Detecting Sub-Clinical Tuberculosis in Face Masks Worn by People Living with HIV Attending Routine HIV Care at Parirenyatwa Group of Hospitals Opportunistic Infections Unit, Harare, Zimbabwe

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ABSTRACT

Tuberculosis (TB) is a bacterial infection spread through inhaling tiny droplets from the coughs or sneezes of an infected person. It is associated with a dynamic spectrum of pathology between adequate containment and progression to active disease, hence it has three main stages of infection namely subclinical stage, latent infection stage and active TB disease stage. Individuals with subclinical tuberculosis (TB) represent a large proportion of all prevalent cases of TB, yet their contribution to Mycobacterium tuberculosis (Mtb) transmission is unknown. Subclinical TB has been increasingly recognised as a separate spectrum of the disease, and evidence on its transmissibility is, however, still inconclusive and unknown. Culture or Xpert MTB/ RIF positivity in bronchoalveolar lavage from sputum-negative or sputum-scarce patients suggests the presence of Mtb in the peripheral airways. This study, therefore, aimed to provide preliminary data on the clinically relevant role of face mask sampling as a novel diagnostic specimen for TB screening and diagnosis in asymptomatic HIV-positive patients. An exploratory cross-sectional study was conducted at Parirenyatwa Group of Hospitals (PGH) Opportunistic Infections (OI) unit, which was purposively selected because of its desirable characteristic of population diversity. A total of 160 asymptomatic HIV-positive individuals were enrolled between September and November 2022 and these were randomly sampled from people living with HIV (PLHIV) attending routine HIV care at PGH OI unit. Participants under the age of 18 were asked to provide parental/ guardian consent. Pretested interviewer administered questionnaires were used to collect data for the patients and a desk review of medical records was done for extra data extraction. Statistical analysis was done on the obtained data and various indicators used in the study. Out of the total 160 asymptomatic HIV-positive individuals who were assessed, Mtb was detected in 2.5% of the pulmonary TB (pTB) face mask samples, and all were Rifampicin resistance negative. All positive cases had been on antiretroviral therapy (ART) for less than one year, and all were at least 60 years old. Findings from this study suggests that subclinical TB contributes to transmission of pTB, and thus needs to be diagnosed and treated for effective progress towards TB elimination. The study also proved face mask sampling as an effective and alternative approach to understanding and diagnosing TB. It offers a simple, highly sensitive, inexpensive, non-invasive and easily deployable tool for the diagnosis of pTB, and stratification of transmission risk from individuals with pTB. This study supports the potential of face mask sampling as a clinical tool to enhance TB control programmes necessary for eradication of TB and provides an epidemiological tool to better characterise Mtb transmission within complex community settings. Widespread application will potentially reduce diagnostic uncertainty and enable improved case-finding with both individual and public health benefits.

Key words: Face mask; HIV; Mycobacterium tuberculosis; Subclinical tuberculosis

INTRODUCTION

Introduction

Tuberculosis (TB) is a bacterial infection caused by the human obligate aerobe Mycobacterium tuberculosis (Mtb). It is the leading cause of hospital admissions and in-hospital deaths among people living with HIV (PLHIV), and it often goes unnoticed (Ford et al., 2016; Msukwa et al., 2022). Mtb is reliant on airborne

transmission for survival, therefore, the identification of individuals emitting the infectious bacilli is key to global eradication of the disease (Yates et al., 2016).

TB is associated with a dynamic spectrum of pathology between adequate containment and progression to active disease hence it consists of three main stages of the disease namely the subclinical disease stage, the latent disease stage and the active TB disease stage (Madhukar., 2007; Drain et al., 2018). In subclinical disease stage patients will have no symptoms of TB but will be culture positive, and transmission of Mtb may occur without the recognizable symptoms (Madhukar., 2007; Drain et al., 2018). In the latent phase patients have Mtb present in the body but with no TB disease. Only a small proportion of those with latent infection will progress to active TB infection. In active TB disease patients present with symptoms such as cough, fever and weight loss, and diagnosis can be confirmed with smear, culture and molecular tests (Madhukar., 2007; Drain et al., 2018).

In 2011 the World Health Organization (WHO) recommended the routine screening of TB in people living with HIV using the World Health Organization four-symptom screening algorithm (W4SS) (Getahun et al., 2011). In 2015 WHO also advocated for the screening of close contacts of pulmonary tuberculosis (pTB) cases as a way of identifying recently infected individuals as part of the TB elimination strategy (WHO., 2015). In high burden settings and high-risk individuals this included active case finding programmes to identify subclinical Tuberculosis, as it is a potential important contributor to the overall burden of TB transmission (Kendall et al., 2021).

Improved diagnostics and access to screening and effective treatment are pivotal in the current efforts to control TB infection (Williams et al., 2020). In 2018, 4.1 million people with TB were missed from WHO's recorded figures (Williams et al., 2020). Mortality is usually high in missed and /or delayed diagnoses. Mathematical models of TB transmission indicates that active case-finding is dependent on the detection of the disease in early or subclinical phase of the infection (Dowdy et al., 2013).

Mtb infection is associated with a dynamic spectrum and the persistence of the epidemic is mostly driven by successive cycles of infection, pulmonary disease, Mtb bioaerosol expulsion and inhalation by susceptible individuals (Patterson et al., 2020). Interventions which can halt this cycle are very critical in curbing the TB epidemic. However, knowledge of the physical process of transmission is still very limited (Patterson et al., 2020). The prediction of infectiousness is currently partial and based on retrospective, epidemiological analyses thus limiting the risk stratification for contact screening (Patterson et al., 2020). Tools to facilitate direct detection of viable aerosolised Mtb in exhaled breath are urgently required to address the knowledge gaps.

Background to the study

Tuberculosis is the leading global infectious killer, claiming approximately 1.4 million lives annually (WHO., 2019). People with latent TB have a 5-15% lifetime risk of developing active TB disease whereas those with Human Immunodeficiency Virus (HIV) have a 5-15% annual risk of acquiring active TB disease (Takarinda et al., 2020; Pawlowski et al., 2012). The TB epidemic in sub-Saharan Africa is mostly driven by the HIV epidemic (Takarinda et al., 2020) and Zimbabwe is amongst the sub-Saharan countries involved. It is also one of 14 countries globally affected by the triple burden of TB, TB/ HIV and Multiple Drug Resistant Tuberculosis (MDRTB) (WHO., 2018).

About 70% of the 36.9 million people living with HIV (PLHIV) globally live in sub-Saharan Africa, 76% of whom are found in the eastern and southern regions (Takarinda et al., 2020). Nine percent of the estimated 10 million people who developed TB disease were PLHIV, of whom 72% lived in Africa region (WHO., 2018). In 2019, Zimbabwe had an adult HIV prevalence of 12.9%, with a TB incidence among PLHIV of 193 per 100000 people and 5900 PLHIV deaths due to TB (UNAIDS., 2020; WHO., 2021). In 2011 WHO recommended the routine screening of TB in PLHIV using the W4SS algorithm (Getahun et al., 2011). However, systematic screening has proved a challenge in Low to Middle income countries, Zimbabwe included, owing to insufficient resources. There is, therefore, a need to come up with tools that can rapidly identify the most infective individuals at a cheaper cost and also to develop a focused contact screening

pathway. Face-mask sampling can be an alternative method for quantifying Mtb exhaled by pTB patients. It is a simple, non-invasive and inexpensive procedure which can be applied in any setting where a mask can be worn thus assisting with early TB diagnosis and transmission control.

Statement of the Problem

Halting the transmission of Mycobacterium tuberculosis by infectious individuals earlier is key to eradicating Tuberculosis (Williams et al., 2021). However, this remains underexplored due to the poor understanding of the events surrounding transfer of Mtb between hosts. Determination of when live, infectious Mtb are released and by who has proven very challenging (Dinkele et al., 2021) and consequentially, transmission chains are inferred retrospectively after new cases have been diagnosed resulting in delayed and missed diagnoses which can contribute to failure in controlling the TB epidemic. The targeting of surveillance to active disease means that the potential contribution of asymptomatic transmission is overlooked (Xu et al., 2019). This brings the relevance of the question whether asymptomatic individuals are able to transmit Mtb.

Main Research Objective

The main objective of the study was to detect the existence of subclinical tuberculosis in PLHIV attending routine HIV care at Parirenyatwa Group of Hospitals Opportunistic Infections Unit, using the face-mask sampling method.

Specific Objectives

The specific objectives of the study were:

- To determine the utility of face mask sampling for TB diagnosis and therapeutic monitoring in PLHIV attending PGH.
- To determine the prevalence of mycobacterium tuberculosis-containing bioaerosols in PLHIV with no clinical symptoms of TB attending PGH.
- To check the Rifampicin resistance status in patients with subclinical Tuberculosis attending PGH.

Research Questions

- What is the prevalence of subclinical TB in PLHIV?
- Are individuals with subclinical TB the source of a large fraction of ongoing TB transmission?
- Should individuals with subclinical TB be prioritized for detection and treatment?
- Can face mask sampling be used for TB diagnosis and therapeutic monitoring?
- What is the prevalence of Rifampicin resistance in patients with subclinical tuberculosis?

Justification of the study

Existing data are increasingly clear that subclinical TB comprises a large proportion of prevalent disease at population level and it is becoming increasingly clear that it has a meaningful infectious potential, it follows a heterogenous clinical trajectory and is difficult to diagnose using passive systems (Kendall et al., 2020). Subclinical TB, therefore, has a greater epidemiological significance than it has been traditionally assigned and a large fraction of the Mtb fraction at population level might be originating from individuals with subclinical TB (Kendall et al., 2020). Secondly, the World Health Organization, in 2011, introduced the routine screening of all people living with HIV using the W4SS algorithm (Dhana et al., 2022). This algorithm has proved to be suboptimal for screening TB in these high-risk groups, there is therefore need for a more appropriate rapid TB diagnostic approach. Thirdly, there are no current reports or published papers on the evaluation of the use of

face-mask sampling as a screening tool for TB diagnosis in Zimbabwe. This study therefore, sought to evaluate the utility of face mask sampling as an alternative screening method for TB and also to identify and confirm the existence of subclinical TB in PLHIV.

Significance of the Study

Although symptoms facilitate transmission, TB transmission can also occur in the absence of noticeable symptoms and this study aimed to prove this claim. The study also increased the understanding of face-mask sampling as a potential new approach to understanding and diagnosing TB. Widespread application of face-mask sampling may be a potential way of reducing diagnostic uncertainty and enabling improved case-finding with both individual and public health benefit. It may provide a cheaper alternative screening method which can also supplement WHO's W4SS algorithm. There is therefore need for more research studies to confirm these claims.

REVIEW OF RELATED LITERATURE

Introduction

This chapter will review related literature as well as examining findings from previous related studies to enable a comparison of these studies with the present study. This chapter will also present the conceptual framework, its relevance, the spectrum of Mtb infection, and an overview of subclinical TB. An insight into the related literature will be done.

