THE PREVALENCE AND DETERMINANTS OF ACTIVE TUBERCULOSIS AMONG DIABETES PATIENTS ATTENDING A PRIMARY HEALTH CARE CLINIC IN CAPE TOWN, SOUTH AFRICA

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Dissertation submitted in partial fulfilment of the requirements for the degree

MASTER OF PUBLIC HEALTH (Epidemiology)

In the School of Public Health and Family Medicine

Faculty of Health Sciences

UNIVERSITY OF CAPE TOWN

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February 2016

DECLARATION

I, ...*Adaeze O'jiaku-Okorie*....., hereby declare that the work on which this dissertation/thesis is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university.

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ACKNOWLEDGEMENTS

I would first like to sincerely thank my supervisor, Dr Tolu Oni for seeing my research potential and giving me the opportunity to undertake this dissertation. It has been an absolute privilege working with you. Your patience, insight, and guidance have been invaluable throughout this process. Your constructive feedback and attention to detail has greatly fostered my critical thinking skills. It has been a truly humbling experience.

I would like to thank the Clinical Infectious Disease Research Initiative (Wellcome Trust Grant) for the funding of this study.

Thank you to members of the Robert Wilkinson Group: Amanda Jackson, Dr Natacha Berkowitz, and the rest of the staff members for their contributions to this study.

Special thanks to Karis Moxley at the Faculty of Health Science's writing centre for your additional help in the revision process.

Thank you to my siblings, friends and colleagues for your emotional support and encouragement throughout this process.

I would also like to express my upmost gratitude to my parents for your endless patience, love, and support and for your unwavering faith in my academic endeavours and life's pursuits.

Finally, I would like to thank the patients who volunteered to participate in this study. This dissertation would not have been possible without your vital contribution.

Philippians 4:13

LIST OF ABBREVIATIONS

AFB	Acid-Fast Bacillus Smear
AIC	Akaike's Information Criterion
ART	Antiretroviral Therapy
BMI	Body Mass Index
CI	Confidence Interval
DM	Diabetes Mellitus
FPG	Fasting Plasma Glucose
HbA_{1c}	Glycated Haemoglobin
HIV/AIDS	Human Immunodeficiency Virus-1/ Acquired Immune Deficiency Syndrome
HR	Hazard Ratio
IQR	Interquartile Range
LMIC	Lower Middle Income Countries
LTBI	Latent Tuberculosis Infection
MDG	Millennium Development Goals
MDR-TB	Multidrug-Resistant Tuberculosis
NCDs	Non-Communicable Diseases
NGO	Non-government Organization
NHLS	National Health Laboratory Service
OR	Odds Ratio
RR	Risk Ratio
SES	Socioeconomic Status
SSA	Sub-Saharan Africa
SDG	Sustainable Development Goals
STEPS	WHO STEPwise approach to surveillance of chronic disease risk factors
T1DM	Type 1 Diabetes Mellitus
T2DM	Type 2 Diabetes Mellitus
TB	Tuberculosis
TST	Tuberculin Skin Test
WHO	World Health Organization

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ABSTRACT

Increasing evidence suggests that there is a link between Tuberculosis (TB) and Diabetes Mellitus (DM) with DM tripling the risk of TB.

Although many observational studies have investigated this association, the research has predominantly taken place in Western and Eastern countries. The number of studies addressing the association between DM and TB in the context of sub-Saharan Africa (SSA) is limited and fewer studies have determined whether DM is associated with TB among patients attending diabetes clinics especially in low-resource settings.

In South Africa, where the TB burden is high, the goal to control the infectious disease continues to be a challenge. With the increasing epidemiological shift of disease burden from communicable to non-communicable diseases (NCDs), diabetes is potentially a growing threat to the control of TB. More research is required to develop evidenced-based guidelines that will help improve TB screening among this particular high-risk group.

Through quantitative and epidemiological methods, this dissertation aimed to further explore the association between TB and DM but in a low-resource/SSA setting. The prevalence and risk factors of active TB were investigated among a population diagnosed with, and receiving treatment for DM.

The research protocol (**Part A**), proposes the investigation of socio-demographic risk factors and prevalence of TB by screening DM patients attending a primary care clinic in Khayelitsha using South Africa's national TB programme guidelines, a combination of Xpert MTB/RIF, smear microscopy and *M.tuberculosis* culture testing. In addition to discussing the study design, research justification and ethical implications, the protocol also describes the methodology of the study.

The literature review (**Part B**) consists of the critical appraisal of previous research on the prevalence and association of TB among DM persons. The epidemiology of TB/DM is summarized, risk factors contributing to TB among persons with DM are identified, TB screening methods among DM patients are evaluated, and the gaps and future direction of research are outlined.

The journal manuscript (**Part C**) presents the data of the study. A multivariate logistic regression was performed to investigate key risk factors that contribute to prevalent TB. The results are summarized, analysed and discussed in a format that would meet submission guidelines for a selected peer-reviewed journal.

The appendices (**Part D**) include: Data collection tools, protocol variable table, ethics approval letters, consent forms, protocol of primary study, a detailed statistical analysis of the results, and journal submission instructions to the author.

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PART A: PROTOCOL

A.1 PROTOCOL SUMMARY

Title

The prevalence and determinants of active tuberculosis among diabetes patients attending a primary health clinic in Cape Town, South Africa

Background

Increasing evidence suggests that there is a link between Tuberculosis (TB) and Diabetes Mellitus (DM), with DM, a non-communicable disease (NCD), as a significant risk factor for TB. Although many studies have found an association between TB and DM, the research setting had predominantly taken place in Western and Asian countries. The number of studies addressing the association between DM and TB in the context of sub-Saharan Africa (SSA) is limited and fewer studies exist in countries with low-resource settings, which are typically characterized by shortage of healthcare personnel and a country's lack of funds to cover health care costs. In countries like South Africa, where TB burden is high, the goal to control the communicable disease continues to be a challenge. With the increasing epidemiological shift of disease burden from communicable to NCDs, diabetes is a growing threat to the control of TB.

Methods

Design:	Cross-Sectional Study, Survey.
Population:	Adult persons diagnosed with Diabetes Mellitus (DM).
Site:	Site B Community Health Clinic in Khayelitsha, a peri-urban township in Cape Town, South Africa.
Study Duration:	Duration of data collection and analysis will be from January 2014 to August 2015.
Instrument & Methods:	Privacy and confidentiality will be ensured through the assignment of a unique ID study number for each participant. Sputum will be tested using the rapid test GeneXpert MTB/RIF and smear microscopy (AFB testing). A sputum culture will be done for patients with HIV. Blood samples will be collected to test for baseline glucose and lipid levels. The STEPS tool, a WHO developed, validated three-part questionnaire, will be administered to collect participants' demographic, lifestyle, and biochemical information.

Multivariate logistic regression will be done to identify key risk factors of TB incidence among persons with DM.

Objectives

Primary:

To determine the prevalence of active TB amongst a population diagnosed with, and receiving treatment for DM.

Secondary: To evaluate TB screening and case-finding strategies among persons with DM.

To examine predictors of TB among persons with DM.

A.2 PURPOSE OF THE STUDY

Tuberculosis (TB) is considered a persistent threat to global public health and South Africa is one of 22 high TB burden nations. As a result, efforts to control TB transmission and reduce its prevalence are of importance. NCDs, such as diabetes mellitus (DM) are also increasing in many low-to middle-income countries [1]. Due to behavioural and lifestyle changes, the double burden of diseases is becoming more prominent in sub-Saharan Africa (SSA) [2]. The combination of an already high prevalence of infectious diseases and a growing presence of NCDs amongst the population, makes it a unique challenge [3]. As a result, different strategies should be evaluated to drive the effort of TB prevention and control. In targeting new risk factors, growing evidence suggests that DM is a risk factor for TB. The effect of DM on the incidence and severity of TB and the relationship between nutrition, obesity, DM, and TB remain unknown especially in the South African context [2]. More research investigations should be done to further assess how TB risk varies by socio-behaviour and demographic information, as well as type, duration, and severity of DM.

A.2.1 Objectives

The overall objective for this cross-sectional study will be to determine the prevalence of active tuberculosis amongst a population diagnosed with, and receiving treatment for, DM. Secondary objectives include evaluating TB screening and case-finding strategies among persons with DM. Risk factors associated with TB prevalence among persons with DM will also be investigated.

A.2.2 Research Questions

Primary Question:

What is the prevalence of active TB among DM patients attending a primary health care clinic in Khayelitsha, Cape Town?

Secondary Question:

What are the measured determinants of active TB among the study population?

A.3 BACKGROUND AND SIGNIFICANCE

According to the World Health Organization (WHO), the incidence rate of TB has fallen, albeit slowly, with a 1.5% annual decline in cases since 1990 [4]. In 2014, the prevalence of active TB decreased globally by 42% and the mortality rate by 47% [4]. While an improvement, the rate of declining cases remains slow. The Millennium Development Goal (MDG), which has since been replaced by Sustainable Development Goals (SDGs) in 2016 [5], aimed at a global 50% reduction and prevention of TB by 2015, but this was not accomplished in every region [4]. As a result, TB remains a major threat to global public health, specifically for populations in low to middle income countries. High quality care for TB has increased and improved in the past two decades, making it a highly treatable and preventable disease. However in 2014, an estimated number of 9.6 million people developed TB (an increase from 2012's estimate of 8.6 million people) and 1.5 million died [4,6]. That same year, SSA had the highest burden of TB cases, with 281 per 100,000 population compared to the global average of 133. For co-morbidities such as HIV, SSA carried 75% of TB-HIV burden, as 12% of new cases were HIV-positive [4].

The strategies to combat TB epidemic include continued improvement in diagnosis, treatment, and prevention, but these strategies must be implemented in an efficient and accelerated rate in order to be effective. Consequently, other alternatives need to be explored to improve diagnostic methods of TB. Due to an epidemiological and demographic transition, communicable diseases and NCDs needs to be acknowledged as part of the strategy [3]. A rapid development in urbanization and an aging population, has amplified the incidence of NCDs [2], causing a double burden of both communicable diseases and NCDs. TB risk factors such as poor nutrition and HIV as a co-infection can impair the immune system increasing the likelihood of activating latent TB (LTBI) [7]. However, NCDs that impair immune function, such as DM should also be investigated as

an additional risk factor for TB [3]. As a result, in order to re-evaluate risk factors for TB new risks factors need to be identified [8].

Due to the changing patterns of diet and physical activity, DM has become increasingly prevalent. As of 2010, there were an estimated number of 12.1 million prevalent cases of DM in sub-Saharan Africa and this figure is projected to increase to 23.9 million by 2030 [9]. Eighty percent of deaths due to DM occurred in low-to middle-income countries [10]. Several systematic reviews on the association of DM and TB have concluded that there is a strong association and having DM increases the relative risk of developing active TB by 3.11 (95% CI: 2.27 to 4.26) [11].

In studies examining health outcomes, there is evidence that TB patients with DM have a higher risk of TB treatment failure, death and recurrence compared to TB patients without DM, resulting in a poorer disease outcome [12–14]. Treatment failure of TB in patients with DM might be due to the severity of the disease originating from an altered immune response caused by DM as opposed to drug resistance or low treatment adherence [15]. In order to improve TB treatment outcomes among persons with DM, DM as a comorbidity for TB needs to be addressed in order to develop new strategies for TB control.

It is hoped that through improved DM diagnosis and management of the disease, the negative effects of DM on TB infection can be diminished. Previous observational studies show an increased risk of TB in people with poorly managed DM compared to patients that have better management. Results of a systematic review [12] determined that TB outcomes were generally worse in patients with poorly managed DM. The relative risk of TB treatment failure or mortality among patients with DM was 1.69 (95% CI, 1.36 to 2.12). It

has been suggested that DM patients with poor glycaemic control could have more critical cases of TB. Although it hasn't been studied in much detail, DM might contribute to poor treatment responses in those with multi-drug resistant TB (MDR-TB). Improved management of DM might diminish the risk of TB infection in people with DM [3]. By eliminating DM associated with TB, there could be an estimated 15% decrease in global TB incidence by 2035 [7].

A.3.1 Research Justification

South Africa, where the shift towards a double disease burden of infectious and NCDs is occurring, has the third highest estimated incidence of TB associated with DM in the world [3]. TB incidence in South Africa remains one of the highest in the world with 834 cases per 100,000 people [4]. When estimating TB burden attributable to DM, it was determined that 15% of TB cases in South Africa could be attributed to DM [3]. Despite these findings, there have been no previous studies done to assess the prevalence of TB among DM patients in South Africa.

A United Kingdom (UK) cohort study [16] was done to estimate the risk of TB in those with and without DM, and determined that there was an increased risk of TB among people with DM. Although many studies have found an association between TB and DM, the research settings has predominantly taken place in Western and Eastern countries [11,17–19] where TB burden is low in some cases. The UK study in particular took place in generally affluent and healthy areas [16].

The number of studies that have addressed the magnitude and direction of association between DM and TB in the context of SSA is limited and information is dearth. In one of the few studies investigating TB prevalence among DM population in SSA setting,

Mtwangambate et al. found that 1.3% of patients with DM in Tanzania had TB, an estimate that was 7-fold greater than the general population [20].

Many existing studies also have not controlled for lifestyle and socio-demographic factors such as alcohol use and smoking, HIV status, ethnicity, and socioeconomic status (SES) in order to investigate possible risk factors and issues of selection bias was also not addressed.

There is a limited amount of research on how best to screen for TB among DM patients in resource-limited settings. In one such setting, a prospective cohort study in Tanzania evaluated "cough-triggered" screening algorithm to determine how to screen for TB in patients with DM [20]. The feasibility of sputum induction in DM clinics was assessed. Future studies should evaluate the screening method for TB symptoms among patients with DM.

Systematic screening for TB in people with DM could improve early detection in settings with high TB incidence/burden. Future studies on the topic should lead to identifying cost-effective strategies for screening and management of both DM and TB. Point of care diagnostics such as the Xpert® MTB/RIF test, should be assessed for their feasibility among DM patients especially in the context of a high burden/low resource setting. Current availability of such data is non-existent. Further studies are needed to provide reliable estimates of TB prevalence in people with DM and to determine whether any association exists between glycaemic control and TB among DM risk factors, and TB contact and malnutrition among TB risk factors. The results from this study are expected to add to the growing body evidence of the association between TB and DM.

A.4 METHODOLOGY

A.4.1 Study Design/Characteristics of the Study Population

This will be a sub-study of a larger cross-sectional epidemiological study and will use data that has been collected from that particular study. The parent study is examining the association of DM, HIV and TB in South Africa and the Western Cape. Primary objectives of the larger study include, investigating the best screening algorithms for DM and TB among newly diagnosed TB patients and DM patients respectively.

The cross-sectional study will be conducted in Khayelitsha, one of the biggest and fastest growing peri-urban townships in Cape Town, South Africa. With a predominantly Black African population of 391,749 (as of 2011 census) [21], it has the highest burden of TB and HIV in the country and globally [22]. In 2010, the prevalence of antenatal HIV was 26%. The TB case notification rate was 1500 per 100,000 population per year [23]. Previous studies have demonstrated a 13% (95% CI: 11.0-15.1) prevalence of DM in South Africa, specifically in the township communities [24]. Site B Community Health Clinic was selected as a study site as it functions as a designated provincial sentinel site, which collects clinical data for surveillance and research. In addition, there is a TB and a diabetes clinic on-site that is in close proximity to each other.

A.4.2 Sample Size

In order to have 80% statistical power in determining TB prevalence among DM patients, a sample size of 457 patients with DM will be needed. This is assuming that the estimated TB prevalence is 5% with a precision/sampling error of 2%, and a two-sided significance level of 95%.

A.4.3 Recruitment and Enrolment

As part of the larger study, to screen for TB in patients with DM, adult patients (18+) attending the DM clinic for chronic disease management in Khayelitsha (Site B) Community Health Clinic will be recruited. An on-site physician will ask patients about potential participation.

A.4.4 Inclusion and Exclusion Criteria

Participants will be included in the study if they have been diagnosed with DM and are over the age of 18 years old. Participants will not be excluded on the basis of culture, religion, race, mental or physical disability, and sexual orientation. Participants will be excluded if they did not provide consent.

A.4.5 Research Procedure and Collection of Data

Patients that are interested in participating will be referred to a community health worker who is bilingual in both English and Xhosa, the predominantly spoken languages in Khayelitsha. After informed consent is obtained, the WHO STEPwise approach to surveillance of chronic disease risk factors (STEPS) [25] will be administered to collect participants' data (Appendix 1). The STEPS instrument is a validated tool that focuses on obtaining core data on the established risk factors that determine major disease burden. The instrument involves three sections: (1) a questionnaire that asks about the socio-demographics of the patient (income, education, etc.), (2) behavioural/lifestyle choices (smoking, alcohol use, diet, physical activity, etc.), and (3) biochemical measurements (blood test results, lipid profile, body mass index (BMI), etc.).

A validated TB screening tool will be used to assess for TB symptoms, which include cough, night sweats, fever, loss of weight or appetite, and will gather information about previous TB and TB contact history. The community health worker at the DM clinic will implement these surveys to the participants.

Spontaneous and induced sputum collected from participants will be tested and a positive diagnosis for TB will be confirmed through the following tests: Xpert MTB/RIF (Xpert), smear microscopy (acid-fast bacilli), *M. tuberculosis* culture, and chest X-ray.

Plasma HbA_{1c} (glycated haemoglobin) and fasting glucose will be measured to determine baseline glycaemic control at the time of sample collection. Patients will also be screened for HIV.

Since this is a cross-sectional study, data will be collected from each participant only once over a 2-hour survey period, when participants attend a booked appointment. If results come back positive for TB and/or HIV, patients will be recalled and debriefed for further care and treatment.

Recruitment of participants began in September 2014 and will end in October of 2015 (See section A.4 on Ethical Considerations for additional information).

A.4.6 Variable Table.

See Part D, Appendix 1 for full variable table.

A.4.7 Data Analysis

Diagnostic test options include: Xpert MTB/RIF test and/or smear microscopy (acid-fast bacilli test). TB screening for all participants in this study will be conducted using the national programmatic guidelines for TB management [26]. Therefore, Xpert will be the primary diagnostic test for TB. If the patient is HIV-positive, they will be subjected to an additional diagnostic test involving a sputum culture test [26].

Demographic data, lifestyle/behavioural data, and biochemical measurements associated with TB will be analysed using multivariate analysis (logistic regression). Variables gathered from the survey (Part D, Appendix 1), will be used to compare TB cases and non-cases. The model will be built manually [27] comparing nested models to determine best fit using the likelihood ratio test. The Akaike's Information Criterion (AIC) will be used to compare non-nested models; an AIC that is significantly lower will indicate an improved model. Model checking will be done to identify outliers and influential observations and potential effect modification will be assessed using interaction variables.

