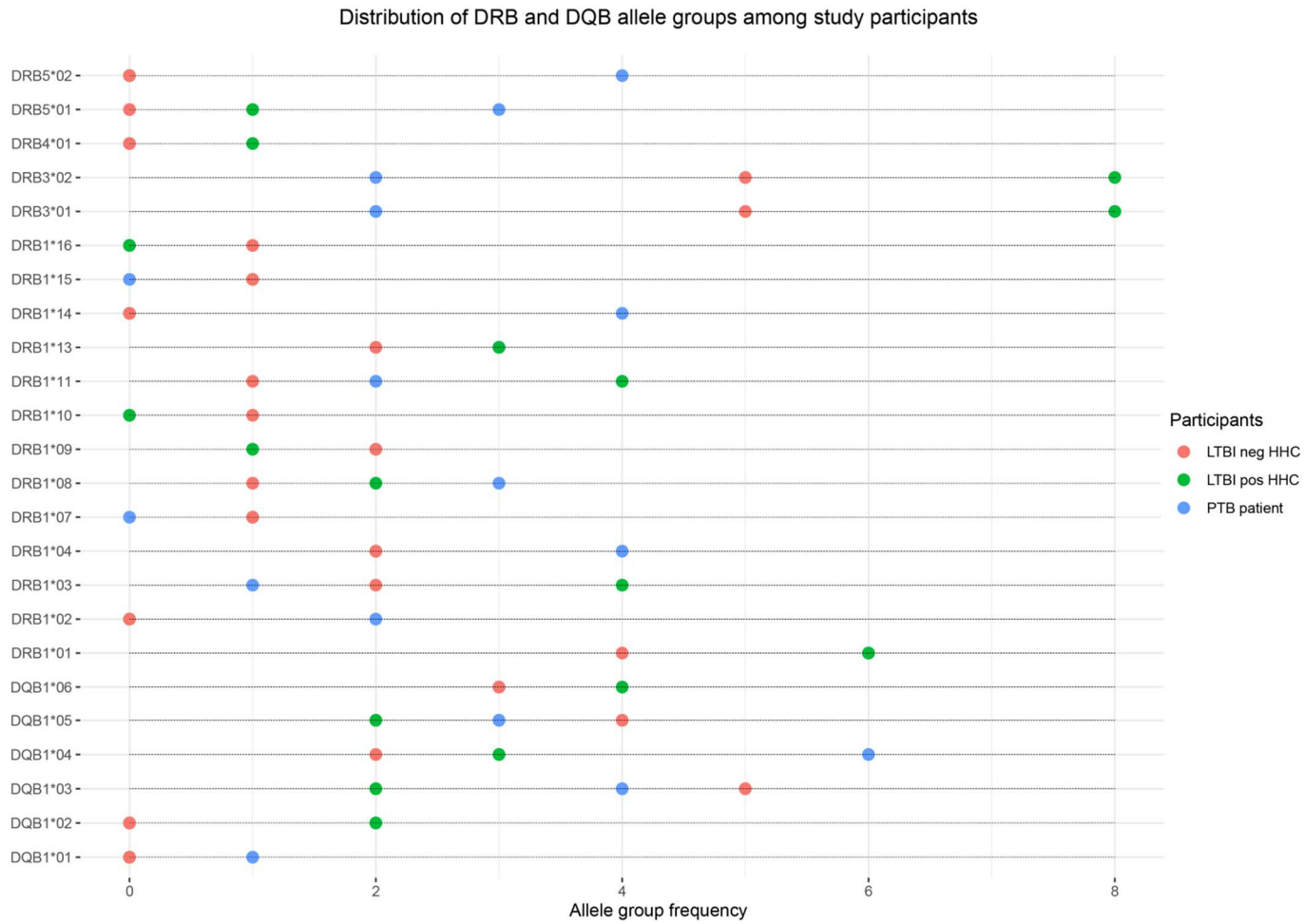

Figure 20. Illustration of the distribution and frequencies of different allele groups across the study participants stratified as PTB patients, LTBI positive HHCs and LTBI negative HHCs.

4.5.3 Distribution of HLA-DRB and HLA-DQB allele groups across the study participants stratified by gender

Compared by gender, frequencies of HLA-DRB allele groups were higher among males than females (12/18) (**Figure 21**).

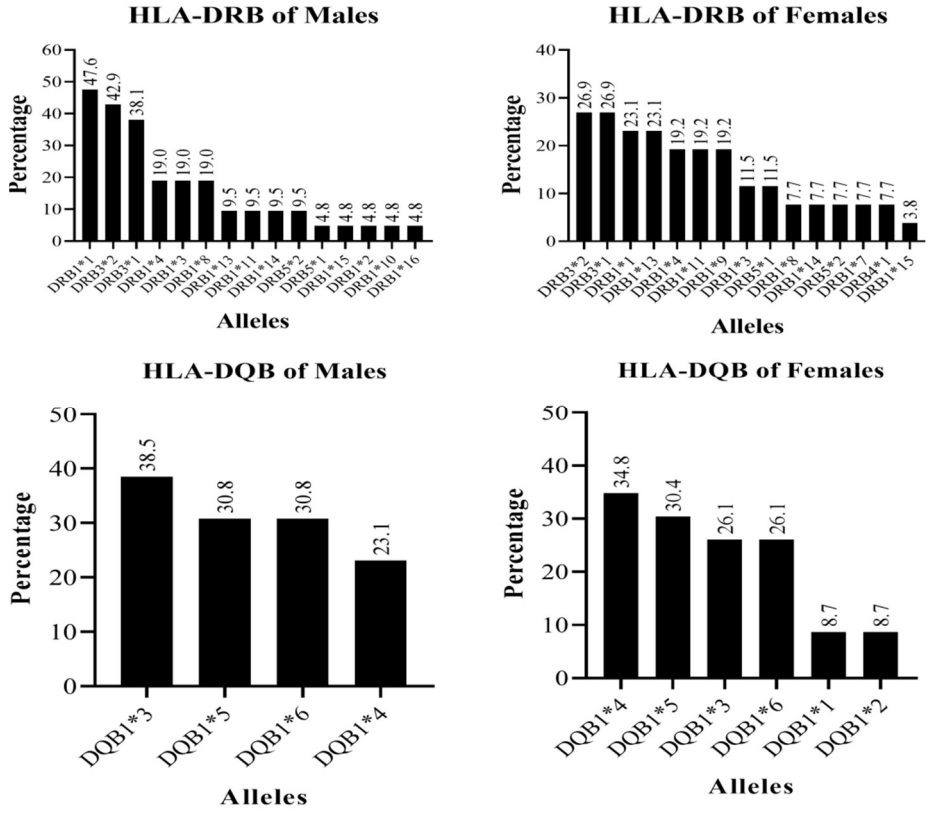

Figure 21. Distribution of HLA-DRB and HLA-DQB allele groups by gender

There were 12 HLA-DRB allele groups with frequencies >5% identified in this study population, while all the identified HLA-DQB allele groups in this study population had frequencies >5% (**Table 10**). However, the differences in frequency counts of the allele groups between the two genders were not statistically significant before adjusting for HIV status (**Table 10**). After adjustment for HIV status, the frequency of the DRB1*9 allele group was significantly higher among females compared to males (p=0.034) (**Table 10**).

Table 10. Distribution of -DRB and -DQB allele groups by gender and HIV status

Allele group	N	%	Male	Female	p value	Adjusted p value*
DRB	**47**	**100**	**N=21**	**N=26**		
DRB1*1	16	34.0	10 (47.6)	6 (23.1)	0.122	0.088
DRB3*2	16	34.0	9 (42.9)	7 (26.9)	0.355	0.270
DRB3*1	15	31.9	8 (38.1)	7 (26.9)	0.533	0.428
DRB1*4	9	19.1	4 (19.0)	5 (19.2)	1.000	0.937
DRB1*13	8	17.0	2 (9.5)	6 (23.1)	0.269	0.169
DRB1*3	7	14.9	4 (19.0)	3 (11.5)	0.684	0.476
DRB1*11	7	14.9	2 (9.5)	5 (19.2)	0.436	0.427
DRB1*8	6	12.8	4 (19.0)	2 (7.7)	0.386	0.249
DRB1*9	5	10.6	0 (0.0)	5 (19.2)	0.056	**0.034**
DRB1*14	4	8.5	2 (9.5)	2 (7.7)	1.000	0.916
DRB5*1	4	8.5	1 (4.8)	3 (11.5)	0.617	0.457
DRB5*2	4	8.5	2 (9.5)	2 (7.7)	1.000	0.096
DRB1*7	2	4.3	0 (0.0)	2 (7.7)	0.495	0.998
DRB1*15	2	4.3	1 (4.8)	1 (3.8)	1.000	0.945
DRB4*1	2	4.3	0 (0.0)	2 (7.7)	0.495	0.998
DRB1*2	1	2.1	1 (4.8)	0 (0.0)	0.447	0.998
DRB1*10	1	2.1	1 (4.8)	0 (0.0)	0.447	0.261
DRB1*16	1	2.1	1 (4.8)	0 (0.0)	0.447	0.998
DQB	**36**	**100**	**N=13**	**N=23**		
DQB1*3	11	30.6	5 (38.5)	6 (26.1)	0.474	0.999
DQB1*4	11	30.6	3 (23.1)	8 (34.8)	0.708	0.999
DQB1*5	11	30.6	4 (30.8)	7 (30.4)	1.000	0.416
DQB1*6	10	27.8	4 (30.8)	4 (26.1)	0.679	0.583
DQB1*1	2	5.6	0 (0.0)	2 (8.7)	0.525	0.959
DQB1*2	2	5.6	0 (0.0)	2 (8.7)	0.525	0.959

4.6 Research Objective 4: Association between HLA -DRB and -DQB allele groups and outcome of exposure to *Mycobacterium tuberculosis*

4.6.1 Bivariate and multivariate logistic regression of Allele group frequencies in the PTB patients and Household Contacts

We analysed the HLA-DRB and HLA-DQB allele groups in 17 patients with active TB and 37 HHCs without clinical evidence of TB. We conducted bivariate and multivariate logistic regression by comparing frequencies of HLA-DRB and HLA-DQB allele groups among study participants grouped as PTB patients and HHCs before and after adjustment for HIV status.

The frequency of the DRB5*2 allele group was significantly higher among PTB patients compared to HHCs (p=0.013) before adjusting for HIV status (**Table 11**). The frequency of DRB3*1 was 0.17 (95% CI=0.03-0.83) times lower among PTB patients compared to HHCs before adjustment for HIV status. However, after adjusting for HIV status, differences in the frequencies of DRB5*2 and DRB3*1 groups were not statistically significant.

After adjusting for HIV status, the frequency of the DRB1*14 allele group was 12-fold (95% CI=1.11-138.2) higher among PTB patients compared to HHCs (p=0.040). Frequencies of DRB1*1, DRB1*2, DRB1*3, DRB1*4, DRB1*7, DRB1*8, DRB1*9, DRB1*10, DRB1*11, DRB1*13, DRB1*14, DRB1*15, DRB1* 16, DRB3*2, DRB4*1, and DRB5*1 and DQB1*1, DQB1*2, DQB1*3, DQB1*4, DQB1*5, and DQB1*6 among PTB and HHCs were comparable before and after adjustment for HIV status (**Table 11**).

Table 11. Comparison of -DRB and -DQB allele groups across PTB patients and HHCs, stratified by HIV status

Allele group	PTB	HHCs	OR (95% CI)	p value	AOR (95% CI) *	p value
DRB	**N=17**	**N=30**				
DRB1*1	6 (35.3)	10 (33.3)	1.09 (0.32-3.60)	1.000	1.33 (0.30-5.83)	0.702
DRB1*2	1 (5.9)	0 (0.0)	-	0.361	-	0.998
DRB1*3	1 (5.9)	6 (20.0)	0.25 (0.02-1.93)	0.395	0.00 (0.00-)	0.999
DRB1*4	5 (29.4)	4 (13.3)	2.70 (0.60-9.88)	0.251	2.78 (0.50-15.4)	0.241
DRB1*7	0 (0.0)	2 (6.7)	-	0.528	0.00 (0.00-)	0.999
DRB1*8	3 (17.6)	3 (10.0)	1.92 (0.40-8.99)	0.652	2.25 (0.31-15.9)	0.416
DRB1*9	2 (11.8)	3 (10.0)	1.20 (0.19-6.39)	1.000	0.00 (0.00-)	0.999
DRB1*10	0 (0.0)	1 (3.3)	-	1.000	-	0.999
DRB1*11	2 (11.8)	5 (16.7)	0.66 (0.12-3.97)	1.000	0.00 (0.00-)	0.999
DRB1*13	3 (17.6)	5 (16.7)	1.07 (0.25-5.24)	1.000	2.14 (0.40-11.2)	0.368
DRB1*14	3 (17.6)	1 (3.3)	6.21 (0.82-82.9)	0.127	12.42 (1.11-138.2)	**0.040**
DRB1*15	0 (0.0)	2 (6.7)	-	0.528	0.00 (0.00-)	0.999
DRB1*16	0 (0.0)	1 (3.3)	-	1.000	-	0.999
DRB3*1	2 (11.8)	13 (43.3)	0.17 (0.03-0.83)	**0.048**	0.00 (0.00-)	0.999
DRB3*2	3 (17.6)	13 (43.3)	0.28 (0.07-1.08)	0.111	0.14 (0.01-1.296)	0.084
DRB4*1	1 (5.9)	1 (3.3)	1.81 (0.09-35.4)	1.000	3.22 (0.18-56.8)	0.424
DRB5*1	3 (17.6)	1 (3.3)	6.21 (0.82-82.9)	0.127	7.25 (0.58-90.5)	0.124
DRB5*2	4 (23.5)	0 (0.0)	-	**0.013**	**-**	0.998
DQB	**N=14**	**N=22**				
DQB1*1	1 (7.1)	1 (4.5)	1.61 (0.07-31.9)	1.000	0.00 (0.00-)	0.999
DQB1*2	0 (0.0)	2 (9.1)	-	0.511	-	0.999
DQB1*3	4 (28.6)	7 (31.8)	0.85 (0.23-3.26)	1.000	0.71 (0.11-4.47)	0.719
DQB1*4	6 (42.9)	5 (22.7)	2.55 (0.64-11.6)	0.273	2.04 (0.35-11.67)	0.423
DQB1*5	5 (35.7)	6 (27.3)	1.48 (0.34-5.83)	0.715	1.60 (0.28-8.85)	0.590
DQB1*6	3 (21.4)	7 (31.8)	0.58 (0.14-2.45)	0.706	0.71 (0.11-4.47)	0.719

4.6.2 Bivariate and multivariate logistic regression of Allele group frequencies in PTB patients, LTBI positive HHCs and LTBI negative HHCs stratified by HIV serostatus.

Further analysis of an association was made between the identified HLA-DRB and HLA-DQB allele groups and 17 PTB patients (active tuberculosis), 19 Latent TB positive HHCs (exposed to *Mycobacterium tuberculosis* and infected) and 18 Latent TB negative HHCs (exposed to *Mycobacterium tuberculosis* but not infected). The frequencies were also compared before and after adjustment for HIV status.

The frequency of HLA-DRB3*1 was 34.4%, significantly higher among LTBI -ve HHCs (46.2%) compared to PTB patients (11.8%) before adjustment for HIV status (p=0.049). After adjustment, the difference was not statistically significant. DRB1*1, DRB1*2, DRB1*3, DRB1*4, DRB1*7, DRB1*8, DRB1*9, DRB1*10, DRB1*11, DRB1*13, DRB1*14, DRB1*15, DRB1*16, DRB3*2, DRB4*1, DRB5*1, and DRB5*2 and DQB1*1, DQB1*2, DQB1*3, DQB1*4, DQB1*5, and DQB1*6 frequencies were comparable between PTBs, LTBI+ HHCs, LTBI -ve HHCs before and after adjustment for HIV status (**Table 12**).

Table 12. Comparison of HLA-DRB and HLA-DQB allele groups between PTB patients, LTBI positive HHCs and LTBI negative HHCs.

	Participants			p value			Adjusted p value*		
Allele group	**PTB**	**LTB+**	**LTB-**	**PTB/LTB+**	**PTB/LTB-**	**LTB+/LTB-**	**PTB/LTB+**	**PTB/LTB-**	**LTB+/LTB-**
DRB	**N=17**	**N=17**	**N=13**						
DRB1*1	6 (35.3)	6 (35.3)	4 (30.8)	1.000	1.000	1.000	0.807	0.646	0.795
DRB1*2	1 (5.9)	0 (0.0)	0 (0.0)	1.000	1.000	-	0.998	0.999	-
DRB1*3	1 (5.9)	4 (23.5)	2 (15.4)	0.335	0.564	0.672	0.999	0.999	0.583
DRB1*4	5 (29.4)	2 (11.8)	2 (15.4)	0.398	0.426	1.000	0.253	0.407	0.773
DRB1*7	0 (0.0)	2 (11.8)	0 (0.0)	0.484	-	0.492	0.050	-	0.999
DRB1*8	3 (17.6)	1 (5.9)	2 (15.4)	0.601	1.000	0.564	0.286	0.773	0.406
DRB1*9	2 (11.8)	3 (17.6)	0 (0.0)	1.000	0.492	0.237	0.999	1.000	0.999
DRB1*10	0 (0.0)	0 (0.0)	1 (7.7)	-	0.433	0.433	-	0.999	0.998
DRB1*11	2 (11.8)	3 (17.6)	2 (15.4)	1.000	1.000	1.000	0.999	0.999	0.869
DRB1*13	3 (17.6)	2 (11.8)	3 (23.1)	1.000	1.000	0.627	0.253	0.708	0.417
DRB1*14	3 (17.6)	1 (5.9)	0 (0.00	0.601	0.237	1.000	0.121	0.999	0.999
DRB1*15	0 (0.0)	0 (0.0)	2 (15.4)	-	0.179	0.179	-	0.999	0.998
DRB1*16	0 (0.0)	1 (5.9)	0 (0.0)	1.000	-	1.000	0.999	-	0.999
DRB3*1	2 (11.8)	7 (41.2)	6 (46.2)	0.117	**0.049**	1.000	0.999	0.999	0.785
DRB3*2	3 (17.6)	7 (41.2)	6 (46.2)	0.258	0.123	1.000	0.114	0.087	0.785
DRB4*1	1 (5.9)	1 (5.9)	0 (0.0)	1.000	1.000	1.000	0.696	0.999	0.999
DRB5*1	3 (17.6)	1 (5.9)	0 (0.0)	0.601	0.237	1.000	0.286	0.999	0.999
DRB5*2	4 (23.4)	0 (0.0)	0 (0.0)	0.102	0.112	-	0.998	0.999	-
DQB	**N=14**	**N=11**	**N=11**						
DQB1*1	1 (7.1)	0 (0.0)	1 (9.1)	1.000	1.000	1.000	1.000	0.999	0.999
DQB1*2	0 (0.0)	2 (18.2)	0 (0.0)	0.183	-	0.476	0.999	-	0.999
DQB1*3	4 (28.6)	4 (36.4)	3 (27.3)	1.000	1.000	1.000	0.601	0.912	0.648
DQB1*4	6 (42.9)	2 (18.2)	3 (27.3)	0.233	0.676	1.000	0.353	0.657	0.613
DQB1*5	5 (35.7)	4 (36.4)	2 (18.2)	1.000	0.406	0.635	0.960	0.353	0.346
DQB1*6	3 (21.4)	3 (27.3)	4 (36.4)	1.000	0.656	1.000	0.912	0.601	0.648

PTB= Pulmonary tuberculosis, LTB+ =Latent TB positive, LTB- = Latent TB negative, *Adjusted for HIV status

4.7 SECONDARY OBJECTIVE 1: To document challenges experienced by Household Contacts of PTB patients in an urban setting.

We documented the experiences and perspectives of the HHC and PTB patients using a structured questionnaire and informal interviews.

4.7.1 Perspectives of the Household contacts of the PTB patients

A summary of the data from the study questionnaire revealed that the majority of the HHCs, 86.6% (154), could access current information on PTB whenever they visited the hospital. Almost all HHCs at 92.0% (161) stated that their patients received counselling after diagnosis, while 82.9% (145) reported that their patients could obtain prescribed medication without any challenges. A majority of HHCs, 94.3% (165) stated that the PTB patients received social support from other household members. The summary of perspectives from HHCs is as shown (**Table 13**).

Table 13. Perspective of the Household contacts of PTB patients patients **N=175 (total number of HHCs interviewed)**

	Frequency	Percent
Access to current information on TB	154	88.6
Availability of prescribed medication	145	82.9
Adequate resources for nutrition	153	87.4
TB patient counselled after diagnosis	161	92.0
Social support from household members	165	94.3

4.7.2 Perspectives of the PTB patients

Analysis of the data from the study questionnaire revealed that 82.5% (137) of the PTB patients indicated that they had access to information on TB. 79.5% (132) and 83.1% (138) had easy access to the diagnostic centre and prescribed medication, while 82.5% (137) and 84.9% (141) had access to adequate nutrition and support from household contacts (**Table 14**).

Table 14. The perspective of PTB patients on care **N=166 (total number of PTB patients interviewed)**

	Frequency	Percent
Access to information on TB	137	82.5
Access to a diagnostic centre	132	79.5
Access to prescribed medication	138	83.1
Adequate nutrition	137	82.5
Social support from household contacts	141	84.9

We explored the level of awareness of LTBI diagnosis and treatment among HHCs of PTB patients. None of the HHCs interviewed had any knowledge of latent tuberculosis. They were unaware of the existence of tests to diagnose infection after exposure to an active TB case, even when an individual did not have any symptoms of TB. They did not also have any knowledge of the availability of treatment to prevent reactivation in those found to be latently infected.

We also documented their experiences of living with PTB patients. The HHCs in our study filled the questionnaire and gave verbal accounts of their experiences as the primary caregivers to the PTB patients at home. Some excerpts were reported verbatim from the recorded responses as follows:

"*This disease has made me very lonely. Since my husband was diagnosed, no one comes to visit, and even my neighbours are not as welcoming as they used to be". Respondent A*

"I wonder if there is something wrong with my family. This is the third person in our family to get this disease. Perhaps there is something we are unaware of that is making us sick". Respondent B

"I don't have much money and worry a lot about how to keep my children safe. Should we use different utensils? We live in one room, and it is impossible to open the window at night for ventilation. Security is not so good where we live". Respondent C
"I am worried for my children. What if my husband does not make it? What if I also get sick? Who will take care of our children? People fear this disease". Respondent D

The themes highlighted below emerged as challenges faced by the HHCs:

Psychosocial challenges: Some HHCs battled personal fears of caring for a patient infected with a disease considered in their immediate community 'dangerous' and 'infectious'. Some noticed

decreased levels of interaction with other family members and neighbours. They noticed that visitors shunned their homes or were reluctant to have direct contact with the patient and refused any food offer from the host. Furthermore, some neighbours did not want their children to mingle during playtime.

Care-giver burnout: Physical exhaustion dealing with the physical needs of the patients impacted the other members of the household and, most significantly, the spouses of the patient and children. This was quite predominant in families that had babies or very young children.

Hygiene needs: Most participants lived with the PTB patient in a one- or two-room house with minimal ventilation. Some HHCs observed that TB patients were isolated from the other patients while in the hospital and wondered how they should minimise the spread of infection at home where available space could not allow for any isolation. Most households had inadequate water for domestic use, which was a significant hurdle when bathing very sick patients, especially in communal facilities. Washing the patients' clothes/bedding that were sometimes visibly soiled and cleaning utensils was mainly done using soap because disinfectants were costly.

Clinical Waste Management: Disposal of waste differed considerably between households from formal estates and informal settlements. The homes in informal settlements where most of the PTB patients resided lacked appropriate means of waste disposal, and the collection, if any, was irregular and random. Some neighbourhoods did not have explicit systems to deal with generated household waste which sometimes ended up in the environment around the house or by the walkways and roadsides. All the household waste, including what would be categorised as clinical waste, such as vomit, sputum, pus, tissue paper, and soiled linen, was disposed of like any other regular waste.

4.8 SECONDARY OBJECTIVE 2: To design a prototype PTB HHC Data card that can be customised in specific TB diagnostic centres for follow-up of PTB patients' household contacts.

A prototype data card- the 'PTB HHC data card 2022' (**Appendix 1**)- was designed to consider useful information pertinent for follow-up of household contacts of index TB patients. We used the 2019 WHO policy document (7) on the updated and consolidated guidelines for programmatic management of LTBI as a reference for investigation of close contacts of PTB patients and as a

benchmark for a suitable algorithm to use in the evaluation of clinical symptoms, diagnosis and follow-up of LTB infected individuals. Further, we incorporated guidelines from the Government of Kenya 2020 policy document (Ministry of Health, 2020a) that offers recommendations on testing and treatment of LTBI in Kenya.

The current document used for follow-up of close contacts with TB patients (the National IPT-SOP Kenya 2015 document) Field (Ministry of Health, 2012) is tailored towards people living with HIV, children under five years exposed to smear-positive TB and prisoners. The data card included in the 2015 SOP was designed based on Isoniazid Preventive Treatment (IPT). It captures the socio-demographic details, HIV status of the individual and adherence to medication which was limited to Isoniazid only. It also documents follow-up at months 6,12,18, and 24, the outcome of the IPT or the reason for discontinuation of treatment.

The proposed PTB HHC data card has been designed to collect data and follow up on a specific at-risk population- the Household contacts of PTB patients. Unlike the previous data form, this card envisages the broad range of socio-demographics that may be present in a household population and the need to offer tailored TB preventive treatment to all members. It has considered the two likely outcomes of HIV serostatus and increases the choices of regimen according to the WHO recommendations for TB preventive treatment of various special populations. The card includes sections identifying the county and facility where the TB patient was diagnosed and the patient's serial number. The number of household contacts affiliated with an index TB case and the HHC socio-demographic characteristics and clinical history are also documented. Subsequent sections in the card follow a proposed algorithm based on the evaluation of the HHC for any of the listed clinical symptoms that would warrant screening for TB disease. For HHCs who do not have any of the clinical signs, the next course of action is guided by the results of the LTBI screening results, chest radiography and HIV serostatus; the choice of treatment for LTBI is selected from the different regimens recommended in the Government of Kenya 2020 policy document (Isoniazid alone daily for 6-9 months; Rifampicin alone daily for 3-4 months; Isoniazid plus Rifampicin daily for 3-4 months and Rifapentine plus Isoniazid weekly for three months). Any adverse reactions and adherence to treatment according to the regimen picked are documented (at 3,6,9,12,24,36 months). Repeat testing for LTBI is then recommended, with positive cases

referred for chest radiography to rule out active TB and other diseases and negative issues documented as a successful treatment for LTBI.