Theoretical/ Conceptual Framework

Conceptual Framework

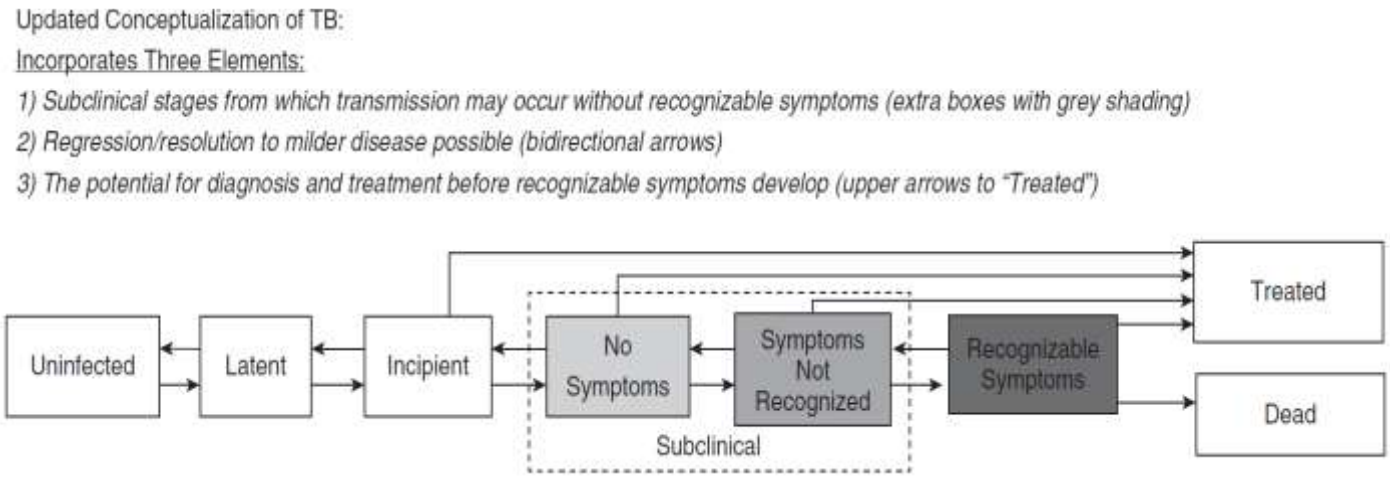
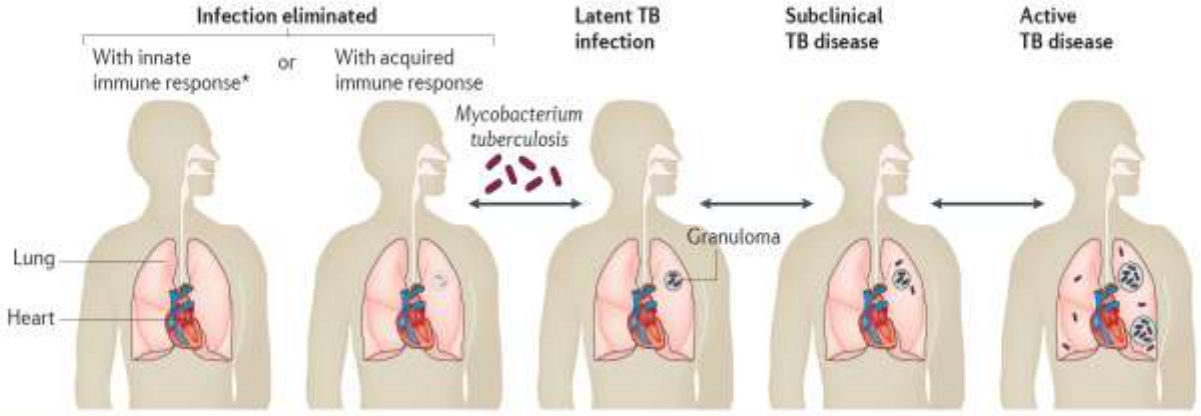


Figure 1 Updated Conceptualization of TB (From USAID., 2022)

The classic conceptualization of TB used to have two forms namely latent TB infection and active TB infection (Madhukar., 2007; Drain et l., 2018). It conceptualized TB as a two-stage entity with inevitable forward progression, transmission occurring only from those with active TB, and diagnosis and treatment implicitly focused on people with active/ symptomatic TB. However, the updated conceptualization of TB is based on the understanding that Mtb infection is associated with a dynamic spectrum of pathology, between adequate containment and progression to active disease (Madhukar., 2007; Drain et al., 2018). It incorporates three elements namely subclinical stages, regression or resolution to milder disease and the potential for diagnosis and treatment before recognizable symptoms develop. It implies that individuals with subclinical TB may be truly asymptomatic or have symptoms that are not recognized, both progression and regression across stages occur without inevitable development of recognizable symptoms, and individuals with milder forms of TB disease can be effectively diagnosed and treated. Shading intensity indicates that both active and subclinical TB states may be infectious and that infectiousness is likely to increase with more advanced

disease, although degree of correlation is uncertain. This is illustrated in figure 1 above. In subclinical TB infection, transmission of Mtb may occur without recognizable symptoms.

The spectrum of Mtb infection



TST	Negative	Positive	Positive	Positive	Usually positive
IGRA	Negative	Positive	Positive	Positive	Usually positive
Culture	Negative	Negative	Negative	Intermittently positive	Positive
Sputum smear	Negative	Negative	Negative	Usually negative	Positive or negative
Infectious	No	No	No	Sporadically	Yes
Symptoms	None	None	None	Mild or none	Mild to severe
Preferred treatment	None	None	Preventive therapy	Multidrug therapy	Multidrug therapy

Figure 2 The spectrum of TB-from Mycobacterium tuberculosis infection to active pulmonary TB disease (From Madhukar., 2007)

As shown in figure 2 above, exposure to Mtb can result in the elimination of the pathogen by innate immune response or acquired T-cell immunity (Madhukar., 2001). Individuals who have eliminated the infection by innate or acquired immune response but without retaining immune memory can have a negative tuberculin skin test (TST) or interferon-gamma release assay (IGRA) results. After elimination of the pathogen, some individuals may still retain a strong memory T-cell response and these will have positive results on TST or IGRA (Madhukar., 2007). However, these individuals will not benefit from latent TB infection treatment. Following elimination of the pathogen, bacteria may persist in a quiescent or latent TB infection which can be detected using positive TST or IGRA results. It is estimated that 25% of the world's population is latently infected, but only a small proportion will progress to active TB infection (Madhukar., 2007; Drain et al., 2018). Patients with subclinical TB have no symptoms but will be culture positive but with smear negative owing to the low bacillary load. Patients with active pTB disease present with symptoms such as cough, fever, weight loss and the diagnosis can be confirmed with smear, culture and molecular tests. Active TB disease is a spectrum ranging from extrapulmonary TB, pTB that is smear negative, pTB that is smear positive and disseminated, severe disease (Madhukar., 2007).

Relevance of the Theoretical/ Conceptual Framework

The TB conceptual framework offers a deep understanding of the TB spectrum from Mtb infection to pulmonary TB disease. It also gives an insight into the magnitude of subclinical TB contribution to transmission. It also shows the potential significance of early TB screening thus allowing the implementation of strategies to prevent transmission. The framework will also assist with an understanding of the appropriate treatment and programmatic management protocols. Early latent TB infection detection may become useless without corresponding interventions for preventing new transmission and unfavourable treatment outcomes in TB patients.

REVIEW OF RELATED LITERATURE

Overview of Tuberculosis

Tuberculosis (TB) is the most common opportunistic infection and is the leading cause of mortality in PLHIV (Oni et al., 2011). Reduction of the morbidity and mortality of TB in HIV co-infected patients requires an improved understanding of the epidemiology of TB, including the prevalence and risk factors for latent TB infections and active TB disease in this population (Oni et al., 2011). TB infection often leads to one of three outcomes: 5% are thought to progress to active TB disease (primary TB), while the majority develop and acquired immune response but no signs and symptoms of the disease (latent TB infection) (Oni et al., 2011). Amongst those with latent TB infection there is a 10% risk per lifetime of progressing to active TB disease (Oni et al., 2011). Figure 3 below shows a clinical review of early stages and progression of TB infection, from primary infection site to pathophysiological and observed clinical outcomes.

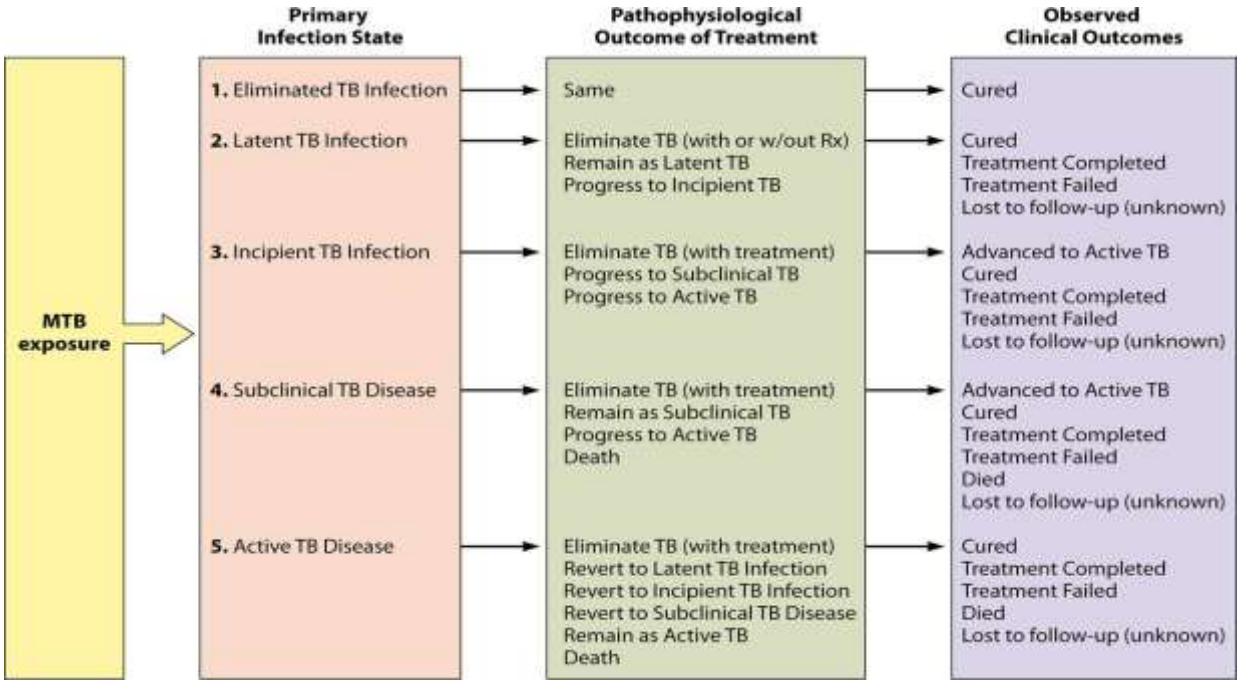


Figure 3 Incipient and subclinical TB: a clinical review of early stages and progression of infection.

The TB epidemic in sub-Saharan Africa is largely being driven by the HIV epidemic which disproportionately affects this region (Takarinda et al., 2020). The incidence of TB is therefore higher in the HIV-positive population as compared to their HIV-negative counterparts (Gupta et al., 2012), and this places PLHIV as a high-risk group. Zimbabwe is one of the sub-Saharan African countries with a largely HIV-driven TB epidemic and it has also adopted WHO's recommendation of 'HIV treat all' approach where PLHIV enrolled in ART care are initiated on isoniazid prevention therapy (IPT) (Takarinda et al., 2020).

Current TB strategies largely focus on the identification and treatment of people with full-blown active TB disease without providing more guidance on how to identify and manage the spectrum of subtle disease (Wong., 2020). Moreover, there is a knowledge gap on the determination of when live, infectious Mtb bacilli are released and by who resulting in delayed and missed diagnoses thus contributing to the failure to control the TB pandemic (Dinkele et al., 2021). This study, therefore, aims to detect the existence of subclinical tuberculosis in PLHIV using the face-mask sampling method.