Significance testing will be done using two-sided p-values ($p<0.05$) and 95% confidence intervals. All data will be analysed using the statistical software STATA 13.0 (StataCorp, College Station, TX, USA).

A.5 ETHICAL CONSIDERATIONS

This study will comply with the latest version of the Declaration of Helsinki (2008) and The Department of Health: Ethics in Health Research: Principles Structures and Processes (2004). Even though the study is not a clinical, trial it will comply with the Guidelines for Good Clinical Practice in the Conduct of Clinical Trials in Human Participants in South Africa (2006). The larger study has already received ethics approval from the University of Cape Town Human Ethics committee (HREC Ref: 403/2011).

A.5.1 Description of Risks and Benefits

The overall risk to participants in this study is estimated to be "minimal" and fulfils the "routine examinations" standard. The probability and magnitude of harm or discomfort anticipated in this study will not be greater than the harms or discomfort ordinarily encountered during physical examinations, especially for those that have been diagnosed with a chronic medical condition.

Potential benefits for participating in the study involve screening of TB and HIV. TB screening is normally not included in general wellness/health assessments, and is not designated as normal standard of care. Late diagnosis and delay in treatment can be avoided if participant tests positive for TB and/or HIV due to early detection.

Participants will be given 30 ZAR in cash to compensate for time spent at the clinic and transport. There will be no follow-up appointments unless test results are abnormal. Abnormal would be defined as a positive TB and/or HIV diagnosis or unusual blood test results.

A.5.2 Informed Consent Process

Respect for the autonomy of participants will be ensured through informed consent. The study doctor and community health worker/nurse will give the written consent form to the participant. The written consent form will be in Xhosa and English, describing the purpose of the study, the research procedure, and will contain information about the potential harm/benefit of participating. The participants' consent to participate is voluntary, and will be free of any coercion or inflated benefits from participation. Participants will be informed that they can withdraw from the study at any time, even after signing the consent form should they decide to.

A.5.3 Privacy and Confidentiality

Participants' confidentiality will be maintained through the assignment of unique identifying numbers (ID). Although patients' personally identifiable data will be stored in a folder, the participant's unique ID will only refer to data that is captured on the database. All blood samples that will be processed by the National Health Laboratory Service (NHLS) will be labelled with the participant's unique study number (ID), their initials, and date of birth. No full names will be attached to the specimens. A transporter will have a logbook to keep track of samples. The surveys will be done in a private setting at the clinic. Data that is recorded into the logbook and participants' folders will be stored in a locked cabinet that can only be accessed by study personnel.

A.6 DISSEMINATION

Stakeholders include high-risk target population, other researchers, government, non-government organizations (NGOs), and hospitals. Results may potentially change the screening processes for TB among persons attending DM clinics, by identifying an additional population that can benefit from screening. Findings from the study can be used to guide interventions related to TB control and prevention especially among “high-risk” groups. In addition to a publication in a peer-reviewed journal and/or abstract presentations at conferences, findings will be disseminated to the Western Cape Department of Health and to the clinic where the study will take place. This study has the potential to contribute evidence of TB/DM association in an SSA setting.

A.7 LOGISTICS

A.7.1 Budget

This study will be funded by the Clinical Infectious Disease Research Initiative, as part of a Wellcome Trust Strategic Grant.

A.7.2 Timetable

Participant recruitment began September 2014 and will conclude October 2015.

Task	Dec. 2013	2014	2015	May	Jun	July	Aug.	Sept.	Oct.	Nov.
Preparatory Phase ➢ Staff recruitment and training ➢ Questionnaire development										
Study recruitment and follow-up ➢ Identification and assessment of participants ➢ Sample collection ➢ Patient follow-up										
Sample and Data Analysis ➢ Data Extraction and Cleaning										
Dissemination ➢ Manuscript Write-up										

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PART B: LITERATURE REVIEW

B.1 BACKGROUND AND OBJECTIVES

TB is an infectious disease caused by *Mycobacterium tuberculosis* that primarily affects the lungs but can also affect other parts of the body. The airborne disease is transmissible through the inhalation of droplets from an infectious person. In healthy individuals, the disease can remain dormant (latent) and is asymptomatic, but in those with a weakened immune system, symptoms can develop resulting in active TB. Classical symptoms include: chronic cough, fever, fatigue, night sweats, weight loss, and chest pains. The 6 to 12 month treatment course of antibiotics makes TB highly treatable [1,2].

According to the WHO, TB accounted for 1.5 million deaths and an estimated number of 9.6 million new cases in 2014. As the leading cause of death, TB remains a major threat to global public health, specifically for populations in low to middle income countries where 95% of cases and deaths have occurred [1]. Further complicating TB control, is the co-infection with HIV as 13% (1.2 million) of new TB cases were also HIV-positive with 74% of them occurring in SSA [1]. Previous studies have shown that those with the co-infection had a higher mortality rate and an increased risk of reoccurrence than HIV-negative TB cases [3–6]. TB diagnosis and treatment has advanced in the past two decades, making it a highly treatable and preventable disease despite its high death rate. Although progress towards TB control is slow, the incident rate of TB has fallen since 2000 by an average of 1.5% per year [1].

During the same year, the WHO along with several stakeholders implemented the ‘End TB Strategy’ to halt the epidemic. From 2016, the strategy hopes to limit TB transmission by 80% and mortality by 90% by 2030 through continued improvements in diagnosis, treatment, and prevention [7,8].

Consequently, other alternatives need to be explored to improve diagnostic methods of TB. Due to a rapid increase in urbanization and an aging population, there has been a shift in the incidence of NCDs resulting in a double burden of both communicable diseases and NCDs [9]. Risk factors associated with TB such as poor nutrition and HIV co-infection can impair the immune system increasing the likelihood of activating latent TB (LTBI) [10,11]. NCDs that can impair immune function, such as DM, should be further assessed as an additional risk factor [12].

DM is a chronic metabolic disease that impairs insulin production and function, resulting in hyperglycaemia. As an NCD, its morbidity and mortality derives from a host of complications that affect the blood vessels (neuropathy and cardiovascular disease) when left untreated [13,14]. Although there are two classifications of DM : type 1 and type 2; type 2 diabetes (T2DM) is the most common form, with incidence rates increasing with age [15].

Due to the changing patterns in diet and physical activity, DM has become increasingly prevalent. As of 2010, there were an estimated number of 12.1 million prevalent cases of DM in sub-Saharan Africa and this figure is projected to increase to 23.9 million by 2030 [16]. Eighty percent of deaths due to DM occurred in low-to middle-income countries [17].

The results of two systematic reviews have concluded that DM strongly influences risk of TB. Those with DM were 3 times as likely to develop TB compared to persons without DM [18]. In 2013, it was estimated that DM was attributable to 15% (1 million) of all new cases of TB, an increase from 10% in 2010[12]. In a systematic review examining treatment outcomes, there is evidence that TB patients with DM have a poorer treatment outcome than non-DM TB patients, with a higher risk of TB treatment failure, death and recurrence compared to TB patients without DM [19]. Through improved diagnosis of TB among DM patients and better chronic disease management of DM, the negative effects of DM on TB can be diminished. By preventing DM-associated TB cases, there could be an estimated 15% decrease in global TB incidence by 2035 [20]

The aim of this dissertation is to further explore the association of TB and diabetes mellitus (DM), in a high burden/low resource setting.

In order to reduce TB burden and optimize active TB case-finding strategies in SSA and low-resource settings, this research aims to explore the prevalence of TB amongst a population diagnosed with and receiving treatment for DM. Research goals also include the investigation and evaluation of significant risk factors associated with TB.

The objectives of this literature review are:

- To summarize the evidence of association between active TB and DM and its associated risk factors. To explore the epidemiology of TB and DM in the sub-Saharan Africa (SSA) context.
- To evaluate risk factors associated with TB incidence and prevalence among DM patients.
- To evaluate TB screening methods & case-finding strategies in DM patients within a high burden/low resource setting.

B.2 METHODOLOGY

B.2.1 Study selection and data extraction

To satisfy the objectives of the review, studies were included for data extraction if they: (1) determined the prevalence and incidence rate of TB amongst a DM population, (2) screening feasibility of TB in people with DM, and (3) reported on risk factors of TB in DM and/or discussed TB/DM associations. Studies not written in the English language, studies where the study population/participants were TB patients and was diagnosed prior to DM diagnosis were omitted from data extraction, in order to specifically analyse the population of interest. Studies that assessed for TB infection (Tuberculin Skin Tests- TST) and individual studies that had undergone meta-analysis in the published systematic reviews [18, 19] were also excluded to reduce redundancy. Studies not included in or published after the systematic reviews [18, 19] were selected. Studies where screening resulted in zero TB cases were also omitted. The following data was extracted in observational studies examining TB incidence among DM population: Study (Author) Region/Country, Study Period, DM diagnosis method, TB diagnosis method, DM status, % male (if available), age distribution (if available), and comparison population (Table B.2). TB prevalence and/or incidence, population size and number needed to screen one case (Table B.1) were also extracted. The cohort/longitudinal studies were analysed specifically to examine characteristics associated with TB prevalence amongst DM patients, while screening methods in addition to characteristics were investigated in cross-sectional studies.

B.2.2 Search strategy

A literature search was conducted in the following databases: PubMed® , Medline via PubMed, Web of Science™, Europe PubMed Central®, and was supplemented with Google Scholar and reference lists of relevant published studies. The search date was from January 1950 to August 2015. The following keywords were included in the search: tuberculosis, diabetes mellitus, detect, screen, diagnosis. ‘Detect*’, ‘screen*’ and ‘diagnos*’ were used as root terms to find screening studies for persons with DM. The key terms produced 835 citations of which 21 (2 systematic reviews, 10 screening studies, and 9 cohort/longitudinal studies) were selected for analysis.

B.3 RESULTS

B.3.1 Epidemiological evidence of association between TB and DM

In evaluating the magnitude of association between TB and DM, a systematic review found that those with DM had a 3-fold risk of developing TB compared to those without DM (95% CI 2.27-4.27) [18]. Those that had a DM diagnosis prior to TB showed a stronger association (RR 2.73) than those without a determined temporal order (RR 2.10) [18]. Longitudinal population-based studies conducted following the systematic review continue to confirm the positive association between DM and TB, although modestly so [21–29] .

In a recent prospective cohort study conducted in the UK, a low TB-burden, high-income country, the relative risk for TB among DM was a modest 1.30 (95% CI 1.01-1.67) [29]. This increased risk was consistent with results of other similar cohort studies, including a population-based cohort study conducted in another low TB burden country where there was a 1.4-fold increased risk of TB in those with DM, compared to those without DM [23].

Kuo et al. [25] conducted a longitudinal cohort study using Taiwan's National Health Insurance Research Database (NHIRD) and found that patients with T2DM had 1.43-fold higher incident of TB than a matched control group with a relative risk of 2.08 (95% CI: 1.23-1.39) compared to 1.45, after adjusting for co-morbidities such as asthma and chronic obstructive lung disorder (COPD) .

A prospective cohort study by Baker et al. [30] using both Taiwan's research database and national data from its 2001 National Health Survey, found a hazard ratio of 2.09 (95% CI: 1.10-3.95), after adjusting for confounding factors such as lifestyle, behaviour, and socio-demographics, which had been previously unmeasured in the Kuo et al. study [25]. This was the first prospective cohort study on DM and TB that was both conducted among a general population and adjusted for socioeconomic and other socio-demographic confounders.

When examining confounding variables the majority of the studies controlled for age, gender, and other co-morbidities such as chronic liver disease or COPD. Pealing et al. and Baker et al. were unique in that they controlled for environmental, educational and socioeconomic, demographic, lifestyle factors in their cohort studies [29,30]. This was unlike other studies, which were missing such measures, as that information was not reported on the databases that they analysed. One study noted that the impact of other TB risk factors such as COPD, and DM associated co-morbidities such as end renal stage disease, liver cirrhosis, and immunosuppressant use, were not considered as including those may mediate the association between TB and DM.

Lee et al. was one of a few cohort studies that also controlled for HIV status, a strong TB risk factor while Kuo et al. completely excluded those who had an HIV diagnosis, but measured contact with known TB cases as a possible confounder ("TB attacker") which had been previously unmeasured in most of the cohort studies [21].

Pealing et al. [29] argued that the reasons for the modest increase in risk of TB with DM is because as the study size increases there is a decrease in the estimate for association between DM and TB seen in both cohort and case control designs, even in countries with high incidence. This is consistent with a retrospective cohort study investigating DM-medication adherence and risk of TB in Taiwan. The sample size was more than 45,000 DM patients and matched controls, and resulted in a lower relative risk of 1.29 (95% CI 1.15-1.45) [21]. However, in another cohort study in a similar setting with a much smaller sample size (8000 DM patients) a higher relative risk was measured (RR 3.5 95% CI 3.0-4.0) [18].

This negative association between study size and TB prevalence estimates is further reflected in the first retrospective cohort study conducted in the UK using a dataset of hospital admissions at two time periods, where the relative risk was 2.63 (95% CI 0.91-6.30) in the second most recent time period (1999-2005) among a sample size of close to 8000 [24].

Pealing et al. [29] however did not control for HIV status, and admitted that there may have been misclassification of the data as there was no validation of TB or DM diagnosis, unlike other longitudinal studies that validated TB and DM diagnosis, which suggests the possibility of an underestimation of the prevalence estimate.

Another factor that may affect TB prevalence estimates among DM patients is the differential diagnosis of DM. The same study [29] used consultation rates as a measurement proxy for chronic disease management and subsequently DM severity. Those who had higher consultation rates had more DM- related complications, related to poor glucose control, and worse health outcomes, leading to higher risk of developing TB. Although it was noted that those with lower consultation rates might be neglecting disease by not attending clinics, which could also lead to poor glucose control and a higher risk of developing TB. As a result, consultation rates at the opposite ends of the spectrum (both high and low) lead to an increased TB risk. Further research involving validating DM diagnosis would need to be conducted to determine true estimate of TB among DM patients.

The estimated relative risk from the systematic review and studies following the review have been based on cohort studies predominantly conducted in Asia, where over half of global TB cases are found [1]. Moderate relative risks from studies conducted outside of Asia and/or in low TB burden areas might just be a reflection of its impact, highlighting geographical and demographic differences.

B.3.2 Prevalence- Does screening TB in persons with DM increase yield of TB cases?

A systematic review of bi-directional screening of TB and DM cases was conducted to assess the yield of active case finding of TB and DM. The review concluded that active screening of DM patients lead to more detection of TB cases. However TB prevalence among DM patients was highly variable, with prevalence ranging from 1.7% to 36%, with greater prevalence in high TB burden areas [31]. These results were consistent in 9 studies that had screened for active TB among DM patients, where the prevalence ratios ranged from 1.6 in a community clinic setting in China [32] to 147 among 15 primary care units in Mexico [33]. All studies reported a TB prevalence among DM patients that was higher than the reported prevalence of the study country's general population. Incidence of new TB diagnosis following screening, ranged from 0.03% to 21.1% (Table B.1), also revealing broad variations in TB incidence among DM population. Although notification rates among persons with DM remained higher than rates in general population, TB incidence was decidedly low overall in high TB burden areas [34,35]. In addition to a narrative, an overview of the population characteristics and screening methods in the selected studies evaluated were also tabulated (Table B.2).

Table B.1 Results of TB screening on DM Patients. Q1-Q3 signifies quarters.

Author/Year	Pop Size of DM	# of TB	# of TB diagnosed before screening	TB Prevalence In DM (per 100,000)	TB prevalence for comparison (per 100,000)	Prevalence ratio	# needed to screen to detect 1 TB case	TB incidence (%)/(per 100,000)
[41] Lin Y-H/2015	3087 T2DM[1]	12	5	389	-	n/a	-	7/3087 (0.22)
[32] Lin Y /2015	2942 DM patients	3	2	102	65	1.6	2702	1/2942 (0.03)
[33] Castellanos-Joya/2014	783 DM patients	38	6	4853	33	147	23	11/783 (1.4)
[50] Mtwangambate/2014	693 DM patients	9	2	1299	177	7.1	89	7/693 (1.01)
[43] Amare/2013	225 DM patients	14	NR	6222	394	15.8	17	14/225 (6.22)
[35] Kumar/2013	Q1[2]-1907, Q2-6393, Q3-5661 DM patients	254	180	Q1 -859, Q2 -956 Q3- 642	107			Q1-105 Q2- 172 Q3- 88
[68] Jali/2013	2072 DM patients	111	109	5357	283	18.9	20	2/2072 (0.10)
[42] Kumptala/2013	7083 DM patients	47	35	706	NR	n/a	-	12/7083 (0.17)
[62] Bates/2012	19 DM patients	4	NR	21052	462	45.6	5	4/19 (21.1)
[34] Lin Y/2012	Q1-3361, Q2-5669, Q3-2300	48	7	Q1 2012- 774 Q4 2011- 352 Q3 2011- 391	31-111	-	-	Q1-595 Q4 -265 Q3 -217

[1] Type 2 Diabetes Mellitus

[2] Q1-Q3 signifies quarter (time period)

Table B.2 Population characteristics and screening method of observational studies.