The card exists in a hard copy and digital format, which can be printed out and filled.
- The digital card can be accessed on any internet-connected device by clicking on the access link and populated accordingly. The use of the digital form will simplify data storage and further analysis. The link to access the card:

https://forms.gle/yrWjofVZzX44QFVE8

-A designated health worker should perform data entry.
-It would also be essential to identify an adult who will be the lead contact household member where the TB patient lives.
The 'PTB HHC Data card 2022' has been divided into five sections: facility identification, index patient identifiers, household contact identification and baseline screening, the latent TB work-up plan and the follow-up schedule as per the treatment regimen selected.

1. **Facility Identification**: County, Sub-county, Facility name
2. **Index patient identifiers**: Name/Serial No. of PTB patient, Age, Sex, Number of household members
3. **Household Contact Identification and Baseline Screening**: Name, Age, Sex, Weight, Height,
   Date of screening: Day/Month/Year
   Clinical history of HHC: Smoking, Alcohol use, Diabetes, Hepatitis test results, Organ transplant history, On dialysis, On immunosuppressants, Neuropathies, Previous/Current TB treatment
4. **Latent TB Work-up**:

(Subsequent sections in the card follow a proposed algorithm based on the evaluation of the HHC for any of the listed clinical symptoms):

- Clinical symptoms: Current cough, Night sweats, Fever, Weight loss
  Any positive clinical symptoms, screen for TB and initiate treatment if found positive

No positive symptom- screen for LTBI using TST or IGRA; if positive, request for chest radiography.

- Chest radiography: Check if chest radiography is normal

  Normal chest radiography- screen and document HIV serostatus; counsel on LTBI treatment

  Abnormal chest radiography- screen for TB and other diseases

- LTBI treatment: Select LTBI treatment

  Daily, Isoniazid alone for 6-9 months

  Daily, Rifampicin alone for 3-4 months

  Daily, Isoniazid plus Rifampicin, for 3-4 months

  Weekly, Rifapentine plus Isoniazid for three months

  If seropositive, initiate IPT for 36 months

- Adverse reactions/symptoms: Document any adverse reactions

  Anorexia, Vomiting, Abdominal discomfort, Dark coloured urine, Jaundice, Persistent fatigue/ weakness

5. **Follow-up schedule** (as per selected LTBI treatment regimen picked 3,6,9,12,24,36 months)

- Adherence to treatment: Document adherencetherapyment according to regimen picked - - Completed, Continued, Lost, Not applicable
- Re-testing for LTBI after 6,12,36 months

  Positive TST/IGRA test- request chest radiography to rule out TB and other diseases. Negative IGRA test- Successful treatment for LTBI.

The prototype PTB-HHC Data Card is displayed on subsequent pages:

NAME OF FACILITY	COUNTY: SUBCOUNTY:

PTB HOUSEHOLD CONTACT CARD

INDEX TB PATIENT DETAILS

Name of Patient	TB Serial No	Age	Gender	Drug Resistance TB YES NO	Treatment Start Date	No. of Household contacts

HOUSE HOLD CONTACT- BASELINE SCREENING

Date of Screening:	CONTACT SCREENING NUMBER:		
Name of Contact			
Age	Gender	Weight	Height
Telephone Number			
Current TB treatment	YES	NO	
Past TB treatment	YES COMPLETED	NO	
Diabetes	YES DURATION	NO	
Known HIV serostatus	YES	NO	
Tested for Hepatitis	YES	NO	
Organ transplant history	YES	NO	
On Dialysis	YES	NO	
Known Neuropathies	YES	NO	
On immunosuppressants	YES	NO	
Smoking	YES STOPPED	NO	
Alcohol use	YES	NO	
Currently Pregnant	YES	NO N/A	

LATENT TB WORK-UP

Presumptive TB Clinical symptoms	Cough	Night sweats	Fever	Weight loss
Positive clinical symptoms	Screen for TB and other diseases and treat			
No clinical symptoms: Screen for LTBI	TST/ IGRA		Positive	Negative
Positive LTBI Screening test: Perform Chest radiography	Chest Radiography Done YES NO		Normal Screen for HIV Counsel on LTBI treatment	Abnormal Screen for TB
Select LTBI treatment regimen	Daily, Isoniazid alone for 6-9 months			
	Daily, Rifampicin alone for 3-4 months			
	Daily, Isoniazid plus Rifampicin, for 3-4 months			
	Weekly, Rifapentine plus Isoniazid for 3 months			

FOLLOW-UP SCHEDULE (AS PER LTBI TREATMENT REGIMEN PICKED AT 3,6,9,12,24,36 MONTHS)

CONTACT SCREENING NUMBER:		
MONTH 3	Follow up screening Due date:	Contact Screened YES NO
	Reason if 'NO'	
	Follow up screening Done DATE:	
	Adverse reactions to treatment: Anorexia, Vomiting, Abdominal discomfort, Dark coloured urine, Jaundice, Persistent fatigue/ weakness	YES NO
	Presumptive TB Clinical symptoms If positive screen for TB	YES NO
	Signature of Medical Officer and Date:	
MONTH 6	Follow up screening Due date:	Contact Screened YES NO
	Reason if 'NO'	
	Follow up screening Done DATE:	
	Adverse reactions to treatment: Anorexia, Vomiting, Abdominal discomfort, Dark coloured urine, Jaundice, Persistent fatigue/ weakness	YES NO
	Presumptive TB Clinical symptoms If positive screen for TB	YES NO
	Repeat LTBI Testing TST/IGRA Positive Negative Positive TST/IGRA test- request chest radiography to rule out TB and other diseases	
	Signature of Medical Officer and Date:	
MONTH 9	Follow up screening Due date:	Contact Screened YES NO
	Reason if 'NO'	
	Follow up screening Done DATE:	
	Adverse reactions to treatment: Anorexia, Vomiting, Abdominal discomfort, Dark coloured urine, Jaundice, Persistent fatigue/ weakness	YES NO
	Presumptive TB Clinical symptoms If positive screen for TB	YES NO
	Signature of Medical Officer and Date:	
MONTH 12	Follow up screening Due date:	Contact Screened YES NO
	Reason if 'NO'	
	Follow up screening Done DATE:	
	Adverse reactions to treatment: Anorexia, Vomiting, Abdominal discomfort, Dark coloured urine, Jaundice, Persistent fatigue/ weakness	YES NO
	Presumptive TB Clinical symptoms If positive screen for TB	YES NO
	Repeat LTBI Testing TST/IGRA Positive Negative Positive TST/IGRA test- request chest radiography to rule out TB and other diseases	
	Signature of Medical Officer and Date:	

MONTH 24	Follow up screening Due date:	Contact Screened YES NO
	Reason if 'NO'	
	Follow up screening Done DATE:	
	Adverse reactions to treatment: Anorexia, Vomiting, Abdominal discomfort, Dark coloured urine, Jaundice, Persistent fatigue/ weakness	YES NO
	Presumptive TB Clinical symptoms If positive screen for TB	YES NO
	Signature of Medical Officer and Date:	
MONTH 36	Follow up screening Due date:	Contact Screened YES NO
	Reason if 'NO'	
	Follow up screening Done DATE:	
	Adverse reactions to treatment: Anorexia, Vomiting, Abdominal discomfort, Dark coloured urine, Jaundice, Persistent fatigue/ weakness	YES NO
	Presumptive TB Clinical symptoms If positive screen for TB	YES NO
	Repeat LTBI Testing TST/IGRA Positive Negative Positive TST/IGRA test- request chest radiography to rule out TB and other diseases	
	Signature of Medical Officer and Date:	

FINAL OUTOCME AT END OF TREATMENT REGIMEN		
No TB	TB	LOST
Reasons for 'Lost'		
Remarks		
Signature of Medical Officer and Date		

CHAPTER FIVE

5 DISCUSSION

TB is a chronic mycobacterial infection with a latency period following most initial infections.The disease mainly affects the lungs after infection with *M. tuberculosis* complex organisms. These acid-fast bacilli spread to new hosts through airborne droplet nuclei after close contact with patients with respiratory tuberculosis. Due to the mode of transmission of the pathogen, individuals in close contact with persons with active PTB have a higher risk of infection as the confirmed cases remain highly infectious before and while in the early stages of treatment. Those predisposed to tuberculosis due to prolonged exposure to *M. tuberculosis* include healthcare workers, HHCs, schoolmates, and workmates (Ganmaa, Khudyakov, Buyanjargal, Baigal, et al., 2019). The HHCs are the patients' primary caregivers while at home, and subsequently, they have a higher cumulative exposure to *M. tuberculosis* through close physical contact and social interactions.

5.1 Latent Tuberculosis Infection

A distinctive feature of tuberculosis's natural history is the diverse infection outcome. There are three possible outcomes of exposure to an infectious TB case: in the first instance, the infection may be cleared by sterilising immunity (Mave et al., 2019) as evidenced by immunological assays (Bifani et al., 2002); secondly, there can be progression to active TB that is clinically evident and thirdly, development of latent tuberculosis infection. TB is a chronic mycobacterial infection with a latency period following most initial infections. Studies have shown that 10% of LTB-infected individuals develop active TB, with the risk of progression to active TB significantly higher in individuals with a weakened immune system, for example, in HIV-coinfection (Tilahun et al., 2019).

People in close contact with TB patients are more likely to be infected with *M. tb.* A study in Ethiopia shows that individuals with a TB household member had an increased risk of TB by 3-fold (Shimeles et al., 2019). Since LTBI contributes significantly to the pool of active TB cases once reactivation occurs, its diagnosis and treatment in high-risk groups, including HHCs of PTB patients, is essential for reducing and ultimately eliminating TB (World Health Organization,

2018a). There is an increasing awareness of the problem LTBI poses to HHCs and, therefore, the need to sensitise and protect such at-risk groups.

In Kenya, however, data on LTBI is mainly anecdotal, which hinders its active management. To fill this gap, we established the prevalence of LTBI and its possible risk factors in a group of adult HHCs of PTB patients seeking health care services in a public hospital in the Kenyan capital, Nairobi. Overall, it was evident that LTBI is a common yet neglected problem for HHCs of PTB patients, with important factors that may contribute to the risk of developing LTBI such as their relationship to the index TB patient, the patient's HIV serostatus and the length of time spent cohabiting with or caring for TB patients leading to repeated and cumulative exposure to *Mycobacterium tuberculosis*.

We reported a high prevalence of LTBI among HHCs, with Interferon Gamma Release Assay results of over half of our participants indicating positivity for LTBI. For HHCs with complete data (174), the prevalence of LTBI was 55.7% (Odera et al., 2020). This finding was similar to a study in Western Kenya (Warria et al., 2020) which reported a TST positivity of 50.1% among household contacts of bacteriologically confirmed TB cases, in Uganda (Martinez et al., 2016), which reported LTBI prevalence of 65.7% using the TST test and Ethiopia, where the prevalence of LTBI was observed at 63.7% (Legesse et al., 2011). However, our finding was slightly higher than Warria et al. Mathematical modelling by Houben and Dodd) reported a global average of 23% (Houben & Dodd, 2016b), while studies in Georgia and Singapore reported 34% and 12.7%, respectively (Baliashvili et al., 2018; Yap et al., 2018). We used the IGRA test to screen for LTBI. Some individuals exposed to *M. tuberculosis* may not produce IFN-gamma as measured by the IGRA test, it is possible that the prevalence of LTBI in this cohort would be higher that the thesis found out.

The wide variations in LTBI prevalence statistics might be associated with the disproportional distribution of TB worldwide. Data from the WHO indicates that 2,480,000 TB cases are reported in Africa annually, with 25% dying because of TB or TB complications(World Health Organization, 2018c). The incidence in Africa is higher than in the Americas, the East Mediterranean, and Europe, where TB is estimated to infect 282,000, 771,000, and 273,000 people annually(TBFacts, 2022). The higher exposure to *M. tuberculosis* in Africa increases the risk of

infection and, therefore, the prevalence of LTBI. Variations in LTBI prevalence reports might also be associated with the methodology used for LTBI diagnosis. Some prevalence studies used the TST assay for LTBI diagnosis, a test whose accuracy measure can be confounded by Bacillus Calmette-Guérin (BCG) vaccination, non-tuberculous mycobacteria (NTM) infections (Sharma et al., 2017), and the HIV serostatus of the individual. Other studies that used the IGRA tests also used different generation kits. The most recent one (QuantiFERON Gold plus) has been further optimised with more tuberculosis-specific antigens that elicit CD4+ and CD8+ T cell responses. This offers the advantage of providing a more precise assessment of a patient's immune response to TB infection after infection with *M. tb*.

The IGRA test is both quantitative and qualitative. Our study population showed a significant difference in the median values of optical density IFN gamma measurements between the LTBI negative and LTBI positive groups (p-value <0.0001). From the comparative analysis of the Optical Densities values in the LTBI positive group and variables identified as potential risk factors for LTBI, we did not find any association with the amount of Interferon-gamma. The clinical utility of IGRA has been discussed, including the use of IGRA to distinguish latent and active TB and investigate its prognostic capability in patients undergoing treatment (Abubakar I et al, 2013). Further studies can evaluate more factors that could influence the amount of Interferon-gamma produced by an LTBI-positive individual. These may include the causative infecting pathogen strain, underlying illnesses, and medication history (Abubakar et al., 2013).

5.2 Risk factors for Latent Tuberculosis Infection in Household Contacts of TB patients

Several known risk factors for LTBI were investigated. They included gender, age group, relationship with the TB patient, duration of association with the patient, sharing a room with the patient, education level, use of cigarettes, alcohol use, and the HIV sero status of both the patient and the HHC. Our finding that 30-39-year-old HHCs had a higher prevalence of LTBI than the younger age group (18-29 years) was consistent with the results of studies in Mongolia and in India (Christopher et al., 2010; Ganmaa, Khudyakov, Buyanjargal, Jargalsaikhan, et al., 2019). In both studies, increasing age was associated with a high risk of LTBI/TB. A 2019 study on the age-specific prevalence of LTBI among HHCs of TB patients (Dolla et al., 2019) observed an increased prevalence of infection in older children. It proposed the need to expand TB preventive therapy to include all HHCs. A review article by Menzies et al. in 2007 (Menzies, Pai, et al., 2007) reported

a higher risk of LTBI among the elderly. According to Zhang *et al.*, older household members are more likely to spend a long time caring for TB patients than younger members of the household (Zhang et al., 2019). The finding was that marital relation to the PTB patient was a significant risk factor for LTBI. Our data showed that other (non-spousal) household members were 91% less likely to test positive for LTBI. Spouses are likely to have a higher cumulative exposure to *M. tuberculosis* through physical contact and social interactions, which predisposes them to a higher risk of infection. A study in Afghanistan (Qader GQ et al., 2021) reported a higher risk of LTBI among married participants than single participants. Similarly, a South African study (Ncayiyana JR et al., 2016) on LTBI in an informal urban settlement identified marriage as an essential risk factor for the development of LTBI.

The prevalence of LTBI was slightly higher among males at 58.8% than females at 54.5%. However, after our univariate analyses, gender was not identified as a risk factor for LTBI, as in a study of health care workers in Kigali, (Rutanga et al., 2015). In China, men were less susceptible to LTBI (Chen et al., 2015), while females had a significantly lower risk of LTBI in South Korea (Lee et al., 2014). The inconsistency in the occurrence of LTBI by gender might be due to behavioural factors or different gender roles in the communities but may also be due to sexual dimorphism. In this regard, women have been reported to exhibit a higher ability to recognise pathogens, recruit more innate immune cells and sustain more robust adaptive immune responses (Klein & Flanagan et al., 2016). A recently published study on gender variation in infectious diseases (Gay et al., 2021) indicated that several epidemiological studies and clinical observations have documented that women have a lower risk than men of developing infectious diseases. Although health behaviours and hormonal differences contribute to the observed gender differences, these studies have shown that sexual dimorphism affects how the immune system fights infection.

In Kenya, most families in urban centres live in rented single rooms or one-bedroom houses, where men and women share rooms (Mwangi, 1997). In such communal settings, Menzies *et al.* propose that the risk of transmission of infectious diseases such as TB might not differ by the occupation or gender of the HHCs (Menzies, Joshi, et al., 2007). Our study showed that room sharing at night with a TB patient was a common practice at 65.7%, with most studied HHCs (66.8%) cohabiting

with TB patients for at least four weeks. These point to increased exposure to the air-borne pathogen in the indoor environment of the home setting.

In our study population, after univariate analysis, level of education was not identified as a risk factor for LTBI. However, in a study conducted in Uganda, limited knowledge on TB among HHCs was recognised as one of the barriers to tuberculosis contact investigation and, therefore, its control (Ayakaka et al., 2017). (Gil et al., 2018) found that a gap in the knowledge about TB among HHCs increased the risk of disease transmission and reiterated the need for education campaigns at the community level to address misconceptions on causation and transmission. This would also reduce the stigma in the community against infected individuals. The WHO recommends routine contact investigation in TB high burden countries through counselling and educating HHCs, the primary caregivers for active TB patients at home (World Health Organization, 2018b).

The HIV serostatus of both the PTB patients and HHCs in our study population were identified as essential factors in the occurrence of LTBI and in diagnosing LTBI via the IGRA test, respectively. HIV infection increases the risk of progression from latency to clinically evident TB disease. However, it remains uncertain whether individuals who are coinfected with HIV and TB have a higher risk of transmitting the infection to their close contacts. Due to variation in reported results, a likely question in TB contact investigation studies is whether HIV seropositivity of an index TB patient would be associated with an increased or reduced risk of latent TB infection. In our study population, the index PTB patient serostatusatus was a significant risk factor ($p=0.002$) for the LTBI status of their HHCs. Our data showed that an HHC living with an HIV seropositive PTB patient was 59% less likely to test positive for LTBI. Our finding agrees with other studies in South Africa (P. MacPherson et al., 2020) and Italy (Carvalho et al., 2001). In both studies, household contacts of HIV-positive PTB patients had substantially lower odds of latent TB infection than household contacts of HIV-negative index cases. Reduced LTBI was also reported in household contacts of seropositive TB cases in Brazil and the Dominican Republic (Martinez et al., 2016). Data suggests that HIV co-infection may reduce the duration of symptomatic pulmonary disease with less cavitation and lower bacillary load; hence several HIV-positive patients present as smear-negative (Yates et al., 2016). This would make the HIV-positive TB patient less infectious than the HIV-negative TB patient. Other studies in Tanzania (Kifai & Bakari, 2009) and Zaire (Klausner

et al., 1993) did not find any significant difference between the HIV status of the index case and TB infection among their household contacts.

Many limitations may make it difficult to conclusively interpret the findings of our study and others mentioned above. The HHCs may have been exposed to other TB cases in the community, and we cannot directly infer direct transmission from the index patient. Similar to our study, other literature reports did not control strain variation in the infecting pathogen. Furthermore, we did not establish the level of immunosuppression by measuring the $CD4^+$ T cell count. Lower cell counts have been reported in patients with lower smear grades. A more robust study would be required to tease out the intricate relationship of immunopathogenesis in HIV/TB co-infection. In most studies evaluating risk factors for progression to TB, the outcome of exposure to the pathogen is a result of a complicated interaction of host and pathogen genetics (susceptibility and virulence), the infectiousness of the PTB index case and environmental factors (Martinez et al., 2016).

Our study also evaluated the serostatus of household contacts as a risk factor for LTBI. Immunosuppression has been reported to be an independent risk factor for LTBI (van Rie et al., 2013). People with HIV have a weakened immune system and are at high risk of infections such as TB. Individuals with no factors associated with immunosuppression can contain infection with *Mycobacterium tuberculosis* in a state of latency. In our study, 90% (159/175) of the HHCs recruited were seronegative. The odds of having a positive LTBI diagnosis using the QFT-GIT test was 2.26 (0.78 - 6.54) when HHCs had an HIV seronegative status, even though the relationship was not statistically significant ($p = 0.123$). This finding might be related to the low specificity of the QFT-GIT test in HIV seropositive patients. In the absence of a reference standard of screening for LTBI in an immunosuppressed individual, it is difficult to establish the actual performance characteristics of IGRA tests. In a study by Legesse et al., 2010 in Ethiopia, the sensitivity of the QFT-GIT test among HIV seropositive patients was 83.3%, while its specificity was lower at 50% (Legesse et al., 2011). The performance of QFT-GIT depends on the stimulation of CD4+ T-cells, which limits its performance in HIV seropositive individuals. These individuals have a reduced capacity for IFN-gamma secretion from CD4+ T-cells. As such, because the QFT-GIT in-tube kit would most likely perform better in people with a negative HIV serostatus, interpretation of IGRA results in the absence of serostatus data would be limited. A newer generation of the QFT assay - the QuantiFERRON^R-TB Gold Plus, has been developed to overcome such limitations. Apart from

antigens found in the QFT-GIT kit, the QFT-Gold plus has shorter peptides of the same antigens, which simulate CD8+ T-cells to improve its sensitivity in HIV seropositive patients. With this improvement on the IGRA test, 2018 WHO guidelines included a new recommendation on using either TST or IGRA to test for LTBI (World Health Organization, 2018b).

Medical management of an individual who tests positive using the IGRA test would involve evaluating epidemiologic and medical history and other clinical information. If the individual is at risk for progression to active TB and has signs and symptoms suggestive of active disease, an additional evaluation would be required. Treatment of selected persons with LTBI using preventive therapy aims at preventing active disease.

The 2018 WHO Guidelines included policy documents for programmatic management of LTBI in people living with HIV and household contacts of TB patients and other at-risk groups. As highlighted in these documents, the cascade of care for managing LTBI would include identifying at-risk populations, ruling out active TB disease, testing for LTBI, providing treatment, monitoring adverse events, and completing the treatment (World Health Organization, 2018b). One of the identified at-risk groups is household contacts of people with bacteriologically confirmed pulmonary TB. In this group, it is necessary to identify the intensity of exposure and the risk for the development of active TB and ascertain infection through testing for LTBI.

There is an increasing awareness of the problem that LTBI poses to HHCs and, therefore, the need to protect such at-risk groups. However, almost all of the HHCs in our study population did not have any knowledge of LTBI. There was much anxiety noticed among most of the HHCs in our study group when we relayed the positive LTBI test result from the Interferon Gamma Release Assay. The HHCs who tested negative wanted to know how often they needed to try for LTBI while living with the PTB patient. Coincidentally many were not aware of their HIV serostatus. At the time of collecting this data, the country's national policy on latent tuberculosis testing and treatment recommended follow-up of household contacts of TB patients who were adults diagnosed as HIV seropositive and children <5 years of age. These groups are at higher risk of developing full-blown tuberculosis disease on exposure due to poor immunity (Esmail et al., 2014). However, in a recent review of these guidelines, the recently launched Kenya Latent

Tuberculosis Infection policy of 2020 by the Ministry of Health enhanced the scope of testing and treatment of LTBI to include all household contacts of diagnosed PTB patients.

The 2020 Government of Kenya policy on testing and treating LTBI allows for the use of Tuberculin Skin Test (TST) and Interferon-Gamma Release Assays (IGRAs). The currently available treatment options recommended by WHO are 6-month isoniazid daily (6H) or 3-month rifapentine plus isoniazid weekly (3HP) or 3-month isoniazid plus rifampicin daily(3RH). The Kenya policy document stipulates that 3HP is the preferred treatment option for LTBI management in individuals above 15 years of age in Kenya, although it is contraindicated for People living with HIV on Nevirapine and PI-based ARVs; 3RH is the preferred treatment option for LTBI management in children below the age of 15 years although it is contraindicated for Children Living with HIV on PIs and Nevirapine based – ARVs and 6H is the preferred treatment option for anyone with a contraindication to the above two regimens (Ministry of Health, 2020b).

Our finding of a high LTBI prevalence in this population of HHCs of PTB patients should be an essential consideration in public health TB control programs in this setting. One of the critical pillars of TB prevention recommended in the WHO END TB strategy is the prevention of reactivation of TB through chemoprophylaxis. Screening individuals for LTBI can be best used to achieve this. In limited resource settings such as this, they are using a focused approach targeting the high-risk populations such as household contacts of TB patients. Essential factors to consider would be the HIV serostatus of the PTB patient and their HHC, the relationship between the patient and the HHC (spousal and other relationships), the duration of association and the number of rooms in the household. Although significant TB transmission might occur outside the home, robust preventive measures in TB-affected households can potentially reduce the burden of TB in the community. Such actions are direr in families in poor settings where infection control measures may be limited. The lack of awareness about latent tuberculosis infection, diagnosis and treatment among all the HHCs indicates an urgent need for increased sensitisation in the community through aggressive public health education and targeted screening.

5.3 Distribution of HLA class II (-DRB and -DQB) allele groups and association of their frequencies with the outcome of exposure to *Mycobacterium tuberculosis*

Several studies have been conducted in high-burden TB areas to identify determinant factors associated with TB in adult populations (Shimeles et al., 2019). They can be broadly categorised as environmental, socioeconomic, and host/pathogen genetics. Although most socioeconomic and ecological factors that contribute to the sustained high prevalence of TB are known, it is not clear to what extent host genetic differences account for the higher levels of infection observed in some countries (Ganmaa, Khudyakov, Buyanjargal, Jargalsaikhan, et al., 2019; Shimeles et al., 2019).

Infectious diseases exert significant selective genetic pressure, and the genes involved in the immune response are exquisitely diverse Fields (Burgner et al., 2006). This diversity explains, in part, why some people resist infection more successfully than others (Coscolla & Gagneux, 2014). The observations suggest a vital role for host genetic variability in the susceptibility to exogenous pathogens (Klebanov, 2018). The frequencies of some host factors most significantly alleles associated with susceptibility or resistance to TB may differ among populations according to ethnic or racial backgrounds. Global data shows that TB occurs at different rates among particular races, ethnicities, and families, indicating a genetic predisposition to TB susceptibility (Hayward et al., 2018b).

Studies have also shown, with a great degree of variation, that specific HLA class II alleles are protective against infection or rapid progression after infection. Several case-control, candidate-gene, family studies, and genome-wide association studies (GWAS) suggest the association of host genetic factors to TB susceptibility or resistance in various ethnic populations. They have reported genetic markers that can predict TB development in human leucocyte antigen (HLA) and non-HLA genes like killer immunoglobulin-like receptor (KIR), toll-like receptors (TLRs), cytokine/chemokines and their receptors, vitamin D receptor (VDR) among others (Harishankar et al., 2018).

The HLA class II alleles play an essential role in the early immune response to TB by presenting antigenic peptides to CD4+ T cells. HLA molecules are among the most polymorphic human gene products known. Polymorphisms affecting antigen processing and presentation, and hence the profile of cytokines secreted, can influence the efficiency of the immune response to infection and

can play a significant role in the host response (Chang et al., 2008). This diversity would also limit the global use of a vaccine candidate if the analysis were based on pathogen strains and allele frequencies of HLA molecules in only specific populations. The distribution and frequency of HLA alleles within a particular population would be valuable baseline data for vaccine design studies and formulation of population-specific intervention strategies, especially in countries with a high TB burden.