The limited understanding of TB's pathogenic spectrum of infection and disease has led it, in part, to being the leading infectious cause of mortality worldwide (Drain et al., 2018). Tuberculosis infection exists as a continuous spectrum of metabolic bacterial activity and antagonistic immunological responses; and there are two main clinical states namely incipient and subclinical tuberculosis (Drain et al., 2018). This helps in dividing TB into latent and active TB along the clinical disease spectrum, and also in the development of

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diagnostic and therapeutic interventions for the prevention of progression to active TB disease as well as transmission of Mtb.

The TST and IGRA have been used to identify individuals with a persistent immune response to TB antigens but they have their limitations of being very poor at predicting those with a greater risk of disease progression (Drain et al., 2018). Traditional diagnostic tests for confirmation of active TB disease which include sputum smear microscopy and mycobacterial culture have low diagnostic sensitivity especially in patients with subclinical TB, since they may be unable to produce a quality sputum specimen as they are assumed to be infected with low numbers of Mtb (Drain et al., 2018; Esmail et al., 2022). There is, therefore, need to develop non-sputum-based approaches for the detection of subclinical tuberculosis.

Overview of Sub-clinical tuberculosis

Subclinical TB can be defined as TB disease due to viable Mtb bacteria that does not cause clinical TB-related symptoms but causes other abnormalities that can be detected using existing radiologic or microbiologic assays.

Subclinical TB represents a substantial proportion of individuals with TB disease, although there is limited evidence available to understand the epidemiological characteristics of these cases in Zimbabwe. Despite the great achievements over the past decades, Zimbabwe still had a high burden of TB with 41% of all prevalent cases found to be subclinical at the time of screening, 82% of which had developed symptoms within a few weeks of follow up (Oni et al., 2011; Corbett et al., 2007). Figure 4 below shows the spatial distribution of Mtb in Harare Metropolitan, Zimbabwe.

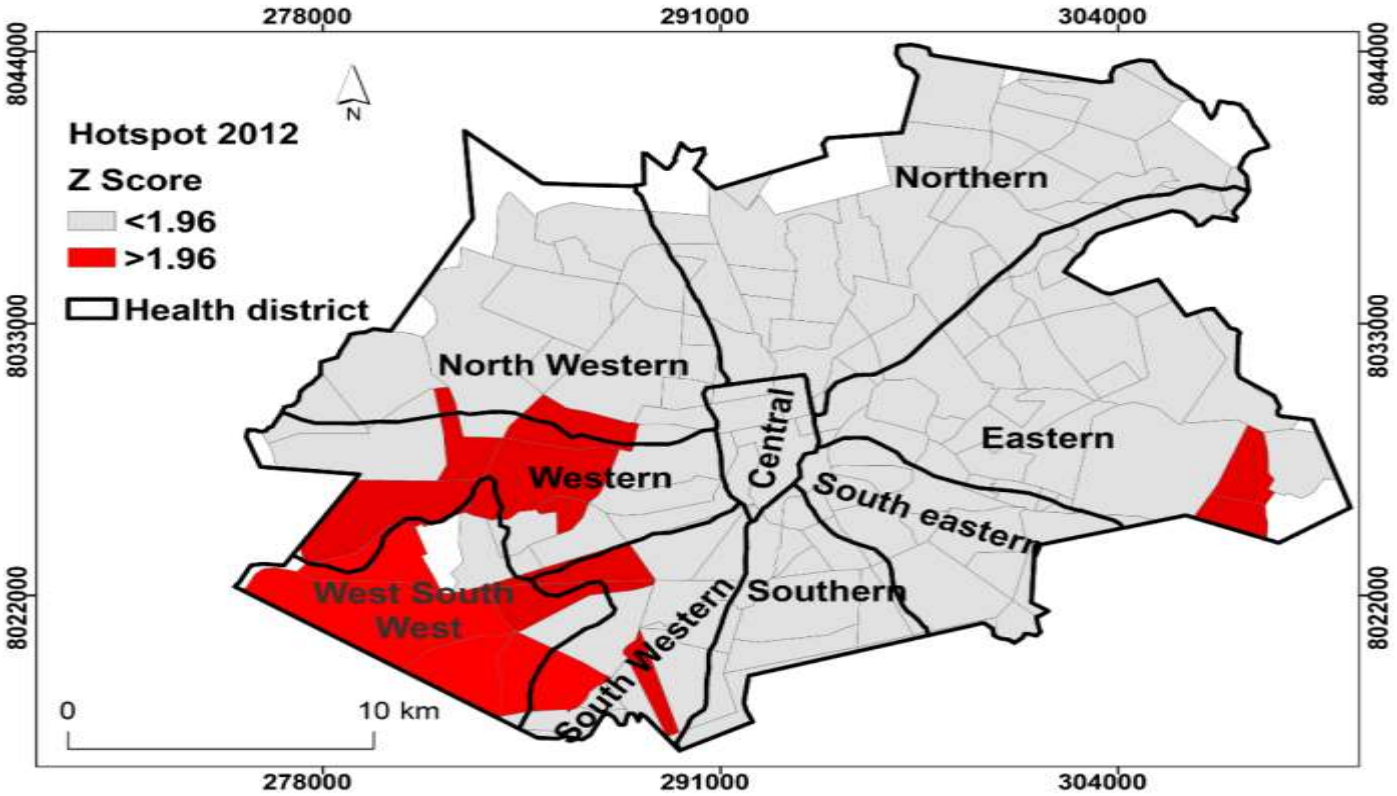

Figure 4 Spatial distribution of Mtb in Harare Metropolitan, Zimbabwe (Corbett et al., 2007).

The high burden of subclinical TB in Zimbabwe can be partly attributed to the new TB algorithm which was introduced in 2017. The Zimbabwe Tuberculosis and Leprosy Management Guidelines recommended a new TB algorithm in 2017 (MoHCC., 2017), where Xpert was recommended as the primary diagnostic for all patients presenting with symptoms suggestive of TB. This new algorithm did not take into consideration patients with no signs and symptoms of the TB, who could have subclinical TB. Figure 5 shows the simplified new TB testing algorithm for Zimbabwe which was introduced in 2017.

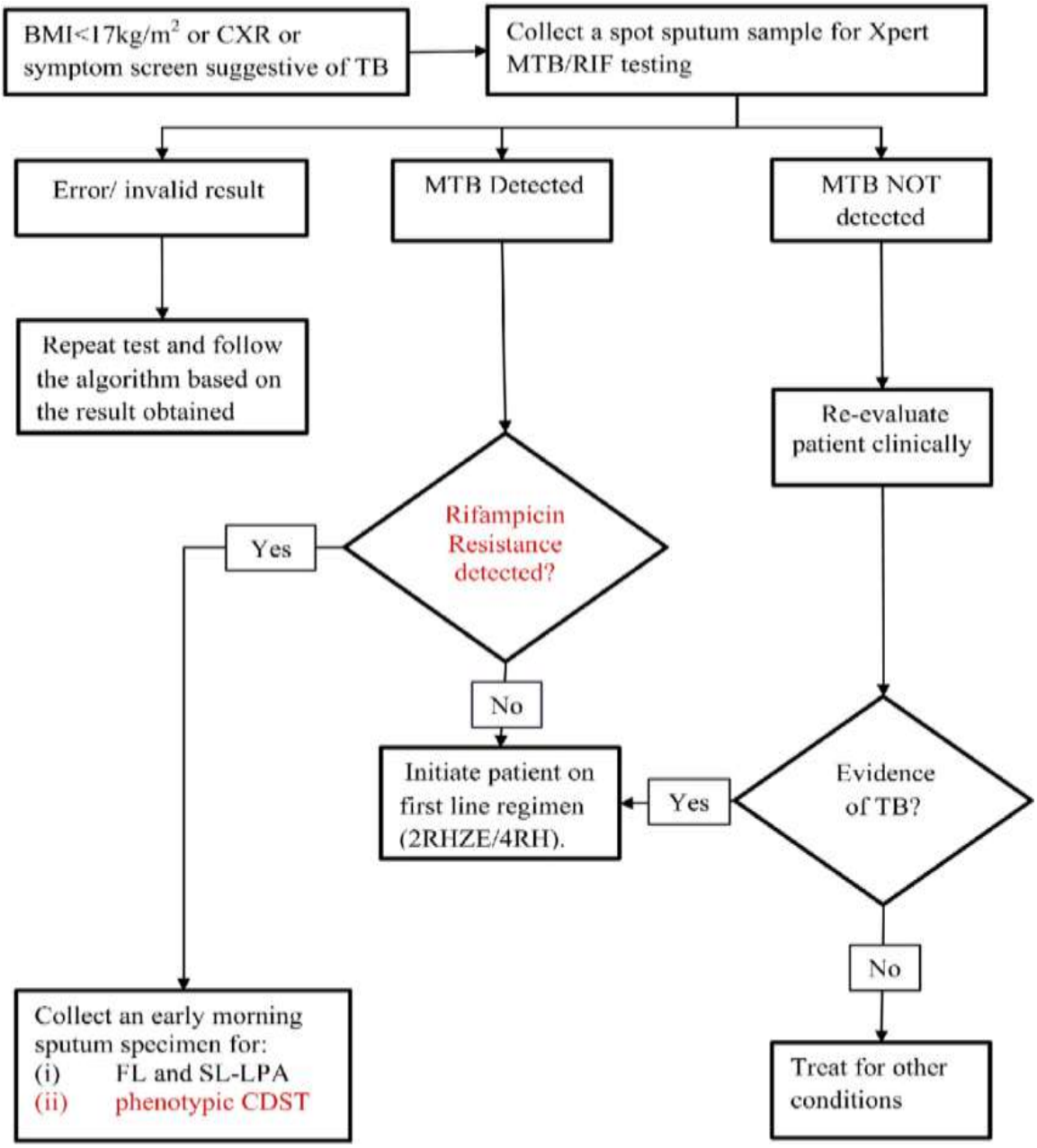

Figure 5 The simplified new TB testing algorithm for Zimbabwe, 2016-18 (MoHCC., 2017)

Several studies have reported subclinical TB as defined by culture-positive TB in the absence of TB symptoms. Swaminathan *et al.* screened HIV-1 infected persons in India and reported a 4% prevalence of unrecognised TB in HIV-1 infected persons with minimal or no symptoms, normal chest x-rays, negative smears and different levels of immunosuppression (Swaminathan et al., 2004). Mtei *et al*. also recruited HIV-1 infected persons with CD4 counts >200 cell/mm^3 and found a 15% prevalence of TB, 29% of which was subclinical (Mtei et al., 2005).

According to a study by Williams *et al* (2020), aerosols potentially detect subclinical tuberculosis better than sputum-based methods. In a 24-hour longitudinal study of patients admitted to one of 3 hospitals in Pretoria, Mtb was detected in 86% of 192 face mask samples and 21% of 184 assessable sputum samples obtained over a 24-hour period (Williams et al., 2020). Face mask sampling thus offers a highly efficient and non-invasive method for detecting exhaled Mtb, informing the presence of active infection, both with greater consistency and at an earlier stage than with sputum samples (Williams et al., 2020). It is a very simple method which is easily deployable, clinically compatible, which can also detect other target organisms and reliably yields a sample and it seems superior to sputum samples for the detection of early TB disease (Williams et al., 2020). However, there is still need for large scale, community-based studies to determine the potential of this method in enhancing early diagnosis and transmission control in tuberculosis.