Author/Year	Region/Country	Study Period	DM diagnosis method	TB diagnosis method	DM Status	% male	Age distribution (average)	Comparison population
[41] Lin Y-H/ 2015	Taiwan	Sept 2012-Nov 2012	Previously diagnosed Patients. Fasting and postprandial samples are collected from known DM cases.	Chest X-ray, Symptom checking and referral TB clinic, Sputum smear microscopy (Acid-fast stain) and culture for *Mycobacterium tuberculosis* for referrals	Among DM: HbA1c(%) 7.46 Among TB: HbA1c(%) 7.71	Among DM: 48% Among TB: 83.3%	Among DM: 74.15 Among TB: 75.08	DM non- TB controls.
[32] Lin Y/2015	China	June 2013-April 2014	Attendance of DM clinic of Patients previously diagnosed with DM. Registered patients with DM managed by NCD control program.	Symptom checking, referral to TB clinic following positive symptom screen, chest x-ray	NR	NR	NR	None
[33] Castellanos-Joya/2014	Mexico	July 2012-April 2013	Previous Diagnosis of DM	Symptom checking, following positive symptom screen: Sputum smear microscopy (Acid-fast stain) and culture for *Mycobacterium tuberculosis* among smear positive	Among DM: 5.1% Insulin Dependent Among TB: 32.4% Insulin Dependent	Among DM: 24.8% TB: 52.6%	Among DM: 49 [56-66] Among TB: 46 [57-66]	DM non-TB controls
[50] Mtwangambate/ 2014	Tanzania	Sept 2011-March 2012	Previous diagnosis	Symptom checking, following positive symptom screen: sputum smear microscopy (Acid-fast stain) and chest x-ray	Among DM: 46.3% Among TB: 71.4% fasting blood glucose > 7 mmol/l or>12 random blood glucose	Among DM: 48.8% Among TB: 71.4%	Among DM: 60 (50-66) Among TB: 45 (40-60)	All adults with DM and cough

[43] Amare/2013	Northeast Ethiopia	Feb 2012-April 2012	Previous Diagnosis-fasting blood glucose by photometer and glucometer	Sputum smear microscopy (Acid-fast stain) and chest x-ray (2 positive smear results) or 1 positive and chest x-ray	Of those with blood glucose (mg/dl) >288 Among DM: 85.7% Among TB: 14.3%	Among DM: 46.67% Among TB: 78.57%	Among DM: 65.4% over the age of 40 Among TB: 64.3%	DM non-TB controls
[35] Kumar/2013	India	Jan 2012 - Sept 2012	Previous Diagnosis	Symptom checking and referral, sputum smear microscopy (Acid-fast stain) and chest x-ray for negative smears following referral	NR	NR	NR	None
[68] Jali/2013	India	Feb 2012-Sept 2012	Previous Diagnosis	Symptom checking and referral to TB services, following positive symptom screen	NR	Among DM: 57% Among TB: NR	Among DM: 46.8 Among TB: NR	None
[42] Kumptala/2013	India	March 2012-Dec 2012	Previous diagnosis. Fasting and postprandial blood amples were collected from known DM cases.	Symptom checking, following positive symptom screen: sputum smear microscopy (Acid-fast stain) and chest x-ray for negative smears for positive symptoms	NR	NR	NR	None
[62] Bates/2012	Zambia	Sept 2010-Dec 2011	In-patient Admission Diagnosis	Sputum smear microscopy (Acid-fast stain) and culture for *Mycobacterium tuberculosis*, and phenotypic Drug Susceptibility Testing (DST) on culture positive samples	NR	NR	NR	None
[34] Lin Y/2012	China	Sept 2011-March 2012	Previous Diagnosis	Symptom checking and referral TB clinic following positive screen. Sputum smear microscopy (Acid-fast stain) and chest x-ray among referrals	NR	NR	NR	None

B.4 MAJOR DETERMINANTS OF TB IN DM PATIENTS

The existing evidence of the major risk factors associated with TB incidence in persons with DM are outlined and summarized below. The major determinants associated with the incidence of TB among persons with DM include: Poor glycaemic control and DM severity, DM duration, Age and gender, BMI, Multi-morbidities (hypertension, COPD, HIV etc.), and lifestyle factors such as smoking. Cross-sectional studies conducted within the SSA and in low resource/high burden areas were also summarized and its screening methods were evaluated.

B.4.1 Poor Glycaemic Control and DM severity

Immunological studies on TB and DM have suggested that DM, through hyperglycaemia, impairs the function of the immune system by indirectly affecting phagocyte and lymphocyte function, which are crucial components in combating TB. DM appears to affect chemotaxis (organism movement in response to a chemical stimulant), macrophage activation, antigen presentation, and phagocytosis[36,37]. Leukocyte bactericidal activity is diminished in people with DM, especially in those with poor glucose control, leading to higher bacterial loads. DM patients with poorer glycaemic control appear to be at a higher risk of TB than those with managed DM, revealing a positive correlation between hyperglycaemia and TB risk [38]. Hyperglycaemic conditions appeared to impair interferon-gamma production, a cytokine involved in anti-bacterial immune response [39]. Levels of interferon-gamma were negatively correlated with levels of HbA1c (a measure of serum glucose levels over time), this was seen in experiments involving mice with "streptozotocin-induced DM." T-cell growth, function, and proliferation were also negatively affected. Interleukin-12, a T-cell stimulating factor produced by macrophages and play a critical role in the immune response, were lower in the lungs and spleen of mice with DM [40].

Results from epidemiological studies, have confirmed the findings that poor glycaemic control among DM patients is associated with an increased risk of TB [21,23,30,41,42]. An HbA1c of more than 7% and high levels of insulin dependence were usually used as markers to indicate poor glycaemic control [31]. However, among the observational studies there wasn't a consistent measurement in evaluating glycaemic control and DM severity. Information about HbA1c or insulin dependence levels from the study population, which could determine glycaemic control, were unavailable in many studies, and instead proxies such as consultation rates, number of DM-associated hospital admissions and complications, and anti-DM medication adherence were used to determine DM severity [21,24,26,29]. In one prospective cohort study, Baker et al. assessed DM severity through a proxy using the number of DM complications and a scoring system (Diabetes Complications Severity Index-DCSI) used to predict DM associated mortality and hospitalizations. Results showed a positive association between increasing number of complications and DCSI score, and hazard ratio for TB among DM patients. The adjusted HR was 1.73 (95% CI: 0.61-4.89) among patients with no or 1 complications of DM) to 3.45 (95% CI: 1.59-7.50) for those with more than 2. Among those with a DCSI score less than 4 adjusted HR was 1.73 (95% CI: 72-4.13) and jumped to 5.05 (95% CI: 2.11-12.04) among those with a DM severity score greater than 4. The increasing number of complications and DCSI score resulted in an increasing hazard ratio for tuberculosis, revealing disease severity as a TB risk among DM patients [30].

Pealing et al. found no evidence that poor glucose control increased TB risk when evaluating HbA1c measurements of T2DM patients and their matched unexposed controls but found TB risk to be associated with chronic disease management, as those with the lowest and highest rates of chronic disease management had the highest risk of developing TB compared to factors such as age, DM duration, and severity of DM [29]. In another

study, glucose control was also found not to be a significant risk factor, except that TB patients had higher low-density lipoprotein (LDL) compared to non-TB among DM [41].

However, one Australian cohort study used insulin use as a measure of severity and found that those using insulin had a 2.3 fold risk compared to general population cohort (RR: 2.27; 95% CI: 1.41-3.66) [23]. Results were also consistent in a screening study conducted in a tertiary care hospital in south India, where HBA1c levels were used and found that the rate of TB incidence increased with increasing HbA1c levels especially in patients with HbA1c over 9.0% [42].

Where other studies used one or two markers, Lee et al. used a combination of adherence to anti-DM medication, and the maximum average daily dose, insulin use, and number of DM-related hospital admissions as measurements for DM severity, as low adherence to DM medication and high number of DM related admissions imply poor glycaemic control. Results showed that an increase in DM-associated admissions paralleled the risk of TB [HR: 1.79; 95% CI: 1.23-2.60] as well as insulin use during admission (HR: 1.46; 95% CI: 1.09-1.97) [21]. Heo et al. could not determine the degree of glycaemic control that directly affected TB development as data on blood glucose levels were not available for DM patients [28].

B.4.2 DM Duration

Many studies did not have data on duration of DM or time of diagnosis, however for studies that measured DM duration, the evidence varied in whether DM duration was associated with TB and if so there was conflicting evidence in whether long-term DM or recently diagnosed DM duration increased TB risk. Knowing the extent of association between TB

and DM duration may help improve the diagnostic algorithm by identifying the appropriate time point to screen DM patients.

Lin et al., found no significant difference in DM duration and TB risk between TB/DM group and control group when screening for PTB among elderly DM patients in a Chinese community hospital [41]. In one study conducted in Ethiopia, long DM duration (more than 10 years) was positively associated with an increased risk of smear positive pulmonary TB (OR: 8.89; 95% CI: 1.88-58.12) [43]. This result was consistent with another screening study conducted in India, where the incidence rate increased among those with DM duration of over 10 years versus 6 years for those without TB [42].

However, in a population aged 20-89, among those that were newly diagnosed with DM within the first year of study (2009), it was discovered that the risk of TB among DM patients increased particularly during the first 12 months after DM diagnosis, with the greatest risk occurring during the first 6 months as the incidence rate was 33/10,000 (95% CI: 30.0-35.6) within the first 6 months compared to 19/10,000 (95% CI: 16.5-20.6) in the latter half of the year until the risk subsequently decreased the following year [28].

These findings were similar to another longitudinal study investigating statin use and TB incidence, of newly diagnosed DM patients, aged 20-99 where the estimated TB incidence was 251/100,000 (95% CI: 243-258) [26]. In a population based follow-up study on risk of TB incidence among DM patients in Denmark, results of the study revealed an inverse association, as it was found that longer DM duration resulted in a reduced TB risk, especially during the first two years of initial DM diagnosis [44]. However, the study did not take into account that there may have been a delay into when DM patients were registered with the national database for DM and unlike the other follow-up studies [26,28] T1DM and T2DM were not distinguished. Carstensen et al. noted the effects of "calendar

time" which suggested that DM duration was impacted by diagnosis date of DM. The effect of DM duration on TB incidence had decreased as the years of the study period examined (1995 to 2009) progressed [44].

In two studies that evaluated TB incidence among newly diagnosed DM patients [26,28], patients with pre-existing DM or were currently on anti-DM medication before study period were excluded from the study, so it is unknown what the incidence or prevalence of that particular group would have been, as there was no formal control group in both studies. It would have been helpful to compare those with pre-existing DM and those categorized with newly diagnosed DM to examine any differences in the TB incident rate between both groups. The lag time between symptom onset and DM diagnosis is also unknown, as time of diagnosis is not the same as time of biological onset, which is much more difficult to determine. For those with newly diagnosed DM, information about the severity of the disease at the time of diagnosis was not available. In the statin study, which had covered a period of three years, there was no distinction made of a patient's year of DM diagnosis and year of TB diagnosis. It was unknown whether some of the patients newly diagnosed with DM in the first year were the same patients that were diagnosed with TB in the subsequent years of the study [26].

Finally, all of the studies did not confirm directionality of TB and DM association, as previous studies have shown that TB can induce hyperglycaemia [45–47]. It has been suggested that the reasons for the subsequent decrease in TB risk, with longer TB duration could be explained by improved diagnosis and treatment of DM, which reverses impaired glucose tolerance and DM-related complications diminishing TB risk. Findings of a positive association between TB risk and DM duration in the screening studies done in Ethiopia and India, could be explained by the small sample sizes of the study, as it might have limited the statistical power of both studies [42,43].

B.4.3 Age and Sex

Several studies have identified age as a risk factor, but whether it is a positive or weak association [21,23,33] remains to be determined. Systematic reviews revealed a negative association between age and TB risk among DM patients, and it was suggested that TB risk decreased with age [18,48].

Among T1DM patients, studies have suggested that they are at a higher risk of developing TB compared to T2DM patients [24,27,49]. In comparing one study examining T2DM patients and one study examining T1DM patients, the incidence rate of TB in T1DM study was higher among younger patients where the average age of the study population was significantly younger than cohort of the T2DM study (mean age: 55.5 years in T2DM compared to 18.9 years in T1DM) [25,27].

This finding was consistent in one population-based cohort study, where age was a significant risk factor for tuberculosis in all participants (HR: 1.04; 95% CI: 1.04-1.04) [25]. The relative risks of developing TB among patients with T2DM were higher in younger age groups than in older age groups. The incidence rate of TB among 30-40 year old females was 1.35[3] (95% CI: 1.03-3.25), which was higher than the incidence rate among non-DM females aged 50-60 (HR: 1.02; 95% CI: 1.03-3.25). Among males in the same age groups, the incidence rate was 2.01 in 30-40 years old DM males compared to older male controls which had an incidence rate of 1.70 [25]. This finding is in agreement with another study that showed a higher incidence of TB among DM patients compared to a non-DM control group. A study with a similar study design found that DM patients with TB were younger than non-DM TB patients. The median age for TB diagnosis in that particular study was nearly five years younger for patients with DM (67.5 years) compared to non-DM patients (71.9 years) [29].

[3] per 1000-person-year

However, in the Kuo et al. study, this finding becomes misleading when age groups within the DM cohort are compared. Older DM patients when compared with their younger counterparts had an increased TB incidence rate. For female DM patients aged 50-60, the incidence rate was 1.80, a much higher rate than the reported rate of younger female DM patients [25].

This positive association was also found in one study, where it was determined that older DM patients had a higher risk of TB. The average age of TB cases was 61.41 years, an average that was much higher than the age of TB cases among the general population [30]. In a screening of elderly patients where the average age was 74 years, the prevalence rate of TB increased from 6 to 7 fold among advanced aged DM patients, compared to national estimates. However in the study's analysis age was found to have a weak association (OR: 1.02; 95% CI: 0.94-1.13) as those with TB had a modestly higher mean age than DM controls (mean age: 75.08 and 74.14 years, respectively) [41].

These results were consistent with other studies where the average age of TB cases among DM patients was over 40 years [26,42,50]. Positive association between age and TB risk could be due to increases in the cumulative prevalence of TB. Immunosuppression as a result of the natural aging process, could make the progression from latent to active TB more likely among the elderly compared to younger patients [51]. Age might also be confounded by type of DM, as some studies have found that T1DM patients had a higher TB risk than T2DM due to poorer glycaemic control [52].

For gender, previous studies show weak associations and more investigations may need to be conducted to confirm. In a systematic review, Stevenson et al., [53] noted that in a case series of patients with both TB and DM, a higher proportion were males at younger ages

but this linear trend was reversed for females as higher proportions of TB were found among women as age increased. However in some studies, gender was a significant factor, as the incidence and risk of TB was higher in males [26,33,42] [21] In a cohort study investigating incidence of TB among T2DM patients, incidence ratio among males with DM was 3.25[4] to 2.19 in male controls (95% CI: 1.35-1.63) [25]. In one screening conducted in Ethiopia a high proportion of DM patients diagnosed with TB were male (78.6%) [43], despite a weak association (adjusted OR: 3.60; 95% CI: 0.77-16.73). These results were consistent in another screening study where 76.7% the odds of developing TB among males was almost 4 times higher compared to non-TB males (OR: 3.66; 95% CI: 2.12-6.33) [41]. These findings seem to confirm sex as a risk factor for TB among DM patients. However, males in general have a higher TB incidence and mortality risk than females, regardless of DM status [54]. Possible differences in exposure risks and rate of progression from latent to active TB, could explain the higher risks among males.

B.4.4 BMI

Historically, increasing body weight has been a significant risk factor in developing T2DM [55] and body mass index (BMI) has been used an indicator in measuring obesity and its association with DM. Not enough studies however have evaluated the association between BMI and TB risk among DM patients. However, evidence suggests that low body weight among DM patients is associated with increased TB risk. Weight loss is one of the clinical symptoms of TB, which could explain the risk. In one study, weight loss was a more commonly reported symptom among DM patients compared to non-DM [56].

[4] per 1000-person-year

In one prospective cohort study conducted in Taiwan, DM patients that were categorized as underweight had a higher risk of TB incidence than DM patients with no TB [30]. When comparing BMIs with non-DM patients, DM patients had on average higher BMIs (24.74; 95% CI: 22.49-27.31 versus 22.49; 95% CI: 20.17-25.10). Among elderly DM patients (over 65 years) there was a negative linear trend between BMI and TB risk, with the risk of TB incidence increasing with decreasing body weight [41]. In a low TB burden area, BMI and TB risk was also found to be inversely related and a high BMI (over 30) had a protective effect (RR=: 0.21; 95% CI: 0.17-0.27) [29]. This finding was consistent in non-DM TB studies, where HIV-positive persons with BMIs over 25 (obese and overweight) had a reduced risk of TB [57]. In a high TB burden area, these findings were similar to results of other studies where the average BMI was lower among DM patients with TB than in non-TB [33,42].

B.4.5 Other socio-demographic/lifestyle factors

While many studies adjusted for age and sex, few studies have evaluated lifestyle and socio-demographic factors such as tobacco smoking, alcohol use, and socioeconomic status (SES) in its association with TB risk among DM patients. In a UK study, TB incidence among DM patients increased in those that were non-drinkers, current smokers and a high index of multiple deprivation, a measure of low socioeconomic status (206/100,000 py) compared to non-DM TB patients [29]. In one of the few observational studies conducted in SSA, living in an urban setting ($p=0.004$) and religion ($p=0.049$) were some of the risk factors associated with TB prevalence among in Ethiopia. However, occupational status, marital status, income, and smoking and alcohol use were not significantly associated with prevalent TB [43]. These findings were different from another lower-middle income country (LMIC), where a high proportion of TB incidence among DM patients occurred in those that had a lower education level ($p<0.001$), an unskilled occupation ($p<0.001$), and consumed more alcohol compared to DM patients without TB ($p<0.001$) [42] . However

compared to this study, the Ethiopian study had a much smaller sample size, and therefore limited statistical power than the Indian study (225 DM patients versus 7000 DM patients), which could explain the non-significance of the listed socio-demographic factors. These findings however were consistent with one of the few longitudinal studies that examined socio-demographic factors, which found that TB risk increased among DM patients that have ever been married or cohabitating and for those receiving government subsidies [30].

Although smoking is a well-known lifestyle risk factor for TB [58] , many studies did not assess smoking status among DM population and in the few studies that investigated smoking, there has been conflicting evidence in its association. Kumpatla et al. identified smoking as a weak, non-significant association [42], but in another study conducted in China [41] smoking ($p<0.001$) among DM patients showed stronger associations for TB compared to non-TB DM patients. Strength of smoking association in the Kumpatla et al. [42] study may actually be underestimated as not all "suspicious" cases adhered to clinic referrals following positive symptom screening.

B.4.6 The role of multi-morbidities in TB risk among persons with DM

In one cohort study, findings showed a positive association between TB incidence and hypertension, heart and lung disease [30] among patients with DM compared to controls. However in a cross-sectional study of elderly DM patients, there was a weak association between DM/TB and hypertension ($p<0.001$) and a strong association of liver cirrhosis and TB incidence ($p=0.004$) [41], leading to inconsistencies about hypertension as an association. However, the use of controls in the study by Baker et al., [30] and its methodology (retrospective cohort) adds strength to the association found between hypertension and DM. COPD and cancers were also common underlying co-morbidities among T2DM patients that developed TB [21,25]. Among T1DM, chronic liver disease and kidney disease were significant risk factors for TB incidence ($p<0.0001$), compared to

controls [27], however knowledge of lifestyle factors were limited in these studies making it difficult to attribute TB incidence to other factors rather than other co-morbidities.

B.5 TB/DM SCREENING IN POOR RESOURCE/HIGH TB BURDEN SETTINGS (SSA CONTEXT)

B.5.1 Epidemiology of TB in DM population in SSA

Despite being a highly endemic area for TB [1], a limited number of studies in the SSA context have investigated the epidemiology of TB among DM population. No studies from SSA were included in the TB/DM systematic review, although a positive association was noted in the ones that were not included [18]. On the few studies that have been conducted in the SSA setting, TB patients were the study population, with DM as the event/disease of interest [59,60]. Very few studies exist in the SSA context, where DM patients were diagnosed prior to the measurement of TB incidence and prevalence. In a systematic review of TB/DM bidirectional screening, only two studies that screened for TB among people with DM were conducted in an SSA setting [18,52,61]. Both studies took place in South Africa, and results from both studies showed high prevalence rates of TB in DM patients, which was much larger than the national estimate.