In this study, the frequencies of HLA class II alleles of the DRB- and DQB- loci were analysed in PTB patients. They compared these frequencies with those of their adult household contacts stratified as LTBI positive and LTBI negative using the Interferon Gamma Assay (IGRA) test. We hypothesised that specific HLA class II alleles might be associated with protection against infection, or upon infection, with failure to progress to active TB.

Analysis of the HLA-DRB locus revealed -DRB1*1 (34.0%) and -DRB3*2 (34.0%) alleles were the highest in the population, followed by -DRB3*1 (31.9%) and -DRB1*4 (19.1%) while the least frequent alleles were -DRB1*2, DRB1*10 and DRB1*16 (all occurring at 2.1%). At the -DQB locus, -DQB1*3, -DQB1*4 and -DQB1*6 were the most frequent, being identified among 30.6% of the study population, while -DQB1*1and -DQB1*2 were the least frequent (both occurring at 5.6%). Comparatively, a 2014 study on the diversity of HLA class I and II alleles in an East African population on the shores of Lake Victoria also identified HLA-DRB3*2 and HLA-DQB1*3 among the most common alleles in the -DRB and -DQB loci (Peterson et al., 2014). Similar to our study, only one HLA-DRB4 allele (-DRB4*1) was identified in both study populations. However, observed differences in the frequency of other alleles in the two studies could be due to variations in the ethnicity of people from different geographical locations. Our study was conducted in an urban setting with a wide variation of the ethnic background of participants. The population in the study by Peterson et al. did not have a similar variation in ethnic background. Furthermore, the 2014 study had a gender bias by recruiting only female participants.

A comparison of the frequency distribution of the allele groups across gender revealed an even distribution of HLA-DQB alleles. Analysis of the distribution of the HLA-DRB allele groups showed a higher frequency in males than females except for the HLA-DRB1*9 allele group, which was significantly higher among females after controlling for the HIV serostatus. Few studies have suggested that gender substantially influences the distribution of HLA-DR and -DQ alleles Field

(Khodaeian et al., 2015). More research on our findings should be explored in a more extensive study. As reported by Klein et al. (Klein & Flanagan, 2016), where the female gender might have a higher ability to sustain more robust adaptive immune responses, likely, a gender variation in the frequency of alleles involved in the presentation of antigenic peptides to immune cells would impact the efficacy of the immune response mounted against an infection.

5.4 Association of the frequencies of HLA class II (-DRB and -DQB) allele groups and outcome of exposure to *Mycobacterium tuberculosis*

Comparative analyses were done, and frequencies and odds ratios were interpreted as the measure of association between HLA allele frequency and susceptibility to tuberculosis infection and progression to active TB. This study did not find significant differences in the frequencies of HLA-DQB alleles in the PTB patients, latent tuberculosis infected, and exposed uninfected (Zhou et al., 2016). HHCs. Therefore, none of the identified HLA- DQB alleles was associated with this population's susceptibility to infection or progression to PTB. This finding was in agreement with the study of Duarte et al. in 2011, who studied a Portuguese population and found no association between the phenotypic distributions of HLA-DQB in the healthy exposed group (healthy exposed positive and healthy exposed negative) and patients who had active TB (178). Other studies by Cao et al. in the USA and Lombard *et al*. in South Africa also found no association, complementing our findings (Cao et al., 2001; Lombard et al., 2006). However, a study in Uganda found a negative correlation between TB susceptibility and the -DQB1*3 allele, which contradicted our findings (Wamala et al., 2016). Studies in India (Selvaraj et al., 1998), Thailand (Vejbaesya et al., 2002), and Iran (Amirzargar et al., 2004) reported a positive correlation between TB susceptibility and expression of DQB alleles. The divergent associations might be due to differences in sample size, study designs, and the genetic heterogeneity of the *Mycobacterium tuberculosis* complex, which were not controlled during analyses. We also had a small sample size that might have underestimated or overestimated the link between the HLA-DQB1 alleles and TB susceptibility or resistance in this population.

The HLA-DRB3*1 allele group occurred at a statistically significant higher frequency (p=0.049) among latent TB-negative HHCs (46.2%) compared to PTB patients (11.8%). The negative association with susceptibility suggests that this allele group is likely to be protective against infection and progression to active TB. Our findings contradicted a 2019 study in Mali, West

Africa, which reported that the HLA-DRB3*01:01 allele, among others, had a significant association with *M. tuberculosis* infection compared to healthy controls (Kone et al., 2019). Unlike our study, which did not control for the strains in the *M. tuberculosis* complex, the Mali study analysed whether the association of HLA with TB susceptibility was strain dependent with epidemiological evidence that *M. africanuum* was more common in West Africa compared to other regions in the continent. Furthermore, the variation in HLA-DRB allele distribution across populations in Africa's Western and Eastern areas could have contributed to the different findings. Because of the paucity of published literature on the association of the HLA-DRB3*1 allele group with TB disease, we could not make further comparisons with our results. Notably, the -DRB3*1 allele group was one of the most common HLA-DRB alleles occurring in 31.9% of our study population. Pathogens often adapt to the most frequent HLA alleles. In contrast, rarer ones have a selective advantage because of host-pathogen interactions (Spinola, 2016). This could explain why the most frequent alleles in the study population were not linked with TB susceptibility /progression.

Furthermore, the HLA-DRB5*2 allele occurred at a statistically significant (p=0.013) higher frequency among PTB patients compared to HHCs. Although its frequency was low in our study population (8.5%), the higher occurrence in PTB patients (23.5%) compared to HHCs (0.0%) hints at a positive association with TB progression and susceptibility to infection. Our finding was in agreement with a 2017 study of susceptibility loci associated with tuberculosis in Han Chinese, which reported that candidate genes that were significantly associated with TB included, among others, genes in the HLA-DRB5 (Qi et al., 2017). The HLA-DRB5*2 allele may have the “rare allele advantage” in this population, which argues that pathogens adapt to HLA alleles that are more prevalent in the people; thus, such alleles would not have a significant role to play in the immune-pathogenicity of those pathogens (Martin & Carrington, 2013).

All the HHCs were HIV negative, while 41.2% of the PTB patients were HIV positive. HIV infection is a known factor that increases the risk of progression to active TB. Therefore, during statistical analysis of the distribution of the HLA alleles, the HIV serostatus of the study participants was controlled for. After controlling for HIV status in our study population, the HLA-DRB1*14 allele was significantly higher (p=0.040) among the PTB patients compared to HHCs, suggesting its potential contribution to the development of active TB. This finding was in

agreement with a study by Duarte et al. in 2011 in a Portuguese population where the frequency of the HLA-DRB1*14 allele was significantly higher in TB patients compared to healthy exposed controls (Duarte et al., 2011). Similar reports from India (Sharma et al., 2017) and Russia (Malkova et al., 2020) also suggested that this could be a susceptibility allele for evolution from infection to active TB. The role of the HLA-DRB1*14 allele in TB immunopathogenesis is also highlighted in a study on serology-based antibodies for TB diagnosis, where researchers documented a significant increase in the frequency of HLA-DRB1*14 among subjects with high antibody response levels compared to those with low levels (Zhou et al., 2016).

Our findings show that the HLA-DRB5*2 and HLA-DRB1*14 alleles were more prevalent in study participants with active TB in our study population. The HLA-DRB5*2 was positively associated with active TB diagnosed individuals before controlling for HIV serostatus. At the same time, the HLA-DRB1*14 allele was a significant factor in the development of active TB after controlling for HIV serostatus. The chances of an individual with recently acquired TB infection progressing to active disease are higher with HIV co-infection, with many studies postulating the potential role that HLA polymorphisms play in the differential development of TB in HIV-infected individuals (Mohammed et al., 2014; Saikia et al., 2015; Shankarkumar et al., 2009). Furthermore, a study in China highlighted the important critical the HLA-DRB1*14 plays in accelerated disease progression in HIV infection (Xue et al., 2018). Closely related to this, our study findings suggest that in our study population, the HLA-DRB1*14 allele plays a synergistic role in disease progression in an individual exposed to both HIV and *M. tuberculosis*.

5.5 Challenges of Household contacts of PTB patients

Tuberculosis (TB) continues to be a global concern because of its high infectivity, mortality, and cost of therapy. In the study population majority of HHCs indicated their patients had easy access to a TB diagnostic facility, counselling sessions for the patient upon diagnosis, and availability of prescribed medication. Diagnosed patients received nursing care and supervised treatment (directly observed treatment support) whenever they attended the outpatient clinic.

Many HHCs doubled up as providers of home-based care for the patient. As has been documented in other studies, depending on the severity of the illness, PTB patient relies a lot on other household members for support in the physical and psychological care (Jeon et al., 2018). In addition, the

patients' families are confronted with extra social and clinical burdens associated with TB disease (Cui et al., 2019b). Informal discussions with the HHCs revealed many challenges they faced living with and caring for PTB patients. These challenges were grouped under four themes: psychosocial, caregiver burnout, hygiene and waste management.

Some HHCs had psychosocial challenges dealing with personal fears of caring for a patient infected with a disease considered in their immediate community as lethal, and decreased social interaction with extended family members or neighbours due to stigma led to increased feelings of isolation and resentment toward both the patient and their HHCs. A study on the effects of stigma in Indonesia found that stigma affects both the TB patient as an individual and also the family and caregivers, a situation described as courtesy stigma (Cui et al., 2019b). A study on TB stigma in a pastoralist population in Kenya (Mbuthia et al., 2020) reported that the association between TB and HIV infection/AIDs increases TB stigma. They also said the female members of the household faced a disproportionately higher level of stigma due to the gender inequalities that exist. The male patients did not experience much stigma at the family level because they still benefited from the caring nature of female household members. On the other hand, females would face stigma from within the family and their immediate community.

The stigma encountered began after diagnosis and sometimes continued even after treatment had been completed. These negative attitudes affected the mental well-being of the HHCs and negatively influenced the patients' self-esteem. Counselling should be offered to the HHCs of PTB patients on managing the challenges encountered in their role as caregivers. Household contacts should be sensitised on available resources for support and how to monitor and document the patient's progress and adherence to medication at home. Since the household members may be aware of the contagious nature of TB disease, they need to be enlightened on latent infection, its diagnosis and available treatment. Furthermore, health care professionals should initiate all-inclusive intervention strategies to reduce stigma in the community for PTB patients and family caregivers.

Many HHCs complained of physical and mental exhaustion that led to caregiver burnout. Relatively weak patients relied on other household members to meet their physical needs. As highlighted in Mbuthia's study (Mbuthia et al., 2020), female household members would continue

being the primary caregivers even when in a state of sickness. This was quite difficult for families with young children. Mental exhaustion arose from anxiety about the patient's progress, the likelihood of getting infected, the need to constantly encourage a PTB patient battling low self-esteem constantly, and the economic impact of TB disease. Most participants did not have private medical health insurance covers and relied on daily wages as casual labourers. Therefore, they were concerned about dwindling resources due to diminished family income arising from job losses in cases of a sick spouse. In a study exploring the experiences of family caregivers of TB patients, caring for them at home necessitated disruptions in their routine or expenditure and increased stress (Fana & Sotana, 2021).

Due to poor housing infrastructure, inadequate water and absent waste disposal systems, many HHCs were concerned about poor hygiene practices within their households. For immunosuppressed individuals, the risk of acquiring an infection is proportional to the dose of tubercle in the air, emphasising the need for robust indoor air quality monitoring (Nardell, 2015).

Community health workers should visit the households and offer advice on clinical waste management in the home environment. The household members would also need training on improving air quality by reducing all air pollution, for example, using clean energy sources for cooking fuel and ensuring adequate ventilation day and night. The government provides TB treatment medication free of charge. The TB programs should go a step further in boosting preventive intervention measures by delivering gloves, masks and disinfectants for infection control to the home caregivers. Many studies have shown a strong association between poverty and TB disease, with the most vulnerable groups and developing countries having higher incidences of TB. Consequently, alleviating poverty within individual households would reduce the risk of tuberculosis transmission and its progression from infection to disease (Wingfield et al., 2018).

5.6 The proposed tool: PTB patient Household Contact Data card

Household contact tracing of index PTB cases has been advocated as a critical component of PTB prevention strategies for many years. Household contacts of PTB patients have a higher cumulative exposure to *M. tuberculosis* through close physical contact and social interactions. The prolonged exposure predisposes them to a higher risk of infection, morbidity, and mortality. The WHO

recommends routine contact tracing and investigation in PTB high burden countries through counselling and education of HHCs, who are the primary caregivers for active PTB patients at home (Wingfield et al., 2018). However, implementing these recommendations in low-resource settings is poor; hence screening of HHCs has remained sub-optimal.

Kenya is recognised as one of the high-burden TB countries. Recent efforts by the World Health Organization to reduce and possibly eliminate TB emphasise the importance of treating the pool of individuals with latent TB infection (LTBI) and putting more effort into researching more efficacious population-specific vaccines. As outlined in WHO guidelines, contact investigation has not yet been fully implemented by the National Tuberculosis program in Kenya. Prior Before the 2020 review of the national guidelines, the program recommended Isoniazid Preventive Therapy administration to household contacts of PTB patients who are either less than five years old or are HIV-infected adults (Burmen, 2019). The current national guidelines reviewed in 2020 recommended that standardised contact investigation include active screening, evaluation and chemoprophylaxis of HHCs of PTB patients, including HIV-negative adults. Household contact tracing and appropriate action would reduce the pool of individuals likely to progress to active TB after reactivation. A systematic investigation would also boost intensive case findings of previously undetected active TB cases.

With this background, we designed a prototype data card- the PTB HHC data card 2022 (**Appendix 1**) that can be piloted for adaptation in different level health facilities involved in TB diagnosis and management. The objective of designing this data card was to have a tool that can be used with ease in a programme that comprehensively and systematically identifies, screens, initiates preventive therapy and documents all findings in follow-up visits of the household contacts of the TB patients. The card would be a valuable tool for health care workers to screen HHCs of PTB patients and make the necessary follow-up based on clinical symptoms, adherence to preventive treatment and adverse drug reactions.

One significant advantage of this card is that it is also available in a digital format which can be easily populated. The digital design also eases follow-up when contacts have travelled or moved from their primary residence. Such movement would not interfere with the course of treatment or

follow-up because the form would be readily available with authorised access to the health worker at a TB management facility.

The card has been designed considering the Government of Kenya 2020 policy document that offers guidelines on testing and treating LTBI. A study in Chennai District, India (Velayutham et al., 2020) noted the usefulness of employing a contact card and register to screen household contacts of PTB patients.

PTB patients on home-based treatment should identify a lead household member who will be the link between the patient, the health care workers, and other household members. This individual should be within reach by phone or other digital channels and will provide needed data for the PTB HHC Data card to be filled by assigned health care workers upon diagnosis of the patient and subsequent follow-ups. Self-evidently, aggressive community sensitisation on TB contact tracing and available latent TB diagnostic and treatment resources will contribute to a successful roll-out of programmes on managing high-risk groups. It is envisaged that the PTB-HHC data card can be piloted, optimised and presented as a policy brief document towards eradicating latent TB infection among household contacts of PTB patients.

Study Limitations

Our study design was a cross-sectional study with a convenience sampling approach. A longitudinal cohort study would have generated more data with follow-up yielding the prognostic value of IGRA in identifying the risk of progression to active TB. Recruitment of HHCs at home would have also yielded a more representative sample of the study population by reducing bias.

The study did not explore the different infecting *Mycobacterium tuberculosis* strains, which could have been associated with the HLA alleles conferring susceptibility to and protection against infection and disease. Future studies should expand their scope to include elements of pathogen genetics and the possible interplay between the bacilli and the host. Research on the genetic theory of infectious disease is quite expensive and requires robust funding for elaborate work in a large sample size. Our study had a limited budget. Hence both IGRA analysis and SSP PCR typing of the HLA alleles were also limited by the number of kits available. Comparative analyses of HLA class II allele distribution between LTBI positive and negative HHCs and PTB patients could have been underpowered because of the small sample size. The HLA typing kit used for the SSP-PCR

procedure was a low-resolution typing that only allowed for identifying the HLA allele group. A higher resolution typing kit would provide more detailed data on the specific alleles in each group. Available funds limited the type of kit used.

CHAPTER SIX

6 CONCLUSION AND RECOMMENDATIONS

6.1 CONCLUSION

The prevalence of LTBI at 55.7% among HHCs of PTB patients in this population was high. Essential factors to consider when designing screening programs would be the relationship (spousal or other) between the HHC and the index TB patient and the HIV serostatus of both the TB patient and their HHCs.

- Spouses have a higher risk of infection because of closer social and physical interaction with the PTB patient and, therefore, a relatively higher cumulative exposure to the air-borne transmitted bacilli. This increases their risk of infection.
- The HIV sero- status of the index PTB patient may influence their level of infectiousness. In our study, contacts of HIV seronegative patients were more likely to be LTBI positive. The presence and severity of immunosuppression in an HIV/PTB co-infected individual should be determined as a potential influence on transmissibility and, therefore, the risk of infection to close contacts. A possible confounder would be the variation in virulence of the infecting *Mycobacterium tuberculosis* strains.
- The HIV serostatus of the HHCs is a critical factor when screening for LTBI using the IGRA assay. Immunosuppressed individuals cannot mount a robust immune response to test antigens. Therefore, the performance of older-generation IGRA tests may not be optimal in immunosuppressed individuals.

The HLA-DRB5*2 and HLA-DRB1*14 allele groups were more prevalent in active PTB study participants. Although the varied contribution of each allele to the risk of infection and progression to active TB before and after controlling for HIV is open to further research, both alleles would make natural candidates for further research on genetic susceptibility to TB in this population.

The study also highlights the potential protective role of the HLA-DRB3*1 allele group against *Mycobacterium tuberculosis* infection in this population. It was more prevalent in the exposed uninfected (LTBI negative) study participants. This protective association can be explored further in the development of population specific vaccines.

6.2 RECOMMENDATIONS

- LATENT TUBERCULOSIS

1. The high prevalence of LTBI in adult household contacts of PTB patients emphasises the need for TB programs to invest more in targeted screening and treatment of household contacts in high transmission settings. In resource-limited settings, programs should target LTBI screening of spouses of PTB patients.
2. The appropriate IGRA test to screen for LTBI in a BCG vaccinated population should be prioritised by policymakers. They should also consider essential characteristics such as the HIV sero status of the individual. Prospects of using IGRA tests for LTBI diagnosis in High Burden Countries should focus on simplification of the tests to minimise necessary laboratory infrastructure and overall cost.
3. There was a significant interaction of terms where a non-spousal relationship seemed to modify the effect of an HHC who was seropositive. We hypothesise that this interaction in the non-spousal group may be due to host genetic factors in contacts with similar genetic ancestry to the PTB patient. Similar observations in infectious diseases have been explored in Familial Aggregation studies. We recommend similar studies focusing on this specific category to investigate the further effect of household contact HIV status as a potential risk factor for LTBI in both spouses and non-spouse groups.
4. We recommend a more elaborate cohort study of PTB patients and their HHCs. This would generate more knowledge of the clinical utility of IGRA tests and potential risk factors, including co-morbidities like Diabetes mellitus. The prognostic use of IGRA measurements to assess the risk of progression to active TB can be evaluated.
5. Pathogen strain variation and host factors can influence the outcome of exposure to the pathogen. We recommend further similar studies focusing on the interplay of circulating strains and genetics and immune response variations in the host.
6. TB prevention efforts should focus on sensitising household contacts, and other high-risk groups, on the importance and availability of LTBI diagnosis and preventive treatment to avoid reactivation. We recommend more aggressive public health education towards this.

- HLA IN TB IMMUNOPATHOGENENSIS

1. We recommend further studies using higher-resolution typing kits on a more extensive study population to investigate the roles of specific alleles in the HLA-DRB5*2, HLA-DRB3*1 and HLA-DRB1*14 allele groups in TB immunopathogenesis in this population.
2. Further experimental studies to explore (HLA) population-specific TB vaccine candidates.

- CHALLENGES OF HHCS OF PTB PATIENTS:

1. Psychological care through individual and group counselling should be offered to the HHCs of the PTB patients.
2. Training of household contacts of PTB patients on home-based care should focus on improving indoor air quality, infection control measures and handling clinical waste.

- THE PTB-HHC data card

Involve stakeholders (National TB Program, MoH, IT, Communication and Legal experts) to optimize and pilot the proposed card digital/hard copy formats.

We recommend piloting the proposed PTB HHC Data Card in the digital and hard copy format in healthcare facilities handling TB patients. Findings will identify gaps and inform the effectiveness of the PTB HHC Data Card as a follow-up tool of contacts PTB patients at the household level.

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APPENDICES

APPENDIX 1: PULMONARY TUBERCULOSIS HOUSEHOUSEHOLDACT CARD

LINK TO ONLINE FORM: https://forms.gle/yrWjofVZzX44QFVE8

NAME OF FACILITY	**COUNTY:** **SUBCOUNTY:**

PTB PATIENT HOUSEHOLD CONTACT CARD

INDEX TB PATIENT DETAILS

Name of Patient	TB Serial No	Age	Gender	Drug Resistance TB YES NO	Treatment Start Date	No. of Household contacts

HOUSEHOLD CONTACT- BASELINE SCREENING

Date of Screening:	CONTACT SCREENING NUMBER:		
Name of Contact			
Age	Gender	Weight	Height
Telephone Number			
Current TB treatment	YES	NO	
Past TB treatment	YES COMPLETED	NO	
Diabetes	YES DURATION	NO	
Known HIV serostatus	YES	NO	
Tested for Hepatitis	YES	NO	
Organ transplant history	YES	NO	
On Dialysis	YES	NO	
Known Neuropathies	YES	NO	
On immunosuppressants	YES	NO	
Smoking	YES STOPPED	NO	
Alcohol use	YES	NO	
Currently Pregnant	YES	NO N/A	

LATENT TB WORK-UP

Presumptive TB Clinical symptoms	Cough	Night sweats	Fever	Weight loss
Positive clinical symptoms	Screen for TB and other diseases and treat			
No clinical symptoms: Screen for LTBI	TST/ IGRA		Positive	Negative
Positive LTBI Screening test: Perform Chest radiography	Chest Radiography Done YES NO		Normal Screen for HIV	Abnormal Screen for TB

		Counsel on LTBI treatment	
Select LTBI treatment regimen	Daily, Isoniazid alone for 6-9 months		
	Daily, Rifampicin alone for 3-4 months		
	Daily, Isoniazid plus Rifampicin, for 3-4 months		
	Weekly, Rifapentine plus Isoniazid for 3 months		

FOLLOW-UP SCHEDULE (AS PER LTBI TREATMENT REGIMEN PICKED AT 3,6,9,12,24,36 MONTHS)

CONTACT SCREENING NUMBER:		
MONTH 3	Follow up screening Due date:	Contact Screened YES NO
	Reason if 'NO'	
	Follow up screening Done DATE:	
	Adverse reactions to treatment: Anorexia, Vomiting, Abdominal discomfort, Dark coloured urine, Jaundice, Persistent fatigue/ weakness	YES NO
	Presumptive TB Clinical symptoms If positive screen for TB	YES NO
	Signature of Medical Officer and Date:	
MONTH 6	Follow up screening Due date:	Contact Screened YES NO
	Reason if 'NO'	
	Follow up screening Done DATE:	
	Adverse reactions to treatment: Anorexia, Vomiting, Abdominal discomfort, Dark coloured urine, Jaundice, Persistent fatigue/ weakness	YES NO
	Presumptive TB Clinical symptoms If positive screen for TB	YES NO
	Repeat LTBI Testing TST/IGRA Positive Negative Positive TST/IGRA test- request chest radiography to rule out TB and other diseases	
	Signature of Medical Officer and Date:	
MONTH 9	Follow up screening Due date:	Contact Screened YES NO
	Reason if 'NO'	
	Follow up screening Done DATE:	
	Adverse reactions to treatment: Anorexia, Vomiting, Abdominal discomfort, Dark coloured urine, Jaundice, Persistent fatigue/ weakness	YES NO
	Presumptive TB Clinical symptoms If positive screen for TB	YES NO
	Signature of Medical Officer and Date:	

MONTH 12	Follow up screening Due date:	Contact Screened YES NO
	Reason if 'NO'	
	Follow up screening Done DATE:	
	Adverse reactions to treatment: Anorexia, Vomiting, Abdominal discomfort, Dark coloured urine, Jaundice, Persistent fatigue/ weakness	YES NO
	Presumptive TB Clinical symptoms If positive screen for TB	YES NO
	Repeat LTBI Testing TST/IGRA Positive Negative Positive TST/IGRA test- request chest radiography to rule out TB and other diseases	
	Signature of Medical Officer and Date:	
MONTH 24	Follow up screening Due date:	Contact Screened YES NO
	Reason if 'NO'	
	Follow up screening Done DATE:	
	Adverse reactions to treatment: Anorexia, Vomiting, Abdominal discomfort, Dark coloured urine, Jaundice, Persistent fatigue/ weakness	YES NO
	Presumptive TB Clinical symptoms If positive screen for TB	YES NO
	Signature of Medical Officer and Date:	
MONTH 36	Follow up screening Due date:	Contact Screened YES NO
	Reason if 'NO'	
	Follow up screening Done DATE:	
	Adverse reactions to treatment: Anorexia, Vomiting, Abdominal discomfort, Dark coloured urine, Jaundice, Persistent fatigue/ weakness	YES NO
	Presumptive TB Clinical symptoms If positive screen for TB	YES NO
	Repeat LTBI Testing TST/IGRA Positive Negative Positive TST/IGRA test- request chest radiography to rule out TB and other diseases	
	Signature of Medical Officer and Date:	

FINAL OUTOCME AT END OF TREATMENT REGIMEN		
No TB	TB	LOST
Reasons for 'Lost'		
Remarks		
Signature of Medical Officer and Date		

APPENDIX 2(a): CONSENT FORM FOR PARTICIPANTS

Title of Study: The role of Human Leukocyte Antigens and *M. tuberculosis* strain variation in susceptibility to infection among TB/HIV co-infected subjects in Kenya.

Investigator: Ms Susan Odera, BSc., MSc. Medical Microbiology
PhD student, Department of Medical Microbiology, University of Nairobi

Explanation of Procedures:
You are being asked to participate in a study designed to determine if an individual likely to be resistant to pulmonary TB because of their genetic make-up, and which strains of the causative agent of tuberculosis are associated with varied disease outcomes. Pulmonary tuberculosis is a disease caused by a bacterium, *M. tuberculosis*. Some individuals infected with the bacilli go on to develop active disease, while others have a clinically latent infection: are infected but do not present with clinical symptoms and are not contagious to others. Different strains of this bacterium are also thought to be responsible for recurrence of disease and resistance to drugs.
If you decide to participate, we will ask you to complete a questionnaire. You will be asked questions about your sex, age, ethnic background, education, income, marital status, duration of illness, compliance with treatment, and challenges you have faced either as a TB patient or while caring for a TB patient. After completing the survey, the TB patients will receive routine treatment given to patients with the disease.
The patients will be required to collect one sputum sample of approximately 2ml in the provided specimen container for identification of the pathogen and drug resistance analysis. Blood specimen of 10ml will also be obtained for analysis of the genetic make-up of the Human Leukocyte Antigens.
The close contacts will be required to give one blood specimen of 10ml for the three assays: HIV sero-status, to determine previous exposure to tuberculosis pathogen and for analysis of the genetic make-up of the Human Leukocyte Antigens.