Risk Factors for subclinical tuberculosis in people living with HIV

People living with HIV are classified as a high-risk group for both active and subclinical TB. Several risk factors are point towards the predictive of subclinical TB disease amongst PLHIV and these include the following:

Lower CD4 count

A low CD4 count is one of the risk factors of subclinical TB in PLHIV. In a previous study by Oni *et al.* a 5% decrease in subclinical TB disease was observed for every increase in the CD4 count of 10 cells/mm^3. It is therefore, important for PLHIV to monitor the CD4 count, as well as to resume ART early to avoid a drop in the CD4 count, and this will assist in curbing the spread of TB. Low CD4 count (OR 0.996, p= 0.060) showed a trend towards being predictive of subclinical TB disease.

Increasing number of days since HIV diagnosis

An increase in the number of days since HIV diagnosis is positively associated with an increased risk of developing subclinical TB. Oni *et al*. reported a 24% increase in the risk of subclinical TB disease for every year after HIV diagnosis (Oni et al., 2011). A longer history of HIV infection (OR 1.006, p= 0.056) had a higher risk of being predictive of subclinical TB disease.

A positive TST

A positive TST is also associated with an increased risk of developing subclinical TB disease. According to a previous study by Oni *et al.* a positive TST (OR 4.96, p=0.064) had a positive trend towards being predictive of subclinical TB disease.

Chapter Summary

Now is the time to shine a bright light on asymptomatic TB. Previously people living with HIV had to wait until they were ill with "full-blown" Acquired Immune Deficiency Syndrome (AIDS) until they were eligible for antiretroviral therapy and now there is clear evidence that such an approach was harmful to individuals' health and also contributed to the ongoing transmission that fuelled the epidemic (Wong., 2020). It is also possible to look back at the era in which people waited to treat TB until patients developed full-blown disease with similar regrets. Therefore, TB strategies must focus on identification and treatment of subclinical TB if the TB epidemic is to be eradicated. It is also imperative to tackle the risk factors associated with an increased risk of subclinical TB disease.

METHODOLOGY

Introduction

The methodology selected for this study is detailed in this chapter. The research methodology, research design, study setting, methods of sample selection, data collection and analysis of results is also explained. The ethical considerations for this research study are also detailed. It is hoped that the chosen methodology generated useful information through the collection and analysis of data on the utilisation of face masks in the detection of sub-clinical tuberculosis in PLHIV.

Research Methodology

Since this topic has not been previously studied in depth in the Zimbabwean context, an exploratory cross-sectional study was conducted with a purposive random sampling technique. PGH was purposively chosen because of its desirable characteristic of population diversity since it is the major referral centre in Zimbabwe.

Research Design

An exploratory cross-sectional study design was used in this study because it was less costly and could be done

in the short space of time which was available, yet it can measure multiple variables at the time of the data snapshot producing findings which can generate a hypothesis which can be tested in further researches. This study design suited the limited time-frame, at the same time allowing a one-time data collection procedure. The existence or absence of Mtb constituted the dependent variable while the independent variables included factors like age, gender, marital status, race, employment history, highest level of education, denomination or belief, and period on ART therapy.

Since data was supposed to be collected from a large pool of all PLHIV coming for routine HIV care, an exploratory cross-sectional study was deemed the ideal study design for the present study, as it also allowed the collection of data at a single point in time.

Study Site

The study was carried out at PGH OI unit in Zimbabwe, which is a developing country in Southern Africa with a population of about 13 million people according to the 2013 census report (ZIMSTAT., 2013). It has a gross national index per capita of US$860 with 72.3% of the population living below the national poverty line (The World Bank., 2015). Sample collection was carried out at PGH OI unit where HIV positive patients were reporting for routine check-up. PGH is the major referral centre in Harare, Zimbabwe, and it is the largest medical centre in Zimbabwe. It has a daily occupancy of between 950 to 1000 patients per day. The facility has general medical and surgical sections, Mbuya Nehanda, the maternity section, Sekuru Kaguvi, which specialises in eye treatment; and an annex for psychiatric patients and several specialist paediatric wards. It has an excess of 5000beds and 12 theatres in the main hospital complex. There is also the opportunistic clinic section where PLHIV come for routine HIV care. PGH also has a 24-hour operating accident and emergency department. All these departments are supported by auxiliary services such as the laboratory, pharmacy and physiotherapy.

Sample processing was carried out at GoPath Clinical Laboratories' main branch in Harare, Zimbabwe using the Xpert MTB/ RIF Ultra platform. GoPath Clinical Laboratories is one of the main private laboratories in Zimbabwe, and it offers pathological testing in all departments which include Microbiology, Serology, Haematology, Biochemistry, Immunochemistry, Histology and Cytology. It offers both microbiological and molecular screening tests for Mtb. The gene Xpert MTB/ RIF Ultra assay is one of the most sensitive and specific tests for the detection of Mtb in sputum and/ or aerosol samples.

Both PGH and GoPath Clinical Laboratories are located in an easily accessible area which are within the researcher's reach.

Figure 6. Study site, PGH

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Study Population

HIV positive patients with no clinical symptoms of tuberculosis or confirmed active TB infection were eligible to participate in the study. PLHIV were selected since they are one of the most vulnerable groups of people to pTB disease. PLHIV exhibiting signs and symptoms of TB were excluded from the study since these were most likely active TB disease carriers.

Inclusion and Exclusion Criteria

Inclusion Criteria

Consenting HIV positive patients with no clinical symptoms of TB or confirmed active TB infection coming for routine check-up at PGH OI unit. Participants under the age of 18 were asked to provide parental/ guardian consent. Participants incapable of reading and signing the written consent were assisted by either their relatives or sister in charge at PGH OI unit.

Exclusion Criteria

Non-consenting HIV positive patients.

HIV positive patients presenting with clinical symptoms of TB or with confirmed active TB infection were excluded.

Sample Size

A minimum sample size of 145 participants was adequate for the study, however, 160 study participants were enrolled into the study to increase the power of the study. The minimum sample size was estimated using WHO's findings in 2016 (WHO, 2016) where 40% of HIV deaths were found to be due to tuberculosis; and the Dobson's formular was used;

$$n = \frac{Z_{\alpha}^{2}\,(P)(1-P)}{d^{2}}$$

Where;

n is the minimum sample size, (n = 145)

P is the prevalence of the condition/ health state based on previous literature, (P=0.4)

d is the precision of the estimate (d=0.08), and,

Z_{α} is the Z-value for the 95% Confidence Interval from the probability tables, that is alpha = 5% (Z = 1.96).

Sampling Procedure

A systematic random sampling technique was utilised, where consenting HIV positive patients with no clinical symptoms of TB, previous history or active pTB infection attending PGH OI unit for routine check-up were enrolled into the study. Every third patient who entered the consultation room was selected for the study, and in the event the participant chose not to participate, the next patient in line was selected. A structured questionnaire was administered through an interview. Face masks worn by PLHIV were exchanged for new and clean ones. The old masks were then sent to GoPath Clinical Laboratories for Mtb screening using the Xpert MTB/RIF Ultra platform. Mtb-positive patients were identified and linked to clinical care. A purposive random sampling technique was used for the selection of records, and a desk review was conducted for data extraction.

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Data collection Instruments

Pretested interviewer administered questionnaires which adopted some components from the WHO Stepwise Survey (WHO Stepwise Survey 3.1 version) were used to collect data for the patients while a desk review of medical records was done for additional data extraction. The questionnaire was divided into two sections. Section A consisted of the demographic information of the patient and this included the name, date of birth, age, gender, ethnicity, religion and identification number. Section B consisted of questions regarding the signs and symptoms of tuberculosis; and the exposure status of the study participants. Study participants were asked if they had encountered any of the common symptoms of TB, and also if they had been in contact with or visited persons with TB or high TB-prone areas in the previous 5 years. Prior to section A, there was a brief explanation of the study topic and instructions. The questionnaire was available in both English and Shona languages.

Validity and Reliability

The data collection tools were first developed in English before being translated to the local language (Shona), and then translated back to English. Comparisons of the original and back-translated versions was done to check for validity, and necessary adjustments were made as per need.

Pretesting of the data collection tool

The interviewer administered questionnaire was pretested prior to data collection using the respondent-driven process. This was done at PGH OI unit to determine the potential effectiveness of the questionnaire and also to check if the respondents were able to understand the questions. Sixteen random PLHIV attending routine HIV care at PGH OI unit were selected and asked to complete the questionnaire, taking note of the time they will take to respond and the way they were responding. Sixteen respondents were selected as they were deemed enough to warrant significant results since they represented 10% of the projected sample size for the study. Necessary adjustments were made based on the constructive feedback obtained.

Data collection Procedure

Data collection was conducted in September and November 2022. Research assistants and nurses at PGH OI unit were trained on data collection procedures prior to the actual data collection process. All were asked to fill in confidentiality forms to ensure privacy and confidentiality during the data collection procedure.

Potential participants were asked questions based on the interviewer administered questionnaire, and this was done at PGH OI unit where a room had been reserved for the exercise. This allowed a proper screening of the respondents so that only those presenting with no clinical symptoms of TB and with no history or active TB infection were enrolled into the study. If confirmed suitable to participate, participants were asked to complete an informed consent. Authority was also sought from Africa University Research and Ethics Committee, the Joint Research and Ethics Committee (JREC), and PGH authorities. The data collection procedure was set at 10 minutes per respondent.

After completion of the Informed consent and questionnaire, the participants were asked to exchange their worn mask with new and clean ones. The old mask was placed in a zip lock biohazard specimen bag, each mask being placed in a different labelled biohazard specimen bag, and these were packed in a biosafety transport box and send to the laboratory for processing. Covid-19 prevention protocols such as social distancing, wearing of face masks and use of alcohol-based hand sanitizers were observed before, during and after the data and sample collection procedures. During sample processing, the researcher observed the use of personal protective equipment such as laboratory coats, nitrile gloves, face masks and googles. A class II Biosafety Cabinet was also used during testing. Wastinnova Biohazard Waste Disposal Services private limited company was responsible for the disposal of the face masks and cartridges after sample processing.

Independent variables/ exposures included gender, marital status, level of education, employment history, denomination/ belief, race, age and period on ART. Dependent variables or outcome was the presence or

absence of Mtb in face masks which concluded whether the participant had subclinical TB disease or not.

Analysis and Organisation of data

Data capturing was done using Microsoft Excel and individual participant data was first analysed separately before being reduced to aggregate data for statistical analysis. Data was analysed by plotting bar and line graphs of the various indicators used in the study. The prevalence of sub-clinical TB, that is the proportion of PLHIV with positive Mtb results on the Xpert MTB/RIF ultra-platform, was calculated. The proportion of patients with Rifambicin resistant Mtb was also calculated. A summary of the main characteristics was compiled and shown in tabular form. Level of significance was set at 5%.

Ethical Considerations

Ethical approval was sought from Africa University Research and Ethics Committee and the Joint Research and Ethics Committee. This was done by submitting the research proposal to both ethical committees. Permission was also sought from PGH authorities and these included the Clinical Director, the Matron and Sister in Charge at OI unit.

Informed Consent

Written informed consent was obtained from the participants prior to sample collection. Participants incapable of reading and signing the written consent were assisted by either their relatives or sister in charge at PGH OI unit. Participants under the age of 18 were asked to seek parental authorisation/ consent before enrolling into the study.