B.5.2 Screening in low resource limited/high TB burden areas (Methodology)

Only two studies screening for TB among persons with DM in SSA were able to determine prevalence, and results of those studies highlighted a prevalence rate that was higher than the general population (see Table B.2). [43, 50]. Findings from a study conducted in Tanzania, showed a prevalence of TB among DM patients that was 7-fold greater than the national average (177/100,000) [50] and results of another study in Ethiopia, where prevalence was measured at 6.2%, compared to the country's estimate of 0.39% [43]. In a study examining the TB burden among adult in-patients at a teaching hospital in Zambia, 19 DM patients that were screened, 7 were diagnosed with TB [62]. Unfortunately the age and sex distribution was not noted and the small sample size and study setting could not produce a reliable estimated prevalence of TB among DM population. Despite the high

reported case notification rates among the general population in determining the burden of TB at a DM clinic in Botswana, no new TB cases among 177 screened DM patients were detected [63]. However, it was noted that TB diagnostic tests were conducted on a small proportion of those who had a positive symptom screening, so misclassification of cases may have occurred. True prevalence among population may have been underestimated, as asymptomatic but possibly infectious patients were not screened.

This underestimation is consistent in another study conducted in Iran, where the estimated prevalence rate of TB in 2014 was 33/100,000 (95% CI: 17-55) [1]. Out of 400 screened DM patients, 4 DM patients were diagnosed with TB following a positive symptom screen. However, 35.8% of patients that were asymptomatic for TB had a positive tuberculin skin test (TST), and those with a positive TB symptom screen were given chest X-rays to confirm TB diagnosis as opposed to other more sensitive and specific methods like sputum testing [64].

As recommended by the WHO, many of the studies conducted TB symptom screening, prior to lab screening. Most sputum samples were collected from only clinically "suspicious" cases, those who tested positive to the TB symptom screening. Evidence has shown that symptom screening is not an effective method in identifying TB cases. In results of one particular TB screening study conducted by the CDC in Southeast Asia, all patients with HIV were lab screened regardless of symptom screening, showed that symptom screening alone failed to detect more than 66% of patients with actual TB [65]. A short time period of screenings may have also been a limitation, as TB has been defined as a seasonal disease, therefore the number of cases identified may be underestimated [41].

Human and organizational factors appeared to be a common issue in some studies. In studies assessing screening in a routine setting, doctors allegedly felt too much pressure to routinely screen, and in many cases screening was not performed at all or as

comprehensively as it should have been. In the Mexico study, only 783 (10%) of the 7.763 DM patients that attended the DM clinic were screened [33]. Lack of staff training, may have also influenced case reporting issues. In a large routine screening study conducted in China, under-reporting of positive symptom screens was a major challenge, as it was admitted that even those that had possible symptoms (coughing, fever, etc.) were reported as having a negative screen [34]. In evaluating specific screening strategies in Tanzania, Mtwangambate et al., [50] determined that cough triggered screening might not be feasible, due to the high percentage of non-productive coughs among the study population. Although 17.5% (121/693) of those screened had a cough, 71.9% (81/121) could not produce sputum spontaneously, making TB diagnosis difficult and resulting in a possible underestimation of TB prevalence among DM study population.

This underestimation seems to exist in studies where not all patients that screened for TB had their sputum selected to bacteriologically confirm TB. From the systematic review on bi-directional screening, in studies that reported both x-ray and bacteriologic diagnosis methods, prevalence was determined to be much higher in x-ray assessments than by culture, suggesting an over diagnosis of TB [31]. However when examining sensitivity of these tests, the majority of studies published after the systematic review, conducted symptom screening and smear microscopy to confirm TB, methods which have been evident for low sensitivity. Due to limited resources, many screening studies did not complete x-rays and culture assessments, which might have led to the detection of more TB cases.

Most DM diagnosis in these observational studies have relied on self-report, previous diagnosis from a health professional, and attendance of a DM clinic. Few studies had clinically validated DM through blood testing. However for those in a resource limited setting, waist circumference might be a better indicator for DM as opposed to BMI, due to

its measurement of abdominal obesity [66]. Furthermore, only two screening studies [33,41] measured waist circumference of its participants, indicating more research needs to be conducted to investigate waist circumference and its role in TB incidence among DM patients.

For studies with large sample sizes and screening in routine settings, calculating the overall prevalence among the study population was a challenge. Studies noted the difficulty in determining the total number of DM patients that attended the facilities, as the cumulative number of patients continued to increase through the duration of the study [34,67,68].

To investigate the epidemiological and clinical characteristics of DM patients with "suspect" TB, some studies gave structured questionnaires and collected blood samples, with other studies evaluating lifestyle and demographic characteristics from all study participants. Most studies had a large screening population and were conducted in high TB burden/resource-limited settings. Screening studies that had been conducted in a routine setting to assess for feasibility could be generalized to other similar settings although the yield of TB cases would vary due to population and/or geographical context. Many cross-sectional studies employed bacteriological confirmation of TB in addition to the use of chest x-rays and symptom screening, resulting in the detection of more TB cases.

B.5.3 TB burden among persons with both HIV and DM

In Sub-Saharan Africa (SSA), HIV is the strongest risk factor for TB and it has been suggested to be responsible for driving the TB epidemic [1]. Individuals with HIV are 25 times more likely to develop TB in SSA than HIV negative individuals in the same setting (Reid et al. 2013). However, little is known about the association between DM and TB among those with HIV [69].

In evaluating the burden of TB in adult inpatients at a teaching hospital in Zambia, 26.6% (95% CI: 23.1-30.3%) of those diagnosed with TB were HIV-positive, however it was unknown what proportion of screened DM patients had HIV [62]. Although South Africa has the highest prevalence of HIV and TB co-infection among TB endemic countries, the country is also grappling with the double burden of NCDs and infectious diseases. T2DM is emerging as an epidemic and a high prevalence of the population classified as overweight and obese [66].

In a study conducted on multi-morbidity in a primary care clinic in Khayelitsha, South Africa where TB/HIV co-infection in this local setting has been estimated at 67% [70], 22.6% of patients had multi-morbidity with the highest prevalence found in DM patients (88.1%). Among HIV-positive patients, 24% had TB as a co-morbidity followed by DM at 17%. Multi-morbidity was highest among younger HIV/ART patients (26% among 18-35 years compared to 30% among 36-45 years), and DM prevalence was higher in the HIV/ART patients than in non-ART patients in all age groups [71]. Results of this study, highlight the growing prevalence of multi-morbidities between DM and infectious diseases such as TB and HIV in SSA.

Very few cohort studies examining the association between TB and DM, included HIV as a confounder. Lee et al. was one of a few cohort studies that controlled for HIV status [21,72]. In the methodology of other related cohort studies, an exclusion of DM patients with an HIV diagnosis were explicitly noted [25], with no particular reason noted, while other studies excluded HIV status due to its small population prevalence [25,29,30].

Among cross-sectional studies, one screening study conducted in India noted an explicit exclusion of DM patients with HIV after evaluating their medical history, with no reason

given and despite its routine setting methodology [68]. In the few studies that examined prevalence of HIV among TB-DM patients in an SSA context, Amare et al. found that 35.7% of TB cases in an Ethiopian clinic were HIV positive. Although smoking, TB history, and TB contact were identified as significant risk factors for TB, the study did not assess HIV as a potential risk factor for TB, so it is unknown whether HIV could have been a significant determinant of TB [43]. In the Tanzanian study, all patients that had a positive symptom screening were given an HIV test, and only one of seven DM patients that were newly diagnosed with TB was also HIV-positive [43,50].

It has been suggested that the use of protease inhibitors and other ARV treatments induce dyslipidaemia and insulin resistance, contributing risk factors of T2DM among persons with HIV [69]. Lipid profiles should be measured to evaluate its role in TB incidence and DM especially among those with HIV.

B.6 CONCLUSION

This review covers the recommendations set out by The World Health Organization and the International Union against Tuberculosis and Lung disease (Union) [73] in order to improve the prevention and control of both diseases. One of its recommendations is to broaden the screening algorithm of TB in persons with DM by sub-clinically screening all persons with DM regardless of symptoms. According to the framework, one of their recommendations has been to establish surveillance of TB among patients with DM in settings where TB burden ranges from medium to high. In evaluating the strengths and limitations of this literature review, several strengths include:

- Its identification and stratification of existing studies on TB burden among patients with DM.
- Its contribution to existing literature regarding TB/DM association.
- Its evaluation of TB risk factors associated with DM, and an overview of TB screening methodologies of DM patients.
- The outlining TB-DM studies conducted in an SSA context, which had not been done previously.
- Review also highlights growing importance of multi-morbidity, especially HIV/TB co-infection among persons with DM.

The literature review was not a systematic review, so not all studies about the TB/DM association were included in the study. Due to the wide variations of screening methodologies and contexts in the cross-sectional studies, comparing the prevalence rates of TB among DM patients was difficult. Multidrug-resistant (MDR) TB and other types of TB among patients with DM, were not evaluated due to the dearth of information regarding strength of association.

To improve the yield of TB diagnosis, more effective diagnostic methods may need to be implemented. Employing advanced diagnostic tools such as the Xpert® MTB/RIF (Cepheid, Inc., Sunnyvale, CA, USA) could improve screening algorithm of DM patients for TB by increasing detection and patient compliance rates due to its high sensitivity and rapid testing. The feasibility of Xpert MTB/RIF should be further assessed especially in the context of a high burden/low resource setting, as the availability of such data is non-existent. To increase validity of TB prevalence, all persons with DM should undergo additional evaluation of TB to confirm TB diagnosis, regardless of results from symptom screening. Finally, more investigations should be conducted to further examine the role of lifestyle/socio-demographic factors in order to best characterise high-risk patients with DM and improve active case finding as a result. When examining lifestyle factors, association of nutritional status and TB among DM patients was limited. Nutritional statuses of persons with DM as a potential risk factor for TB, as poor nutrition has been indicated as a risk factor for TB and improving care and prevention among high-risk patients with DM can diminish TB incidence [20].

There is also a paucity of information regarding TB contact and its association with TB incidence among persons with DM. Minimal evidence exists around whether previous TB contact is associated with TB incidence among DM patients, as only a couple studies have measured it and with non-significant results [33,43].

Finally, the findings from the review revealed major literature gaps on the epidemiological features of TB among persons with DM. Lack of existing knowledge about TB burden and screening methods within the DM population remain limited, especially in SSA. Many of the observational studies investigating the association of TB and DM were conducted in high resource, low burden areas such as Denmark, UK, Australia, and in Asian countries

(China, India, Korea and Taiwan) where half the TB burden is located [1]. The existing research highlights a wide variation in TB prevalence/incidence among patients with DM.

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PART C: JOURNAL MANUSCRIPT

The prevalence and determinants of active tuberculosis among diabetes patients attending a primary health care clinic in Cape Town, South Africa

Running head: A cross-sectional study

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Formatted for the Lancet: Diabetes and Epidemiology[5]

Word count (excluding footnote, tables, figures, and abstract): 3,030

Key words TB, diabetes mellitus, prevalence, risk factors

Conflicts of interest: The authors have no conflicts of interest to declare

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[5] For readability, figures and tables are inserted in the text rather than appended at the end of the article. Although article is written according to guidelines set by the *journal*, texts, tables and figure numbering are formatted to align with dissertation. Supervisor's contributions are recognized in the acknowledgments. Detailed instructions for journal can be found in Part D, Appendix 9

C.1 ABSTRACT

C.1.1 Background

The number of studies addressing the association between diabetes mellitus (DM) and tuberculosis (TB) in the context of sub-Saharan Africa (SSA) is limited and fewer studies have determined whether DM is associated with TB among patients attending DM clinics. We aimed to assess the prevalence of TB among a population, diagnosed and receiving treatment for DM at a primary care clinic, and to identify significant risk factors of prevalent TB cases.

C.1.2 Methods

In this cross-sectional study, adult DM patients attending an outpatient clinic at a South African township were evaluated for TB using Xpert MTB/RIF testing (Xpert) and other conventional methods—clinical symptom screening, smear microscopy, chest x-ray, and culture. Socio-demographic and biochemical information were collected using the WHO's STEPwise approach to surveillance of chronic disease risk factors.

C.1.3 Findings

451 DM patients were screened for TB. 16 DM patients were diagnosed with TB, following screening giving a prevalence rate of 3·55% or 35·48 per 1000 people [95% CI: 2·18 - 5·72]. 37·50% (n=6) of TB cases reported at least one clinical symptom of TB [95% CI: 17·34- 63·11%]. 62·5% (n=10; 95% CI :) of TB cases were HIV-positive. In multivariate analysis, HIV (OR: 14·58, p<0·001) and haemoptysis (OR 24·48, p<0·001) were strongly associated with prevalent TB. Identified associations were not modified by

age or gender. There were no significant differences in either fasting plasma glucose or HbA1c levels between TB and non-TB DM participants.

C.1.4 Discussion

Prevalence of TB among DM population was higher than in the general population based on national estimates, highlighting an important DM –TB association in an SSA setting. HIV as a significant risk factor for TB confirms its position as a major driver in TB epidemic overall and in T2DM patients. Two-thirds of prevalent TB cases reported no TB symptoms, suggesting further research is needed to identify more accurate TB screening strategies for DM patients, particularly in HIV-infected persons, to facilitate early detection and treatment of prevalent TB in this population group.

C.1.5 Funding

Clinical Infectious Disease Research Initiative, as part of a Wellcome Trust Strategic Grant.

C.2 BACKGROUND

Previous studies have suggested that DM increases the risk of TB. While its magnitude is still being investigated, little is known about the association of TB and DM among persons with DM. Most importantly, few screening studies exist in SSA where the prevalence of TB among DM population as well as the clinical and/or socio-demographic characteristics that potentially contribute to TB prevalence has been investigated. This study comes on the heels of a new chapter in TB elimination and control. The Sustainable Development Goals (SDGs), which replaced the Millennium Development Goals (MDGs) [1], have made ending the TB epidemic a top priority. In addition the WHO's 'End TB Strategy,' through the integration of the SDGs, hopes to eliminate TB completely by reducing the number of deaths by 95% by 2035 and to reduce the incidence rate by 80% by 2030[2]. With these ambitious goals in place, new strategies need to be employed to meet these targets. Early detection of TB, can lead to prompt treatment, which can increase the chances of survival and diminish transmission risk. Large-scale screenings in China and India have tested and shown the feasibility of screening in routine and low-resource healthcare settings, but it is still unclear how effective it is in detecting new TB cases. There is limited knowledge on the prevalence of TB among DM in the SSA context,

as a consequence the true impact of DM and TB as a co-morbidity remains to be determined.

We aimed to assess the prevalence of TB among a population, diagnosed with and receiving treatment for DM at a local DM clinic and to identify significant risk factors of prevalent TB cases.

C.3 METHODS

C.3.1 Study design and patient population

Between September 2014 and October 2015, we conducted a cross-sectional study at a primary care clinic in Khayelitsha, one of the largest and fastest growing peri-urban townships in Cape Town, South Africa. With a predominantly Black African population of 391,749 (as of 2011 census)[1], it has one of the highest burdens of TB and HIV nationally and globally [2]. In 2010, the prevalence of antenatal HIV was 26% and the TB case notification rate was 1500 per 100,000 people annually [3]. A previous study demonstrated a 13·1% (95% CI: 11·0-15·1) prevalence of DM in Cape Town, specifically in the township communities [4]. A sample size of 457 was determined, based on an estimated TB prevalence of 5% with a precision/sampling error of 2%, and a two-sided significance level of 95%.

To evaluate the prevalence of TB in a routine clinical setting, adult patients diagnosed with DM and attending a DM clinic for chronic disease management at a community health clinic were prospectively recruited and subsequently investigated. Patients were eligible to participate if they had previously received a DM diagnosis and were aged ≥18 years at the time of the study. Patients were excluded if they did not provide consent.

The study received ethics approval from The University of Cape Town Human Research Ethics Committee (HREC Ref: 377/2015) and respect for patients' autonomy was ensured through the informed consent process. Privacy and confidentiality of the patients were maintained through the assignment of unique identifying numbers (ID).

C.3.2 Data Collection

Data were collected from each patient over a 2-hour survey period during a scheduled appointment. Community health workers who were bilingual in both English and Xhosa, predominantly spoken languages in Khayelitsha, administered questionnaires to collect socio-demographic and behavioural/lifestyle information, such as dietary habits and education level and biochemical measurements, such as glucose levels using the WHO STEPwise approach to surveillance of chronic disease risk factors [7] .

A validated TB suspect screening tool (adapted from the Practical Approach to Lung Health and HIV/AIDS in South Africa, a symptom-based clinical management guideline[8]) was used to assess TB symptoms, which included: cough, night sweats, fever, and loss of weight and additional information about HIV status and chronic disease history. Respiratory and pulse rates; temperature, blood pressure, height, and weight measurements; and waist circumference were also collected using standardized techniques. Following an overnight fast of 8-12 hours, blood samples were collected and used to measure plasma HbA1c (glycated haemoglobin) and fasting plasma glucose (FPG) to determine baseline glycaemic control.

TB screening and diagnosis were conducted using the national TB management guidelines [9]. Spontaneous or induced sputum samples were collected and processed for Xpert and drug susceptibility testing regardless of symptomatic status. Additional samples underwent repeat testing using smear microscopy (acid-fast bacilli) if Xpert result was positive and/or sputum culture, or if patient was HIV positive. An accredited national laboratory (National Health Laboratory Service) that adhered to standardised protocols and followed quality assurance measures processed collected samples.

If test results came back positive for TB and/or HIV, patients were recalled and referred for further clinical care and treatment.

C.3.3 Case Definitions

Patients that tested positive for *M. tuberculosis* by either Xpert, smear microscopy, or culture testing were defined as bacteriologically confirmed TB cases.

C.3.4 Data Analysis

The study population was characterized by descriptive statistics using univariate and bivariate analysis. Categorical and continuous variables gathered from the questionnaires were used to test associations between the group with TB and the group without TB using Pearson's $\chi 2$ test and Wilcoxon rank-sum test, respectively. Body mass index (BMI), was categorized into intervals "<24" "25-29" and ">30." Those that refused HIV testing or had an unknown HIV status were re-categorized into HIV-negative category. DM status was categorized into "controlled" and "poor control," with controlled status having an HbA1c level of less than 7%.