Risks and Discomforts:
You may experience discomfort from answering questions of a sensitive nature about your health and treatment but you are at liberty to refuse to answer any questions that you do not wish to answer. The research team includes a counselor who will be available to offer advice on any matter that may concern you. You are encouraged to seek clarification from the research team on any issue that is not clear to you. The members of the research team will readily clarify any queries you may have and will give you useful references for further help if required. You may experience normal minor discomfort from the needle prick as a blood specimen is being collected. However, this will not be any different from a routine veni-puncture for blood specimen and will be performed by experienced trained personnel following the recommended WHO guidelines on blood specimen collection.

Benefits and Compensation:
All specimen obtained from patients with PTB will undergo comprehensive tests to confirm presence of the pathogen and susceptibility pattern to antimicrobial agents. In the event that a drug resistant isolate is identified, your primary care provider will be notified for better management of your condition. The study will also document challenges that you as PTB patients and close

contacts face as patients or primary care givers. The results of this study will benefit the policy makers in their approach to prevention, diagnosis and control of tuberculosis, distribution of resources to infected and affected individuals and improve guidelines on diagnosis and management of multi-drug resistant tuberculosis.

Confidentiality:
Although this study involves investigation of genetic aspects, the findings will not be directly linked to you because all samples and questionnaires will be assigned specific study numbers. All information that we collect from your responses to the questionnaire used in this study will be kept private, under lock and key and access will be limited to the principal researcher and supervisors. Officials from KNH/UoN Research and Ethics Committee will be able to inspect the records and progress of the study at any time. The clinical and laboratory results of this study will be shared with health officials from Mbagathi District Hospital and compiled analyses may be published for scientific purposes. However, your identity will not be revealed at any stage of the study and any finding will not be linked directly to you in any way.

Refusal, Withdrawal and Cost of Participation:
Participation in this study is completely voluntary. Please be assured that should you refuse to participate, your care at the hospital will not be affected in any way. Refusal to participate will have no effect on the service that you will receive from Mbagathi District Hospital. You are also free to withdraw your consent and to end your participation in this study at any time. There will be no extra cost incurred by you from participation in this research.

Questions and Contact:
If you have concerns now or in the future regarding your rights in this study or research related injury, you may contact me, Susan Odera or my supervisor through this number: 0723470211, or Prof. Guantai, the Chairperson of Kenyatta National Hospital/University of Nairobi Research and Ethics committee, P.O Box 20273, Nairobi.TEL. NO 020726300 ext. 9.

SIGNATURE:
The above details about the study and the basis of participation have been explained to me in English/ Kiswahili and I agree to take part in the study. I understand that I am free to choose to be part of the study or not.
Patient signature/ Thumb mark: …….
Witness's signature……..
Research assistant's signature…….
Date: ……..

APPENDIX 2(b): CONSENT FORM FOR PARTICIPANTS (SWAHILI)
FOMU YA KUKUBALI KWA WASHIRIKI

Kichwa cha Utafiti: Wajibu wa Antijeni za Binadamu za Leukocyte na aina ya bakteria ya *M. tuberculosis* katika kumfanya mtu kuathirika kwa urahisi na ambukizo miongoni mwa wagonjwa wa TB ya mapafu na watu wanaoshirikiana nao, nchini Kenya.

Mtafiti: Ms Susan Odera, BSc., MSc. Medical Microbiology
Mwanafunzi wa PhD (Medical Microbiology), Cho kikuu cha Nairobi

Maelezo ya Utaratibu Utakaotumiwa:
Unaombwa kushiriki katika utafiti unaokusudia kutambua iwapo mtu anaweza kuwa na ukinzani kwa TB ya mapafu kwa sababu ya hali yake ya jenetiki, na aina za dalili zinazosababisha TB zinahusishwa na matokeo mbalimbali ya ugonjwa huo. TB ya mapafu ni ugonjwa unaosababishwa na bakteria inayoitwa *M. tuberculosis*. Wagonjwa wengine wanaoambukizwa na backteria hii hupata ugonjwa unaoonekana wazi, ilhali wengine hupata ambukizo lililojificha: huwa wameambukizwa lakini hawaonyeshi dalili zilizo wazi na hawawezi kuwaambukiza watu wengine. Aina mbalimbali za bakteria hii hudhaniwa kusababisha kutokea tena kwa magonjwa na kuwa na ukinzani kwa dawa.
Ukiamua kushiriki katika utafiti huu, tutakuomba ujaze fomu ya udadisi. Utaulizwa maswali kuhusu jinsia yako, umri, kabila, elimu, mapato, hali ya ndoa, muda wa ugonjwa, kufuata matibabu, na changamoto ulizopata kama mgonjwa wa TB au ulipokuwa ukimtunza mgonjwa wa TB. Baada ya kumaliza uchunguzi huu, wagonjwa wa TB watapokea matibabu ya kawaida yanayotolewa kwa wagonjwa walio na ugonjwa huu. Watu wote watakaochaguliwa kushiriki katika utafiti huu watafanyiwa eksirei ya kifua, na matokeo yoyote yasiyo ya kawaida yakionekana, watatumwa kwa tabibu ili uchunguzi zaidi uweze kufanywa.
Wagonjwa watahitajika kuleta sampuli moja ya kohozi kiasi cha ml 2 katika chombo cha sampuli watakachopewa ili kuweza kutambua pathojeni na kufanya utafiti wa ukinzani kwa dawa. Sampuli ya damu kiasi cha ml 10 itachukuliwa pia ili kufanya utafiti wa hali ya jenetiki ya Antijeni za Binadamu za Leukocyte.
Watu wanaoshirikiana na mgonjwawatazungumza na daktari kabla na baada ua uchunguzi na pia kuhusu jambo lolote kwenye uchunguzi hii. Watafanyiwa uchunguzi wa eksirei na watahitajika kutoa sampuli moja ya damu kiasi cha ml 10 ili kubainisha iwapo kuna virusi vya HIV, kama waliweza kupatwa na pathojeni za TB hapo awali na kufanya utafiti wa hali ya jenetiki ya Antijeni za Binadamu za Leukocyte.
Miezi 6 itakayofuata baada ya sampuli kuchukuliwa, kutakuwa na ufuatiliaji wa washiriki wa utafiti huu. Wakati wa maamkuzi hayo, data inayohusu hali ya kimatibabu ya wagonjwa itarekodiwa pamoja na ile ya watu wanaoshirikiana na mgonjwa huyo, eksirei ya pili ya kifua pamoja na sampuli ya damu kiasi cha ml 4 itachukuliwa ili kurudia uchanganuzi wa IGRA unaotumiwa kutambua maambukizi ya pathojeni ya TB.

Hatari na Usumbufu:
Huenda ukawa na usumbufu katika kujibu maswali ya siri kuhusu afya yako na matibabu lakini una uhuru wa kukataa kujibu maswali usiyotaka kujibu. Kikundi cha utafiti pia iko na mshauri atakayekupa ushauri kwa swala lolote. Unahimizwa kupata maelezo zaidi kutoka kwa kikundi cha utafiti kuhusu suala lolote usilolielewa. Washirika wa kikundi cha utafiti watakuwa tayari kujibu

swali lolote utakalouliza na watakupa marejeleo muhimu yatakayokusaidia zaidi iwapo utayahitaji. Unaweza kuhisi uchungu kidogo kutokana na kudungwa sindano wakati wa kutolewa sampuli ya damu. Kwa hali yoyote, hii haitakuwa tofauti na udungaji sindano wa kawaida katika mishipa ya damu ili kutoa sampuli ya damu. Udungaji huo utafanywa na watu walio na ujuzi na waliopewa mafunzo kwa kufuata mwongozo wa Shirika la Afya Duniani unaohusu utoaji wa sampuli ya damu.

Faida na Fidia:
Sampuli zote zitakazochukuliwa kutoka kwa wagonjwa wa TB ya mapafu zitafanyiwa uchunguzi mpana ili kuthibitisha kuwepo kwa pathojeni pamoja na mwelekeo wa kuathirika kwa urahisi na nguvu za asili za dawa zinazotumiwa kutibu vijiumbe. Iwapo kutapatikana na ambukizo lililo na ukinzani kwa dawa, mtu anayekutunza ataarifiwa ili aweze kuitunza vyema hali yako. Watu wanaoshirikiana na mgonjwa ndani ya nyumba na wanaoshiriki katika utafiti huu watafanyiwa uchunguzi wa eksirei ya kifua na yeyote atakayepatikana na matokeo yasiyo ya kawaida atatumwa kwa tabibu ili achunguzwe zaidi. Utafiti huu utatoa maandishi kuhusu changamoto ambazo wewe kama mgonjwa wa TB ya mapafu pamoja na watu unaoshirikiana nao au wanaokutunza hupata. Matokeo ya utafiti huu yatawafaidi waundaji wa sera katika njia ya kuzuia, kubainisha na kuzuia TB, ugawaji wa fedha za kuwashughulikia walioambukizwa na walioathirika na vilevile kuendeleza mwongozo wa ubainishaji na ushughulikiaji wa TB iliyo na ukinzani kwa dawa nyingi.

Usiri:
Ingawa utafiti huu unahusiana na uchunguzi wa hali ya jenetiki, matokeo yake hayatahusishwa moja kwa moja nawe kwa sababu sampuli zote na maswali ya udadisi yatapewa namba maalum za utafiti. Habari yoyote tutakayotoa katika majibu yako ya maswali ya udadisi, yanayotumiwa katika utafiti huu yatawekwa kama siri, yatahifadhiwa vizuri na watakaoyatumia ni mtafiti mkuu pamoja na wasimamizi wake pekee. Maofia wa Kamati ya Utafiti na Maadili kutoka Hospitali Kuu ya Kenyatta au Chuo Kikuu cha Nairobi wataweza kuchunguza rekodi hizo na maendeleo ya utafiti huu wakati wowote. Matokeo ya kliniki na ya maabara ya utafiti huu yatatolewa kwa maofisa wa afya wa Hospitali ya Wilaya ya Mbagathi na utafiti utakaokusanywa unaweza kuchapishwa kwa makusudi ya kisayansi. Kwa hali yoyote, habari kukuhusu wewe binafsi haitafichuliwa katika hatua yoyote ya utafiti huu na matokeo yoyote hayatahusishwa nawe moja kwa moja.

Kukataa, Kujiondoa na Gharama ya Kushiriki:
Kushiriki katika utafiti huu ni kwa hiari ya mtu. Tafadhali, unahakikishwa kwamba ukikataa kushiriki, matibabu yako kutoka hospitalini hayataathirika kwa njia yoyote. Kukataa kushiriki hakutaathiri huduma utakayopokea kutoka Hospitali ya Wilaya ya Mbagathi. Pia, una uhuru wa kujiondoa katika utafiti huu wakati wowote. Hutahitajika kulipa malipo yoyote kwa kushiriki katika utafiti huu.

Maswali na Njia ya Mawasiliano:
Iwapo una maswali yoyote wakati huu au katika siku za baadaye kuhusu haki yako katika utafiti huu au madhara yoyote yanayotokana na utafiti huu, wasiliana name, Susan Odera au msimamizi wangu kupitia kwa nambari hii: 0723470211, au Prof. Guantai, Mwenyekiti wa Hospitali Kuu ya Kenyatta/Kamati ya Utafiti na Maadili ya Chuo Kikuu cha Nairobi, S.L.P 20273, Nairobi. Nambari ya Simu 020726300 ext 9.

SAINI:
Nimepewa kikamilifu maelezo kuhusu utafiti huu pamoja na msingi wa kushiriki, katika lugha ya Kiingereza/Kiswahili na ninakubali kushiriki katika utafiti huu. Ninaelewa kwamba nina uhuru wa kuamua kushiriki katika utafiti huu au la..
Saini ya mgonjwa/ Alama ya kidole gumba: …….
Saini ya shahidi……
Saini ya wasaidizi wa utafiti……
Tarehe: …….

APPENDIX 3: QUESTIONNAIRE FOR PARTICIPANTS (PTB PATIENTS)

Title of Study: The role of Human Leukocyte Antigens and *M. tuberculosis* strain variation in susceptibility to infection among TB/HIV co-infected subjects in Kenya.

Study No: ☐☐☐ Date of interview ☐☐☐ File No: ☐

Patient initial: ☐

Sex ☐

Date of Birth: ☐☐☐
dd mm yy

Nationality _____

Ethnic Background _____

Marital Status: Single ☐ Married ☐ Separated ☐
Widowed ☐ Divorced ☐

Residential area _____

No. of rooms in your house _____

Educational Level: None ☐ Primary ☐ Secondary ☐ College ☐

Occupation: Professional ☐ Housewife ☐ Unemployed ☐

Smoker ☐ Previous smoker ☐
Consume Alcohol ☐

New case (no previous treatment) ☐
Follow ups (those currently on treatment) ☐

Previous case (treatment) treatment failure ☐ Defaulter ☐

Others specify _____

Main challenges faced by patient
- Access to information on TB ☐
- Access to adequate diagnostic centers ☐
- Access to prescribed medication ☐
- Adequate nutrition ☐

Social support from household contacts and community ☐

Others
……
……
……
……..

Sign …….. Date…….

APPENDIX 4: QUESTIONNAIRE FOR PARTICIPANTS (HOUSEHOLD CONTACTS)

Title of Study: The role of Human Leukocyte Antigens and *M. tuberculosis* strain variation in susceptibility to infection among TB/HIV co-infected subjects in Kenya.

Study No: ☐☐☐ Date of interview ☐☐☐

Sex ☐

Date of Birth: ☐☐☐
dd mm yy

Nationality _____

Ethnic Background _____

Marital Status: Single ☐ Married ☐ Separated ☐
Widowed ☐ Divorced ☐

Educational Level: None ☐ Primary ☐ Secondary ☐ College ☐

Occupation: Professional ☐ Housewife ☐ Unemployed ☐

Residential area _____

No. of rooms in your house _____

Do you share a room with the patient _____

Duration of association with the patient _____

Number of PTB patient(s) in household ☐
Relation with PTB patient(s) ☐

Smoker ☐ Previous smoker ☐
Consume Alcohol
Duration of associa☐with PTB patient(s) ☐
Challenges faced by contacts of PTB patients (tick where it applies)

- Access to current information on TB ☐
- Availability of prescribed medication ☐
- Adequate resources for nutrition ☐
- Counseling services offered after diagnosis of patient ☐
- Social support from household members ☐

Others

……
……
……
……..

Sign …….. Date……..

APPENDIX 5: LABORATORY METHODS PROTOCOLS

Appendix 5a: QuantiFERON TB Gold test for IGRA

APPENDIX 5b: DNA Extraction from blood

Fifth Edition May 2016

QIAamp® DNA Mini and Blood Mini Handbook

For DNA purification from whole blood, plasma, serum, buffy coat, lymphocytes, dried blood spots (QIAamp DNA Mini Kit only), body fluids, cultured cells, swabs, and tissue (QIAamp DNA Mini Kit only)

QIAGEN

Sample & Assay Technologies

APPENDIX 5c: HLA SSP-PCR typing

HLA Typing Using Olerup SSP® Kits and the QIAxcel® Advanced System

For analysis and typing of PCR products from different HLA loci using the QIAxcel Advanced and the Helmberg-SCORE software

Introduction

Human leukocyte antigens (HLA) are a group of proteins that are present on each cell surface and allow the immune system to recognize "self" from "foreign" (1). HLA antigens therefore play a fundamental role in organ transplantation as fully or nearly fully matched organs (e.g., kidneys) will result in a lower risk for rejection and in longer graft survival (2). HLA alleles have also been associated with a number of diseases, cancer and drug hypersensitivity (3).

HLA genes are found on the short arm of chromosome 6 and are highly polymorphic with paternal and maternal alleles being coexpressed in each individual. In 1992, Olerup SSP AB pioneered the field of molecular HLA typing with the introduction of its invention PCR-SSP (PCR amplification with sequence-specific primers) (4). This technology enabled for the first time determination of accurate HLA types at the DNA level within a few hours. The method is based on DNA amplification with up to 96 highly multiplexed primer mixes and results in a complex pattern of positive and negative reactions specific for the alleles present in the sample. The method usually requires the separation of the SSP products on a standard agarose gel with gel documentation and manual data entry into the typing software. The QIAxcel Advanced System enables automated and fast separation of PCR fragments, analysis and digital data transfer to the Helmberg-SCORE™ analysis software. The QIAxcel is a highly automated capillary electrophoresis system that provides reliable results with minimal hands-on time and no exposure to toxic gel stains like ethidium bromide. PCR products from different HLA loci can be analyzed and typed in a few minutes.

The following procedure is for Research Use Only. Not for use in diagnostic procedures.

QIAxcel Advanced System

The QIAxcel Advanced instrument is a capillary electrophoresis system used for the separation, detection and analysis of nucleic acids (DNA and RNA). Convenient, ready-to-use cartridges provide an array of 12 capillaries and a reservoir containing proprietary gel polymers mixed with the fluorescent dye. Automated sample loading and analysis limit error-prone manual steps, thereby ensuring reproducibility of measurements. As no hazardous compounds need to be

APPENDIX 6: UoN/KNH UNIVERSAL HEALTH CONFERENCE 2019

University of Nairobi | MBAGATHI HOSPITAL | BIO-ZEQ KENYA LTD. | KAVI-ICR KAVI-Institute Of Clinical Research UNIVERSITY OF NAIROBI

LATENT TUBERCULOSIS INFECTION AMONG HOUSEHOLD CONTACTS OF PULMONARY TUBERCULOSIS CASES IN NAIROBI, KENYA: PREVALENCE AND ASSOCIATED FACTORS

Susan Odera*, Julias Oyugi*, Marianne Mureithi*, Isabella Mwangi**, Meshack Obwogi***, Comfort Nyando****,Omu Anzala*

*Department of Medical Microbiology, University of Nairobi

**Qiagen laboratory, Biozeq Kenya Limited- KAVI-ICR

***Mbagathi District Hospital

**** Maseno University

Introduction:

Tuberculosis is a communicable disease caused by infection with *M. tuberculosis* complex. Latent tuberculosis infection (LTBI) is a non-communicable asymptomatic condition that can persist after infection and can develop into active TB. One of the pillars of the World Health Organization END-TB strategy promotes testing and treatment of LTBI in groups at high risk of reactivation. Close contacts of active TB patients are at risk of both active and LTBI.

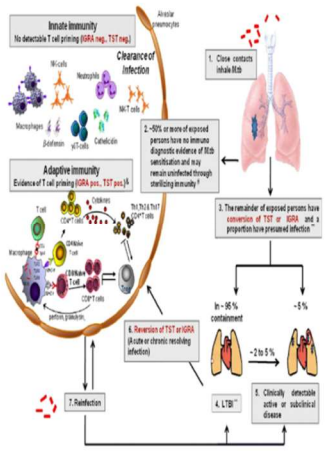


Figure 1: Image of immune response to *M. tuberculosis* infection

Objective:

This study aimed at screening household contacts of smear positive pulmonary tuberculosis patients, in Nairobi, Kenya to estimate the prevalence of latent tuberculosis and to identify predominant socio-demographic risk factors associated with infection. It is nested in a larger study investigating host genetic factors and susceptibility to TB infection.

Methods:

An analytical cross-sectional study was conducted at Mbagathi Hospital, Nairobi between July 2016 and September 2016. Household contacts traced from PTB patients visiting the hospital were recruited after written informed consent was obtained.

They were tested for LTBI using the QuantiFERRON -TB Gold In-Tube (QFT-G). This is a test for Cell Mediated Immune response to peptide antigens (ESAT-6. CFP-10 and TB7.7 proteins) that simulate mycobacterial proteins. These proteins are absent from all BCG strains and most non-tuberculosis mycobacteria except *M. kansasii, M. szulgai and M. marinum.* CD4+ T cells in infected individuals recognize these antigens leading to secretion of the cytokine IFN-γ. The detection and subsequent quantification of IFN-γ forms the basis of this test.

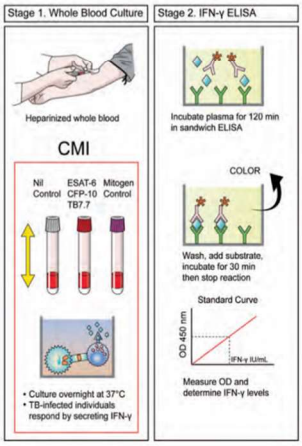


Figure 2 Test procedures of QuantiFERRON-TB Gold In-Tube test. Modified from package insert and website of Cellestis Ltd (Carneige, Australia)
CMI: Cell Mediated Immunity

Results:

Of the 175 household contacts traced from 166 PTB patients, 55% (98/175) tested positive. A test was considered positive if an IFN-γ release assay response to the TB antigen tube was significantly above the Nil IFN-γ value (>0.35IU/ml).

Table 1: QuantiFERRON-TB Gold In-Tube Assay results IGRA Positive

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	no	77	43.8	44.0	44.0
	yes	98	55.7	56.0	100.0
	Total	175	99.4	100.0	
Missing	System	1	.6		
Total		176	100.0		

Table 2: Summary of Study participants Socio-demographics

	PERCENTAGES		
DEMOGRAPHIC	DESCRIPTION	CONTACTS	PATIENTS
SEX	Male	30	48.2
	Female	70	51.8
AGE	18-29	30.3	24.7
	30-39	37.7	33.1
	40-49	16.3	22.3
	50-59	12.6	15.1
	60-69	2.3	4.2
	67-79	0.6	0.6
MARITAL STATUS	Single	4.6	28.0
	Married	41.0	64.0
	Separated	6.6	1.7
	Widowed	7.2	5.1
	Divorced	0.6	1.1
EDUCATION LEVEL	None	3.4	9.0
	Primary	31.4	39.2
	Secondary	38.9	39.2
	College	26.3	12.7
OCCUPATION	Professional	39.4	27.7
	Unemployed	42.9	63.9
	Home self-employed	17.7	8.4
ROOMS IN HOUSEHOLD	Share one room	55	
	More than one room	45	
ALCOHOL USE	Yes	17	25.9
	No	83	74.1
CIGARETTE SMOKERS	Previous	2	17.5
	Current smoker	5	12.7
	Non smoker	93	69.9
RELATION WITH PATIENT BLOOD RELATION	Parent	29.1	
	Sibling	31.4	
	Cousin	2.9	
	Aunt/Uncle	8.6	
NON-BLOOD RELATION	Spouse	11.4	
	Others	16.6	
HIV STATUS	Positive	9 (16)	65.1
	Negative	90 (159)	34.9

In this study, there wasn't any statistically significant association between these socio-demographic factors and presence of LTBI.

Conclusion:

- The prevalence of LTBI among household contacts of active pulmonary TB patients was high.
- Testing for latent TB in high risk individuals should be part of the strategy towards eliminating TB and identified individuals should be offered preventive treatment

NOTE: Performance of QFT-GIT depends on stimulation of CD4+ T-cells which limits its performance in HIV-positive individuals who have reduced capacity for IFN-gamma secretion from CD4+ T-cells. A new generation of the QFT assay, the QuantiFERRON-TB Gold plus, has been developed. It has the same antigens in QFT-GIT, as well as shorter peptides of the same designed to specifically stimulate CD8+ T-cells, and this modification could improve sensitivity in immunosuppressed subjects.

On-going Research:

This data is part of an on-going study on pulmonary tuberculosis patients and their household contacts. The study is evaluating host genetic factors, specifically the Human Leukocyte Antigen (HLA), and susceptibility to infection.

The HLA system plays an important role in modulation of the immune response in infectious diseases. Such modulation may be subjected to immunogenetic predisposition because studies have shown that certain infectious diseases occur more frequently among individuals carrying particular HLA alleles. Studies have also shown that the effect of polymorphisms in the HLA alleles and risk of infection or disease could be modified by HIV-1 co-infection.

References:

1: WHO 2018: Latent TB infections Updated and Consolidated guidelines for programmatic Management

2: Stephan Scwander and Keertan Dheda. Human Lung Immunity against *Mycobacterium tuberculosis:* Insights into pathogenesis and protection. Am J Respir Crit Cate Med. 2011 Mar 15: 183 (6): 696-707

APPENDIX 7: AMERICAN SOCIETY FOR MICROBIOLOGY CONFERENCE CHICAGO, USA. 2020

University Of Nairobi | Mbagathi Hospital | BIO-ZEQ KENYA LTD. | KAVI-ICR KAVI-Institute Of Clinical Research UNIVERSITY OF NAIROBI

LATENT TUBERCULOSIS AMONG HOUSEHOLD CONTACTS OF PULMONARY TUBERCULOSIS CASES IN NAIROBI, KENYA

S. A. ODERA, M. MUREITHI, O. ANZALA, J. OYUGI
UNIVERSITY OF NAIROBI, NAIROBI, KENYA

INTRODUCTION

- Tuberculosis (TB) is a major health problem in sub-Saharan Africa and other developing countries including Kenya because of its high infectivity, mortality, and cost of therapy.
- A hallmark of the natural history of tuberculosis is the diverse outcome of infection,which includes Latent Tuberculosis Infection (LTBI)

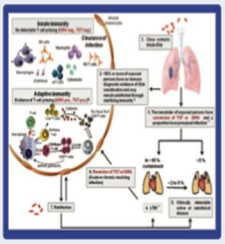


Image 1: Immune response of Mycobacterium tuberculosis infection Am J Respir Crit Care Med. 2011 Mar 15; 183 (6): 696-707 © 2011, American Thoracic Society

BACKGROUND

- Close contacts of pulmonary tuberculosis patients are at a high risk of infection
- Individuals who have LTBI are assymptomatic and are not infectious
- LTBI contributes significantly to the pool of active TB cases once reactivation occurs, therefore its diagnosis and treatment in high-risk groups, including HouseHold Contacts (HHCs) of Pulmonary Tuberculosis (PTB) patients is essential for reduction and ultimately elimination of TB.

OBJECTIVE

This study aimed at screening household contacts of smear positive pulmonary tuberculosis patients, in Nairobi, Kenya to estimate the prevalence of latent tuberculosis and to identify predominant socio-demographic risk factors associated with infection.

METHODS

- 175 household contacts of PTB patients at Mbagathi District Hospital, Nairobi were invited to participate in the study and informed consent was obtained.
- A structured questionnaire was used to capture socio-demographic data
- 3 blood samples were collected by venipuncture from each household contact directly into the blood collection tubes provided in the QuantiFERON®-TB Gold In-Tube kit (QFT-GIT), (Qiagen).
- The blood samples were precessed for the Interferon-gamma release assays (IGRAs) at the University of Nairobi.
- Univariate analysis using the Statistical Package for Social Scientists (SPSS) was used to determine the prevalence of LTBI and risk factors at 95% Confidence Interval (CI).