Voluntarism

Participation in this study was voluntary and this was clearly stated to the prospective participants. Prospective research participants were fully informed about the procedures and any potential risks involved in the study and they were asked to consent to participate. Participants were free to withdraw their consent and discontinue from participating in the study at any given time without being penalised.

Confidentiality

Participant information, records and results were treated with confidentiality. Password protected documents were used to ensure data security. Individual responses were not linked with participants' identities and sample identifiers were only be accessible to the researcher. The completed questionnaires were kept in files which were stored in lockable cupboards and these were only accessible to the researcher.

Risks and Benefits

No foreseeable risks, discomforts or inconveniences were expected to be encountered by the participants in this study. However, tuberculosis positive participants were linked to healthcare and possible counselling, where necessary. The main benefit of this study to the participants is that they got to know their tuberculosis status without paying any amount for the service, and this also helped in protecting their friends, family and relatives from the infection. If confirmed to have tuberculosis it was an opportunity for the participants to quickly seek medical attention as they were linked to healthcare. This study also assisted the general population by identifying the burden of sub-clinical tuberculosis in at-risky individuals and also indicating whether tuberculosis screening and detection should be prioritised amongst the same population. It also helped by identifying whether face mask sampling can be regarded as a possible sampling method for tuberculosis diagnosis and therapeutic monitoring. Participants also received USD$2 as a participating incentive.

Non-discrimination Participation

All HIV positive patients reporting for routine check-up at PGH OI unit were considered for the study. An interviewer administered questionnaire was used to screen participants without discriminating any individuals

based on sex, race, ethnicity or any other factors.

Chapter Summary

This chapter detailed the study methodology that was used by describing the study design, study setting, population under study, sampling technique, data collection tools, procedure and data analysis, together with the ethical considerations which guided the study.

DATA PRESENTATION, ANALYSIS AND INTERPRETATION

Introduction

This chapter presents the findings and data analysis from the study. The main purpose of the study was to detect and confirm the existence of subclinical tuberculosis in PLHIV attending routine HIV care at PGH OI unit, using the face-mask sampling method.

Demographic characteristics of the participants

A total of 160 participants were enrolled into the study, and 56 (35%) were males whilst the remaining 104 (65%) were females giving a 1:1.9 male to female ratio. The ages of the participants ranged from 18 to 80 years old with a mean age of 42±9 years. Ninety-nine (61.9%) of the participants belonged to the Pentecostal church, 28 (17.5%) were from the apostolic sect, and 20 (12.5%) were from orthodox churches (Table 1). The majority (61.3%) of the participants were formally employed, 22.5% were self-employed, and 16.3% were unemployed. The majority (68.8%) of the participants were married or cohabiting, whilst 26 (16.3%) were widowed, 20 (12.5%) were divorced or separated, and 4 (2.5%) were single (never married). The highest level of education of the participants at the time of the study ranged from none 4 (2.5%) to Master's degree level 3 (1.9%) as shown in Table 1.

Table 1 Demographic characteristics of the participants

Variable		**Frequency n = 160**	**Percentage (%)**	
Age (years)	Mean			42
	Standard deviation			9
Gender	Males	56	35.0	
	Females	104	65.0	
Marital Status	Single (Never married)	4	2.5	
	Married or cohabiting	110	68.8	
	Divorced or separated	20	12.5	
	Widowed	26	16.3	
Race	African	160	100	
	Other	0	0.0	
Religion	Pentecostal	99	61.9	
	Apostolic	28	17.5	
	Orthodox	20	12.5	
	Traditional	13	8.1	
Occupation	Formally employed	98	61.3	
	Self-employed	36	22.5	

	Unemployed	26	16.3	
Highest level of education	None	4	2.5	
	Primary level	8	5.0	
	Ordinary level	96	60.0	
	Advanced level	22	13.8	
	Diploma	17	10.6	
	Bachelor's degree	10	6.3	
	Master's degree	3	1.9	

Participants' place of residence

Of the 160 participants, 68.1% (109) were from the high-density suburb, 2.4.4% (39) were from the medium-density suburb, 3.1% (5) were from the low-density suburb, and 4.4% (7) were from the rural area, as shown in table 2 below.

Table 2 Study participants by place of residence

Variable	**Characteristic**	**n**	**%**
Residence	High Density	109	68.1
	Medium Density	39	24.4
	Low Density	5	3.1
	Rural	7	4.4

Participants' Body Mass Index (BMI)

The weight and height of the participants was used to calculate the Body Mass Index (BMI). Sixty-seven (41.8%) of the participants were overweight, 59 (36.9%) had normal weight and 34 (21.3%) were underweight, as illustrated in figure 7.

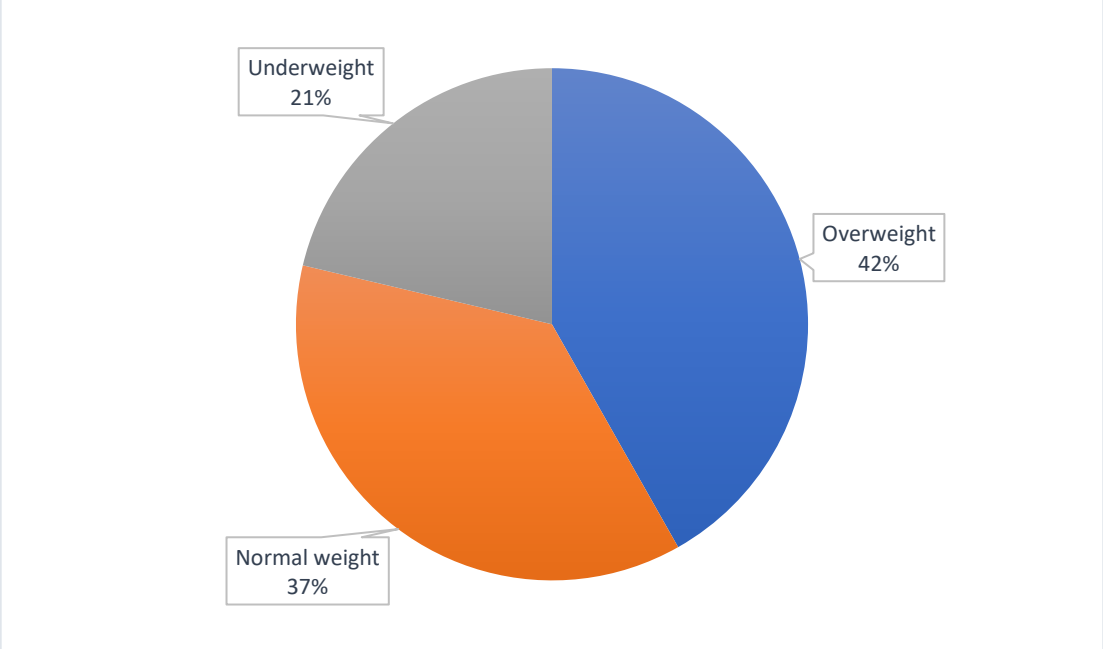

Figure 7 Participants' BMI

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Participants' physical activities

Data on participants' physical activities was also collected during data collection, and the majority of the participants, 80.0% (128) reported doing low intensity physical activities like walking, 11.3% (18) reported doing middle intensity physical activities like jogging, 8.1% (13) reported doing high intensity physical activities like running, and 0.6% (1) reported doing no physical activities. Of those who exercised, 48.0% reported that they exercised daily, 20.0% exercised at least once in 2 days, 22.0% exercised at least twice a week, and 10% exercised at least once a week. Figure 8 below shows the distribution of the participants' physical activities.

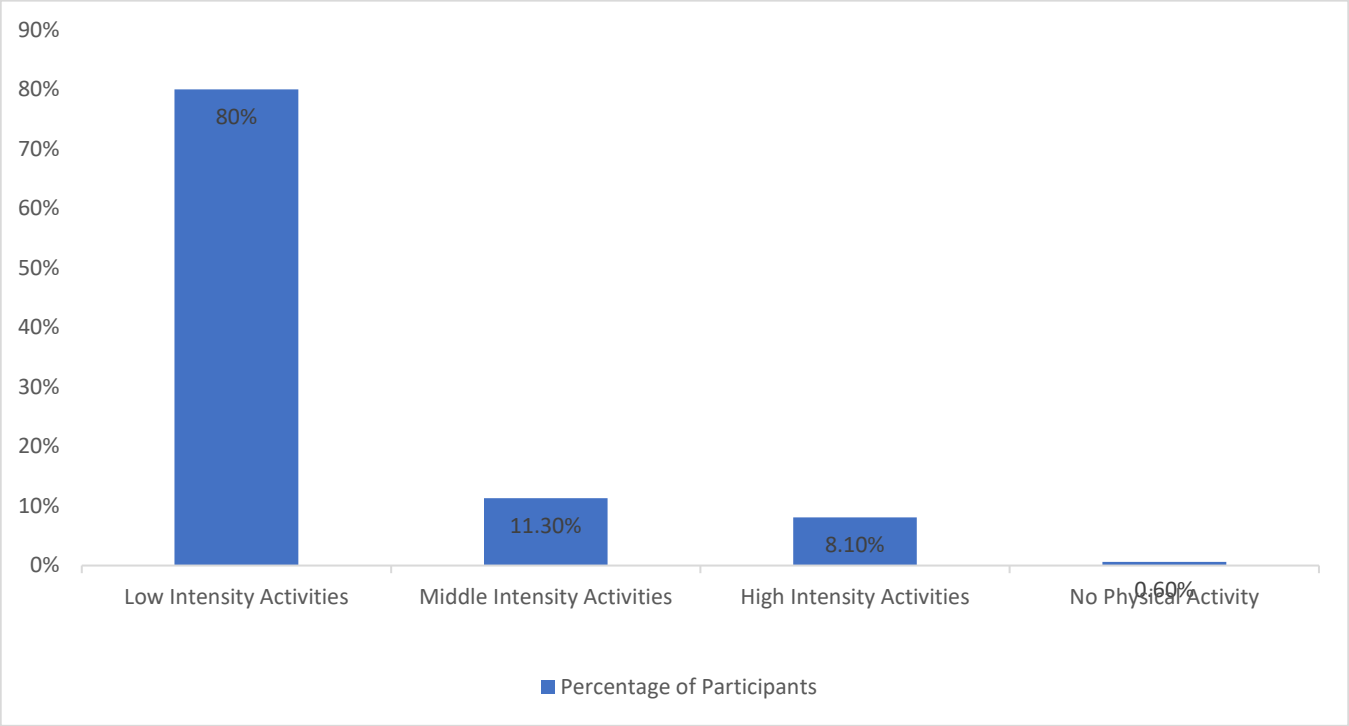

Figure 8 Participants' physical activities

Tuberculosis Diagnoses

Forty percent (64) of the participants were on antiretroviral therapy (ART) for 1-2 years, whilst 48 (30%) apiece were on treatment for less than a year and for more than 2 years (Figure 9).