Risk factors (variables with a p-value of ≤ 0.25 [10]) associated with TB were further analysed using multivariate logistic regression. Significance testing was two-sided at p-values ≤ 0.05. Significant risk factors to be included in the final model were evaluated by the stepwise regression function, a modified version of the forward-selection function, to control for possible confounders [11]. Odds ratios (OR) and 95% confidence intervals were also estimated. Model validation was completed to identify any outliers and influential observations and fit was evaluated using Pearson's goodness of fit test. Potential effect modification was assessed using interaction variables. Prevalence of TB was also calculated.

All data analyses were conducted using the statistical software STATA version 13.

C.4 FINDINGS

Demographic and clinical characteristics were stratified by TB and non-TB DM patients and both groups were compared to evaluate significance. The distributions between remaining measured characteristics in this study were similar and not significantly different between TB and non-TB DM groups. Characteristics between both groups, along with the overall distribution of their estimates are summarized in Table C.1.

C.4.1 Prevalence of TB

Of 492 DM patients included in the study, 451 were screened for TB (73·9% female). Sixteen DM patients were diagnosed with TB (prevalence rate 3·6% [95% CI: 2·2 - 5·7]. Prevalence of TB was higher in female participants, (56·3%; CI 31·6- 78·1, versus 43·8%; CI 21·9 - 68·4 in male participants). The median age of TB cases was 54·5 [IQR 45·4-60·5]. This was not significantly different in non-TB patients (p=0·706) where median age was 54·5, [IQR: 46·6-62·1]. There was also no significant difference in gender (p=0·122) between TB cases and non-TB cases.

The majority of TB cases (68·8%; CI: 42·4 - 86·8) were detected by Xpert testing; 12·5% (CI: 3·0- 39·9) were confirmed through smear microscopy, and 25·0% (CI: 9·4- 51·8) through *M.tb.* culture. TB cases who were HIV positive, was 62·5% (CI: 36·9- 82·6) compared to 11·5% of non-TB cases (CI: 8·8-14·9).

C.4.2 TB symptoms

62·5% of TB cases were asymptomatic for TB (CI: 36·9- 82·6%). In reporting TB symptoms, 46·6% of all screened participants reported at least one TB symptom (CI: 42·0 -51·2). Among TB cases: cough, fever, and body weight loss were the most commonly reported symptoms (18·8%; CI 5·9- 45·9). Fatigue was the most common

symptom reported among non-TB cases (32%; CI: 27·7- 36·5) followed by body weight loss (16·8%; CI 13·5 - 20·6) and night sweats (16·3%; CI 13·1 - 20·1).

C.4.3 DM management

Median HbA1c was 9·15 [IQR 7·3-11] and fasting plasma glucose (FPG) was 8·2 [IQR 6·1- 11·6] for all participants. There was no significant difference in either FPG (p=0·943) or HbA1c% (p=0·898) levels between TB cases and non-TB cases. Although 20% (CI: 16·5-23·9) of screened patients had "controlled" DM, all TB cases were on medication prior to TB diagnosis.

Although non-significant, median BMI in TB cases was 27·94 [IQR 24·95 - 44·1]; lower than the median BMI in non-cases [32·78; IQR 28·6 - 38·64]. 65·9% (CI 61·2 -70·3) of non-cases was classified as "obese" (BMI >30) compared to 37·5% (CI: 17·4 - 63·1) in TB cases.

Hypertension was the most common co-morbidity among all participants (60·4% (CI: 55·8- 64·9), but was higher in non-TB cases (60·8%; CI: 56·1-65·3) than in TB cases (50%; 95% CI: 26·6-73·4).

C.4.4 Risk factors associated with prevalent TB

In a univariate analysis, HIV (X^2 34·81, p<0·001), ARV medication (X^2 30·52; p<0·001), haemoptysis (X^2 15·77; p=0·013) and categorical BMI (X^2 6·17; p=0·034) were associated with prevalent TB. In a multivariate analysis, HIV (adjusted OR: 14.58; CI: 4·47- 36·83) and haemoptysis (adjusted OR: 24·48; CI: 3·12- 192·26) remained significantly associated with prevalent TB (p<0·001). The odds of having TB were 15 times larger for DM patients living with HIV (95% CI: 4·77 -44·57), compared to DM patients without an HIV diagnosis when controlling for other confounders. Patients reporting haemoptysis symptom were 25 times more likely to have TB compared to those

that did not report the symptom (95% CI: 3·12 -192·26). Both the univariate and multivariate analysis of significant risk factors are summarized in Table C.2.
There were no statistically significant interactions between age or gender and haemoptysis or HIV associations. However, 70% (CI: 57·2-80·3) of HIV-positive DM patients were younger (<54 years old) and female compared to 30% (CI: 19·7- 42·8) of older and male patients, leading to increased relative odds of having TB among younger female patients.

Table C.1 Demographic and clinical characteristics of 451 DM patients with and without TB attending a DM clinic at Khayelitsha (Site B) Community Health Clinic from September 2014 to October 2015.

Characteristic	**DM patients w/ TB (n=16) N (%)**	**DM patients w/o TB (n=435) N (%)**	**Total (n=451) N (%)**	**P-value**
Age (years) Median (n=451)	54.51 [45.41-60.51]	54.37 [46.62-62.07]	54.37 [46.61-61.97]	0.706
Gender				0.122
Female	9 (56.25)	324 (74.48)	333 (73.84)	
Marital Status (n=449)				0.718
Single	5 (31.3)	115 (26.6)	120 (26.73)	
Married	8 (50.0)	239 (55.2)	247 (55.0)	
Divorced	1 (6.25)	4 (0.92)	5 (1.11)	
Widowed	2 (12.5)	75 (17.3)	77 (17.2)	
HIV (n=451)				<0.001
Positive	10 (62.5)	50 (11.5)	60 (13.3)	
ARV medication* (n=60)				<0.001
Yes	8 (80.0)	35 (70.0)	43 (72.7)	
Number of People in Household, median	2 [1-3]	2 [1-3]	2 [1-3]	0.870
Average monthly income (ZAR)(n=391) median (IQR)	1500 [1300 – 2200]	1400 [1300 – 2600]	1400 [1300 – 2600]	0.029
Education level (n=450)				0.873
University	0 (0.00)	5 (1.15)	5 (1.11)	
Primary	10 (62.5)	250 (57.6)	260 (57.8)	
Secondary	5 (31.3)	152 (35.0)	157 (34.9)	
None	1 (6.25)	27 (6.22)	28 (6.22)	
Employment status				0.568
Employed	6 (37.5)	144 (33.3)	150 (33.5)	
Unemployed	7 (43.8)	177 (41.0)	184 (41.1)	
Retired	3 (18.8)	111 (25.7)	114 (25.5)	

TB Contact				0.608
Yes	3 (18.8)	106 (24.4)	109 (24.2)	
Previous TB (n=445)				0.971
Yes	3 (18.8)	82 (19.1)	85 (19.1)	
TB Symptom				0.462
Yes	6 (37.5)	204 (47.0)	210 (46.6)	
Cough >2 weeks				0.458
Yes	3 (18.8)	54 (12.4)	57 (12.6)	
Fever				0.170
Yes	3 (18.8)	37 (8.51)	40 (8.9)	
Weight Loss				0.836
Yes	3 (18.8)	73 (16.8)	76 (16.9)	
Fatigue				0.119
Yes	2 (12.5)	139 (32.0)	141 (31.3)	
Blood-stained sputum				0.003
Yes	2 (12.5)	4 (0.92)	6 (1.3)	
Chest pain (n=451)				0.757
Yes	2 (12.5)	44 (10.1)	46 (10.2)	
Night sweats (n=451)				0.685
Yes	2 (12.5)	71 (16.3)	73 (16.19)	
BMI (kg/m^2) (n=445)	27.94 [24.95 - 44.1]	32.78 [28.6 - 38.64]	32.72 [28.23- 38.65]	0.426
<24	4 (25.0)	52 (12.1)	56 (12.6)	
25-29	6 (37.5)	95 (22.1)	101 (22.7)	
>30	6 (37.5)	282 (65.7)	288 (64.7)	
HbA1c (%) (n=446)	9.35 [7.65-10]	9.1 [7.3-11]	9.15 [7.3-11]	0.898
<5	1 (6.3)	9 (2.1)	10 (2.2)	
5.5-6.9	3 (18.8)	77 (18.0)	80 (18.0)	
>7	9 (56.3)	278 (64.8)	287 (64.5)	
Fasting Plasma Glucose (mmol/L) (n=445)	8 [5.4-10.6]	8.2 [6.1-11.6]	8.2 [6.1-11.6]	0.943
<5	4 (25.0)	74 (17.3)	78 (17.5)	
5.5-6.9	3 (18.8)	77 (18.0)	80 (18.0)	
>7	9 (56.3)	278 (64.8)	287 (64.5)	
Triglycerides (mmol/L) (n=447)	1.40 [1.05-1.95]	1.3 [1-1.8]	1.3 [1-1.8]	0.183

Smoking (past or current, n=449)				0.547
Yes	2 (12.5)	80 (18.5)	82 (18.3)	
Alcohol Consumption (n=449)				0.642
Yes	3 (18.8)	63 (14.6)	66 (14.7)	
Family History of DM (n=446)				0.236
Yes	11 (68.8)	230 (53.5)	241 (54.0)	
DM Medication				NA
Yes	16 (100.0)	421 (96.8)	437 (96.9)	
DM Status				
Poorly controlled	16(100.0)	430 (98.9)	446 (98.9)	NA
Hypertension (n=447)				0.782
Yes	8 (50.0)	169 (60.8)	270 (60.4)	
Co-morbidity				0.795
Yes	15 (93.8)	400 (92.0)	415 (92.0)	
Fruit & Vegetable Servings (n=449)				NA
>5 servings	0 (100.0)	7 (1.6)	7 (1.6)	

Table C.2 Risk factors associated with TB in univariate and multivariate analysis.

Characteristic	OR (95% CI)	P-value	AOR (95% CI)	P-value
HIV		<0.001	14.58(4.77- 44.57)	<0.001
Yes	12.83 (4.47- 36.83)			
No	1.00		1.00	
			<0.001	
		11.08 (3.93- 31.28)		
ARV medication				
Yes				
No	1.00			
Blood-stained sputum		0.003	24.48(3.12- 192.26)	0.002
Yes	15.39 (2.60- 91.17)			
No	1.00		1.00	
Fatigue		0.119		
Yes	0.30 (.068-1.36)			
No	1.00			
Fever		0.170		
Yes	2.48 (0.68-9.11)			
No	1.00			
Mines		0.169		
Yes	2.96. (0.63-13.94)			
No	1.00			
Gender		0.113		
Male	1.00			
Female	0.44 (0.16-1.21)			
Family history of DM		0.236		
Yes	1.91 (0.65-5.60)			
No	1.00			
Average monthly income	1.00	0.029		
Triglycerides	1.28 (0.89-1.83)	0.183		
BMI (kg/m^2)(n=445)		0.028		
<24	1.00			
25-29	0.82 (0.22 -3.04)			
>30	0.28 (0.08-1.01)			

C.5 DISCUSSION

The prevalence of TB among DM patients in this study was 4-fold greater than the national estimate of 834 per 100,000 at a prevalence rate of 3·55%. Previous screening studies conducted in similar high TB burden settings have reported prevalence rates ranging from 0·10% to 6·22%. Concluding evidence suggests that DM patients should be targeted as a "high-risk" group and therefore screened for TB.

HIV and haemoptysis were significantly associated with TB. DM patients with HIV were 15 times more likely to have TB than DM patients without an HIV diagnosis (OR: 12·83 95% CI: 4·47 -36·83). Although not statistically significant, this association was higher in the younger age group (<54 years old) and in females.

In one of the few studies examining the prevalence of HIV among TB/DM patients in a high TB/HIV burden setting, 35·7% of newly diagnosed TB cases were HIV-positive [12]. However while smoking, TB history, and TB contact were identified as significant risk factors for TB, the study did not assess HIV as a potential risk factor for TB, so it is unknown whether HIV could have been a significant determinant of TB.

A high proportion of TB cases in our study were female (n=9, 56·3% CI 31·6-78·1%), however the odds of having TB were higher in males (OR: 2·26 p>0·113 CI: 0·824-6·22%). Of the total screened, 26·22% (n=118) were male. This finding was consistent in other studies where the risk of TB was higher in DM males compared to controls. [12–15].

In this study, elevated glycosylated haemoglobin (HbA1c %) and fasting plasma glucose assessing glycaemic control did not attain significant results. This was comparable to other studies evaluating HbA1c measurements of DM patients, that found no association between poor glucose control and increased TB risk [16,17]. However, this was inconsistent with a screening study conducted in a tertiary hospital in India, where there

was a positive association between the rate of TB incidence and HbA1c levels especially in patients with HbA1c over 9.0% [14].

Previous studies have suggested a positive association between TB and hyperglycaemia[18,19] [20]. There is biological evidence that DM patients having poor glycaemic control have a higher risk of TB than those with well-controlled glycaemic levels [21]. Out of all screened participants, 98·9% had poorly controlled DM [95% CI: 97·4 - 99·5]. There was no significant difference in glycaemic control between TB and non-TB DM patients (p>0·666), with an overall median HbA1c level of 9·15% [IQR: 7·3 – 11%]. There has been conflicting evidence regarding the use of HbA1c, as a diagnostic tool for DM. Previous clinical studies have shown that Black Africans had higher HbA1c levels than Caucasians with similar plasma glucose levels [22,23] or despite having normal fasting glucose levels [24]. This could explain the non-significant correlation between elevated Hba1c levels and prevalent TB, as our study population was predominantly Black African. Levels of FPG are also indicative of DM status, with elevated levels increasing odds of prevalent TB. However, TB cases had a lower median FPG level than non-TB cases. Although not significant, the magnitude of association between glycaemic control and TB remains to be seen, as there was no significant correlation between FPG levels and prevalent TB.

Proxies to determine DM status could be used as an alternative method to further evaluate the association between poor glycaemic control and TB risk. Although one longitudinal study conducted in a low TB burden setting found no association between glycaemic control and TB risk, chronic disease management was measured and those with the

highest and lowest numbers of consultations or clinic follow-ups had the strongest association with TB risk, compared to other significant risk factors [17].

Although smoking and alcohol dependence are well-known lifestyle risk factors for TB [25–27] smoking and alcohol were not identified as significant risk factors in our study. A large percentage (81·7%) of participants in our study did not report tobacco use [95% CI: 77·9- 85·1].

In addition to using body mass index (BMI), poor diet was assessed as a proxy for nutritional status. Inadequate intake of fruits and vegetables has been associated with poor nutrition [28]. Only 1.6% of DM patients received the daily-recommended servings of fruits and vegetables [96% CI: 0.74-3.24]. Previous research have assessed nutritional status among persons with DM and TB, as malnutrition is a known risk factor for TB and improving care and prevention among high-risk patients with DM can diminish TB incidence [29][30]. While studies have used BMI as a proxy for nutritional status, few studies have assessed dietary intake as a risk factor. Although 64.7% of DM patients screened were categorized as obese (BMI > 30 kg/m^2) [95% CI: 60.3-69.2] BMI, was not a significant risk factor for TB, which was consistent with results from another screening study [15,31]. However other studies have shown a negative correlation between BMI and TB risk [14,16,17,32], suggesting that a higher BMI may have a protective effect [31] against TB risk. Despite lack of significance ($p>0.072$), our study is consistent in that obese patients were 0.27 times as likely to have TB compared to underweight and overweight DM patients [95% CI: 0.213 - 2.93].

Our study was unique in that all DM patients were sub clinically screened for TB regardless of reported symptoms. In similar screening studies, sputum samples were collected from only clinically “suspicious” DM patients [12,16,33–37].. However,

evidence has shown that symptom screening may not be an effective method in detecting TB cases, in those with HIV [38,39] and DM [40]. In one particular TB screening study conducted by the Centers for Disease Control and Prevention (CDC) in Southeast Asia, all patients with HIV were sub clinically screened regardless of symptoms. Results revealed that symptom screening alone failed to detect more than 66% of patients with actual TB [39]. Similar results were found in a South African study screening HIV-1 patients, which found a high prevalence of asymptomatic TB disease in co-infected persons [38]. In one particular screening in Iran [40], 24 "suspicious" DM patients were clinically screened, leading to the detection of 4 TB cases. However 35·8% (143) of study population screened had a positive tuberculin skin test (TST), which suggests that the actual prevalence of TB disease among study population might have been underreported despite being asymptomatic

Several limitations were identified in this study. Selection bias may be present due to convenience sampling of the participants, as the sampling frame excluded individuals that have been diagnosed with DM but do not attend the clinic. Prevalence rate might have been underestimated as individuals with DM that don't attend clinics for follow-ups may have poorly managed DM, and therefore poor glycaemic control, which is associated with TB risk.

In addition, we did not objectively obtain information on reported symptoms in order to confirm or validate them. There is a possibility that patients might have misinterpreted or misperceived definition of TB symptoms that might have resulted in an over- or under-estimation of reported symptoms leading to respondent/recall bias. Other than cough and

fever, the temporality and duration of these classical TB symptoms were not reported. For TB contact, date of contact was also not obtained.

Lastly the wide confidence intervals of HIV and haemoptysis produces uncertainty and imprecision about its true magnitude of association with TB.

C.5.1 Conclusion

Prevalence rate of TB in DM patients was much higher than the national estimate. The high prevalence rate among DM patients adds to the growing body evidence that DM is strongly associated with TB. DM patients are a “high-risk” population and should undergo TB screening as part of their routine care.

The majority of TB cases were asymptomatic for TB at the time of diagnosis. This finding poses a serious threat to TB control especially among this high-risk population. The low sensitivity of symptom screening highlights the need of using a point of care diagnostic tool such as the Xpert to detect more TB cases among an asymptomatic population. HIV as a significant risk factor for prevalent TB confirmed its position as a major driver in the TB epidemic. This finding also shows the growing trend of multi-morbidity, and the need to focus on the care and management of both chronic and infectious diseases. Future research needs to focus on the feasibility of Xpert MTB/Rif, in order to establish the most effective screening algorithm for DM patients. The use of a rapid, point of care diagnostic such as Xpert can increase the number of detections, which can lead to early detection and treatment of TB.

C.6 FUNDING

This study was funded by the Clinical Infectious Disease Research Initiative, as part of a Wellcome Trust Strategic Grant.