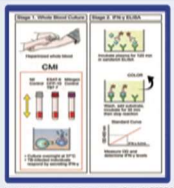


Image 2: Test procedure for Interferon-gamma release assays QuantiY FERRON-TB Gold In-Tube test. Modified from package insert and website of Cellestis Ltd (Carnelge, Australia). A positive QFT GiT test has ELISA values for interferon gamma (IFN-γ) above the Nil IFN-γ value (> 0.35 IU/ml).

RESULTS

Overall, it was evident that LTBI is a common yet neglected problem for HHCs of PTB patients, with factors such as their age, relation to the patient, and the length of time spent cohabiting with or caring for TB patients leading to repeated exposure to Mycobacterium tuberculosis and thus the risk of developing LTBI.

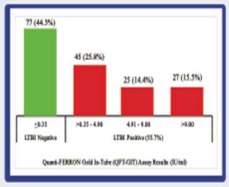


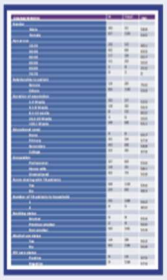
Figure 1: summary of the distribution of the concentration of IFN-γ, measured by ELISA, across household contacts. The Quanti-FERRON Gold In-Tube (QFT-GIT) assay results indicated that 55.7% of contacts were positive for LTBI. A test was positive if ELISA value for interferon gamma (IFN-γ) was significantly above the Nil IFN-γ value (> 0.35 IU/ml).

Sociodemographic characteristics

Table 1: Socio-demographic characteristics of household contacts of TB patients, N = 175

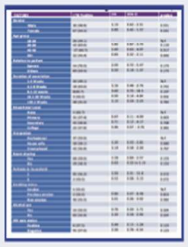
Analysis of LTBI prevalence stratified by socio-demographics

Table 2: Prevalence of LTBI stratified by socio-demographics of household contacts N-174



Potential risk factors for LTBI

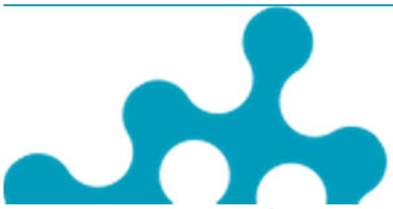
Table 3: Risk factors for LTBI among TB household contacts, N = 174



CONCLUSION

- The high prevalence of LTBI in adult household contacts of TB patients in this population emphasizes the need for TB programs to invest more in the screening and treatment of household contacts in high transmission settings
- TB prevention efforts should also focus on sensitizing household contacts on the importance of preventive treatment to avoid reactivation.

APPENDIX 8: European Congress of Clinical Microbiology and Infectious Disease- 2020 CONFERENCE PARIS



3018 ABSTRACT BOOK – **30th ECCMID 2020**

Latent tuberculosis infection among household contacts of pulmonary tuberculosis cases in Nairobi, Kenya

Susan Akinyi Odera*[1], Marianne Mureithi[1], Omu Anzala[1], Julius Oyugi[1;1]
[1]*University of Nairobi, Nairobi, Kenya*

Background: Tuberculosis (TB) is a significant health problem in sub-Saharan Africa and other developing countries including Ke- nya, which is ranked among the top 30 countries with the highest burden TB. Household Contacts (HHCs) of Pulmonary Tuber- culosis (PTB) patients have a higher risk of LTBI. However, its prevalence and risk factors among adults living with PTB patients are poorly documented in Kenya.
Materials/methods: An analytical cross-sectional study was conducted to establish the prevalence and risk factors of LTBI in a cohort of adult HHCs of active PTB patients who were seeking health care services in a per-urban public district hospital in the Kenyan capital, Nairobi. Informed consent was obtained. A structured questionnaire was used to capture socio-demographic data of the study participants and thereafter 3 blood samples collected by venipuncture from each HHCs directly into the blood collection tubes provided in the QuantiFERON®-TB Gold In-Tube kit (QFT-GIT) for Interferon-gamma release assays (IGRAs).
Results: A total of 166 PTB patients yielded 175 adult HHCs of whom 29.7% (52/125) were males and 70.3% (123/125) were females. A majority of HHCs [65.7% (115/175)] lived in a single-room house with the patient, and [37.7% (66/175)] were in the age group 30-39 years. The overall prevalence of LTBI was 55.7%, peaking among spouses of the patients [70.0% (14/20) and the 30-39 year age group [63.5% (42/66)]. Potential risk factors for LTBI included cohabiting with a PTB patient for 8 to 12 weeks [OR= 3.6 (0.70-18.5), p=0.107], being a spouse of the patient [OR=2.0 (0.72-5.47), p=0.173] and sharing a single room with the patient [OR=1.58 (0.84-2.97), p=0.158].
Conclusions: There is an increasing awareness of the problem LTBI poses to HHCs of PTB patients. In Kenya, however, data on LTBI is mainly anecdotal, which hinders its active management. Overall, it was evident that LTBI is a common yet neglected problem for HHCs, with factors such as age, relation to TB patients, and the length of time spent cohabiting with or caring for TB patients leading to repeated exposure and thus the risk of developing LTBI. The findings demonstrate the need for targeted contact-screening programs in high TB transmission settings.
Presenter email address: sueodera@yahoo.com

APPENDIX 9: PUBLICATION 1 PAN AFRICAN MEDICAL JOURNAL

Article

PanAfrican Medical Journal

Research

Latent tuberculosis among household contacts of pulmonary tuberculosis cases in Nairobi, Kenya

Susan Odera, Marianne Mureithi, Andrew Aballa, Noel Onyango, Omu Anzala, Julius Oyugi

Corresponding author: Susan Odera, Department of Medical Microbiology, University of Nairobi, Nairobi, Kenya. sueodera@yahoo.com

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Latent tuberculosis among household contacts of pulmonary tuberculosis cases in Nairobi, Kenya

Susan Odera[1,&], Marianne Mureithi[1,2], Andrew Aballa[3], Noel Onyango[4], Omu Anzala[1,2], Julius Oyugi[1,5]

[1]Department of Medical Microbiology, University of Nairobi, Nairobi, Kenya, [2]KAVI-Institute of Clinical Research, University of Nairobi, Nairobi, Kenya, [3]Department of Medical Laboratory Sciences, School of Medicine, Kenyatta University, Nairobi, Kenya, [4]Department of Clinical Medicine and Therapeutics, University of Nairobi, Kenya, [5]University of Nairobi Institute of Tropical and Infectious Diseases, University of Nairobi, Nairobi Kenya

[&]**Corresponding author**
Susan Odera, Department of Medical Microbiology, University of Nairobi, Nairobi, Kenya

Abstract

Introduction: *Household Contacts (HHCs) of Pulmonary Tuberculosis (PTB) patients have a higher risk of latent tuberculosis infection (LTBI). However, its prevalence and risk factors among adults living with PTB patients are poorly documented in Kenya.* ***Objective****: to determine the prevalence and risk factors for LTBI among adult HHCs of PTB patients in Kenya.* ***Methods:*** *this was an analytical cross-sectional study of HHCs of PTB patients in Nairobi, Kenya. Socio-demographic data was captured on questionnaires and blood samples drawn for Interferon gamma (IFN-γ) quantification. Univariate and multivariate analyses using the Statistical Package for Social Scientists (SPSS) was used to determine the prevalence of LTBI and risk factors at 95% Confidence Interval (CI).* ***Results:*** *a total of 166 PTB patients yielded 175 HHCs of whom 29.7% (52/125) were males and 70.3% (123/125) were females. A majority of HHCs [65.7% (115/175)] lived in a single-room house with the patient and [37.7% (66/175)] were in the age group 30-39-years. The overall prevalence of LTBI was 55.7%, peaking among spouses of the patients [70.0% (14/20) and the 30-39 year age group [63.5% (42/66)]. Potential risk factors for LTBI included cohabiting with a PTB patient for 8 to 12 weeks [OR = 3.6 (0.70-18.5), p = 0.107], being a spouse of the patient [OR = 2.0 (0.72-5.47), p = 0.173] and sharing a single room with the patient [OR = 1.58 (0.84 - 2.97), p = 0.158].* ***Conclusion:*** *the high prevalence of LTBI among adult HHCs of PTB patients in this population demonstrates the need for targeted contact-screening programs in high TB transmission settings.*

Introduction

Tuberculosis (TB) is a major health problem in sub-Saharan Africa and other developing countries including Kenya, which is ranked among the top 30 countries with the highest burden TB [1, 2]. TB is a communicable disease caused by infection with *M. tuberculosis* complex organisms, which typically spreads to new hosts via airborne droplet nuclei from patients with respiratory tuberculosis disease.

A hallmark of the natural history of tuberculosis is the diverse outcome of infection. There are three possible outcomes of exposure to an infectious TB case: the infection may be cleared by sterilizing immunity [3] as evidenced by immunological assays [4]; development of active TB and development of latent tuberculosis infection (LTBI). Studies have shown that 10% of LTB infected individuals develop active TB and because of the underlying immune deficiency, HIV-infected individuals with LTBI are at 26-fold higher risk for TB reactivation [5].

Since LTBI contributes significantly to the pool of active TB cases once reactivation occurs, its diagnosis and treatment in high-risk groups, including HHCs of PTB patients is essential for reduction and ultimately elimination of TB [2]. It is therefore important to establish the prevalence and risk factors of LTBI among HHCs of PTB patients in this population, since this data will inform policy on the need for targeted contact-screening programs.

Methods

Study design and setting

An analytical cross-sectional study conducted in Mbagathi District Hospital (MDH), Nairobi Kenya.

Study population

Patients with a definitive TB diagnosis through sputum smear examination, chest X-ray, and Xpert MTB/RIF technique were recruited at the outpatient and inpatient TB wards of the hospital. HHCs were defined as adults who shared meals and rooms with the patient, and were living together. The HHCs were identified during the patient visiting hours or as they accompanied their patients to the outpatient clinic.

Sample size and data collection

One hundred and sixty six (166) TB patients were identified and from these, 175 HHCs who agreed to participate in the study gave their informed consent and were recruited. A structured questionnaire was used to capture data on the socio-demographic characteristics of the study participants and 3 blood samples collected by venipuncture from each HHCs directly into the blood collection tubes provided in the QuantiFERON®-TB Gold In-Tube kit (QFT-GIT), (Qiagen, Germany) for Interferon-gamma release assays (IGRAs). These samples were then transported within 15 minutes to research laboratories, at College of Health Sciences, University of Nairobi for further processing.

Interferon-gamma release assays (IGRAs)

Diagnosis of LTBI infection can be made using immunodiagnostic tests such as the Tuberculin Skin Test (TST) or Interferon Gamma (IFN- γ) Release Assays (IGRAs). Both tests are used to identify individuals with previous sensitization to mycobacterial antigens. However, the sensitivity of TST is compromised in individuals with immunosuppression, cross reactions from infection with non-tuberculous mycobacteria or BCG vaccination. IGRAs identify a memory of an adaptive immune response against mycobacterial antigens, and are more sensitive than TST. They are not affected by prior BCG vaccination and look for the body´s response to TB antigens not present in other forms of mycobacteria [6].

The QuantiFERON® - TB Gold In-Tube test that was used in this study is an IGRA test for Cell Mediated Immune (CMI) responses to peptide antigens that simulate mycobacterial proteins. These proteins, ESAT-6, CFP-10, and TB 7.7 are absent from all BCG strains and from most non-tuberculous mycobacteria with the exception of *M. kansasii, M. szulgai and M. marinum*. Individuals infected with *M. tuberculosis* complex organisms usually have lymphocytes in their blood that recognize these and other mycobacterial antigens. This recognition process involves the generation and secretion of the cytokine, IFN-γ. The detection and subsequent quantification of IFN-γ forms the basis of this test. The QuantiFERON® - TB Gold In-Tube test (QFT GIT) (Qiagen, Germany) test was performed according to the manufacturers instructions. Briefly, blood was collected by venipuncture from each HHCsinto 3 blood collection tubes one containing M. tuberculosis peptide antigens ESAT-6, CFP-10, and TB 7.7; one containing a mitogen, and one nil tube, with no antigen. The Mitogen-stimulated plasma sample served as an IFN-γ positive control for each specimen tested. Whole blood was incubated for 16 hours at 37°C and transferred to 4oC until processing, but for no longer than 48 hours. Tubes were centrifuged at 2000g for 15 min, and then supernatants were stored at 80oC until the QFT ELISA could be conducted. Supernatants and IFN-? standards (50 ml) plus conjugate (50 ml) were incubated for 2 hours, washed 6 times, and incubated for 30 min with substrate solution. After 30 min, 50 ml stop solution was added and the wells and the plates were read at 450 nm with a 650 nm reference filter. Concentrations of IFN-? were calculated based on the standard curve and test outcomes (positive, negative, or indeterminate) determined using a mathematical algorithm from the manufacture. Samples of participants with indeterminate QFT results were rerun and positive or negative outcomes on second run interpreted as their final results. Patients with indeterminate on second run were scored as QFT indeterminate. A test was positive if the ELISA value for interferon gamma (IFN-γ) was above the Nil IFN-γ value (> 0.35 IU/ml).

Statistical analysis

Socio-demographic data were extracted from the study questionnaires and entered into a worksheet using the Statistical Package for Social Scientists (SPSS) software (version 21). Then, data were scrutinised for inconsistencies, typing errors and missing data, and the dataset cleaned following the published guidelines on data cleaning [7]. Descriptive statistics were explored and presented as tables, the prevalence of LTBI computed

following the guidelines by Ward, 2013, and risk factors for LTBI determined by testing for fitness by Chi-square tests at 95% CI. $p < 0.05$ was significant [8].

Ethical considerations

This data presented here was obtained in a larger study on "The role of Human Leukocyte Antigens and *M. tuberculosis* strain variation in susceptibility to infection among pulmonary tuberculosis patients in Kenya". The Kenyatta National Hospital/University of Nairobi Ethics and Research Committee (KNH/UoN ERC) reviewed and approved the study, Reference No. KNH-ERC/A/392. This manuscript is original and is not currently under consideration by another journal.

Results

Socio-demographic characteristics of household contacts

Of the one hundred and seventy-five (175) HHCs recruited, 29.7% (52/175) were male and 70.3% (123/175).were female. Approximately 37% (66/175) were 30-39 years old, while 39.4% (69/175) had lived with the TB patients for over 20 weeks. About 38.9% (68/175) had secondary education, 42.9% (75/175) were unemployed, while 65.7% (115/175) lived in a one-room structure with the TB patient. Cigarette smoking, alcohol consumption, and HIV sero-positivity constituted 5.1% (5/175), 16.6% (29/175), and 9.1% (16/175) of the HHCs respectively (Table 1).

Prevalence of LTBI among household contacts

Of the 175 HHCs recruited only 174 had data on the QFT-GIT assay used for diagnosis of LTBI, and therefore were included in our univariate analyses. The overall prevalence of LTBI among the HHCs was found to be 55.7% (97/174). A test was positive if the ELISA value for interferon gamma (IFN-γ) using the QFT-GIT assay was above the Nil IFN-γ value (> 0.35 IU/ml). A majority of LTBI cases 46.4% (45/97) had an interferon gamma (IFN-γ) ELISA value of between 0.36 and 4.9; while 27.8% (27/97) had a value greater than 9.0 IU/ml (Figure 1). The HHCs were stratified according to socio-demographic characteristics and the prevalence of LTBI computed in each group. Analysis by gender revealed an LTBI prevalence of 58.8% (30/51) in males and 54.5% (67/123) in females. By age, LTBI exhibited a dome-shaped frequency curve, which peaked at 63.6% (42/66) among HHCs aged 30-39 years old. The 40-49-year-old group was the second most affected group with 60.7% (17/28) testing positive while 50% (11/22) of 50-59 year olds were affected. Among the HHCs who had a spousal relationship with the TB patient, the prevalence for LTBI was 70.0% (14/20) while the other relations had a prevalence of 53.9% (83/154).Analysis of the duration of association with the TB patient revealed a higher prevalence of LTBI at 80% (8/10) among HHC who were living with TB patients for 8.1-12 weeks, compared to the categories of duration of weeks of association which ranged from 50 to 56.3% prevalence (Table 2). HHCs that had no formal education had an LTBI prevalence of 66.7% (4/6) while those who had tertiary level education had a prevalence of 47.8% (22/46). Most of the HHCs lived in a single room house with the TB patient and as such had a close proximity to the TB patient. A prevalence of 59.6% (68/114) was reported amongst the HHCs who shared a room with the patient (Table 2).

Risk factors for LTBI

Several risk factors for LTBI were investigated in this study. Although a slightly higher prevalence of LTBI was reported in males, the risk of LTBI did not vary significantly between males and females [OR = 1.09 (0.62 - 2.31, (p = 0.531)].].The risk of LTBI was higher among older HHCs, with the 30-39 year old age group [OR = 1.82 (0.87 - 3.79), p = 0.110] and 40-49 year old age group [OR = 1.60 (0.63 - 4.07), p = 0.317] having a higher odds of infection in reference to 18-29 year old. Finally, the odds of LTBI were higher among spouses than other household members [OR = 2.0 (0.72 - 5.47), p = 0.173], HHCs who shared rooms with TB patients [OR = 1.58 (0.84 - 2.97), p = 0.153], and HHCs who cohabited with TB patients for 8.1-12 weeks [OR = 3.60 (0.70 - 18.5), p = 0.107]. A tertiary level of education [OR = 0.46 (0.07 - 2.76), p = 0.385] was protective against infection with TB (Table 3).

Discussion

Tuberculosis (TB) continues to be a global concern because of its high infectivity, mortality, and cost of therapy. In addition, the patients´ families are confronted with extra social and clinical burdens associated with TB disease [9]. There is an increasing awareness of the problem LTBI poses to HHCs and therefore the need to protect such at-risk groups. In Kenya, however, data on LTBI is mainly anecdotal, which hinders its active management. To fill this gap, we established the prevalence of LTBI and its possible risk factors in a cohort of vulnerable HHCs of active TB patients who were seeking health care services in a per-urban public district hospital in the Kenyan capital, Nairobi. Overall, it was evident that LTBI is a common yet neglected problem for HHCs, with factors such as their age, relation to active TB patients, and the length of time spent cohabiting with or caring for TB patients leading lead to repeated exposure to *Mycobacterium tuberculosis* and thus the risk of developing LTBI.

We reported a high prevalence of LTBI among HHCs, with the QFT-GIT results of over half our participants indicating positivity for LTBI. For HHCs with complete data (174), the prevalence of LTBI was 55.7%, which was higher than the global average of 23% [10], and 34% in Georgia and 12.7% in Singapore [11, 12]. However, similar results have been reported in Ethiopia, where the prevalence of LTBI is observed as 63.7% [13]. The discrepancies in prevalence might be associated with the methodology used for LTBI diagnosis and the disproportional distribution of TB worldwide. Some of the prevalence studies used the TST assay for LTBI diagnosis, a test whose accuracy measure has been confounded by Bacillus Calmette - Guérin (BCG) vaccination and non-tuberculous mycobacterial (NTM) infections [14]. Data from the WHO indicating that 2,480,000 TB cases are reported in Africa every year, with 25% of these cases dying because of TB or TB complications [15]. The incidence in Africa is significantly higher than in the Americas, the East Mediterranean, and in Europe, where the TB is estimated to infect 282,000, 771,000, and 273,000 people annually [16]. The higher exposure to *M. tuberculosis* in Africa increases the risk of infection and therefore the prevalence of LTBI. People with other mycobacterial infections also often have false positive reactions to ESAT-6, CFP-10, and TB7.7 antigens, as the genes encode the proteins found in *Mycobacterium kansasii*, *Mycobacterium szulgai*, and *Mycobacterium marinum* [17]. Even though the prevalence of Non-Tuberculous Mycobacteria (NTM) is estimated to be approximately 5-15% in Africa [18], we did not control for NTM infections in this study.

Several known risk factors for LTBI from literature were investigated. They included gender, age group, and relationship with the TB patient, duration of association with the patient, sharing a room with the patient, education level, use of cigarettes, alcohol use, as well as the HIV sero-status. Our finding that 30 - 49year old HHC were susceptible to LTBI than younger ones (18-29 years) was consistent with the findings of in Mongolia and in India [19, 20]. In both studies, an increasing age was associated with a high risk of LTBI/TB. An age specific prevalence of LTBI among HHCs of TB patients observed an increased prevalence of infection in older children and young adults [21], and proposed the need to expand TB preventive therapy to include all HHCs. Menzies et al., 2007 reviewed article also reported a higher risk of LTBI among the elderly - a finding that was corroborated by in Ghana and in a tuberculosis-prevalent country [22, 23]. According to Zhang *et al.* 2019, elderly caregivers are more likely to spend a longer time caring for TB patients than younger ones in hospitals and homes [24]. As such, these individuals have a higher cumulative exposure to M. tuberculosis through physical contact and or social interactions, which predisposes them to a higher risk of infection, morbidity, and mortality. Moreover, because the immunity of humans

diminishes with increasing age, elderly HHCs are less likely to clear the *Mycobacterium tuberculosis* infections that they are exposed to in their high TB settings, which increase the risk of persistent infection further.

The prevalence of LTBI was slightly higher among males at 58.8%than females at 54.5%. However, after our univariate analyses, gender was not identified as a risk factor for LTBI, as was the case in a cross sectional study of final year medical students in Kenya [25] and HCWs in Kigali, Rwanda [26]. In China, men were less susceptible to LTBI [27], while females had a significantly lower risk of LTBI in South Korea [28]. The inconsistence in the occurrence of LTBI by gender might be due to the variability in the structure of households in different regions and not differences in the susceptibility of the two genders to TB infections. In Kenya, most families live in rented single or one bedroom houses, where men and women share rooms [29]. In such communal settings, Menzies et al., 2007 proposes that the risk of transmission of infectious diseases such as TB might not differ by the occupation or gender of HHCs, if their risk status (such as the length of stay with active TB patients) is comparable [22]. In our study, room sharing was common (65.7%), with most HHCs cohabiting with TB patients for over five months (39.4%). A tertiary level of education seemed to be protective against LTBI. In reference to HHCs with no formal education, the odds of LTBI was 0.46 (0.07 - 2.76, p = 0.385 among HHCs with a tertiary education and 0.71(0.12 - 4.17), p = 0.71 among HHCs with secondary education. Similar results have been reported from Uganda, identifying a limited knowledge on TB among HHCs as one of the barriers of for tuberculosis contact investigation and therefore its control [30]. Gil *et al.* in 2018 [31] reported that a significant gap in knowledge about TB among HHCs increased the risk of disease transmission and reiterated the need for education campaigns at the community level, which can address misconceptions on causation and transmission of TB. The WHO recommends routine contact investigation in TB high burden countries through counseling and education of HHCs, who are the primary caregivers for active TB patients at home (WHO, 2018). Even though a majority of our respondents indicated that they had access to recent information on TB (88.6%), social support (94.3%), and counseling sessions for TB patients upon diagnosis (92%), this were not tested formally.

Immunosuppression is an independent risk factor for LTBI [32]. People with HIV have a weakened immune system and therefore are at high risk of infections such as TB. In our study, 90% (159/175) of the HHCs recruited were sero-negative. The odds of having a positive LTBI diagnosis using the QFT-GIT test was 2.26 (0.78 - 6.54) when HHCs had a HIV sero-negative status, even though the relationship was not statistically significant (p= 0.123). This discrepancy might be related to the low specificity of the QFT-GIT test in HIV seropositive patients. In the study by Legesse *et al.* 2010 in Ethiopia, the sensitivity of the QFT-GIT test among HIV seropositive patients was 83.3%, while its specificity was significantly lower at 50% [13]. The performance of QFT-GIT depends on stimulation of CD4+ T-cells, which limits its performance in HIV-positive individuals, especially if they have a reduced capacity for IFN-gamma secretion from CD4+ T-cells. As such, because the QFT-GIT in-tube kit would most likely perform better in people with a negative HIV sero-status, interpretation of results in the absence of sero-status data would be limited. A newer generation of the QFT assay - the QuantiFERON®-TB Gold Plus, has been developed to overcome such limitations. In addition to the antigens found in the QFT-GIT kit, the QFT-Gold plus has shorter peptides of the same antigens, which simulate CD8+ T-cells to improve its sensitivity in HIV seropositive patients. The 2018 WHO guidelines included a new recommendation on the use of TST or IGRA to test for LTBI [2].

Medical management of an individual who tests positive using the IGRA test would involve an evaluation of epidemiologic and medical history and other clinical information. If the individual is at risk for progression to active TB and has signs and symptoms suggestive of active disease, additional evaluation would be required. Treatment of selected persons with LTBI using preventive therapy aims at preventing active disease. The 2018 WHO Guidelines included policy documents for programmatic management of LTBI in people living with HIV and household contacts of TB patients and other at-risk groups. As highlighted in these documents, the cascade of care for managing LTBI would include identification of at-risk populations, ruling out active TB disease, testing for LTBI, providing treatment, monitoring adverse events, adherence and completion of treatment [15]. One of the identified at risk groups are household contacts of people with bacteriologically confirmed pulmonary TB. A study done in Ethiopia showed that individuals that had a TB household member had an increased risk of developing TB by 3-fold [33]. In such groups it is necessary to identify the intensity of exposure, the risk for development of active TB and ascertainment of infection through testing for LTBI.

Kenya is recognized as one of the high burden TB countries. Contact investigation as outlined in WHO guidelines has not yet been fully implemented by the National Tuberculosis program in Kenya. The TB program in Kenya recommends Isoniazid Preventive Therapy administration to household contacts of PTB patients who are either less than 5 years old or are HIV infected adults [34]. The current national guidelines should be expanded to implement the standardized contact investigation that would include active screening, evaluation and considered chemoprophylaxis of HHCs of PTB patients, including HIV negative adults.

Conclusion

The high prevalence of LTBI among HHCs of PTB patients in this population demonstrates the need for targeted contact-screening programs in high TB transmission settings. This would enable early detection of LTBI and initiation of treatment of at-risk groups such as HHCs. Investigations should be targeted on close contacts of PTB patients such as spouses, elderly HHCs, and HHCs who share rooms with PTB patients for weeks. Efforts should also focus on airborne infection control measures in homes and on sensitizing at-risk HHCs on LTBI and the importance of preventive treatment to avoid reactivation.