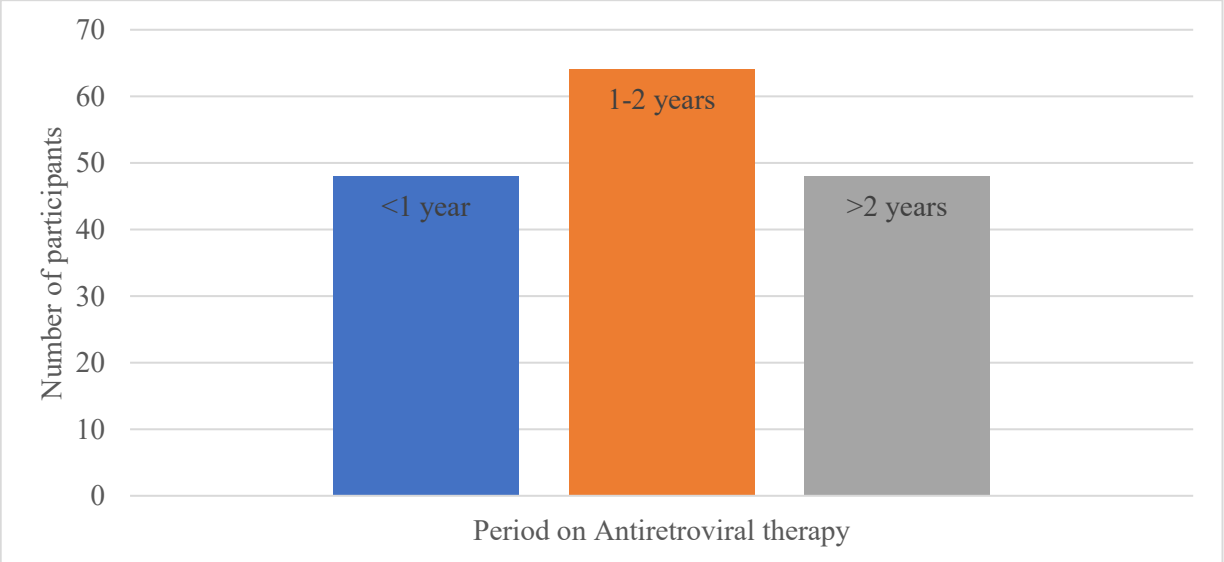

Figure 9 Participants' number of years on Antiretroviral therapy (ART)

All participants were asymptomatic for tuberculosis. Using the Gene Xpert MTB/RIF ultra-platform, 4 (2.5%) of the participants were positive for MTB gene Xpert, and 156 (97.5%) were negative as shown in Figure 10. All 4 positive cases were Rifampicin resistance negative, had been on ART for less than 1 year, and were at least 60 years old.

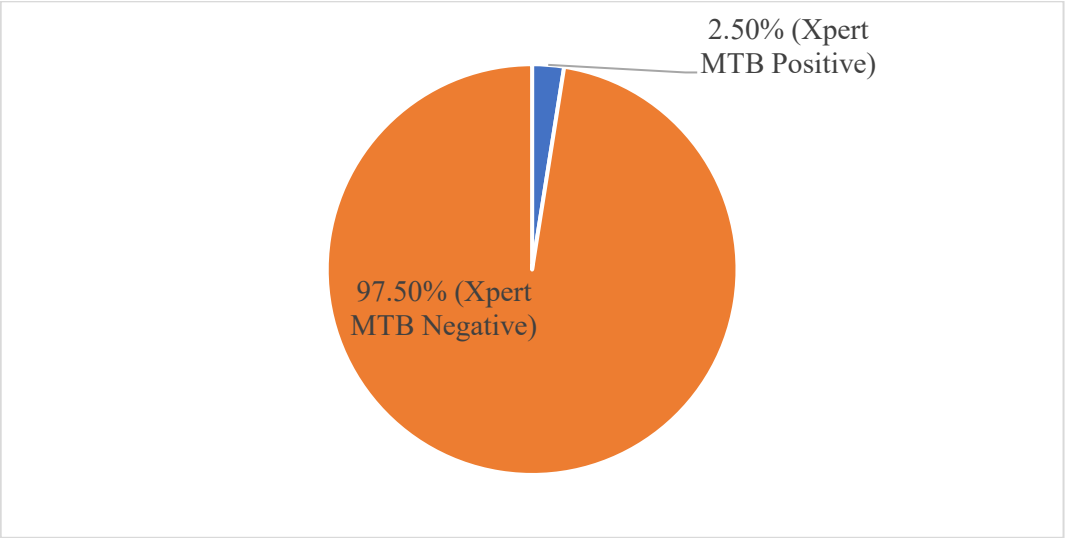

Figure 10 Xpert MTB/RIF outcome

Chapter Summary

In summary, 4 (2.5%) of the 160 study participants had Mtb detected in their face masks. All were at least 60 years old and had been on ART for less than one year. All positive cases were Rifampicin resistance negative.

DISCUSSION, CONCLUSION AND RECOMMENDATIONS

Introduction

Published studies on Mtb bioaerosl samples have been limited to individuals with sputum-positive pTB, however, this study proposed the use of face mask sampling to investigate the prevalence of Mtb-containing bioaerosols in asymptomatic but at-risk individuals, at the same time evaluating the potential to identify unrecognised transmitters of TB. This chapter highlights the discussion of the major findings, conclusion and recommendations based on the study findings.

Discussion

Subclinical TB cases are increasingly gaining attention due to their high potential to exacerbate TB transmission (Oni et al., 2011, Kendall et al., 2021). Accurate estimates of their contribution to the transmission of infectious Mtb bacilli are hampered by the limited available evidence. There are no current studies available to describe the prevalence of subclinical TB in PLHIV, and the potential of face mask sampling in diagnosing these patients.

Tuberculosis diagnosis is mostly based on clinical symptoms and laboratory findings, yet individuals with subclinical TB represent around half of all prevalent TB cases (Emery et al., 2022; Patterson et al., 2020), and their contribution to Mtb transmission is, however, unknown (Emery et al., 2022). Findings from the current study demonstrate that face-mask sampling provides a simple and non-invasive tool to support clinical assessment of pTB in high-risk and high burden settings, for the diagnosis and stratification of the risk of TB transmission amongst HIV-positive patients.

Our data demonstrated that four (2.5%) of the 160 study participants were Mtb positive thus highlighting face-mask sampling as a potential alternative method for screening and diagnosing asymptomatic pTB in HIV patients, at the same time confirming the presence of subclinical TB in PLHIV.

A growing body of literature indicates that individuals without recognizable symptoms of TB are capable of producing infectious droplets to potentially accumulate community transmission of infectious pathogens (Bai et al., 2020). Findings from the present study support the hypothesis that subclinical TB is responsible for a fraction of Mtb transmission, given that Mtb bacilli could be detected by the existing molecular diagnostics in 2.5% of individuals with subclinical TB. Similar findings were also reported by William *et al* (2020) in Gambia, in a longitudinal 24-hour sampling study, where facemask sampling detected Mtb more than 4 times more frequently than in sputum analyses (William et al., 2020). William *et al* (2020) also further supported the proposition by noting that facemask sampling had greater sensitivity than both sputum and chest radiography with an added advantage of sampling at one timepoint (William et al., 2020). Consistent results were also reported in Cape Town, South Africa by Dhana *et al* (2022) in a study where TB screening was conducted among HIV-positive inpatients (Dhana et al., 2022). Dhana *et al* (2022) reported that 2% of HIV-positive inpatients who did not meet eligibility for Xpert testing were positive for Xpert MTB/ RIF, a finding which is similar to findings from the current study. This further highlights the importance of early TB screening in asymptomatic HIV-positive patients.

Sixty five percent of the study participants in the current study were females and this could be due to the poor health-seeking behaviours by their male counterparts. Previous studies have suggested that males have a reluctance to seek access to health services as compared to females (Galdas et al., 2005), and this could be the reason for the low turnout of males as study participants.

All positive cases were at least 60 years old and had been on ART for less than a year. This, therefore, implies that late commencement of ART could have contributed to the TB incidence. ART is associated with a 67% reduced risk of developing active TB disease among PLHIV (Lawn et al., 2010; Takarinda et al., 2020). ART has a TB protective effect among PLHIV (Takarinda et al., 2020), and it has also been shown to reduce the population incidence of TB by between 27% and 80% (Miranda et al., 2007; Middlekoop et al., 2011). The fact that all positive cases were at least 60 years old, and with less than one year on ART, implies that the cases might have taken long to seek access to health services. It also brings the relevance of an increase in the number of days since HIV diagnosis was made. Oni *et al.* reported an increase of 24% in the risk of TB for every year after HIV diagnosis.

To our knowledge, TB screening has not been performed in asymptomatic HIV-positive patients, despite evidence of transmission from this group of individuals (Behr et al., 1999; Hernandez-Garduno et al., 2004; Tostmann et al., 2008; Lawn et al., 2009). This study, therefore aimed to extend attempts to detect Mtb in asymptomatic HIV-positive patients. Expectorated sputum, which is the number one specimen for pTB diagnosis has significant limitations as highlighted by Fennelly *et al* (2019) hence the need for an alternative method. Observations from multiple studies conducted in different high-burden settings indicate that only 1 – 30% of new Mtb infections can be linked with known TB cases (Crampin et al., 2006; Glynn et al., 2015; Middlekoop et al., 2015; Verver et al., 2004). This is consistent with the existence of many unrecognised transmitters in TB endemic communities. Based on findings from the current study, we therefore hypothesize that asymptomatic individuals represent a major driver of transmission. However, it has been historically difficulty to investigate the infectiousness of this subpopulation, partially owing to the dependence on sputum for diagnosis (Patterson et al., 2020).

Globally, only 55% of pTB cases are bacteriologically confirmed by sputum testing (WHO., 2019; Patterson et al., 2020). The remaining cases are either diagnosed using clinical symptoms or by chest radiography, or they occur without Mtb bacilli accumulation in sputum (Patterson et al., 2020). Some patients do not produce sputum, others are not able to expectorate adequately, whilst children and HIV-infected individuals are usually associated with poor sputum sample quality leading to sub-optimal diagnostic sensitivity (Patterson et al., 2020). Xpert MTB/RIF or culture positivity in bronchoalveolar lavage from sputum-negative or sputum-scarce patients (Barnard et al., 2015; Lee et al., 2013; Kim et al., 2020) suggests the presence of Mtb in the peripheral airways. This study, therefore, provides preliminary data on the clinically relevant role of face mask sampling as a novel diagnostic specimen for TB screening and diagnosis.

A further key question is an extent to which individuals at high risk for subclinical TB should be prioritized for testing and treatment. Several studies have reported that individuals at high risk for subclinical TB are similar

to those for active TB, including residence in high-incidence settings and persons with a history of TB (Oni et al., 2011; Drain et al., 2018). In a recent modelling study where PLHIV comprise a small proportion of subclinical TB cases conducted by Kendall and colleagues showed that HIV-positive individuals progress more rapidly from infection to disease onset (Kendall et al., 2021).

Limitations of the study

Since all data was collected in Harare, Zimbabwe, the generalisability of this study's findings to other geographical regions and low TB prevalence settings remains unclear. No modified masks were used in this study and this might have impacted the Mtb yield. Modified face masks with a gelatine sampling filter would have improved the Mtb yield thus reducing the chances of missed diagnoses. However, surgical masks were used as these were the ones mostly available and which were being used by the majority in Zimbabwe. The unavailability of previous data or literature in the Zimbabwean context to support a reliable sample size calculation was also another major limitation. Time factor was a limitation; this study was supposed to be completed within 8 months and thus it was not possible to conduct a prospective cohort study within the specified period. A prospective cohort study would have allowed a follow up of the study participants especially those who would have shown face mask sampling positivity to characterise the natural history of subclinical Mtb shedding. Resampling of the study participants over a specified period would have increased the accuracy of the study. A prospective cohort study would also have made it possible to give participants modified face masks for a specified period before sample collection was done thus increasing the Mtb yield.