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PART D: APPENDICES

D.1 APPENDIX 1: PROTOCOL-VARIABLE TABLE

Category	Response	Variable Name
Age (years)	Numeral discrete (mean or median) Categorical: <30yrs 0 31-40 1 41-50 2 >50 3	age agecat
Gender	Categorical: Male = 0 Female =1	sex
BMI (kg/m^2)	Numeral discrete Categorical: <18 1 underweight 18-24 2 normal 25-29 3 overweight >30 4 obese	bmi catbmi
Waist Circumference	Numerical	
Tobacco use	Categorical: Yes 1 No 0	smokerever 1/smokerever
<u>Smoking History</u>	Yes 1	smoker

Current smoker Previous smoker	No 0	pastsmoker
Alcohol Consumption	Yes 1 No 0	alcoholEVER
Marital Status	Categorical: Single 1 Married 2 Divorced 3 Widowed 4	maritalstatus
Education	Categorical: University 1 Secondary 2 Primary 3 No School 4	education
Employment Status	Categorical: Employed 1 Unemployed 2 Retired 3	employment
Socioeconomic status (SES)	Numerical discrete	avgincomemonth
Household Number	Numerical discrete Categorical: <3 people 1 4-6 people 2	Householdnumber householdcat

	7 people 3	
Family History of DM	Categorical: Yes 1 No 0	familyhist
HbA1c (%)	Numerical Categorical: 0/5.64 0 normal 5.69/6.44 1 impaired 6.5/18.4 2 diabetes	HBA1C hba1c
Fasting glucose	Numerical Categorical: 0/5.54 0 normal 5.59/6.99 1 impaired 7.0/23.5 2 diabetes	HbA1cFASTINGGLUCOSEmmol hba1cmmol
DM meds	Yes 1 No 0	DMmeds
Poorly controlled diabetes	Categorical: poor 1 good 0	DM33
TB	Categorical: Yes 1 No 0	TB

Previous TB	Categorical: Yes 1 No 0	prevTB
TB contact	Categorical: Yes 1 No 0	contact
MDR/XDR contact	Categorical: Yes 1 No 0	MDRcontact
<u>Exposure Risk</u> Prisoner HCW Mines Other	Categorical: Yes 1 No 0	prisoner healthworker mines other
<u>TB Symptoms</u> Cough >2 weeks Fever >2 weeks Weight Loss Fatigue Sputum (blood) Night Sweats Chest pain	Categorical: Yes 1 No 0	sym_cough sym_fever sym_weightloss sym_fatigue sym_sputum sym_sweats sym_chestpain
Positive TB symptom screen	Categorical: Yes 1 No 0	TBsympt

<u>Comorbidities</u>		
Hypertension	Categorical:	hbp
	Yes 1	HBP (w/meds)
	No 0	
High Cholesterol		Cholest
Asthma/COPD		Asthma
Depression		Depression
Epilepsy		Epilepsy2
Comorbidity Status		Chronic
HIV	Categorical:	HIVARV (w/ARV)
	Yes 1	HIVstatus
	No 0	
HIV medication		arv
<u>Diet</u>		
Fruit Servings	Categorical:	fruitservings
Fruit Servings/ week		fruitweek
	Servings	
Veg Servings	More than 5 1	vegservings
Veg servings/week	Less than 5 0	vegweek
Fruit and Veg Servings	*#Days*	fruitvegservings
	0-3 days per week 0	
	4-6 days per week 1	
	"Daily 2	
Meal Prep	1 "vegoil"	mealprep1
	2 "animalfat"	

	3 "butter" 4 "margarine" 5 "Other" 6 "none” 77 "77"		
Outside meals during week	Numerical		outsidemeal
	Yes 1 No 0		outsidemealbinary
<u>Physical Activity</u> Any	Categorical: Yes 1 No 0		physicalactivity
Days/week	<2 days per week 3-4 days >5 days per week	0 1 2	physicaldays1
Cycle/Walk	Yes 1 No 0		bikewalk

D.2 APPENDIX 2- DETAILED STATISTICAL ANALYSIS & MODEL BUILDING

RESULTS

Demographic characteristics of DM patients

A total of 492 DM patients were enrolled to participate in this screening study. The number of DM patients screened for TB was 451, giving an overall response rate of 91.7%. The mean age of those screened was 54.6 years and the majority of those screened were female (n=332; 73.78%). In terms of HIV status, 60 patients were HIV-positive, with 71.67% (43) currently on antiretroviral therapy (ART). More than half of the participants (261) were married (54.0%). The average number of people in the household was 2 [IQR: 1-3]. In terms of employment status, 184 were unemployed (41.1%), 150 were employed (33.5%), and 114 were retired (24.5%). Average monthly income was 1400 ZAR [IQR: 1300 – 2600 ZAR]. In terms of education level, primary school was the highest level completed by most participants (n=260; 57.8%).
With regards to biochemical measurements, the average body mass index (BMI) was 32.72 [IQR: 28.23- 38.65], with 64.7% (288) classified as "obese" (BMI > 30 kg/m^2). Of those screened, 14 (3.1%) were not on DM medication. More than half (n= 241; 54.0%) of participants reported having a family history of DM. The average level of HbA1c (%) was 9.28 ±2.36% with fasting glucose of….. Only 1.11% (5) had controlled DM. In terms of co-morbidities, 92% (415) had one or more of the following co-morbidities: hypertension, depression/anxiety, asthma/COPD, epilepsy, and high cholesterol. Hypertension was the most common co-morbidity, with 60.4% (270) having high blood pressure and/or taking medication for hypertension.

In terms of lifestyle/behavioral habits, 14.7% (66) of participants reported alcohol consumption and 18.3% (82) had a history of smoking. In terms of dietary habits, 288 reported eating non-home cooked one or more times during the week. The majority of patients were not receiving the daily-recommended servings (>5) of fruit and vegetables a day. In terms of physical activity, more than half did not engage daily in any form (moderate, vigorous, walking/biking for more than 10 minutes) of physical activity.

In terms of TB symptoms, 210 (46.6%) reported at least one TB symptom. Fatigue was the most common symptom reported (n= 141; 31.3%) followed by body weight loss (n=76; 16.9%) and night sweats (n=73; 16.19%). The most common exposure risk was mines (n=22, 4.88%) followed by prisons (n=24, 5.3%). Of those screened, 19.1% (85) previously had TB and 24.2% (109) had a known previous contact with a TB patient, a time frame was not collected.

Prevalence of TB among DM patients

TB prevalence rate was 3.55% or 35.48 per thousand people [95% CI: 2.18 - 5.72]. Patients that were HIV positive and reported a bloody sputum symptom were significantly associated with increased odds of having TB. Patients living with HIV were 15 times more likely to have TB than patients without an HIV diagnosis (AOR=14.58 [95% CI: 4.77 -44.57]). Patients reporting a bloody sputum symptom were 26 times more likely to have TB compared to those that did not report the symptom (AOR= 25.5 [95% CI: 3.12 -192.26]). Among younger patients, 18.5% (42) were HIV positive. Among older patients 8.0% (18), were HIV positive.

Among females, 12.7% (42) were HIV positive. Among males, 15.3% (18) were HIV positive. Among females, 0.90% (3) reported bloody sputum. Among males, 2.54 (3) reported bloody sputum. Among younger patients, 1.32% (3) reported bloody sputum. Among older patients, 1.34% (3) reported bloody sputum.

Demographic characteristics of Cases

Sixteen DM patients were diagnosed with TB, based on clinical examination and test results. Of these cases, 11 (68.75%) were detected by Xpert MTB/RIF assay, and 5 (31.25%) were confirmed through smear-positive/culture-positive results. Among the 16 cases, 10 (62.5%) were HIV positive with the majority on ART (n=8; 80.0%).The majority of Cases were female (n=9; 56.25%) and the mean age was 53.6 years. Half were married (n=8; 50%) and 43.8% (7) were unemployed. Average HbA1c was 9.21 2.29%, and all cases were on DM medication. All DM patients with TB also had uncontrolled DM, 1.15% (5) from non-TB DM patients had controlled DM. A high proportion of cases (68.8%) had a family history of DM. Average BMI was 27.95 [24.95-44.1], with 75% (12) classified as either "overweight" or "obese." Three (18.8%) had a previous history of TB, and 18.75% (3) had contact with a TB patient. The most common reported exposure risk was mines (n=2, 12.5%). TB symptoms were reported by 37.5% (6) of cases, with cough > 2 weeks (n=3, 18.8%), fever (n=3, 18.8%), and body weight loss (n=3, 18.8%) as the most commonly reported symptoms. Among cases, 2 (12.5%) reported smoking. In terms of diet, all cases did not receive the daily-recommended servings of fruit and vegetables and a high proportion ate non-home cooked meals one or more times a week. In terms of physical activity, 75.0% (12) did not engage daily in any form of physical activity. In terms of co-morbidity, majority of cases had one or more of the listed co-morbidities (n=15; 93.8%). Half of cases were hypertensive and/or taking medication for hypertension (n=8; 50%).

Among HIV positive males, 27.8% (5) had TB. Among HIV positive females, 11.9% (5) had TB. Among both males and females who reported a bloodstained sputum symptom, 33.3% (1) had TB respectively. Among older patients who were HIV positive, 27.8% (5) had TB. Among younger patients who were HIV positive, 11.9% (5) had TB. Among both older and younger patients that reported a bloodstained sputum symptom, 33.3% (1) had TB respectively.

Table D.1 Demographic and clinical characteristics of 451 DM patients with and without TB attending a DM clinic at Khayelitsha (Site B) Community Health Clinic from September 2014 to October 2015.

Characteristic	DM patients w/ TB (n=16) N(%)	DM patients w/o TB (n=435) N(%)	Total (n=451) N(%)	P-value
Gender				0.113
Male	7 (43.75)	111 (25.58)	118 (26.22)	
Female	9 (56.25)	323 (74.42)	332 (73.78)	
Age (years) Mean±SD (n=451)	53.56 ± 8.5	54.59 ±10.80	54.55± 10.73	0.706
<40 years	1 (6.25)	52 (11.95)	53 (11.8)	
41-50 years	5 (31.25)	121 (27.8)	126 (27.9)	
>50 years	10 (62.5)	262 (60.23)	272 (60.3)	
Marital Status (n=449)				0.718
Single	5 (31.3)	115 (26.6)	120 (26.73)	
Married	8 (50.0)	239 (55.2)	247 (55.0)	
Divorced	1 (6.25)	4 (0.92)	5 (1.11)	
Widowed	2 (12.5)	75 (17.3)	77 (17.2)	
BMI (kg/m^2) median (IQR)(n=445)	27.94 [24.95 - 44.1]	32.78 [28.6 - 38.64]	32.72 [28.23- 38.65]	0.426
<24	4 (25.0)	52 (12.1)	56 (12.6)	
25-29	6 (37.5)	95 (22.1)	101 (22.7)	
>30	6 (37.5)	282 (65.7)	288 (64.7)	
HIV (n=451)				<0.001
Positive	10 (62.5)	50 (11.5)	60 (13.3)	
Negative	6 (37.5)	385 (88.5)	391 (86.7)	
ARV medication* (n=60)				<0.001
Yes	8 (80.0)	35 (70.0)	43 (72.7)	

No	2 (20.0)	15 (30.0)	17 (28.3)	
Number of People in Household, median (IQR) (n=429)	2 [2-3]	2 [1-3]	2 [1-3]	0.870
<3 people	13 (86.7)	311 (75.12)	324 (75.5)	
4-6 people	2 (13.3)	96 (23.2)	98 (22.8)	
>7 people	0 (0.00)	7 (1.7)	7 (1.63)	
Average monthly income (ZAR)(n=391) median (IQR)	1500 [1300 – 2200]	1400 [1300 – 2600]	1400 [1300 – 2600]	0.029
Education level (n=450)				0.873
University	0 (0.00)	5 (1.15)	5 (1.11)	
Primary	10 (62.5)	250 (57.6)	260 (57.8)	
Secondary	5 (31.3)	152 (35.0)	157 (34.9)	
None	1 (6.25)	27 (6.22)	28 (6.22)	
Employment status				0.568
Employed	6 (37.5)	144 (33.3)	150 (33.5)	
Unemployed	7 (43.8)	177 (41.0)	184 (41.1)	
Retired	3 (18.8)	111 (25.7)	114 (25.5)	
Smoking (past or current, n=449) Yes	2 (12.5)	80 (18.5)	82 (18.3)	0.547
No	14 (87.5)	353 (81.5)	367 (81.7)	
Alcohol Consumption (n=449)				0.642
Yes	3 (18.8)	63 (14.6)	66 (14.7)	
No	13 (81.3)	370 (85.5)	383 (85.3)	
Family History of DM (n=446)				0.236
Yes	11 (68.8)	230 (53.5)	241 (54.0)	
No	5 (31.3)	200 (46.5)	205 (46.0)	
HbA1c (%) (mean ±SD) (n=446)	9.21± 2.29	9.28 ± 2.37	9.28 ± 2.36	0.898
<5	1 (6.3)	9 (2.1)	10 (2.2)	
5.5-6.9	3 (18.8)	77 (18.0)	80 (18.0)	
>7	9 (56.3)	278 (64.8)	287 (64.5)	
Fasting plasma glucose (mmol/L) (mean ±SD) (n=445)	9.08 ± 4.71	9.15 ± 3.86	9.15 ± 3.89	0.943
<5	4 (25.0)	74 (17.3)	78 (17.5)	
5.5-6.9	3 (18.8)	77 (18.0)	80 (18.0)	

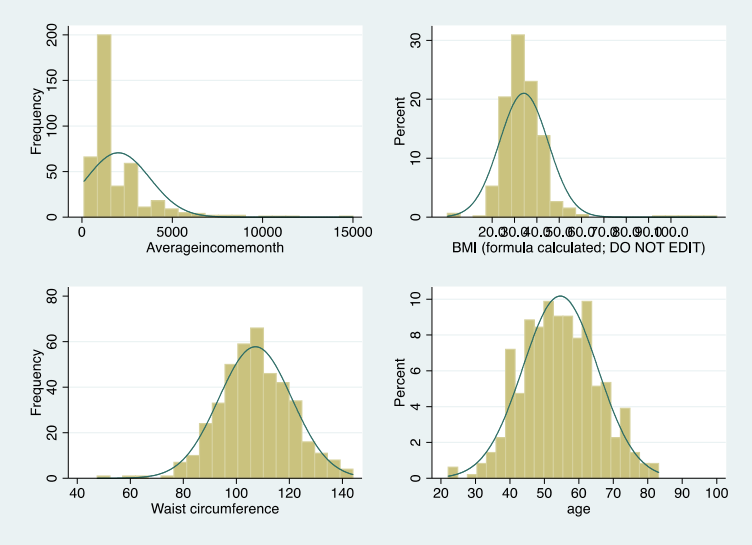
>7	9 (56.3)	278 (64.8)	287 (64.5)	
HDL (mmol/L) (n=447)	1.19± .338	1.23 ± .551	1.22± .545	0.778
Total Cholesterol(mmol/L) (n=447)	4.30 ±1.26	4.38 ±1.02	4.37 ±1.028	0.754
Triglycerides (mmol/L) (n=447)	1.89 ±1.343	1.55 ± .974	1.56 ±.989	0.183
DM Medication				NA
Yes	16 (100.0)	421 (96.8)	437 (96.9)	
No	0 (0.00)	14 (3.2)	14 (3.1)	
TB Contact				0.608
Yes	3 (18.8)	106 (24.4)	109 (24.2)	
No	13 (81.3)	329 (75.6)	342 (75.8)	
Previous TB (n=445)				0.971
Yes	3 (18.8)	82 (19.1)	85 (19.1)	
No	13 (81.3)	347 (80.9)	360 (80.9)	
Prisoner				0.866
Yes	1 (6.3)	23 (5.3)	24 (5.3)	
No	15 (93.8)	412 (94.7)	427 (94.7)	
Mines				0.2206
Yes	2 (12.5)	20 (4.6)	22 (4.88)	
No	14 (87.5)	415 (95.4)	429 (95.1)	
TB Symptom				0.462
Yes	6 (37.5)	204 (47.0)	210 (46.6)	
No	10 (62.5)	231 (53.1)	241 (53.4)	
Cough >2 weeks				0.458
Yes	3 (18.8)	54 (12.4)	57 (12.6)	
No	13 (81.3)	381 (87.6)	394 (87.4)	
Fever				0.170
Yes	3 (18.8)	37 (8.51)	40 (8.9)	
No	13 (81.3)	398 (91.5)	411 (91.1)	
Weight Loss				
Yes	3 (18.8)	73 (16.8)	76 (16.9)	0.836
No	13 (81.3)	362 (83.2)	375 (83.2)	
Fatigue				0.119
Yes	2 (12.5)	139 (32.0)	141 (31.3)	
No	14 (87.5)	296 (68.1)	310 (68.7)	

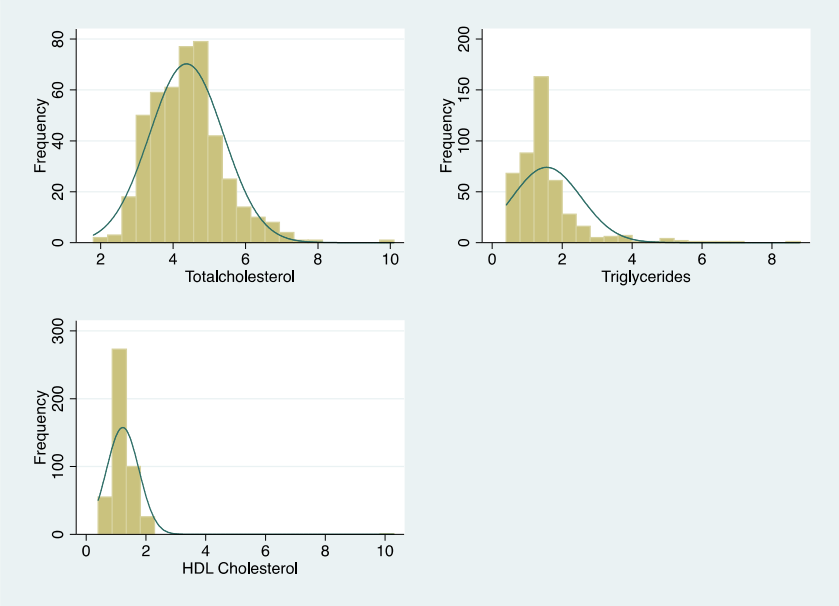
Blood-stained sputum				0.003
Yes	2 (12.5)	4 (0.92)	6 (1.3)	
No	14 (87.5)	431 (99.1)	445 (98.7)	
Chest pain (n=451)				0.757
Yes	2 (12.5)	44 (10.1)	46 (10.2)	
No	14 (87.5)	391 (89.9)	405 (89.8)	
Night sweats (n=451)				0.685
Yes	2 (12.5)	71 (16.3)	73 (16.19)	
No	14 (87.5)	364 (83.8)	378 (83.8)	
Hypertension (n=447)				0.782
Yes	8 (50.0)	262 (39.2)	270 (60.4)	
No	8 (50.0)	169 (60.8)	177 (39.6)	
Asthma/COPD				NA
Yes	0 (0.0)	18 (4.1)	18 (4.0)	
No	16 (100.0)	417 (95.9)	433 (96.0)	
Comorbidity				0.795
Yes	15 (93.8)	400 (92.0)	415 (92.0)	
No	1 (6.3)	35 (8.1)	36 (8.0)	
Fruit/Veg Servings (n=449)				NA
>5 servings	0 (0.0)	7 (1.6)	7 (1.6)	
<5 servings	16 (100.0)	426 (98.4)	442 (98.4)	
Outside meals during week (n=445)				0.908
Yes	11 (68.8)	277 (64.4)	288 (64.6)	
No	5 (31.3)	152 (35.4)	157 (35.2)	
Physical Activity (n=448)				0.435
Yes	4 (25.0)	149 (34.5)	153 (34.2)	
No	12 (75.0)	283 (65.5)	295 (65.9)	

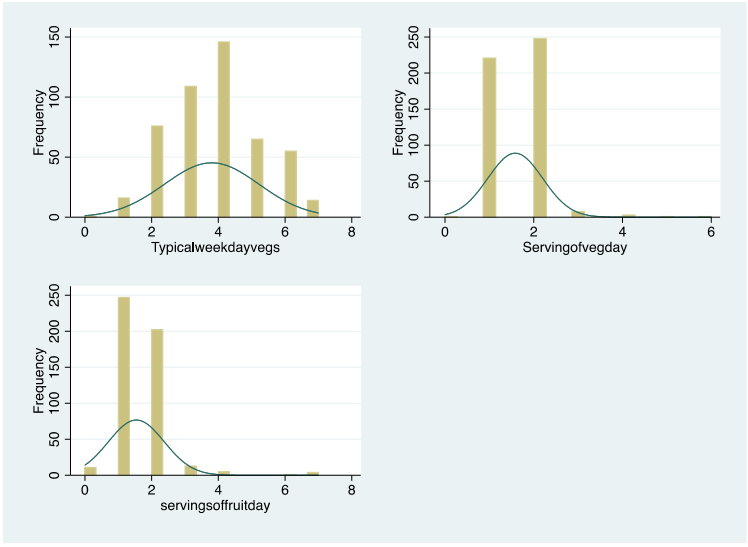
Table D.2. Association of clinical and demographic characteristics with TB as the outcome, using univariate and multivariate analysis.