What is known about this topic

- *Close contacts of pulmonary tuberculosis patients are at a high risk of infection because the highly infectious etiologic agent Mycobacterium tuberculosis is spread airborne;*
- *Individuals who have LTBI are not infectious and do not show any clinical symptoms but are important reservoirs for disease reactivation;*
- *The diagnosis and treatment of LTBI in high-risk groups is essential for reduction and ultimately elimination of TB.*

What this study adds

- *The high prevalence of LTBI in adult household contacts of TB patients emphasizes the need for TB programs to invest more in the screening and treatment of household contacts in high transmission settings;*
- *TB programs in resource limited settings should target LTBI screening of spouses of TB patients and HHCs residing in a one-room structure with the patient;*
- *TB prevention efforts should focus on sensitizing household contacts on the importance of preventive treatment to avoid reactivation.*

Competing interests

The authors declare no competing interests.

Authors' contributions

Susan Odera participated in recruitment of study participants, processing clinical specimen, data entry and analysis, and manuscript writing. Julius Oyugi contributed towards the development of the proposal, study oversight, data analysis and critical

review of the manuscript. Omu Anzala and Marianne Mureithi provided study oversight and critical review of the manuscript. Andrew Aballa and Noel Onyango analyzed the data and reviewed the manuscript. All the authors approved the final version to be published. All the authors have read and agreed to the final manuscript

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Tables and figure

Table 1: socio-demographic characteristics of household contacts of TB patients
Table 2: prevalence of LTBI and demographics of household contacts
Table 3: risk factors for LTBI among TB household contacts
Figure 1: distribution of ELISA test values for the QFT-GIT test among household contacts

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Table 1: socio-demographic characteristics of household contacts of TB patients, N = 175

	N	%
Gender		
Male	52	29.7
Female	123	70.3
Age group		
18-29	53	30.3
30-39	66	37.7
40-49	29	16.6
50+	27	15.4
Relation to patient		
Spouse	20	11.4
Others	155	88.6
Duration of association		
1-4 Weeks	58	33.1
4.1-8 Weeks	32	18.3
8.1-12 weeks	10	5.7
16.1-20 Weeks	6	3.4
>20.1 Weeks	69	39.4
Educational Level		
None	6	3.4
Primary	55	31.4
Secondary	68	38.9
College	46	26.3
Occupation		
Professional	69	39.4
House wife	31	17.7
Unemployed	75	42.9
Room sharing		
Yes	115	65.7
No	60	34.3
Number of patients in household		
1	170	97.1
>1	5	2.9
Smoking status		
Smoker	9	5.1
Previous smoker	4	2.3
Non smoker	162	92.6
Alcohol use		
Yes	29	16.6
No	146	83.4
HIV sero-status		
Positive	16	9.1
Negative	159	90.9

Table 2: prevalence of LTBI and demographics of household contacts

Characteristic	N	Total	(%)
Gender			
Male	30	51	58.8
Female	67	123	54.5
Age group			
18-29	26	53	49.1
30-39	42	66	63.6
40-49	17	28	60.7
50-59	11	22	50.0
60-69	1	4	25.0
70-79	0	1	0
Relationship to patient			
Spouse	14	20	70.0
Others	83	154	53.9
Duration of association			
1-4 Weeks	30	57	52.6
4.1-8 Weeks	18	32	56.3
8.1-12 weeks	8	10	80.0
16.1-20 Weeks	3	6	50.0
>20.1 Weeks	38	68	55.1
Educational Level			
None	4	6	66.7
Primary	31	54	57.4
Secondary	40	68	58.8
College	22	46	47.8
Occupation			
Professional	37	69	53.6
House wife	18	31	58.1
Unemployed	42	74	56.8
Room sharing with TB patients			
Yes	68	114	59.6
No	29	60	48.3
Number of TB patients in household			
1	95	168	56.2
2	2	5	40.0
Smoking status			
Smoker	5	9	55.6
Previous smoker	2	4	50.0
Non smoker	90	161	55.9
Alcohol use status			
Yes	14	28	50.2
No	83	146	56.8
HIV sero-status			
Positive	6	16	37.5
Negative	9	158	57.6

Article

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Table 3: risk factors for LTBI among TB household contacts, N = 174

	LTBI Positive	OR	95% CI	p-value
Gender				
Male	30 (58.8)	1.19	0.62 - 2.31	0.531
Female	67 (54.5)	0.89	0.42 - 1.57	0.531
Age group				
18-29	26 (49.1)			Ref.
30-39	42 (63.6)	1.82	0.87 - 3.79	0.110
40-49	17 (60.7)	1.60	0.63 - 4.07	0.317
50+	12 (44.4)	0.83	0.32 - 2.11	0.696
Relation to patient				
Spouse	14 (70.0)	2.00	0.72 - 5.47	0.173
Others	83 (53.9)	0.50	0.18 - 1.37	0.173
Duration of association				
1-4 Weeks	30 (49.1)			Ref.
4.1-8 Weeks	18 (63.6)	1.16	0.48 - 2.76	0.742
8.1-12 weeks	8(60.7)	3.60	0.70 - 18.5	0.107
16.1-20 Weeks	3 (50.0)	0.90	0.16 - 4.84	0.902
> 20.1 Weeks	38 (25.0)	1.10	0.54 - 2.23	0.784
Educational Level				
None	4 (66.7)			Ref.
Primary	31 (57.4)	0.67	0.11 - 4.00	0.663
Secondary	40 (58.8)	0.71	0.12 - 4.17	0.708
College	22 (47.8)	0.46	0.07 - 2.76	0.385
Occupation				
Professional	37 (53.6)			Ref.
House wife	18 (58.1)	1.20	0.50 - 2.82	0.680
Unemployed	42 (56.8)	1.14	0.58 - 2.20	0.707
Room sharing				
Yes	68 (59.6)	1.58	0.84 - 2.97	0.153
No	29 (48.3)	0.63	0.33 to 1.19	0.153
Patients in household				
1	95 (56.2)	1.93	0.31 - 11.8	0.472
2	2 (40.0)	0.52	0.08 - 3.19	0.472
Smoking status				
Smoker	5 (55.6)			Ref.
Previous smoker	2 (50.0)	0.80	0.07 - 8.48	0.853
Non smoker	90 (55.9)	1.01	0.26 - 3.92	0.984
Alcohol use				
Yes	14 (50.0)	0.76	0.33 - 1.71	0.504
No	83 (56.8)	1.32	0.58 - 2.96	0.504
HIV sero status				
Positive	6 (37.5)	0.44	0.15 - 1.28	0.123
Negative	91 (57.6)	2.26	0.78 - 6.54	0.123

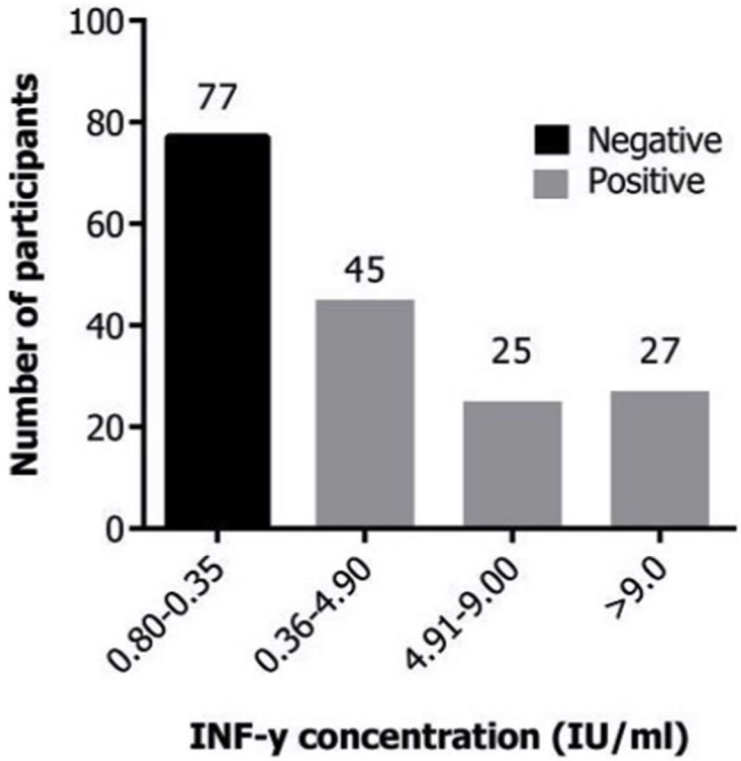

Figure 1: distribution of ELISA test values for the QFT-GIT test among household contacts

APPENDIX 10: PUBLICATION 2 PAN AFRICAN MEDICAL JOURNAL

Article

PanAfrican Medical Journal

Research

Association between human leukocyte antigen class II (HLA-DRB and -DQB) alleles and outcome of exposure to *Mycobacterium tuberculosis*: a cross-sectional study in Nairobi, Kenya

Susan Odera, Marianne Mureithi, Andrew Aballa, Noel Onyango, Samwel Kazungu, Simon Ogolla, George Kaiyare, Omu Anzala, Julius Oyugi

Corresponding author: Susan Odera, Department of Medical Microbiology and Immunology, University of Nairobi, Nairobi, Kenya. sueodera@yahoo.com

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Association between human leukocyte antigen class II (HLA-DRB and -DQB) alleles and outcome of exposure to *Mycobacterium tuberculosis*: a cross-sectional study in Nairobi, Kenya

Susan Odera[1,&], Marianne Mureithi[1,2], Andrew Aballa[3], Noel Onyango[4], Samwel Kazungu[2], Simon Ogolla[2], George Kaiyare[5], Omu Anzala[1,2], Julius Oyugi[1,6]

[1]Department of Medical Microbiology and Immunology, University of Nairobi, Nairobi, Kenya, [2]Kenya Aids Vaccine Initiative (KAVI) Institute of Clinical Research, University of Nairobi, Nairobi, Kenya, [3]Department of Medical Laboratory Sciences, School of Medicine, Kenyatta University, Nairobi, Kenya, [4]Department of Clinical Medicine and Therapeutics, School of Medicine, University of Nairobi, Nairobi, Kenya, [5]Biozeq Kenya Molecular

Laboratory, Nairobi, Kenya, [4]Institute of Tropical and Infectious Diseases, University of Nairobi, Nairobi, Kenya

[&]Corresponding author
Susan Odera, Department of Medical Microbiology and Immunology, University of Nairobi, Nairobi, Kenya

Abstract

Introduction: *human leukocyte antigen (HLA) class II alleles play an important role in the early immune response to tuberculosis (TB) by presenting antigenic peptides to CD4+ T cells, hence polymorphisms in those genes can influence the efficiency of the immune response to infection and progression to active disease.* ***Methods:*** *an analytical cross-sectional study of adult pulmonary tuberculosis (PTB) patients at Mbagathi County Hospital, Nairobi and their HHCs. Sociodemographic data were captured on questionnaires and clinical data extracted from patient files. Intravenous blood samples were drawn for interferon-gamma release assay (IGRA) to determine latent tuberculosis infection (LTBI) among HHCs, and for extraction of DNA used in typing of HLA-DQB1 and HLA-DRB1 alleles by PCR sequence specific primer amplification. Chi-square and Fisher's exact test were used to compare the HLA type II allele frequencies of LTBI negative HHCs, LTBI positive HHCs and active TB patients. Logistic regression was used to adjust for HIV status.* ***Results:*** *the HLA-DQB1 and HLA-DRB1 alleles were analyzed in 17 PTB and 37 HHCs. Nineteen (19) HHCs were LTBI positive, while 18 were LTBI negative. The frequency of DRB3*1 was 0.17-fold lower [95% CI=0.03-0.83] among PTB patients compared to HHCs before adjustment for HIV status (p=0.048). The frequency of the DRB5*2 allele was significantly higher (p=0.013) among PTB patients (23.5%) compared to HHCS (0.00%). After adjusting for HIV status, the frequency of DRB1*14 was 12-fold higher [95% CI=1.11-138.2] among PTB patients compared to HHCs (p=0.040).* ***Conclusion:*** *the higher frequencies of HLA-DRB5*2 and HLA-DRB1*14 alleles in PTB patients suggest a likely association with progression to active PTB. The higher frequency of HLA-DRB3*1 allele among LTBI negative HHCs shows its likely protective role against M. tuberculosis infection in this population.*

Introduction

Tuberculosis (TB) continues to be an infectious disease of public health concern, with the highest-burden afflicting sub-Saharan countries. Recent efforts by World Health Organization to reduce and possibly eliminate TB emphasize the importance of treating the pool of individuals with latent TB infection (LTBI) and putting more effort into researching more efficacious population-specific vaccines. Pathogen, host, and environmental factors all play a role in the outcome of exposure to *Mycobacterium tuberculosis*. Although many environmental factors that contribute to the sustained high prevalence of TB are known, it is not clear to what extent host genetic differences may account for the higher levels of infection observed in some countries [1,2]. Infectious diseases exert significant selective genetic pressure, and the genes involved in the immune response are exquisitely diverse [3]. This diversity explains, in part, why some people resist infection more successfully than others [4]. The observations suggest a vital role for host genetic variability in the susceptibility to exogenous pathogens [5]. The frequencies of some host factors, most significantly alleles associated with susceptibility or resistance to TB, may differ among populations according to ethnic or racial backgrounds. Global data shows that TB occurs at different rates among particular races, ethnicities, and families, indicating a genetic predisposition to TB susceptibility [6].

Studies have also shown that certain HLA class II alleles are protective against infection or rapid progression after infection, with a great degree of variation. Several case-control, candidate-gene, family studies, and genome-wide association studies (GWAS) suggest the association of host genetic factors to TB susceptibility or resistance in various ethnic populations. They have reported genetic markers that can predict TB development in human leukocyte antigen (HLA) and non-HLA genes like killer immunoglobulin-like receptor (KIR), toll-like receptors (TLRs), cytokine/chemokines and their receptors, vitamin D receptor (VDR), among others [7]. The HLA class II alleles play an important role in the early immune response to TB by presenting antigenic peptides to CD4 T cells. HLA molecules are among the most polymorphic human gene products known. Polymorphisms affecting antigen processing and presentation, and hence the profile of cytokines secreted, can influence the efficiency of the immune response to infection and can play a significant role in the host response [8]. This diversity would also pose a limitation on the global use of a vaccine candidate if the analysis was based on pathogen strains and allele frequencies of HLA molecules in only specific populations. Therefore, the distribution and frequency of HLA alleles within a certain population would be very useful baseline data for vaccine design studies and in formulation of population-specific intervention strategies, especially in countries classified as having a high TB burden. Here, we analyzed the frequencies of HLA class II alleles of the DRB- and DQB- loci in pulmonary tuberculosis (PTB) patients and compared these frequencies with those of their adult household contacts stratified as LTBI positive and LTBI negative using the Interferon Gamma Assay (IGRA) test. We hypothesized that certain HLA class II alleles might be associated with protection against infection, or upon infection, with failure to progress to active TB.

Objective: to evaluate the association between Human Leukocyte Antigen Class II (HLA-DRB and -DQB) alleles frequencies and outcome of exposure to *Mycobacterium tuberculosis*.

Methods

Study setting and design: the study participants consisted of adult PTB patients and their household contacts (HHCs), and all were enrolled at Mbagathi County Hospital in 2016. The hospital is a public health facility under the County Government of Nairobi´s Department of Health Services. It provides a broad range of services, including an outpatient clinic and in-patient wards for PTB patients. All the participants gave informed consent before being enrolled into the study. Thereafter, a study questionnaire was administered after which biological specimens were collected. An analytical cross-sectional design was adopted for this study. Consenting individuals were enrolled using a convenience sampling technique.

Study population

Pulmonary tuberculosis (PTB) patients: inclusion and exclusion criteria: the PTB patients enrolled were adults diagnosed at Mbagathi County Hospital and confirmed to have TB by sputum smear microscopy and GeneXpert test with complementary information from chest X-rays. One hundred and sixty-six (166) PTB patients were enrolled from the outpatient clinic and in-patient TB wards, and 175 HHCs who provided informed consent were also enrolled. Sociodemographic data on age, gender, marital status, level of education, and clinical data such as HIV sero-status was collected using a questionnaire and from patient files. All HHCs were analysed for LTBI. Genotyping of the HLA-DRB and human leukocyte antigen (HLA-DRB) loci was carried out on 20 randomly selected PTB patients´ samples and alleles were successfully identified in 17 patients. Despite repeated analysis of extracted DNA, some of the DNA samples did not yield results in the sequence-specific amplification polymerase chain reaction (SSP-PCR) analysis.

Household contacts (HHC): inclusion and exclusion criteria: household contacts were persons living with, shared meals and slept under the same roof with a PTB patient. Adult HHCs were enrolled as they accompanied patients to the out-patient TB clinic or hospital visiting hours in the wards. They showed no signs or symptoms of TB, were apparently in good health, were not pregnant, were not on immunosuppressive drugs, had no history of TB infection and had no prior preventive therapy for LTBI. An IGRA test was used to identify HHCs

who had latent TB infection, and based on this, the HHCS were grouped as LTBI positive and LTBI negative. Sociodemographic data was collected using a questionnaire. Confounding factors like race and genetic background in the ethnically heterogeneous urban setting was reduced by recruiting HHCs of the patients, most of whom were relatives of the patient. The presence or absence of HIV in the samples was determined in the laboratory as the IGRA analysis performed. Genotyping of the HLA-DRB and HLA-DRB loci was carried out on 40 randomly selected HHCs´ samples and alleles were successfully identified in 37 HHCs. Despite repeated analysis of extracted DNA, some of the DNA samples did not yield results in the SSP-PCR analysis.

Blood sample collection: blood specimen was used to diagnose LTBI from HHCs and for HLA genotyping. For the IGRA test, 5ml of blood was collected aseptically from HHCs through venous puncture into three QuantiFeron tubes (nil, antigen, and mitogen tubes) in the QuantiFeron®-TB Gold in-tube kit (QFT-GIT), (Qiagen, Germany) and transported immediately to the Biozeq- KAVI-ICR laboratory for processing. For HLA typing, 5ml of blood was collected in EDTA blood collection tubes from the PTB patients and the HHCs. The samples were centrifuged at 3000rpm for 10min. Buffy coat were aliquoted into 2ml screw-cap tubes and stored at -20°C till needed for DNA extraction.

The interferon-gamma release assay (IGRA) test: the QuantiFeron®-TB Gold in-tube test (QFT GIT) was used to perform the IGRA test according to the manufacturer's (Cellestis Qiagen, Germany) instructions where the HHCs were categorized as LTBI positive and negative based on the Elisa value for interferon gamma (IFN-γ). Diagnosis of LTBI among HHCs was described as reported by Odera *et al.* where the overall prevalence of LTBI was 55.7% [9].

Extraction of DNA from blood: deoxyribonucleic acid (DNA) was extracted from the buffy coat of samples using the QIAmp DNA mini kit (qiagen, strasse 1, Hilden Germany) following the manufacturer's instructions. Briefly, 200 µl of the buffy coat was lysed with proteinase K and DNA molecules adsorbed onto silica-gel membranes with a high chaotropic salt concentration. The resulting DNA were washed and eluted in 200 µl of buffer AE and quantified using standard ultraviolet (UV) spectrophotometric analysis. The A260/A280 ratio was between 1.6 - 2.0 via UV spectrophotometry for optimal band visualization.

HLA-DQB and HLA-DRB genotyping: the HLA SSP (sequence specific primers) technique was used to identify HLA-DQB1 and -DRB1 alleles from DNA using the PCR method. Genotyping was performed using the -DR and -DQ low resolution kit (Olerup SSP®, AB Franzengatan 5, SE- 112 51 Stockholm, Sweden) following the manufacturer's instructions. The PCR master mix was prepared using PCR water, deoxynucleotide triphosphate (DNTPs), magnesium chloride ($MgCl_2$) and Taq-polymerase and aliquoted into 96-well plates with primer mixes. The method allowed simultaneous genotyping of two loci on the same plate (multiplex). The extracted DNA (75ng) was added into each well and PCR amplification done. Gel-electrophoresis was done using a qiaxcel automated machine which uses a special commercial cartridge. The PCR products were displayed on the screen after running for 25 minutes on the Qiaxcel machine. After PCR amplification, the gel picture was uploaded into the Olerup SSP Analysis Software where positive bands were marked in the input data section and analyzed. Genotypes were evaluated using the Olerup SSP HLA allele typing software.

Statistical analysis: statistical package for social scientists (SPSS) software (version 25) was used as the statistical analysis tool. The outcomes of exposure to *Mycobacterium tuberculosis* were sterilizing immunity against infection (LTBI negative), clinically latent infection (LTBI positive) and active TB disease. Chi-square test was used to compare HLA alleles frequencies among the infected (PTB patients and LTBI positive HHCs) and the uninfected (LTBI negative HHCs) stratified by gender and HIV status. The Fisher's exact test was used when an expected absolute cell frequency was less than five. Logistic regression was used to control HIV status. Odds ratios (OR) and adjusted odds ratios (AOR) were evaluated as measures of association with a p value <0.05 considered to be statistically significant at 95% confidence interval (CI).

Ethical considerations: the Kenyatta National Hospital/the University of Nairobi Ethics and Research Committee (KNH/UoN ERC) reviewed and approved the study, Reference No. KNH-ERC/A/392. Written informed consent was obtained from TB patients and their HHCs before enrolment in the study. Mbagathi District Hospital/Nairobi County granted permission to conduct the study.

Results

Demographic and clinical characteristics of the study participants: we analyzed the DNA extracts of 60 study participants and identified alleles in HLA-DQB and -DRB loci in 54 study participants comprising 17 PTB patients and 37 HHCs. The DNA extracts from the unyielding samples were re-submitted to the typing protocol but it was unsuccessful. The mean age of the HHCs (36.3±11.2 years) was slightly older than that of PTB patients (32.9±9.8 years) although this was not statistically significantly (p=0.26). Most of the participants were female in both the PTB patients and HHCs groups, at 58.8% and 56.8% respectively. More PTB patients had a past history of smoking (5.9%) compared to HHCs (2.7%) while alcohol consumption was higher among the HHCs (16.2%) than the PTB patients (5.9%). The differences in gender, past history of smoking and alcohol consumption were also not statistically significant. We observed that 41.2% of the PTB patients were HIV positive compared to 100% of HHCs who were all HIV negative (p<0.01) (Table 1).

Distribution of HLA-DRB and HLA-DQB alleles among the study participants by gender: from a total of 54 samples analyzed through SSP-PCR genotyping, we determined the HLA-DRB genotypes in 47 samples from which 18 allelic groups were identified while the HLA-DQB genotypes were determined in 36 samples from which 6 allelic groups were identified. We repeated the PCR typing in the unyielding samples for the specific loci but the typing was unsuccessful. HLA-DRB alleles with frequencies >5% were 12 while all identified HLA-DQB alleles had frequencies >5% (Table 2). We compared the frequency of distribution of alleles across gender (Figure 1) and noted that while the frequency most HLA-DRB alleles was higher among males compared to females (12/18), the difference was not statistically significant before adjusting for HIV status (Table 2). The frequency of DQB alleles was comparable among females and males before adjusting for HIV status. After adjustment for HIV status, the frequency of the DRB1*9 allele was significantly higher among females (19.2%) compared to males (0.0), p=0.034 (Table 2).

Distribution of HLA-DRB and HLA-DQB alleles among pulmonary tuberculosis patients compared to household contacts: when we compared the distribution of the HLA-DRB and HLA-DQB alleles between the patients and HHCs before adjustment for HIV status, the frequency of the DRB5*2 allele was 23.5% higher among PTB patients compared to HHCS (p=0.013). The frequency of DRB3*1 was 0.17-fold lower (95% CI=0.03-0.83) among PTB patients compared to HHCs before adjustment for HIV status. After adjusting for HIV status, the frequency of DRB1*14 was 12-fold higher (95% CI=1.11-138.2) among PTB patients compared to HHCs (p=0.040). The frequencies of other -DRB and -DQB alleles between PTB patients and HHCs were comparable (Table 3).

Distribution of HLA-DRB and HLA-DQB alleles among study participants stratified as pulmonary tuberculosis patients, latent TB negative household contacts, and latent TB positive household contacts: allele distribution between PTB, LTBI-ve, and LTBI +ve HHCs was compared (Figure 2). The results of the IGRA test revealed 19 HHCs were infected (latent TB positive) while 18

were uninfected (latent TB negative). The frequency of the HLA-DRB3*1 allele was 34.4% significantly higher among LTBI -ve HHCs than PTB patients (p=0.049) before adjustment for the HIV status of patients. The frequency of other DRB alleles and all the DQB alleles evaluated was comparable between PTB and LTB+, PTB and LTBI -ve and LTBI +ve and LTBI -ve. After adjusting for HIV status, the frequency of -DRB and -DQB alleles was comparable between PTB and LTBI+, PTB and LTBI -ve and LTBI +ve and LBVI -ve (Table 4).

Discussion

The overall goal of this study was to evaluate the association between HLA-DRB and HLA-DQB alleles frequencies and susceptibility to *Mycobacterium tuberculosis* infection in a Kenyan population. The study participants' mean age and gender distribution was comparable across the patients and HHCs. The mean age of the PTB patients (32.9 years) mirrored the 2019 WHO report of TB in Kenya which documented that the majority of TB infected individuals are in the 25-34 age group (WHO 2019 TB global data report Kenya). Analysis of HLA-DRB locus revealed -DRB1*1 (34.0%) and -DRB3*2 (34.0%) alleles were the highest in the population, followed by -DRB3*1 (31.9%) and -DRB1*4 (19.1%) while the least frequent alleles were -DRB1*2, DRB1*10 and DRB1*16 (all occurring at 2.1%). At the DQB locus, DQB1*3, DQB1*4 and DQB1*6 were the most frequent, being identified among 30.6% of the study population DQB1*1and DQB1*2 were the least frequent (both occurring at 5.6%). A 2014 study report on the diversity of HLA class I and II alleles in an East African population also identified HLA-DRB3*2 and HLA-DQB1*3 among the most common alleles in the -DRB and -DQB loci [10]. Similar to our study, only one HLA-DRB4 allele (DRB4*1) was identified in both study populations. However, observed differences in frequency of other alleles in the two studies could be due to variations in the ethnic background of the populations in the different geographical locations and the gender bias of female participants only in the East African study. A comparison of the frequency distribution of the alleles across gender revealed an even distribution of HLA-DQB alleles. Analysis of the distribution of the HLA-DRB alleles revealed a higher frequency in males compared to females except the HLA-DRB1*9 alleles which was significantly higher (p=0.034) among females after controlling for the HIV sero-status. Few studies have suggested that gender significantly influences the distribution of HLA-DR and -DQ alleles [11], and more research on our study finding should be explored in a larger study.