Conclusion

Face mask sampling offers a new approach to understanding and diagnosing tuberculosis. It is simple, clinically compatible, capable of detecting other target organisms, reliably yields an adequate sample, and is superior to sputum samples in the detection of early TB infection. It also shows potential for the screening and diagnosis of TB, especially in difficult-to-reach communities. This study, therefore, supports the potential of face mask sampling as a clinical tool which can be used to enhance TB control programmes necessary for eradication of TB, and provides an epidemiological tool which can be used to better characterise Mtb transmission within complex community settings.

Recommendations

This exploratory cross-sectional study acts as a proof-of-concept which provided valuable observational data to inform further work. Large scale and community-based studies are recommended to determine the potential of face mask sampling to enhance early TB diagnosis and transmission control. Since low CD4 count and TST status is associated with an increased risk of developing subclinical TB in PLHIV, a further follow up on the subjects to determine the CD4 counts and TST status will further explore the assumptions.

Dissemination of results

The study findings or outcomes of the analysis will be submitted for publication following all appropriate research reporting guidelines. The study outcome information will also be disseminated to Africa University department of Public Health and Nursing, Africa University library, Parirenyatwa Group of Hospitals management, Parirenyatwa Group of Hospitals Opportunistic Infections Unit and to GoPath Clinical Laboratories.

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Finally, I would like to thank my wife, Gracious Chawasarira, for her encouragement and unwavering support throughout this research study.

Acronyms and Abbreviations

AIDS	Acquired Immune Deficiency Syndrome
ART	Antiretroviral therapy
CDC	Centres for Disease Control and Prevention
HIV	Human Immunodeficiency Virus
IGRA	Interferon-gamma Release Assay
IPT	Isoniazid preventive therapy
MDRTB	Multiple Drug Resistant Tuberculosis
MOHCC	Ministry of Health and Child Care
Mtb	Mycobacterium tuberculosis
OI	Opportunistic Infections
PCR	Polymerase Chain Reaction
PGH	Parirenyatwa Group of Hospitals
PLHIV	People Living with HIV
pTB	Pulmonary Tuberculosis
TB	Tuberculosis
TST	Tuberculin Skin Test
W4SS	WHO four-symptom screening
WHO	World Health Organisation

Definition Of Terms

Tuberculosis It is a bacterial infection spread through inhaling tiny droplets from the coughs or sneezes of an infected person.

Mycobacterium tuberculosis It is a species of pathogenic bacteria in the family Mycobacteriaceae and the causative agent of tuberculosis.

Active tuberculosis It is an infection in which Mycobacterium tuberculosis multiply in the body, causing noticeable symptoms.

Subclinical tuberculosis It is a disease due to viable Mycobacterium tuberculosis bacteria that does not cause clinical tuberculosis-related symptoms but causes other abnormalities that can be detected using existing radiologic or microbiologic assays.

Latent tuberculosis It is a condition where Mycobacterium tuberculosis is present in the body but the person does not have tuberculosis.

Epidemic It refers to a widespread occurrence of an infectious disease in a community at a particular time.

People living with HIV It refers to people infected with the human immunodeficiency virus (HIV), a retrovirus which if untreated may progress to acquired immunodeficiency syndrome (AIDS).

Tuberculin Skin Test (TST) It is a skin test to detect if you have been infected with Mycobacterium tuberculosis.

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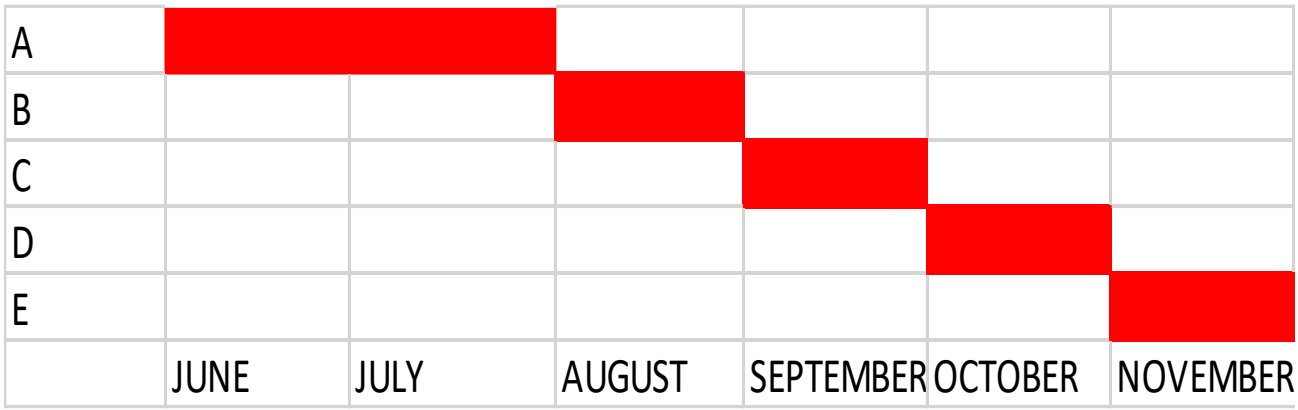
APPENDICES

Appendix 1 Budget and Resource Availability

BUDGETARY ALLOCATION	AMOUNT (USD)
Travelling/ Fuel costs	$100
Printing and photocopying	$50

Mobile Data	$10
Face Masks	$10
Data collection	$400
Sample collection incentive	$400
Statistician	$200
Sample Biohazard Bags	$20
Xpert MTB/ RIF catridges	$1500
TOTAL	**$2690**

Appendix 2 Dissertation Timeline



Key

A: Dissertation proposal and AUREC approval

B: Sample collection and processing

C: Data compilation and analysis

D: Discussion and conclusion

E: Supervisor's review, adjustments and submission

Appendix 3 Participant Informed Consent in English

AFRICA UNIVERSITY
A United Methodist-Related Institution

Participant Informed Consent

PROTOCOL TITLE: Detection of sub-clinical tuberculosis in face masks worn by people living with HIV.

NAME OF RESEARCHER: Courage Kumbirai Mpandawana.

PHONE NUMBER: +263773512571

Project Description

This study aims to detect sub-clinical tuberculosis in face masks worn by people living with HIV. Patients found to have tuberculosis will be linked to healthcare.

Your Rights

Before you decide whether or not to volunteer for this study, you must understand its purpose, how it may help you, the risks to you, and what is expected of you. This process is called informed consent.

Purpose Of The Research

The purpose of the study is to identify and confirm the existence of sub-clinical tuberculosis in people living with HIV using their face masks and also to determine the utility of face mask sampling for tuberculosis diagnosis and therapeutic monitoring. You were selected for the study because you are at risk of contracting tuberculosis and hence early detection will help you quickly get the treatment, at the same time preventing its transmission to your friends, family and relatives. One hundred and ninety-nine other study participants will also be recruited into the study.

Procedures Involved In The Study

If you decide to participate you will be asked a few questions, after which your old face mask will be exchanged with a new one. The old face mask will be sent to the Laboratory for tuberculosis screening. It is expected that this procedure will take about 10 minutes per respondent and your results will be available within 7 days from sample collection.

Discomforts And Risks

No foreseeable risks, discomforts or inconveniences are expected to you in this procedure. However, if your results for tuberculosis are positive, you will be linked to healthcare and possible counselling, where necessary.

Potential Benefits

The main benefit of this study to you is that you will get to know your tuberculosis status without paying any amount for the service, and this will help in protecting your friends, family and relatives from the infection. If you are confirmed to have tuberculosis it will be an opportunity for you to quickly seek medical attention and you will be linked to healthcare. This study will also assist the general population by identifying the burden of sub-clinical tuberculosis in at-risky individuals and also indicating whether tuberculosis screening and detection should be prioritised amongst the same population. It will also help by identifying whether face mask sampling can be regarded as a possible sampling method for tuberculosis diagnosis and therapeutic monitoring. You will also get USD$2 as a participating incentive.

Study Withdrawal

Participation in this study is voluntary and if you decide not to participate in this study, your decision will not affect your future relationship with Parirenyatwa Group of Hospitals. Similarly, if you choose to participate,

you will be allowed to withdraw your consent and discontinue participation at any given time without any penalty.

Confidentiality Of Records

Participant information, records and results obtained in this study will not be disclosed to anyone without your permission. Your name or any other identification will not be asked for in the questionnaires and sample identifiers will not be shared to anyone without your permission.

Problems/ Questions

Before you sign this form, please ask any questions on any aspect of this study that is unclear to you. You may take as much time as necessary to think it over and if you have any questions in future, please feel free to ask.

Authorization

I have read this paper about the study or it was read to me. I understand the possible risks and benefits of this study. I know participation in this study is voluntary. I choose to be in this study: I know I can stop being in this study and I will not lose any benefits entitled to me. I will get a copy of this consent form. (Initial all the previous pages of the consent form).

_____ _____

Name of Research Participant (please print) Date

_____

Signature of Research Participant or legally authorised representative

……

……..

Signature of Researcher Date

…… ……...

Witness Signature Date

If you have any questions concerning this study or consent form beyond those answered by the researcher including questions about the research, your rights as a research participant, or if you feel that you have been treated unfairly and would like to talk to someone other than the researcher, please feel free to contact any of the following:

1. Africa University Research Ethics Committee on telephone (020) 60075 or 60026 extension 1156 email aurec@africau.edu
2. Joint Research Ethics Committee for University of Zimbabwe College of Health Sciences and Parirenyatwa Group of Hospitals (JREC) on +263 242 708140
3. Medical Research Council of Zimbabwe (MRCZ) on (+263242) 2791792
4. Dr E Mugomeri, Project Supervisor on +263776167964

Name of Researcher _____

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Appendix 4 Participant Informed Consent in Shona

AFRICA UNIVERSITY
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Mvumo Yetsvakurudzo

MUSORO WETSVAKURUDZO: Tsvakurudzo yechirwere cherurinhi kuburikidza nekushandisa chifukidzo chepameso (Face mask) kune avo vane denda reHIV.

ZITA REMUTSVAKURUDZI: Courage Kumbirai Mpandawana.

RUNHARE RWEMUTSVAKURUDZI: +263773512571.

Tsanangudzo Yetsvakurudzo

Tsvakurudzo ino ndeyekutsvaga chirwere cherurinhi kuburikidza nekushandisa chifukidzo chepameso kune avo vanedenda reHIV. Avo vanenge vaonekwa vaine chirwere ichi vachabatsirwa kuti vakurumidze kuwana rubatsiro kunanaChiremba.

Kodzero Yenyu

Musati mazvipira kuve nhengo mutsvakurudzo ino, munofanirwa kunzwisisa chinangwa chetsvakurudzo ino, rubatsiro rwaichakupai, njodzi nekusagadzikana, zvipundutso zvingangowanikwa uye zvamunotarisirwa kuzoita. Iyi ndiyo inonzi mvumo yetsvakurudzo.

Chinangwa Chetsvakurudzo Ino

Chinangwa chetsvakurudzo iyi ndechekuona nekutsinhira kuwanikwa kwechirwere cherurinhi mune avo vasina zviratidzwa zvingave zvakarerekera kuchirwere ichi asi vachirarama nedenda reHIV, kuburikidza nekushandisa chifukidzo chepameso (face mask). Tsvakurudzo iyi ichatarisawo zvakare kukwanisika kwekushandisa chifukidzo chepameso senzira yekutsvaga nayo chirwere cherurinhi. Munokurudzirwa kuva nhengo yemutsvakurudzo iyi sezvo muri panjodzi yekubatira chirwere cherurinhi uye kuti mubatsirikane nekukurumidza kuziva pamumire maererano nechiwere ichi zvinova zvinobatsira kuti mukurumidze kuwana rubatsiro pamwe nekuchengetedza hama neshamwari dzenyu kubva kuchirwere ichi. Tsvakurudzo iyi ichange iine nhengo mazana maviri.