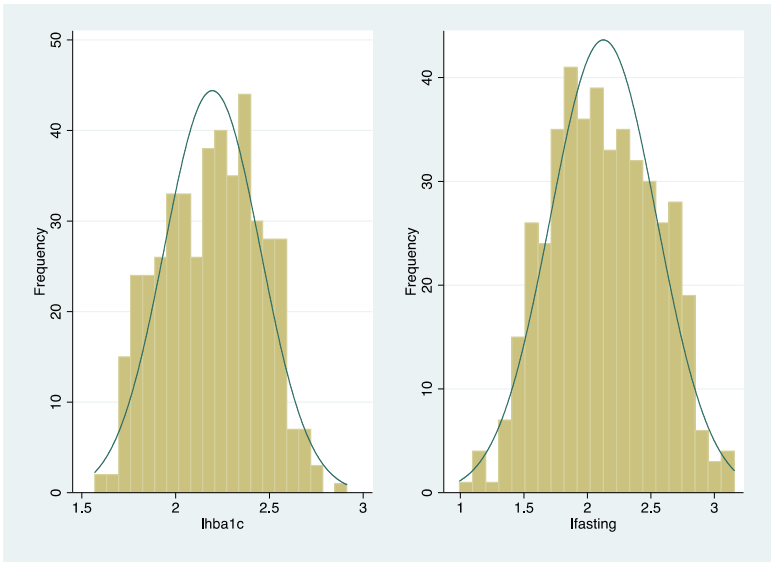
Characteristic	OR (95% CI)	P-value	AOR (95% CI)	P-value
HIV		<0.001	14.58(1.56- 3.80)	<0.001
Yes	12.83 (4.47- 36.83)			
No	1.00		1.00	
ARV medication		<0.001		
Yes	11.08 (3.93- 31.28)			
No	1.00			
Blood-stained sputum		0.003	24.48(1.14- 5.26)	0.002
Yes	15.39 (2.60- 91.17)			
No	1.00		1.00	
Fatigue		0.119		
Yes	0.30 (.068-1.36)			
No	1.00		1.00	
Fever		0.170		
Yes	2.48 (0.68-9.11)			
No	1.00			
Mines		0.169		
Yes	2.96. (0.63-13.94)			
No	1.00		1.00	
Gender		0.113		
Male	1.00		1.00	
Female	0.44 (0.16-1.21)			
Family history of DM		0.236		
Yes	1.91 (0.65-5.60)			
No	1.00		1.00	
Average monthly income	1.00	0.029		
Triglycerides	1.28 (0.89-1.83)	0.183		
BMI (kg/m^2) median (IQR)(n=445)		0.028		
<24	1.00			
25-29	0.82 (0.22 -3.04)			
>30	0.28 (0.08-1.01)			

Univariate analysis of continuous variables

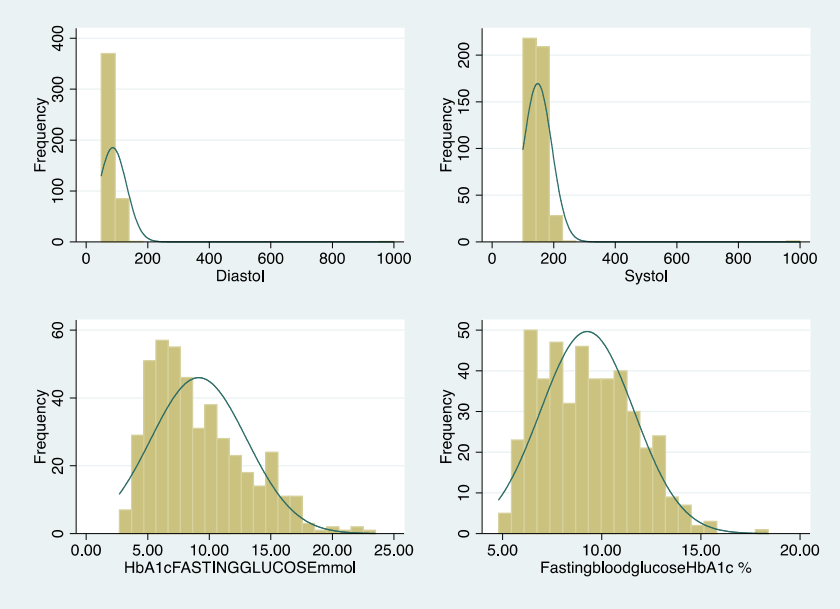


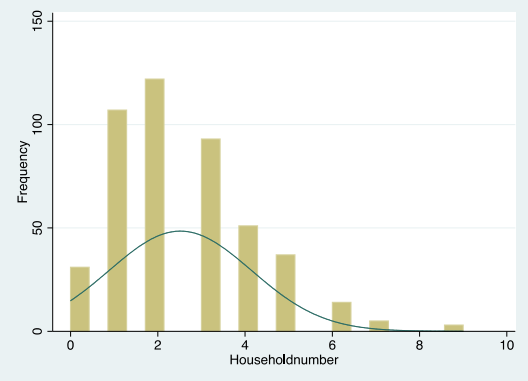






Log transformation HbA1c and Fasting plasma glucose


Frequency
0
100
200
300
400
0
200
400
600
800
1000
DiastoI
Frequency
0
50
100
150
200
0
200
400
600
800
1000
SystoI
Frequency
0
20
40
60
0.00
5.00
10.00
15.00
20.00
25.00
HbA1cFASTINGGLUCOSEmmoI
Frequency
0
10
20
30
40
50
5.00
10.00
15.00
20.00
FastingbloodglucoseHbA1c %


Frequency
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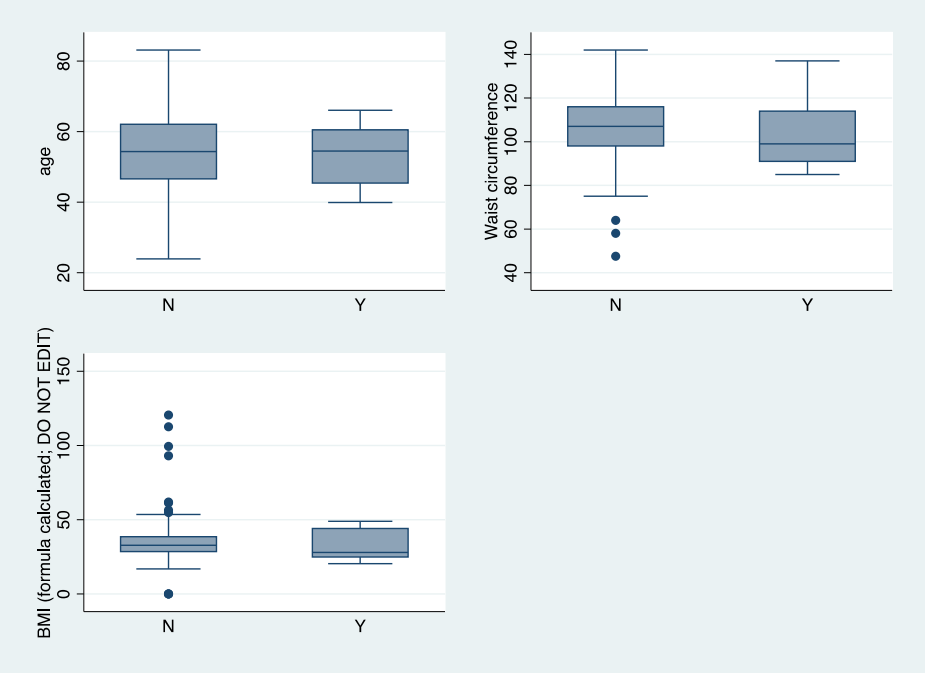
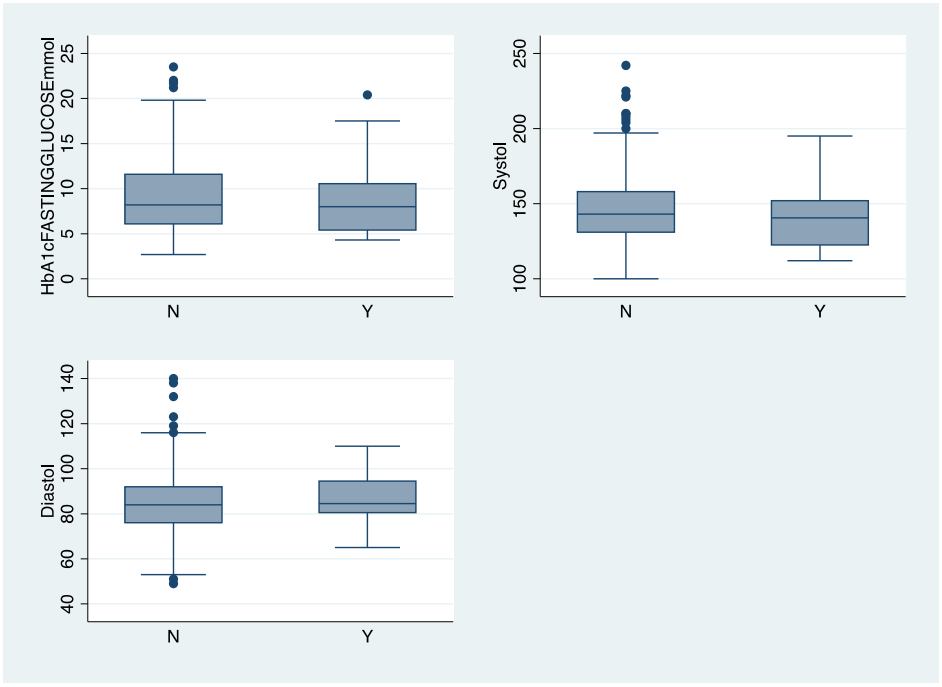
50
100
150
0
2
4
6
8
10
Householdnumber

Box plot of continuous variables by TB outcome (Bivariate analysis)




HbA1cFASTINGGLUCOSEmmol
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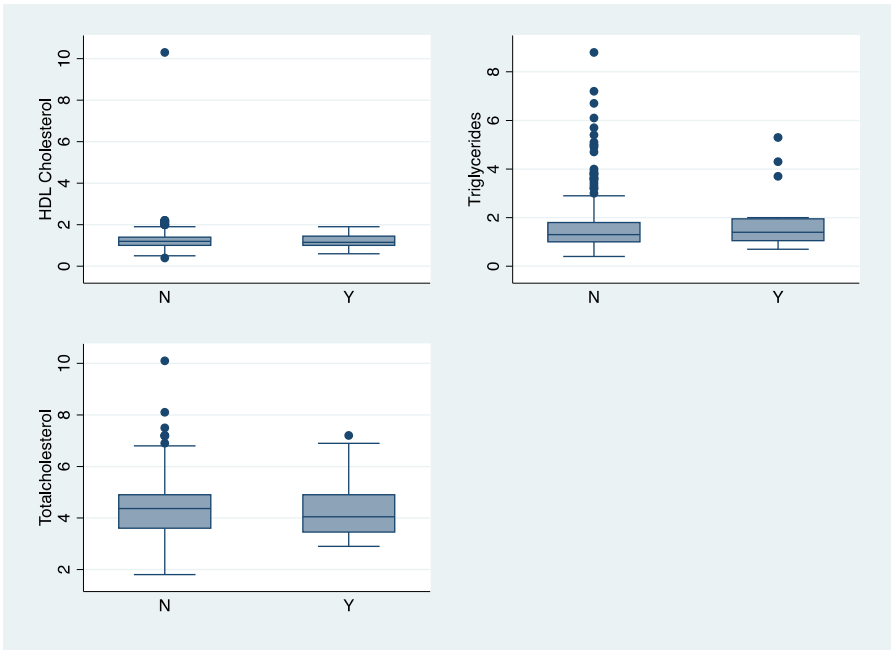
5
10
15
20
25
N
Y
Systol
100
150
200
250
N
Y
Diastol
40
60
80
100
120
140
N
Y


HDL Cholesterol
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2
4
6
8
10
N
Y
Triglycerides
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2
4
6
8
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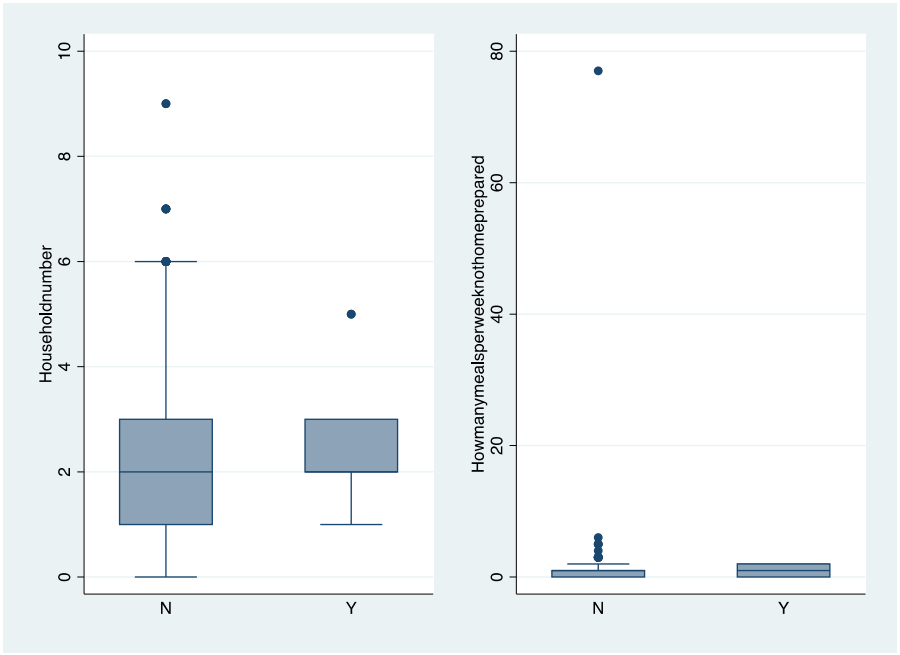
Y
Totalcholesterol
2
4
6
8
10
N
Y


Householdnumber
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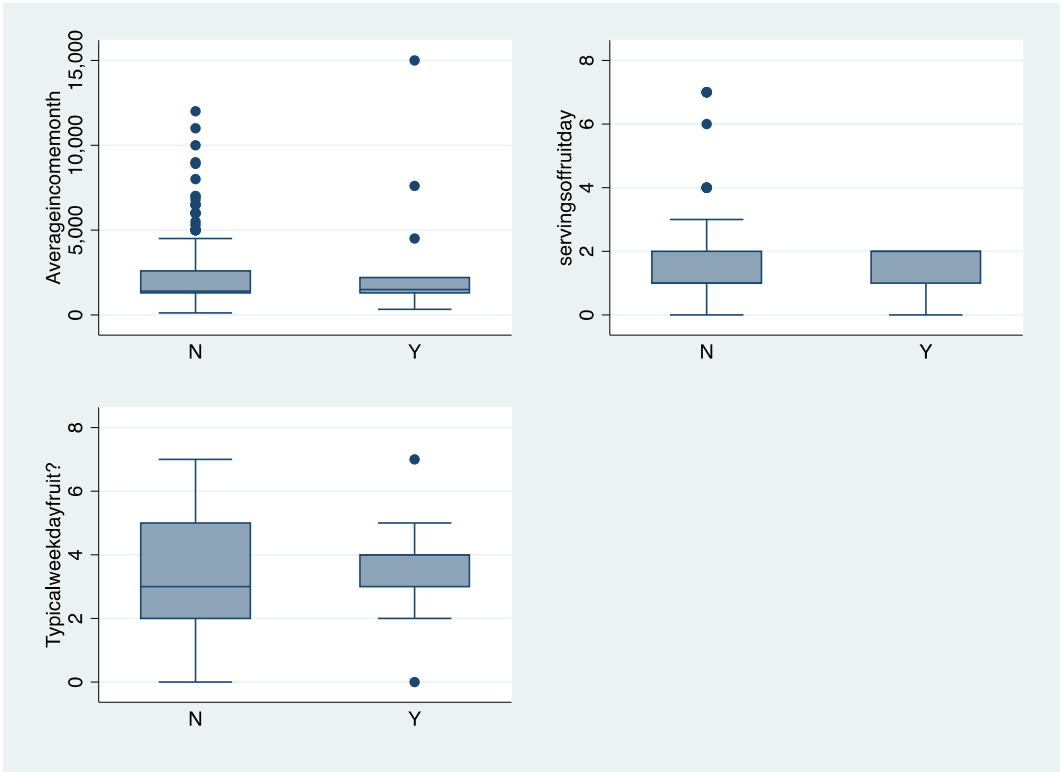
4
6
8
10
N
Y
Howmanymealsperweeknothomeprepared
0
20
40
60
80
N
Y