Comparative analyses were done and frequencies and odds ratios interpreted as the measure of association between HLA allele frequency and susceptibility to tuberculosis infection and/or progression to active TB. This study did not find significant differences in the frequencies of HLA-DQB alleles in the PTB patients, latent tuberculosis-infected, and latent tuberculosis negative HHCs. Therefore, none of the identified HLA- DQB alleles were associated with infection susceptibility or progression to PTB in this population. This finding was in agreement with the study of Duarte *et al.* in 2011 who studied a Portuguese population and found no association between the phenotypic distribution of HLA-DQB in the healthy exposed group (healthy exposed positive and healthy exposed negative) and patients who had active TB [12]. Other studies by Cao *et al.* in the USA and Lombard *et al.* in South Africa also reported no association, complementing our findings [13,14]. However, a study in Uganda reported a negative correlation between TB susceptibility and the DQB1*3 allele, which contradicted our findings [15]. Additionally, other studies in India [16], Thailand [17], and Iran [18] reported a positive correlation between TB susceptibility and expression of DQB alleles. From the available data, the divergent associations might be due to differences in sample size, study designs, and the genetic heterogeneity of the *Mycobacterium tuberculosis* complex, which were not controlled during our analyses. We also had a small sample size that might have underestimated or overestimated the link between the HLA-DQB1 alleles and TB susceptibility or resistance in this population.

The HLA-DRB3*1 allele occurred at a statistically significant higher frequency (p=0.049) among latent TB negative HHCs (46.2%) compared to PTB patients (11.8%). The negative association with susceptibility suggests that this allele could be protective against infection and progression to active TB. Our findings contradicted a 2019 study in Mali, West Africa which reported the HLA-DRB3*01: 01 allele among others had significant association with *Mycobacterium tuberculosis* infection compared to healthy controls [19]. Unlike our study which did not control for the strains in the *Mycobacterium tuberculosis* complex, the Mali study analyzed whether the association of HLA with TB susceptibility was strain dependent with epidemiological evidence that *M. africanum* was more common in West Africa compared to other regions in the continent. Furthermore, the variation in HLA-DRB allele distribution across populations in Africa's Western and Eastern regions could also contribute to the different findings. Because of the paucity of published literature on the association of the HLA-DRB3*1 allele with TB disease, we could not make further comparisons with our findings. Notably, the DRB3*1 allele was one of the most common HLA-DRB alleles occurring in 31.9% of our study population. Pathogens often adapt to the most frequent HLA alleles, while rarer ones have a selective advantage because of host-pathogen interactions [20], which could explain why the most frequent alleles in the study population were not linked with TB susceptibility/progression. Furthermore, the HLA-DRB5*2 allele occurred at a statistically significant (p=0.013) higher frequency among PTB patients compared to HHCs. Although its frequency was low in our study population (8.5%), the higher occurrence in PTB patients (23.5%) compared to HHCs (0.0%), hints at a positive association with T.B. progression and/or susceptibility to infection. Our finding was in agreement with a 2017 study of susceptibility loci associated with tuberculosis in Han Chinese which reported that candidate genes that were significantly associated with TB included among others genes in the HLA-DRB5 [21]. The HLA-DRB5*2 allele may have the "rare allele advantage" in this population which argues that pathogens adapt to HLA alleles that are more prevalent in the population, thus such alleles would not have a significant role to play in the immune-pathogenicity of those pathogens [22].

All HHCs in this study population were HIV negative, while 41.2% of the PTB patients were HIV positive. HIV infection is a known factor that increases the risk of progression to active TB. Therefore, during statistical analysis on the distribution of the HLA alleles, the HIV sero-status of the study participants was controlled for. After controlling for HIV status in our study population, the HLA-DRB1*14 alleles were significantly higher (p=0.040) among the PTB patients compared to HHCs, suggesting its likely contribution to development of active TB. This finding was in agreement with a study by Duarte *et al.* in 2011 in a Portuguese population where the frequency of the HLA-DRB1*14 allele was significantly higher in TB patients compared to healthy exposed controls [12]. Similar reports from India [23] and Russia [24] also suggested that this could be a susceptibility allele for evolution from infection to active TB. The role of the HLA-DRB1*14 allele in TB immunopathogenesis is also highlighted in a study on serology-based antibodies for TB diagnosis where researchers documented a significant increase in the frequency of HLA-DRB1*14 among subjects with high antibody response levels compared to those with low levels [25]. Our findings suggest the HLA-DRB5*2 and HLA-DRB1*14 alleles may be risk factors for progression to active TB in our study population. The HLA-DRB5*2 had a positive association with progression to active TB before controlling for HIV sero-status while the HLA-DRB1*14 alleles were a significant factor in development of active TB after controlling for HIV sero-status. The chances of an individual with recently acquired T.B. infection progressing to active disease are higher with HIV coinfection. Many studies postulate the likely role of HLA polymorphisms in the differential development of TB in HIV infected individual [26-28]. Furthermore, a study conducted

in China highlighted the important role that the HLA-DRB1*14 plays in accelerated disease progression in HIV infection [29]. Closely related to this, our study findings suggest that in our study population, the HLA-DRB1*14 allele plays a synergistic role in disease progression in an individual exposed to both HIV and *Mycobacterium tuberculosis*.

Conclusion

This study provides an important insight into the nature of HLA class II alleles and *Mycobacterium tuberculosis* infection and active TB in this population. The findings suggest the HLA-DRB5*2 and HLA-DRB1*14 alleles may be risk factors for developing active TB. Although the varied contribution of each allele to risk of latent or active TB before and after controlling for HIV is open to further research, both alleles would make natural candidates for further research on genetic susceptibility to TB. The study also highlights the potential protective role that the HLA-DRB3*1 allele may have against *Mycobacterium tuberculosis* infection. However, the evidence from this study is limited by the small sample size. We recommend further studies to investigate the roles of these alleles in TB immunopathogenesis as predictive biomarkers of infection outcome and vaccine candidate development in this population.

What is known about this topic

- *The human leucocyte antigen (HLA) class II alleles play an important role in the early immune response to tuberculosis (T.B.) by presenting antigenic peptides to CD4+ T cells, hence polymorphisms in those genes can influence the efficiency of the immune response to infection and progression to active disease;*
- *The nature and localization of HLA polymorphism observed in populations is likely to be functionally significant in terms of disease susceptibility because different populations exhibit frequency distribution of alleles and extended haplotypes particular to that group.*

What this study adds

- *This study contributes to the pool of knowledge on the distribution of the HLA-DRB and HLA-DQB alleles in this population, by highlighting the most frequent and least frequent alleles;*
- *This study also provides important insight into the HLA class II alleles of the -DRB locus that would make natural candidates for further research on genetic susceptibility to T.B. in this population.*

Competing interests

The authors declare no competing interest.

Authors' contributions

Conception and study design: Susan Odera, Julius Oyugi, Omu Anzala, Marianne Mureithi; data collection: Susan Odera, Simon Ogolla, George Kaiyare; data analysis and interpretation: Samwel Kazungu, George Kaiyare, Simon Ogolla, Andrew Aballa, Noel Onyango, Susan Odera, Julius Oyugi; manuscript drafting: Susan Odera, Julius Oyugi, Andrew Aballa, Omu Anzala, Marianne Mureith; manuscript revision: Andrew Aballa, Noel Onyango, Simon Ogolla, Omu Anzala, Marianne Mureithi, Susan Odera, Julius Oyugi. All the authors have read and agreed to the final manuscript.

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Table 1: demographic and clinical characteristics of PTB patients compared to HHCs

Parameters		PTB (17)	HHC (37)	OR (95% CI)	p value
Age in years	Mean±SD	32.9±9.8	36.3±11.2	-	0.26
Gender	Male	7 (41.2)	16 (43.2)	0.99 (0.29- 3.13)	1.00
	Female	10 (58.8)	21 (56.8)	1.09 (0.32-3.41)	1.00
Cigarette smoking		1 (5.9)	1 (2.7)	2.25 (0.11-43.7)	0.53
Alcohol consumption		1 (5.9)	6 (16.2)	0.32 (0.03- 2.44)	0.41
HIV status	Positive	7 (41.2)	0 (0.0)	-	<0.01
	Negative	10 (58.8)	37 (100)	-	<0.01

HIV: human immunodeficiency virus; PTB: pulmonary tuberculosis; HHC: household contacts; OR: odds ratio; CI: confidence interval

Table 2: distribution of HLA-DRB and HLA-DQB alleles among the study participants by gender

	N	%	Male	Female	p value	Adjusted p value*
DRB alleles	**47**	**100**	**N=21**	**N=26**		
DRB1*1	16	34.0	10 (47.6)	6 (23.1)	0.122	0.088
DRB3*2	16	34.0	9 (42.9)	7 (26.9)	0.355	0.270
DRB3*1	15	31.9	8 (38.1)	7 (26.9)	0.533	0.428
DRB1*4	9	19.1	4 (19.0)	5 (19.2)	1.000	0.937
DRB1*13	8	17.0	2 (9.5)	6 (23.1)	0.269	0.169
DRB1*3	7	14.9	4 (19.0)	3 (11.5)	0.684	0.476
DRB1*11	7	14.9	2 (9.5)	5 (19.2)	0.436	0.427
DRB1*8	6	12.8	4 (19.0)	2 (7.7)	0.386	0.249
DRB1*9	5	10.6	0 (0.0)	5 (19.2)	0.056	0.034
DRB1*14	4	8.5	2 (9.5)	2 (7.7)	1.000	0.916
DRB5*1	4	8.5	1 (4.8)	3 (11.5)	0.617	0.457
DRB5*2	4	8.5	2 (9.5)	2 (7.7)	1.000	0.096
DRB1*7	2	4.3	0 (0.0)	2 (7.7)	0.495	0.998
DRB1*15	2	4.3	1 (4.8)	1 (3.8)	1.000	0.945
DRB4*1	2	4.3	0 (0.0)	2 (7.7)	0.495	0.998
DRB1*2	1	2.1	1 (4.8)	0 (0.0)	0.447	0.998
DRB1*10	1	2.1	1 (4.8)	0 (0.0)	0.447	0.261
DRB1*16	1	2.1	1 (4.8)	0 (0.0)	0.447	0.998
DQB alleles	**36**	**100**	**N=13**	**N=23**		
DQB1*3	11	30.6	5 (38.5)	6 (26.1)	0.474	0.999
DQB1*4	11	30.6	3 (23.1)	8 (34.8)	0.708	0.999
DQB1*5	11	30.6	4 (30.8)	7 (30.4)	1.000	0.416
DQB1*6	10	27.8	4 (30.8)	4 (26.1)	0.679	0.583
DQB1*1	2	5.6	0 (0.0)	2 (8.7)	0.525	0.959
DQB1*2	2	5.6	0 (0.0)	2 (8.7)	0.525	0.959

Table 3: distribution of DRB and DQB alleles among PTB patients and HHCs

	PTB	HHCs	OR (95% CI)	p value	AOR (95% CI) *	p value
DRB alleles	**N=17**	**N=30**				
DRB1*1	6 (35.3)	10 (33.3)	1.09 (0.32-3.60)	1.000	1.33 (0.30-5.83)	0.702
DRB1*2	1 (5.9)	0 (0.0)	-	0.361	-	0.998
DRB1*3	1 (5.9)	6 (20.0)	0.25 (0.02-1.93)	0.395	0.00 (0.00-)	0.999
DRB1*4	5 (29.4)	4 (13.3)	2.70 (0.60-9.88)	0.251	2.78 (0.50-15.4)	0.241
DRB1*7	0 (0.0)	2 (6.7)	-	0.528	0.00 (0.00-)	0.999
DRB1*8	3 (17.6)	3 (10.0)	1.92 (0.40-8.99)	0.652	2.25 (0.31-15.9)	0.416
DRB1*9	2 (11.8)	3 (10.0)	1.20 (0.19-6.39)	1.000	0.00 (0.00-)	0.999
DRB1*10	0 (0.0)	1 (3.3)	-	1.000	-	0.999
DRB1*11	2 (11.8)	5 (16.7)	0.66 (0.12-3.97)	1.000	0.00 (0.00-)	0.999
DRB1*13	3 (17.6)	5 (16.7)	1.07 (0.25-5.24)	1.000	2.14 (0.40-11.2)	0.368
DRB1*14	3 (17.6)	1 (3.3)	6.21 (0.82-82.9)	0.127	12.42 (1.11-138.2)	0.040
DRB1*15	0 (0.0)	2 (6.7)	-	0.528	0.00 (0.00-)	0.999
DRB1*16	0 (0.0)	1 (3.3)	-	1.000	-	0.999
DRB3*1	2 (11.8)	13 (43.3)	0.17 (0.03-0.83)	0.048	0.00 (0.00-)	0.999
DRB3*2	3 (17.6)	13 (43.3)	0.28 (0.07-1.08)	0.111	0.14 (0.01-1.296)	0.084
DRB4*1	1 (5.9)	1 (3.3)	1.81 (0.09-35.4)	1.000	3.22 (0.18-56.8)	0.424
DRB5*1	3 (17.6)	1 (3.3)	6.21 (0.82-82.9)	0.127	7.25 (0.58-90.5)	0.124
DRB5*2	4 (23.5)	0 (0.0)	-	0.013	-	0.998
DQB alleles	**N=14**	**N=22**				
DQB1*1	1 (7.1)	1 (4.5)	1.61 (0.07-31.9)	1.000	0.00 (0.00-)	0.999
DQB1*2	0 (0.0)	2 (9.1)	-	0.511	-	0.999
DQB1*3	4 (28.6)	7 (31.8)	0.85 (0.23-3.26)	1.000	0.71 (0.11-4.47)	0.719
DQB1*4	6 (42.9)	5 (22.7)	2.55 (0.64-11.6)	0.273	2.04 (0.35-11.67)	0.423
DQB1*5	5 (35.7)	6 (27.3)	1.48 (0.34-5.83)	0.715	1.60 (0.28-8.85)	0.590
DQB1*6	3 (21.4)	7 (31.8)	0.58 (0.14-2.45)	0.706	0.71 (0.11-4.47)	0.719

PT: pulmonary tuberculosis; HHCs: household contacts; HLA: human leukocyte antigen; OR: odds ratio; CI: confidence interval; AOR: adjusted odds ratio; *adjusted for HIV status of participants

Table 4: distribution of HLA-DRB and HLA-DBQ alleles between PTB patients, LTBI positive HHCs and LTBI negative HHCs

	Group			p value			Adjusted p value*		
	PTB	LTB+	LTB-	PTB/ LTB+	PTB/ LTB-	LTB+ /LTB-	PTB/ LTB+	PTB/ LTB-	LTB+ /LTB-
DRB alleles	**N=17**	**N=17**	**N=13**						
DRB1*1	6 (35.3)	6 (35.3)	4 (30.8)	1.000	1.000	1.000	0.807	0.646	0.795
DRB1*2	1 (5.9)	0 (0.0)	0 (0.0)	1.000	1.000	-	0.998	0.999	-
DRB1*3	1 (5.9)	4 (23.5)	2 (15.4)	0.335	0.564	0.672	0.999	0.999	0.583
DRB1*4	5 (29.4)	2 (11.8)	2 (15.4)	0.398	0.426	1.000	0.253	0.407	0.773
DRB1*7	0 (0.0)	2 (11.8)	0 (0.0)	0.484	-	0.492	0.050	-	0.999
DRB1*8	3 (17.6)	1 (5.9)	2 (15.4)	0.601	1.000	0.564	0.286	0.773	0.406
DRB1*9	2 (11.8)	3 (17.6)	0 (0.0)	1.000	0.492	0.237	0.999	1.000	0.999
DRB1*10	0 (0.0)	0 (0.0)	1 (7.7)	-	0.433	0.433	-	0.999	0.998
DRB1*11	2 (11.8)	3 (17.6)	2 (15.4)	1.000	1.000	1.000	0.999	0.999	0.869
DRB1*13	3 (17.6)	2 (11.8)	3 (23.1)	1.000	1.000	0.627	0.253	0.708	0.417
DRB1*14	3 (17.6)	1 (5.9)	0 (0.00	0.601	0.237	1.000	0.121	0.999	0.999
DRB1*15	0 (0.0)	0 (0.0)	2 (15.4)	-	0.179	0.179	-	0.999	0.998
DRB1*16	0 (0.0)	1 (5.9)	0 (0.0)	1.000	-	1.000	0.999	-	0.999
DRB3*1	2 (11.8)	7 (41.2)	6 (46.2)	0.117	0.049	1.000	0.999	0.999	0.785
DRB3*2	3 (17.6)	7 (41.2)	6 (46.2)	0.258	0.123	1.000	0.114	0.087	0.785
DRB4*1	1 (5.9)	1 (5.9)	0 (0.0)	1.000	1.000	1.000	0.696	0.999	0.999
DRB5*1	3 (17.6)	1 (5.9)	0 (0.0)	0.601	0.237	1.000	0.286	0.999	0.999
DRB5*2	4 (23.4)	0 (0.0)	0 (0.0)	0.102	0.112	-	0.998	0.999	-
DQB alleles	**N=14**	**N=11**	**N=11**						
DQB1*1	1 (7.1)	0 (0.0)	1 (9.1)	1.000	1.000	1.000	1.000	0.999	0.999
DQB1*2	0 (0.0)	2 (18.2)	0 (0.0)	0.183	-	0.476	0.999	-	0.999
DQB1*3	4 (28.6)	4 (36.4)	3 (27.3)	1.000	1.000	1.000	0.601	0.912	0.648
DQB1*4	6 (42.9)	2 (18.2)	3 (27.3)	0.233	0.676	1.000	0.353	0.657	0.613
DQB1*5	5 (35.7)	4 (36.4)	2 (18.2)	1.000	0.406	0.635	0.960	0.353	0.346
DQB1*6	3 (21.4)	3 (27.3)	4 (36.4)	1.000	0.656	1.000	0.912	0.601	0.648

PTB: pulmonary tuberculosis; LTB+: latent TB positive; LTB-: latent TB negative; *adjusted for HIV status

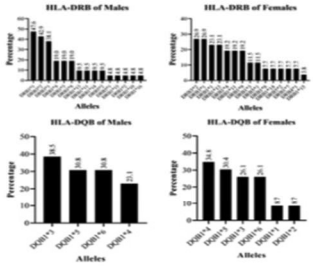

Figure 1: distribution of HLA-DRB and HLA-DQB alleles among the study participants across gender

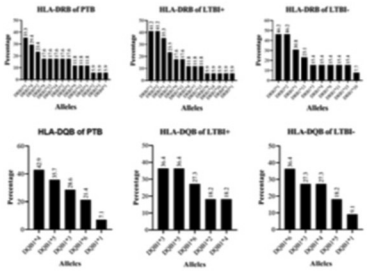

Figure 2: illustration of the distribution of HLA-DRB and HLA-DBQ alleles between PTB patients, LTBI positive HHCs and LTBI negative HHCs

APPENDIX 11: COMMENTARY ARTICLE: Challenges faced by House-Hold Contacts of Pulmonary Tuberculosis Patients in an urban setting in Nairobi, Kenya

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SCIENTIFIC Research and Community

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Challenges Faced by House-Hold Contacts of Pulmonary Tuberculosis Patients In An Urban Setting In Nairobi, Kenya

Susan Odera[1], Julius Oyugi[1,2], Angeline Kirui[3], Andrew Abalia[4], Noel Onyango[5], Comfort Nyando[6], Meshack Obwogi[7], Idah Ombura[8], Onu Anzalaa[3,9] and Marianne Mureithi [3,9]

[1]Department of Medical Microbiology, University of Nairobi, Kenya

[2]University of Nairobi Institute of Tropical and Infectious Diseases, University of Nairobi, Kenya

[3]School of Nursing, University of Nairobi, Kenya

[4]Department of Medical Laboratory Sciences, School of Medicine, Kenyatta University, Kenya

[5]Department of Clinical Medicine and Therapeutics, School of Medicine, University of Nairobi, Kenya

[6]School of Public Health and Community Development, Maseno University, Kenya

[7]Mbagathi County Hospital, Kenya

[8]Directorate of Health Standards and Quality Assurance and Regulations, Ministry of Health, Kenya

[9]KAVI-Institute of Clinical Research, University of Nairobi, Kenya

ABSTRACT

Close contacts of active pulmonary tuberculosis (PTB) patients are at higher risk of infection as the confirmed cases remain highly infectious before and while in the early stages of treatment. This work highlights the encounters and perspectives of household contacts (HHCs) of PTB patients in an urban setting in Kenya, with a focus on accessibility to health services, interactions within the community, and the risk of infection at home. A multimethod study design involving descriptive cross-sectional analysis and informal interviews was used. The study participants were recruited from tuberculosis (TB) isolation wards and outpatient clinics of Mbagathi County Hospital in Nairobi, Kenya. Data was collected using structured questionnaires and informal interviews. Results revealed improved access to treatment by PTB patients. However, the global goal of eliminating TB infections by minimizing latent tuberculosis reactivation remains a challenge in this population primarily because most of the HHCs lacked knowledge on diagnosis and treatment of latent tuberculosis infection (LTBI). Most participants were residents of informal settlements in Nairobi characterized by small and poorly planned housing structures with poor waste management systems. In most houses, the living space doubled as cooking and sleeping area. There was therefore a high exposure of spouses, children and other persons living with the patients. We recommend that further education be provided to HHCs to increase awareness on available testing and preventive treatment for LTBI, and infection prevention practices at the household level. Furthermore, additional resources should be offered to economically disadvantaged patients to support their social and treatment needs.

***Corresponding author**

Susan Odera, Department of Medical Microbiology, University of Nairobi. P.O. Box 19676, code 00200, Nairobi, Kenya. Email: sueodera7@gmail.com

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Introduction

Tuberculosis is an infectious disease caused by M. tuberculosis, an acid-fast bacillus that is transmitted primarily by inhalation of infectious droplet nuclei. Due to the mode of transmission of the pathogen, individuals who are close contacts of persons with active PTB have a higher risk of infection as the confirmed cases remain highly infectious before and while in the early stages of treatment.

The World Health Organization (WHO) has classified TB as one of the top 10 causes of death worldwide and the leading cause of death from a single infectious agent, ranking above HIV/AIDS. Several studies have been conducted in high burden TB areas to identify determinant factors associated with TB in adult populations and they can be broadly categorized as environmental, socioeconomic, and host/pathogen genetics [1].

Individuals who are predisposed to infection due to prolonged exposure periods include healthcare workers, HHCs, schoolmates, and workmates [2]. The HHCs are the primary caregivers of the patients while they are at home, and subsequently, have a higher

patients and their household contacts in Kenya". The Kenyatta National Hospital/the University of Nairobi Ethics and Research Committee (KNH/UoN ERC) reviewed and approved the study, Reference No. KNH- ERC/A/392. Permission to conduct the study was obtained from Mbagathi County Hospital, Nairobi, Kenya.

Competing Interests

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Authors' Contributions

Susan Odera, Julius Oyugi, Omu Anzala and Marianne Mureithi participated in the development of the study proposal, recruitment of study participants, processing of clinical specimen, data entry and analysis, and manuscript writing. Angeline Kirui, Andrew Aballa, Noel Onyango and Idah Ombura conducted data analysis and critically reviewed the manuscript. Comfort Ananda and Meshack Obwogi participated in collection and review of sociodemographic data and manuscript writing. All the authors approved the final version to be published.

Acknowledgments

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cumulative exposure to M. Tuberculosis through close physical contact and social interactions. In this report, we explored the level of awareness on LTBI diagnosis and treatment among HHCs of PTB patients and document their experiences of living with and caring for PTB patients at home. The data will be useful in identifying important gaps that could inform policies and strategies for TB prevention strategies at the household level.

Methods

Study Site: The study participants were recruited at Mbagathi County Hospital in Nairobi, Kenya. It provides broad range of services that include an outpatient clinic and in-patient wards for PTB patients. The majority of the patients are from Kibera slums, the largest informal settlements in Africa.

Study Participants: The PTB patients were diagnosed by chest radiography, smear microscopy and GeneXpert at Mbagathi Hospital. Household contacts were defined as individuals who were living under the same roof with a PTB patient and have a common living room and cooking arrangement. Household contacts were approached during ward visits and at the outpatient clinic as they accompanied their patients. Participants were informed about the study, and those who gave their approved consent were recruited.

Study Size: One hundred and sixty six (166) TB patients were identified and from these, 175 HHCs who agreed to participate in the study gave their informed consent and were recruited. A structured questionnaire was used to record data on socio-demographic characteristics of the study participants. Informal interviews of individual HHCs for supplementary information on their experiences and clinical data was abstracted from the patient files.

Study Questionnaire: The structured questionnaire was designed to evaluate likely risk factors for LTBI based on published literature, and also included open ended questions to capture individual perspectives of the study participants on care of PTB patients. It was piloted on 10 participants before being used in the study during which all ambiguous questions were reframed for clarity.

Study Design: This was a multimethods study that involved a descriptive cross-sectional study design and informal interviews. Data was collected through structured interviews guided by a questionnaire and through unstructured conversations where the participants shared their experiences living with and caring for a PTB patient at home.

Results

Socio-Demographics of the Household contacts of PTB patients: A total of 175 HHCs of 166 PTB patients were recruited. Most of the HHCs [65.7% (115/175)], shared a one or two-roomed house with the patient and 0.03% (5/175) of them had more than one PTB patient in their household. Of the total participants, 11.4% (20/175) were spouses of the PTB patients while the rest were siblings, members of extended family, or friends. The majority of HHCs were female 70.3% (123/175) and those in the productive age of 30-39 years were 37.7% (66/175). Regarding schooling, most of the participants [96.6% (69/175)] had attained formal education with 26.3 % (46/175) attaining college-level education. Only 39.4% (69/175) were employed although most were on casual terms. History of cigarette smoking, alcohol consumption, and HIV seropositivity was reported among 5.1% (5), 16.6% (29/175), and 9.1% (16/175) of HHCs respectively.

Perspectives of the PTB patients: About 82.5% (137/166) of the patients indicated they had access to information on PTB, while 79.5% (132/166) and 83.1% (138/166) had easy access to the diagnostic center and prescribed medication respectively. Access to adequate nutrition was 82.5% (137/166), while support from the community and family members was received by 84.9% (141/166) of PTB patients. The summary of perspectives of PTB patients is as shown (refer to Table 1).

Table 1: The Perspective of PTB Patients on Care

	n	% (n/N)
Access to information on TB	137	82.5
Access to a diagnostic centre	132	79.5
Access to prescribed medication	138	83.1
Adequate nutrition	137	82.5
Social support from household contacts & community	141	84.9

N=166 (total number of PTB patients)

Perspectives of HHCs of PTB patients

A majority of the HHCs at 86.6% (154/175) were able to access current information on PTB whenever they visited the hospital. Almost all HHCs at 92.0% (161/175) stated that their patients received counseling after diagnosis while a majority of the 82.9% (145/175) reported that their patients were able to obtain prescribed medication without any challenges. A majority of HHCs at 94.3% (165/175) stated that the PTB patients received social support from other household members. The summary of perspectives from HHCs is as shown (refer to Table 2).