Hwendaenda Nenguva Ichatorwa

Kana muchinge mazvipira kupinda mutsvakurudzo iyi muchabvunzwa mibvunzo mishoma shoma mozopiwa chifukidzo chemeso (face mask) chinyowani. Chifukidzo chepameso chamanga makapfeka chichaendeswa kurabhoritari kunotariswa kuti hachina utachiona hunokonzeresa denda rerurinhi here. Nhengo imwe neimwe ichatora maminitsi gumi mutsvakurudzo iyi uye tsvakurudzo yekurabhoritari inozobuda mushure memaawa makumi mana nemasere.

Njodzi Nekusagadzikana Kungangowanikwa Kubva Mutsvakurudzo Iyi

Hapana njodzi kana kusagadzikana kunotarisirwa kuva kuchizokonzereswa netsvakurudzo ino. Kana tsvakurudzo dzekurabhoritari dzikabuda dzichitaridza kuti mune denda rerurinhi, muchazobatsirwa

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netsanangudzo yematanho amunokwanisa kutora kuti mukurumidze kuwana rubatsiro kunanaChiremba.

Zvipundutso Kana Muripo Uchawanikwa Kubva Mutsvakurudzo Iyi

Tsvakurudzo iyi ichakubatsirai kuziva pamumire maererano nedenda rerurinhi pasina mari yamuchabvisa zvinova zvichazobatsira kudzivirira hama neshamwari dzenyu kubva kuchirwere ichi. Kune vanenge vabatwa vaine chirwere ichi muchabatsirwa kuti mukurumidze kuwana rubatsiro kubva kunanaChiremba. Tsvakurudzo iyi ichabatsirawo nekuratidza huwandu hwevanhu vane chirwere cherurinhi asi vasingazvizivi kupfurikidza nekunge vasina zviratidziro zvechirwere ichi uye ichataridzawo kukosha kwekuchekwa chirwere ichi kuvanhu ava. Tsvakurudzo iyi icharatidzawo zvakare kuti chifukidzo chepameso chingave imwe yenzira inogona kukwanisa kushandiswa here kutsvaga chirwere cherurinhi. Mumwe nemumwe achave nhengo yetsvakurudzo ino achapiwawo madhora maviri (USD$2).

Kuva Nhengo Yetsvakurudzo Nokuzvidira

Hapana munhu achamanikidzwa kuzova nhengo yetsvakurudzo ino uye vanenge varamba kuva nhengo dzetsvakurudzo ino havasikuzoramba kubatsira pachipatara pano. Nhengo dzichabatsira netsvakurudzo ino dzinobvumidzwa kuchinja pfungwa chero ipi zvayo nguva yadzinenge dzada pasina anodzirambidza.

Kuvanzika

Mazita kana ruzivo rwevanhu vachave nhengo dzetsvakurudzo ino hazvisikuzoratidzwa ani nani zvake pasina mvumo kubva kunhengo idzi uye mazita enyu haasikuzobvunzwa pamibvunzo yamuchabvunzwa mutsvakurudzo ino

Kupindurwa Kwemibvunzo

Musati masaina gwaro rino munobvumidzwa kubvunza mibvunzo yose yamungangodaro muinayo uye munokwanisa kutora nguva yenyu kufunga mibvunzo iyi.

Mvumo

Ndaverenga kana kuverengerwa gwaro retsvakurudzo ino uye ndanzwisisa njodzi nezvipundutso zvingangowanikwa kuburikidza nekuva nhengo mutsvakurudzo ino. Zvakare ndave kuziva kuti ikodzero yangu kusarudza kuva nhengo mutsvakurudzo ino uye ndinokwanisa kuchinja pfungwa chero ipi zvayo nguva pasina anondirambidza. Nokudaro ndinosarudza kuva nhengo mutsvakurudzo ino. Ndichapiwawo gwaro rangu remvumo yesarudzo ino randichagara naro. (Isai runyoro rwenyu pazasi apo senzira yekutaridza kuti maverenga mukanzwisisa uye mukazvipira kuva nhengo mutsvakurudzo ino).

_____ _____

Zita renhengo (Nyorai) Musi

_____

Siginecha yenhengo kana mumiriri wenhengo

_____ _____

Siginecha yemutsvakurudzi Musi

_____ _____

Siginecha yemufakazi Musi

Kana muine mibvunzo maererano netsvakurudzo ino kana bvumidzo yetsvakurudzo ino isina kunge yapindurwa nemutsvakurudzi ivai makasununguka kuchayira panhare dzinotevera:

1. Komiti inoona nezvetsika mutsvakurudzo paAfrica University (AUREC) panhamba dzinoti (020) 60075 kana kuti 60026 extension 1156 tsambambozha aurec@africau.edu
2. Komiti inoona nezvetsika mutsvakurudzo paUniversity of Zimbabwe College of Health Sciences neParirenyatwa Group of Hospitals (JREC) panhamba dzinoti +263 242 708140
3. Kanzuru inoona nezvetsvakurudzo dzezvoutano muZimbabwe (MRCZ) panhamba dzinoti (+263242) 2791792
4. Dr E Mugomeri, Foromani wetsvakurudzo ino panhamba dzinoti +263776167964

Zita remutsvakurudzi _____

Appendix 5 Tuberculosis Screening Questionnaire in English

Study Topic: Detecting sub-clinical Tuberculosis in Face masks worn by people living with HIV.

Study Instructions: Participation in this study is voluntary; and all participants are required to read, understand and sign in the informed consent prior to sample collection. After signing the consent form you are required to fill in this questionnaire which is available in both English and Shona languages. After completing the questionnaire, you are required to exchange the face mask you are wearing with a clean and new one. The old mask will be sent to the laboratory for Tuberculosis screening and your results will be send back to your health care setting within 7 days. Patients found to have Tuberculosis will be linked to clinical care.

SECTION A: Demographic information

Name of Patient: ……

Date of Birth: …….

Age: …….

Gender: ……...

Ethnicity: ……...

Religion: ……..

ID Number: ……

SECTION B: Tuberculosis (TB) Screening Questionnaire

1. Do you have any of the following symptoms? (Mark Yes or No for each)

   Cough (especially if lasting for 3 weeks or longer

   with or without sputum production) Yes ☐ No ☐

	Coughing up blood (haemoptysis)	Yes ☐	No ☐
	Chest pain	Yes ☐	No ☐
	Loss of appetite	Yes ☐	No ☐
	Unexplained weight loss	Yes ☐	No ☐
	Night sweats	Yes ☐	No ☐
	Fever	Yes ☐	No ☐
2.	Have you ever had a BCG vaccine?	Yes ☐	No ☐
3.	Have you ever had a positive PPD or IGRA blood test?	Yes ☐	No ☐
4.	Have you ever had close contact with persons known or suspected to have active TB disease?	Yes ☐	No ☐

5. List the country in which you were born …….

6. List the countries in which you have spent 2 weeks or more in the past five years……
……
…….

7. Have you been a resident, volunteer, and/ or employee of high-risk congregate settings (e.g., correctional facilities, long-term care facilities, homeless shelters, medically underserved, low-income, or abusing drugs or alcohol? Yes No

8. If any yes answers, please explain:
……
……☐…… ☐
……
……

Researcher's Signature: _____ Date: _____

Reviewed by: _____ Date: _____

Appendix 6 Tuberculosis Screening Questionnaire in Shona

AFRICA UNIVERSITY
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Musoro wetsvakurudzo: Tsvakurudzo yechirwere cherurinhi kuburikidza nekushandisa chifukidzo chepameso (Face mask) kune avo vanedenda reHIV.

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Mirayiridzo yetsvakurudzo ino: Hapana munhu achamanikidzwa kuzova nhengo yetsvakurudzo ino uye kana muchinge mazvipira kuva nhengo yetsvakurudzo ino munofanira kutanga maverenga, kunzwisisa nekusaina mvumo yetsvakurudzo ino. Munofanirwawo zvakare kuverenga nekupindura mibvunzo iri mugwaro rino; gwaro rino rinowanikwa mururimi rwaamai nemuchirungu. Kana muchinge madaro muchapiwa chifukidzo chepameso (face mask) chinyowani mosiya chamanga makapfeka icho chichaendeswa kurabhoritari kunotariswa kuti hachina utachiona hunokonzeresa denda rerurinhi here. Tsanangudzo dzekurabhoritari dzinotarisirwa kuzoendeswa kuchipatara chenyu mazuva manomwe asati apfuura uye avo vachaonekwa vainedenda rerurinhi vachazobatsirwa netsanangudzo dzematanho ekuti vatore kuti vakurumidze kuwana rubatsiro kunanaChiremba.

CHIKAMU CHEKUTANGA: Ruzivo pamusoro penhengo dzetsvakurudzo ino

Zita: ……..

Zuva rekuberekwa: ……...

Zera: ……

Murume/ mukadzi: ……...

Rudzi: ……..

Chitendero: ……

Nhamba dzechitupa: …….

CHIKAMU CHECHIPIRI: Mibvunzo neongororo yedenda rerurinhi

1. Makambosanganawo nezviratidzo zvinotevera here? (Makai Hongu kana kwete pane chimwe nechimwe)

   Kukosora (kwemasvondo matatu kana kupfuura muchibuditsa

   kana kusabuditsa gararwa) Hongu ☐ Kwete ☐

   Kukosora muchibuditsa ropa Hongu ☐ Kwete ☐

   Kurwadziwa nechipfuwa Hongu ☐ Kwete ☐

   Kushaya chido chokudya Hongu ☐ Kwete ☐

   Kudzikira uremu zvisina tsanangudzo Hongu ☐ Kwete ☐

   Kubuda ziya wakarara Hongu ☐ Kwete ☐

   Kudziya muviri Hongu ☐ Kwete ☐

2. Makambobaiwa nhomba yekudzivirira chirwere

   Cherurinhi here? Hongu ☐ Kwete ☐

3. Makamboitwa tsvakurudzo yechirwere cherurinhi

   paganda (PPD) kana muropa (IGRA) here? Hongu ☐ Kwete ☐

4. Makambosanga nemunhu kana vanhu vanozikanwa kana kuti vanofungirwa kuti vangangove vaine chirwere cherurinhi here?

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Hongu ☐ Kwete ☐

5. Makaberekerwa munyika ipi …….

6. Ndedzipi nyika dzamakambogara kwemasvondo maviri kana kudarika mumakore mashanu apfuura …….

   ……
   ……
   …….

7. Makambogara kana kuzvipira kana kushanda munzvimbo inenjodzi yakawanda yekubatira chirwere cherurinhi here (mungava mujeri kana kunochengeterwa vasina dzimba kana vakaremara kana kuti kune vanoshandisa zvinodhaka)? Hongu Kwete

8. Kana paine mhinduro imwechete yamapa mhinduro yekuti hongu, munokwanisa kutsanangura zvizere: ……

   ☐ ☐

   ……
   ……
   ……
   ….

   Siginecha yemutsvakurudzi: _____ Zuva/ musi: _____

   Siginecha yemuongorori: _____ Zuva/ musi: _____