Table 2: Perspective of the HHCs of PTB patients

	n	% (n/N)
Access to current information on TB	154	88.6
Availability of prescribed medication	145	82.9
Adequate resources for nutrition	153	87.4
TB patient counselled after diagnosis	161	92.0
Social support from household members	165	94.3

N=175 (total number of HHCs interviewed)

Additionally, the HHCs shared their experiences as caregivers to PTB patients during data collection that were recorded and transcribed. This was done through unstructured interviews. Some excerpts were reported verbatim from the recorded responses as follows:

"This disease has made me very lonely. Since my husband was diagnosed no one comes to visit and even my neighbors are not as welcoming as they used to be". Respondent A

"I wonder if there is something wrong with my family. This is the third person in our family to get this disease. Perhaps there is something we are not aware of that is making us sick". Respondent B

"I don't have much money and worry a lot about how to keep my children safe. Should we use different utensils? We live in one room and it is impossible to open the window at night for ventilation. Security is not so good where we live". Respondent C

"I am worried for my children. What if my husband does not make it? What if I also get sick? Who will take care of our children? People fear this disease". Respondent D

Discussion

Household contact tracing of index PTB cases has been advocated as a key component of PTB prevention strategies for many years due to the higher cumulative exposure to M. tuberculosis through close physical contact and social interactions. The prolonged exposure predisposes HHCs to a higher risk of infection, morbidity, and mortality. The WHO recommends routine contact tracing and investigation in PTB high burden countries through counseling and education of HHCs, who are the primary caregivers for active PTB patients at home [3,4]. However, implementation of these recommendations in low-resource settings is poor hence screening of HHCs has remained sub-optimal.

An investigation of LTBI in this population reported a high prevalence among the HHCs, with the Interferon Gamma Release Assay results for over half (55.7%) of the participants indicating positivity for LTBI, which was higher than the global average of 23% [5]. A study in Ethiopia showed that individuals that lived with a PTB patient in the household had a three-fold increased risk of developing the infection [6]. Interestingly, almost all of the HHCs in our study did not have any knowledge of LTBI. There was a lot of anxiety noticed among majority of the HHCs in our study group when we relayed the results of a positive LTBI test result from the Interferon Gamma Release Assay. The HHCs who tested negative wanted to know how often they needed to test for LTBI while living with the PTB patient. Coincidentally many were also not aware of their HIV serostatus. As at the time of collecting this data, the country's national policy on LTBI testing and treatment considered HHCs who were HIV positive adults and children <5 years of age. These groups are at higher risk of developing full-blown tuberculosis disease on exposure due to poor immunity [7,8]. The recently launched Kenya Latent Tuberculosis Infection policy of 2020 by the Ministry of Health enhanced the scope of testing and treatment of LTBI to include all household contacts of known PTB patients. The policy allows for use of both Tuberculin Skin Test (TST) and Interferon-Gamma Release Assays (IGRAs). The proposed treatment options are based on WHO recommendations of 6-month isoniazid daily (6H) or 3-month rifapentine plus isoniazid weekly (3HP) or 3-month isoniazid plus rifampicin daily(3RH). In the Kenya policy document 6H is the preferred treatment option for anyone with a contraindication to the above two regimens [8].

In the study population majority of HHCs were satisfied with the health care resources that were available to their patients including diagnosis, counseling, and availability of prescribed medication. Many HHCs doubled up as providers of home-based care for the patient. As has been documented in other studies, depending on the severity of the illness, the PTB patient relies a lot on other members of the household for support in physical and psychological care [9]. In addition, most of the patients' families in informal settlements are confronted with extra social, financial, and clinical burdens associated with TB disease. Consequently, alleviation of poverty would reduce the risk of tuberculosis transmission and the risk of its progression from infection to disease [3].

The HHCs in our study discussed the challenges they faced living with and taking care of PTB patients and were classified as follows: Psychosocial challenges: HHCs had challenges dealing with personal fears of caring for a patient infected with a disease considered in the community as 'dangerous'. Some faced stigma from other family members and neighbors. They noticed that visitors shunned their homes, limited social interaction or refused any food offer from the host. A study on the effects of stigma in Indonesia found that stigma affects both the TB patient as an individual and also the family and caregivers, a situation described as courtesy stigma [10]. These negative attitudes affected the mental well-being of the HHCs and negatively influenced the patients' self-esteem.

Care-giver burnout: Physical exhaustion dealing with the physical needs of the patients impacted the other members of the household, and most significantly the spouses of the patient and children. Mental exhaustion arose from anxiety about the patient's progress, the likelihood of getting infected, and the need to constantly encourage a PTB patient battling low self-esteem. They also had concerns about dwindling resources due to diminished family income arising from job loses in cases of sick spouse(s). Majority of the study participants did not have private medical health insurance covers and relied on daily wages as casual laborers.

Hygiene needs: Most participants lived with the PTB patient in a one or two room house that had minimal ventilation. Some HHCs observed that TB patients were isolated from the other patients while in the hospital and wondered how they should minimize the spread of infection at home where available space could not allow for any isolation. Water for domestic use was inadequate in most households and this was a major hurdle when bathing very sick patients especially in communal facilities. Washing the patients' clothes/bedding that were sometimes visibly soiled and cleaning of utensils used was mainly done using soap because disinfectants were costly. Waste management practices differ considerably between formal and informal households where collection of waste, if any, remain irregular and random. In some instances, generated household waste ended up in the environment around the house or by the walkways and roadsides. All the household waste, including what would be categorized as clinical waste such as vomit, sputum, pus, tissue paper, soiled linen was disposed off like any other normal waste.

Conclusion

While most of our study participants acknowledged adequate accessibility to PTB diagnosis and treatment services offered in public facilities, several gaps in home based care persisted from which the following recommendations were drawn:

i. Sensitization on available testing and treatment of LTBI for all HHCs of PTB patients;
ii. Psychological care through individual and group counseling of PTB patients, their HHCs, and caregivers;
iii. Training of HHCs on home-based care with the focus on minimizing exposure and risk of infection from the index case, reduction of air pollution in the home and safe handling of clinical waste;
iv. Adoption of a PTB HHC Card Notification system by health facilities handling TB patients; and
v. Further research on the effectiveness of the PTB HHC card notification system for those in contact with PTB patients at the household level.

Ethical Considerations

The data was extracted from a larger study on "The role of Human Leukocyte Antigens and M. tuberculosis strain variation in susceptibility to infection among pulmonary tuberculosis

Citation: Susan Odera, Julius Oyugi, Angeline Kirui, Andrew Abalia, Noel Onyango, et al (2021) Challenges Faced by House-Hold Contacts of Pulmonary Tuberculosis Patients in an Urban Setting in Nairobi, Kenya. Journal of Infectious Diseases & Case Reports. SRC/JIDSCR-165. DOI. doi.org/10.47363/JIDSCR/2021(2)146.

APPENDIX 12: KNH-UoN ETHICS AND RESEARCH COMMITTEE DOCUMENTS

APPENDIX 12a: Study Approval document

UNIVERSITY OF NAIROBI
COLLEGE OF HEALTH SCIENCES
P O BOX 19676 Code 00202
Telegrams: varsity
(254-020) 2726300 Ext 44355

KNH/UON-ERC
Email: uonknh_erc@uonbi.ac.ke
Website: www.uonbi.ac.ke

KENYATTA NATIONAL HOSPITAL
P O BOX 20723 Code 00202
Tel: 726300-9
Fax: 725272
Telegrams: MEDSUP, Nairobi

KENYATTA NATIONAL HOSPITAL APPROVED 05 DEC 2013 ETHICS & RESEARCH COMMITTEE

Ref: KNH-ERC/A/392 Link:www.uonbi.ac.ke/activities/KNHUoN 5th December 2013

Susan Odera
Dept.of Medical Microbiology
School of Medicine
University of Nairobi

Dear Susan

Research proposal: The role of Human Leukocyte Antigens and M. *tuberculosis* strain variation in Susceptibility to infection among Pulmonary Tuberculosis patients in Kenya (P649/12/2012)

This is to inform you that the KNH/UoN-Ethics & Research Committee (KNH/UoN-ERC) has reviewed and **approved** your above proposal. The approval periods are 5th December 2013 to 4th December 2014.

This approval is subject to compliance with the following requirements:

a) Only approved documents (informed consents, study instruments, advertising materials etc) will be used.
b) All changes (amendments, deviations, violations etc) are submitted for review and approval by KNH/UoN ERC before implementation.
c) Death and life threatening problems and severe adverse events (SAEs) or unexpected adverse events whether related or unrelated to the study must be reported to the KNH/UoN ERC within 72 hours of notification.
d) Any changes. anticipated or otherwise that may increase the risks or affect safety or welfare of study participants and others or affect the integrity of the research must be reported to KNH/UoN ERC within 72 hours.
e) Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. (*Attach a comprehensive progress report to support the renewal*).
f) Clearance for export of biological specimens must be obtained from KNH/UoN-Ethics & Research Committee for each batch of shipment.
g) Submission of an *executive summary* report within 90 days upon completion of the study
This information will form part of the data base that will be consulted in future when processing related research studies so as to minimize chances of study duplication and/or plagiarism.

For more details consult the KNH/UoN ERC website **www.uonbi.ac.ke/activities/KNHUoN.**

"Protect to Discover"

APPENDIX 12b: Extension of Study Approval

UNIVERSITY OF NAIROBI
COLLEGE OF HEALTH SCIENCES
P O BOX 19676 Code 00202
Telegrams: varsity
(254-020) 2726300 Ext 44355

KNH/UON-ERC
Email: uonknh_erc@uonbi.ac.ke
Website: www.uonbi.ac.ke
Link:www.uonbi.ac.ke/activities/KNHUoN

KENYATTA NATIONAL HOSPITAL
P O BOX 20723 Code 00202
Tel: 726300-9
Fax: 725272
Telegrams: MEDSUP, Nairobi

Ref: KNH-ERC/ R/24

3rd March, 2015

Susan Odera
Dept of Medical Microbiology
School of Medicine
University of Nairobi

Dear Susan

Re: Approval of annual study renewal –The role of Human leukocyte antigens and M. tuberculosis strain variation in susceptibility to infection among pulmonary tuberculosis patients in Kenya (P649/12/2012)

Your communication of 3rd February, 2015 refers.

This is to acknowledge receipt of the study progress report and hereby grant you annual extension of approval for ethical research protocol P649/12/2012.

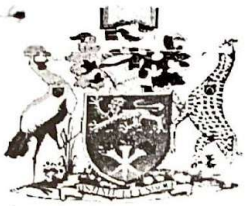
The study renewal dates are 3rd March, 2015 to 2nd March, 2016.

This approval is subject to compliance with the following requirements:

a) Only approved documents (informed consents, study instruments, advertising materials etc) will be used.
b) All changes (amendments, deviations, violations etc) are submitted for review and approval by KNH/UoN ERC before implementation.
c) Death and life threatening problems and severe adverse events (SAEs) or unexpected adverse events whether related or unrelated to the study must be reported to the KNH/UoN- ERC within 72 hours of notification.
d) Any changes, anticipated or otherwise that may increase the risks or affect safety or welfare of study participants and others or affect the integrity of the research must be reported to KNH/UoN ERC within 72 hours.
e) Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. (*Attach a comprehensive progress report to support the renewal*).
f) Clearance for export of biological specimens must be obtained from KNH/UoN-Ethics & Research Committee for each batch of shipment.

g) Submission of an *executive summary* report within 90 days upon completion of the study
This information will form part of the data base that will be consulted in future when processing related research studies so as to minimize chances of study duplication and/or plagiarism.

APPENDIX 12c: Approval of Study Modifications



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COLLEGE OF HEALTH SCIENCES
P O BOX 19676 Code 00202
Telegrams: varsity
(254-020) 2726300 Ext 44355

KNH/UON-ERC
Email: uonknh_erc@uonbi.ac.ke
Website: www.uonbi.ac.ke
Link:www.uonbi.ac.ke/activities/KNHUoN

KENYATTA NATIONAL HOSPITAL
P O BOX 20723 Code 00202
Tel: 726300-9
Fax: 725272
Telegrams: MEDSUP, Nairobi

Ref: KNH-ERC/ MOD/95

3rd March, 2015

Susan Odera
Dept of Medical Microbiology
School of Medicine
University of Nairobi

Dear Susan

Re: Approval of modifications: The role of Human leukocyte antigens and M. tuberculosis strain variation in susceptibility to infection among pulmonary tuberculosis patients in Kenya (P649/12/2012)

Refer to your communication of 3rd February, 2015.

The KNH/UoN-ERC has reviewed and approved modification of the following:

1. In the methodology section: An additional assay, the HIV sero-status of close contacts of pulmonary tuberculosis patients will be included. This is based on new literature pointing out the need to establish the sero-status of individuals when using the IGRAS assay in investigation of latent tuberculosis, and has been included in the literature write up on latent tuberculosis (page 10, 3rd paragraph). The initial proposal had omitted this. References supporting this have been included in the reference section (Ni Cheallaigh et al, 2013) and amendments have also been made in the consent form (English and Swahili versions) to be signed by the study participants
2. The latest data on WHO statistics of tuberculosis in Kenya: World Health Organization (2013). Global tuberculosis control- surveillance, planning, financing. Geneva, Switzerland when the initial proposal was made, the discussion was based on the 2010 report. This has been highlighted in the first paragraph of the introduction (page 8), first paragraph of literature review (page 9), and the write up on the statistics of Multi Drug Resistance Tuberculosis in Kenya (page 14).
3. Additional material retrieved from recent publications and considered relevant to the study has been included in the literature section:
    - Fang Wu et al, 2013 highlighting how polymorphisms in some host genes can influence the clinical phenotype of tuberculosis in different ethnic groups (discussed on page 8)
    - Stein CM2011 highlighting study designs evaluating host genetic factors and susceptibility disease (discussed on page 10, last paragraph)
    - Latha jaganathann et al, 2011 highlighting the relationship between host gene polymorphism and failure to respond to treatment (discussed page 10, first paragraph)

APPENDIX 13: Mbagathi County Hospital Permission to Conduct Study

NAIROBI CITY COUNTY

Tel: 2724712, 2725791, 0721 311 808
Email: mdhnairobi@yahoo.co.uk

Mbagathi Hospital
P.O. Box 20725- 00202
Nairobi

COUNTY HEALTH SERVICES

5th May 2016

Susan Akinyi Odera
University of Nairobi

Dear Madam,

RE: RESEARCH AUTHORIZATION

This is in reference to your application for authority to carry out a research on ***"The role of Human Leukocyte Antigens and M. Tuberculosis strain variation in susceptibility to infection among Pulmonary Tuberculosis patients in Kenya"***

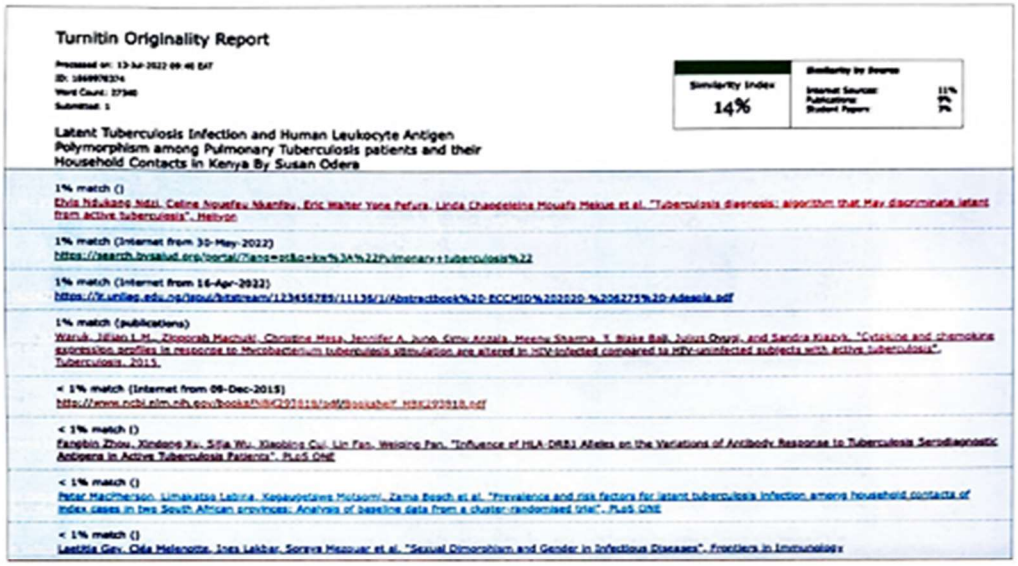
I am pleased to inform you that your request to undertake the research in the hospital has been granted.

On completion of the research you are expected to submit one hard copy and one soft copy of the research report / thesis to this office.

MBAGATHI HOSPITAL P.O. Box 20725 - 00202 NAIROBI 05 MAY 2016

Dr. A. J. Suleh
Medical Superintendent
Mbagathi Hospital

APPENDIX 14: Turnitin Report



Turnitin Originality Report

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Submitted: 1

Latent Tuberculosis Infection and Human Leukocyte Antigen Polymorphism among Pulmonary Tuberculosis patients and their Household Contacts in Kenya By Susan Odera

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The following articles were excluded in the Similarity scan:

Susan Odera et al. Latent tuberculosis among household contacts of pulmonary tuberculosis cases in Nairobi, Kenya. Pan African Medical Journal. 2020;37(87). 10.11604/pamj.2020.37.87.21102 https://www.panafrican-med-journal.com//content/article/37/87/full

Susan Odera et al. Association between human leukocyte antigen class II (HLA-DRB and -DQB) alleles and outcome of exposure to Mycobacterium tuberculosis: a cross-sectional study in Nairobi, Kenya. Pan African Medical Journal. 2022;41(149). 10.11604/pamj.2022.41.149.30056 Available online at: https://www.panafrican-med-journal.com//content/article/41/149/full

Challenges faced by House Hold Contacts of Pulmonary Tuberculosis Patients in an urban setting in Nairobi, Kenya: Susan Odera, Julius Oyugi, Angeline Kirui, Andrew Aballa, Noel Onyango, et al (2021). Journal of Infectious Diseases & Case Reports. SRC/JIDSCR-165. DOI: doi.org/10.47363/JIDSCR/2021(2)146.

13/7/2022
Supervisor

13/7/22

Appendix 15. Sample photographs of Qiaxcel screen displaying PCR products processed in the cartridge gel electrophoresis

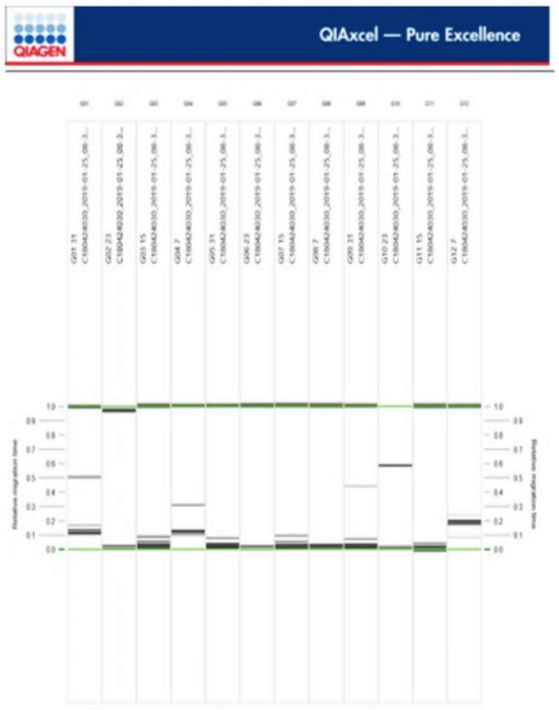

Figure: 7

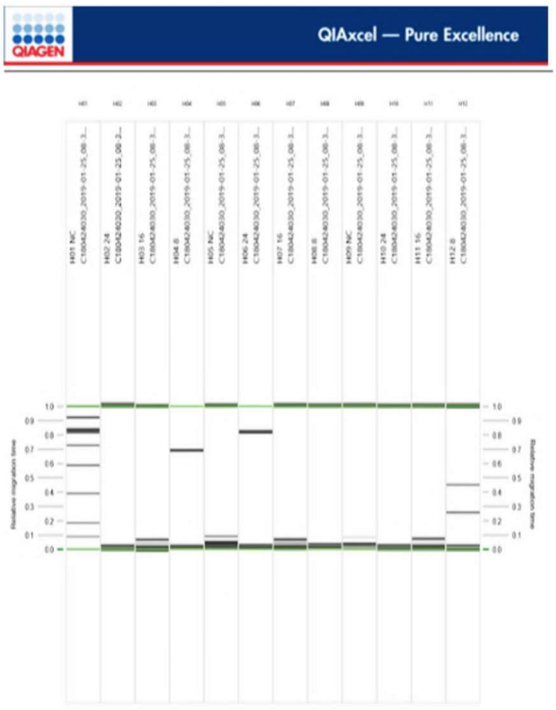

Figure: 8

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FACULTY OF HEALTH SCIENCES
P O BOX 19676 Code 00202
Telegrams: varsity
Tel:(254-020) 2726300 Ext 44355

KNH-UON ERC
Email: uonknh_erc@uonbi.ac.ke
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P O BOX 20723 Code 00202
Tel: 726300-9
Fax: 725272
Telegrams: MEDSUP, Nairobi

Ref. No.KNH/ERC/R/209

7th December 2022

Susan Odera
Department of Medical Microbiology
Faculty of Health Sciences
University of Nairobi

Dear Susan

Re: Approval of Annual Renewal – The role of Human Leukocyte antigens and M.tuberculosis strain variation in susceptibility to infection among pulmonary tuberculosis patients in Kenya (P649/12/2012)

Refer to your communication dated 30th November 2022.

This is to acknowledge receipt of the study progress report and hereby grant annual extension of approval for ethical research protocol P649/12/2012.

The approval dates are 3rd March 2022 – 2nd March 2023

This approval is subject to compliance with the following requirements:

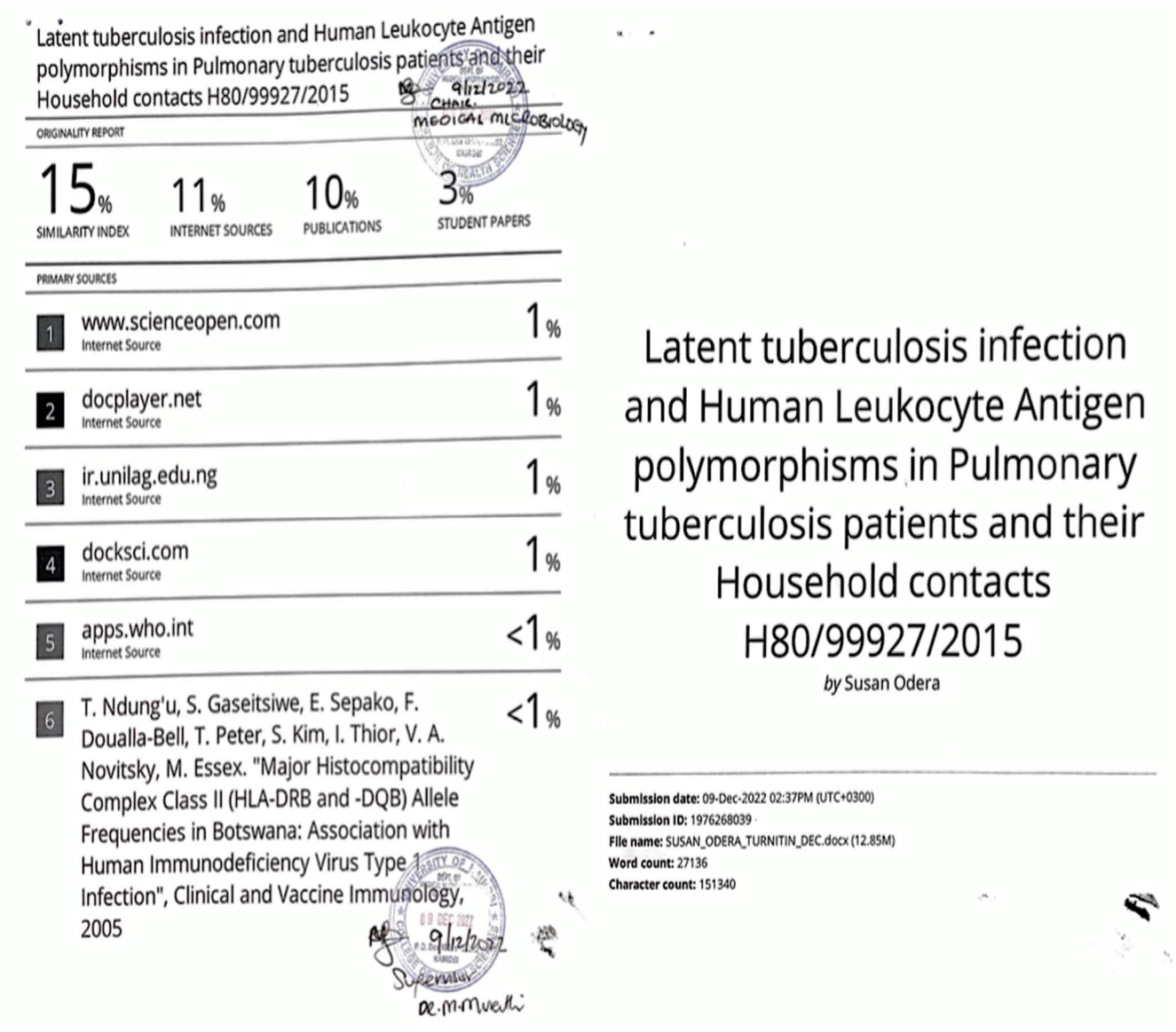
a) Only approved documents (informed consents, study instruments, advertising materials etc) will be used.
b) All changes (amendments, deviations, violations etc.) are submitted for review and approval by KNH- UoN ERC before implementation.
c) Death and life threatening problems and severe adverse events (SAEs) or unexpected adverse events whether related or unrelated to the study must be reported to the KNH- UoN ERC within 72 hours of notification.
d) Any changes, anticipated or otherwise that may increase the risks or affect safety or welfare of study participants and others or affect the integrity of the research must be reported to KNH- UoN ERC within 72 hours.
e) Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. (*Attach a comprehensive progress report to support the renewal*).
f) Clearance for export of biological specimens must be obtained from KNH- UoN-Ethics & Research Committee for each batch of shipment.
g) Submission of an executive *summary* report within 90 days upon completion of the study.

This information will form part of the data base that will be consulted in future when processing related research studies so as to minimize chances of study duplication and/or plagiarism.

Yours sincerely,

DR. BEATRICE K.M. AMUGUNE
SECRETARY, KNH- UoN ERC

cc. The Dean, Faculty of Health Sciences, UoN
The Senior Director, Clinical Services, KNH
The Chair, KNH-UoN ERC



Latent tuberculosis infection and Human Leukocyte Antigen polymorphisms in Pulmonary tuberculosis patients and their Household contacts H80/99927/2015

9/12/2022
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09 DEC 2022
P.O. Box 30197-00100
NAIROBI

9/12/2022
Supervisor
Dr. M. Mwatha

Latent tuberculosis infection and Human Leukocyte Antigen polymorphisms in Pulmonary tuberculosis patients and their Household contacts H80/99927/2015

by Susan Odera

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