Averageincomemonth
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5,000
10,000
15,000
N
Y
servingsoffruitday
0
2
4
6
8
N
Y
Typicalweekdayfruit?
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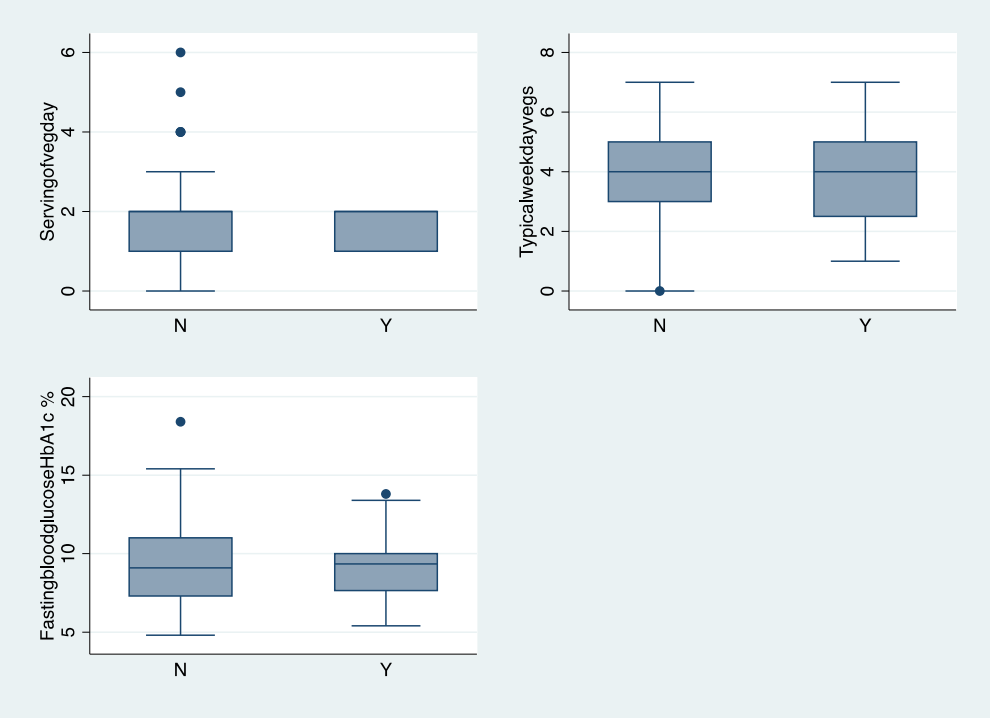
2
4
6
8
N
Y



MODEL BUILDING

Significant risk factors/independent variables to be included in final model were determined through forward selection. Univariate models were fitted for the following baseline variables listed in table 1 in order to identify possible risk factors (explanatory) variables. Variables with a p-value less than 0.20 were fitted for multivariate analysis. HIV status (p=0.000), ARV use (p=0.000), bloody sputum symptom (p=0.0126), average income per month (p=0.0555) were found to be statistically significant risk factors for TB, while previous TB (p=0.9709), HbA1c (%) (p=0.6438), smoking (p=0.5249), cough symptom (p=0.4790), BMI (p= 0.4030), body weight loss (p= 0.8386), and hypertension (p=0.3913) were strongly not associated with TB.

	Model	Log Likelihood	AIC	X2	P-value	Vs
A	TB (n=451)	-69.13483	140.2697	N/A	N/A	N/A
B	hiv (n=451)	-58.04909	120.0982	22.17	0.0000	A
C	ARV (n=451)	-60.21796	124.4359	17.83	0.0000	A
D	sym_sputum (n=451)	-66.02275	136.0455	6.22	0.0126	A
E	sym_fatigue (n=451)	-67.54118	139.0824	3.19	0.0742	A
F	sym_fever (n=451)	-68.34495	140.6899	1.58	0.2088	A
G	mines (n=451)	-68.38462	140.7692	1.50	0.2206	A
H	Sex* (n=451)	-67.90965*	139.8193*	2.38	0.1231	a
I	Familyhist (n=446)	-68.20735*	140.4147*	1.49	0.2220	b
J	Averageincomemonth (n=391)	-55.19703	114.3941*	3.67	0.0555	c
y	Triglycerides (n= 447)	-68.27238	140.5448	1.43	0.2310	z
z	BMI category (n=446)	-66.33242	138.6648	5.17	0.0755	

After fitting univariate model it was determined that HIV had the lowest AIC after a likelihood ratio test was completed. Variables were then added one at a time to "Model B" (HIV). The model was improved with the addition of bloody sputum symptom (Model L). A likelihood ratio test of Model B and L revealed a further decrease in AIC from 120.10 to 115.25. Log likelihood also increased significantly compared to other models. Gender, family history, income, triglycerides, and BMI had missing data, with income having the most missing data (n=391) while still remaining a significant risk factor. Models with missing data were fitted against a theoretical sample size of improved model. In the end, there was no significant improvement in the model with the addition of gender, family history or income, triglycerides, and BMI category.

	Model	Log Likelihood	AIC	X2	P-value	Vs
K	TB + hiv + ARV	-57.84254	121.6851	0.41	0.5204	B
L	TB + hiv + sym_sputum	-54.62476	115.2495	6.85	0.0089	B
M	TB + hiv + sym_fatigue	-57.20827	120.4165	1.68	0.1947	B
N	TB + hiv + sym_fever	-57.4797	120.9594	1.14	0.2859	B
O	TB + hiv + mines	-57.79473	121.5895	0.51	0.4757	B
P	TB + hiv + sex*	-57.05668	120.1134	1.95	0.1622	d
Q	TB + hiv + familyhist*	-57.36885	120.7377	0.52	0.4690	e
R	TB + hiv + Averageincomemonth*	-44.37713	94.75427 (compared to Model f AIC= 95.02589)	2.27	0.1318	f
	TB + hiv + Triglycerides*	-57.81851	121.0623	22.92	0.0000	
	TB + hiv + BMI category	-56.652198	121.3044	24.53	0.0000	

Variables were once again added one at a time to "Model L" (HIV and bloody sputum symptom). There were no further improvements to the model following the addition of the other variables. Adding fatigue symptom improved the model slightly but this improvement was not statistically significant (p=0.1502).

	Model	Log Likelihood	AIC	X2	P-value	Vs
S	TB + hiv + sym_sputum + ARV	-54.495	116.99	0.26	0.6104	L
T	TB + hiv + sym_sputum + sym_fatigue	-53.58969	115.1794	2.07	0.1502	L
U	TB + hiv + sym_sputum + sym_fever	-54.61445	117.2289	0.02	0.8858	L
V	TB + hiv + sym_sputum + mines	-54.26782	116.5356	0.71	0.3982	L
W	TB + hiv + sym_sputum +sex*	-54.03331	116.0666	1.16	0.2819	g
X	TB + hiv + sym_sputum + familyhist*	-54.00434	116.0087	0.48	0.4903	h
Y	TB + hiv + sym_sputum + Averageincomemonth*	-40.52121	89.04241 (Model L AIC=89.54911, lrtest p-value=0.1134)	2.51	0.1134	i
	TB + hiv + sym_sputum + Triglycerides	-54.12314	116.2463	29.73	0.0000	
	TB + hiv + sym_sputum + BMI category	-53.043318	116.0866	31.75	0.0000	

```
. logistic TB hiv sym_sputum sym_fatigue

Logistic regression                             Number of obs   =        451
                                                LR chi2(3)      =      31.09
                                                Prob > chi2     =     0.0000
Log likelihood = -53.589687                     Pseudo R2       =     0.2249

------------------------------------------------------------------------------
          TB | Odds Ratio   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
         hiv |   13.18047   7.582641     4.48   0.000     4.268155    40.70256
  sym_sputum |    31.3889   35.27189     3.07   0.002      3.46957    283.9726
 sym_fatigue |   .3491085   .2827614    -1.30   0.194     .0713719    1.707629
       _cons |    .016258   .0076267    -8.78   0.000     .0064828    .0407729
------------------------------------------------------------------------------
```

```
. lrtest TBfull fatigue, stats

Likelihood-ratio test                                 LR chi2(1)  =      2.07
(Assumption: TBfull nested in fatigue)                Prob > chi2 =    0.1502

Akaike's information criterion and Bayesian information criterion
```

Model	Obs	ll(null)	ll(model)	df	AIC	BIC
TBfull	451	-69.13483	-54.62476	3	115.2495	127.5839
fatigue	451	-69.13483	-53.58969	4	115.1794	131.6252

Note: N=Obs used in calculating BIC; see [R] BIC note

EFFECT MODIFICATION AND INTERACTIONS

HIV and blood-stained sputum

With the addition of an hiv and sym_sputum interaction (hiv*sym_sputum), the model ran into estimation problems likely due to separation and sparseness of the data. The addition of an effect modifier into the model produced a "perfectly" predicted observed outcome. As a result the model was unable to fit and parameter estimates were not obtained. Interaction was subsequently dropped from model. A Mantel-Haenszel test was done to further stratify HIV to confirm confounding status. A zero count cell was observed, under HIV positive. Of those that did not have TB and were HIV positive no one had reported a bloody sputum symptom. Of those that had both TB and HIV only one had reported having a bloody sputum symptom. Increasing sample size may have helped to avoid/ decrease sparseness in data. Presence of effect modification between HIV and bloody sputum symptom, therefore could not be determined/evaluated.

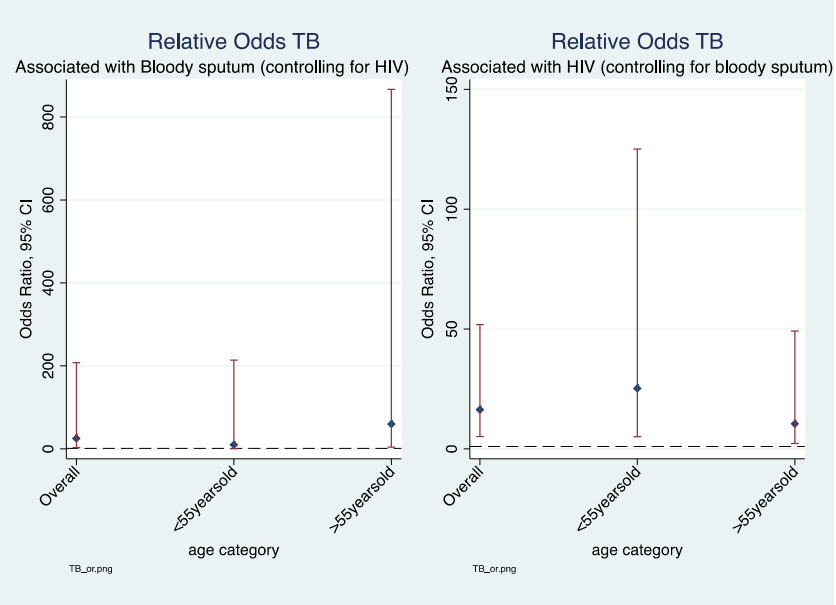
Effect Modification by age

There is little evidence that age categories (>55/<55 years old) is an effect modifier. The association between sputum (controlling for HIV) and TB ($p>0.383$), as well as the association between HIV (controlling for bloody sputum) and TB is not different

depending on age category. Overall, there is no statistically significant interaction between age categories and the associations between bloody sputum, HIV and TB.

For bloody sputum (controlling for HIV): The event of having TB is equally likely for younger DM patients or older DM patients that reported a bloody sputum and for younger DM patients or older DM patients that did not report a bloody sputum.

For HIV (controlling for bloody sputum): The event of having TB is equally likely for younger DM patients or older DM patients that are HIV positive and for younger DM patients or older DM patients who are not.



Effect Modification by gender

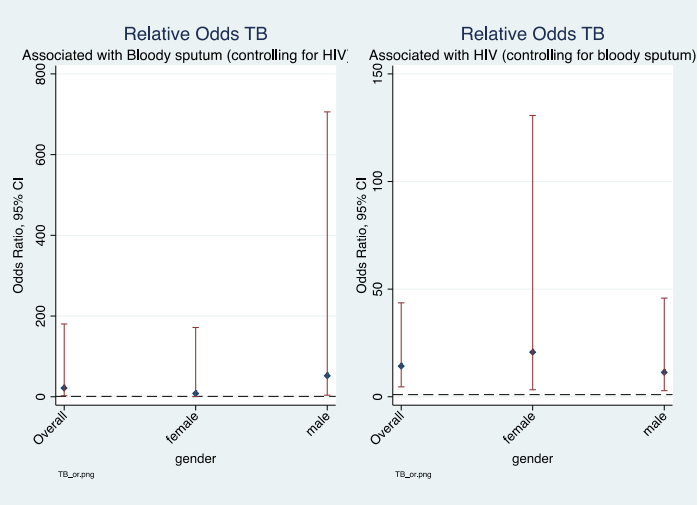
There is little evidence that gender (male/female) is an effect modifier. The association between sputum (controlling for HIV) and TB, as well as the association between HIV (controlling for bloody sputum) and TB is not different depending on gender. Overall,

there is no statistically significant interaction between gender and the associations between bloody sputum, HIV and TB.

For bloody sputum (controlling for HIV): The event of having TB is equally likely for females or males that reported a bloody sputum and for female DM patients or male DM patients that did not report a bloody sputum.

For HIV (controlling for bloodysputum): The event of having TB is equally likely for female DM patients or male DM patients that are HIV positive and for female DM patients or male DM patients who are not.

```
. logistic TB hiv##sex sym_sputum

Logistic regression                             Number of obs   =        450
                                                LR chi2(4)      =      30.41
                                                Prob > chi2     =     0.0000
Log likelihood =  -53.89583                     Pseudo R2       =     0.2200

------------------------------------------------------------------------------
          TB | Odds Ratio   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
         hiv |
         Pos |   20.68842   19.4596     3.22   0.001     3.274039    130.7286
             |
         sex |
           F |   .7696207   .6920386   -0.29   0.771     .1320934     4.48407
             |
     hiv#sex |
       Pos#F |   .5480347   .6344088   -0.52   0.603     .0566828    5.298646
             |
  sym_sputum |   21.83838   23.90115    2.82   0.005     2.556336    186.5619
       _cons |   .0154866   .0119021   -5.42   0.000     .0034338     .069846
------------------------------------------------------------------------------

. logistic TB hiv##male sym_sputum

Logistic regression                             Number of obs   =        450
                                                LR chi2(4)      =      30.41
                                                Prob > chi2     =     0.0000
Log likelihood =  -53.89583                     Pseudo R2       =     0.2200

------------------------------------------------------------------------------
          TB | Odds Ratio   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
         hiv |
         Pos |   11.33797   8.07854     3.41   0.001     2.805669    45.81779
       1.male |   1.299341   1.168361    0.29   0.771     .2230117    7.570402
             |
     hiv#male |
       Pos#1 |   1.824702   2.112288    0.52   0.603     .1887275    17.64204
             |
  sym_sputum |   21.83838   23.90115    2.82   0.005     2.556336    186.5619
       _cons |   .0119188   .0063143   -8.36   0.000     .0042198    .0336648
------------------------------------------------------------------------------
```



Effect Modification by Gender (HIV):

Variable	Odds Ratio	Std. error	P-value	95% CI	Z-test
HIV (hiv)	14.2154	8.13889	0.000	4.628191 43.66232	4.64
HIV OR^{female}	20.68842	19.4596	0.001	3.274039 130.7286	3.22
HIV OR^{male}	11.33797	8.07854	0.001	2.805669 45.81779	3.41
Gender (sex)	.5403305	.303377	0.273	.1797786 1.623981	-1.10
Female*hiv	.5480347	.634408	0.603	.0566828 5.298646	-0.52
Male*hiv	1.824702	2.11228	0.603	.1887275 17.64204	0.52

Effect Modification by Gender (bloody sputum)

Variable	Odds Ratio	Std. error	P-value	95% CI	Z-test
Bloody sputum (sym_sputum)	21.52189	23.35833	0.005	2.564758 180.5985	2.83
Sputum OR^{female}	8.072097	12.59104	0.181	.379545 171.6759	1.34
Sputum OR^{male}	52.09065	69.28222	0.003	3.842683 706.1304	2.97
gender	.5403305	.3033776	0.273	.1797786 1.623981	-1.10
Female*sym_sputm	6.453174	13.12391	0.359	.1198597 347.4351	0.92
Male*sym_sputm	.1549625	.3151494	0.359	.0028782 8.343091	-0.92

Effect Modification by Age category (HIV)

Variable	Odds Ratio	Std. error	P-value	95% CI	Z-test
hiv (HIV)	16.31171	9.624547	0.000	5.131656 51.84916	4.73
HIV OR^{old}	10.45235	8.252991	0.003	2.223926 49.12557	2.97
HIV OR^{young}	25.23131	20.6121	0.000	5.088288 125.1146	3.95
agecat	1.622781	.9144805	0.390	.5377556 4.897052	0.86
old*hiv	2.413937	2.700814	0.431	.2693802 21.63148	0.79
young*hiv	.4142611	.4634928	0.431	.0462289 3.712226	-0.79

Effect Modification by Age category (bloody sputum)

Variable	Odds Ratio	Std. error	P-value	95% CI	Z-test
Bloodysputum (sym_sputum)	24.84964	26.93624	0.003	2.969232 207.9678	2.96
Sputum OR^{old}	59.49908	81.31512	0.003	4.085126 866.5926	2.99
Sputum OR^{young}	9.604779	15.20906	0.153	.431141 213.9712	1.43
Agecat (agecat3)	1.622781	.9144805	0.390	.5377556 4.897052	0.86
old*sym_sputum	.1614274	.3371643	0.383	.0026923 9.679084	-0.87
young*sym_sputum	6.194737	12.9386	0.383	.1033156 371.4325	0.87

FINAL MODEL

Model		Log Likelihood	AIC	X2	P-value	Vs
S	TB + hiv + sym_sputum + hiv*sym_sputum	ND	ND	ND	0.6104	L

As a result, "Model L" was chosen as the best model as it had the lowest AIC and a higher likelihood without running into estimation problems while containing the most statistically significant risk factors associated with TB development.

```
. logistic TB hiv sym_sputum

Logistic regression                             Number of obs   =        451
                                                LR chi2(2)      =      29.02
                                                Prob > chi2     =     0.0000
Log likelihood = -54.624763                     Pseudo R2       =     0.2099
```

TB	Odds Ratio	Std. Err.	z	P>|z|	[95% Conf.	Interval]
hiv	14.57931	8.312539	4.70	0.000	4.768877	44.57158
sym_sputum	24.47586	25.73971	3.04	0.002	3.1159	192.2616
_cons	.0126411	.0057201	-9.66	0.000	.0052073	.0306868

```
. lrtest TB TBfull, stats

Likelihood-ratio test                                 LR chi2(2)  =     29.02
(Assumption: TB nested in TBfull)                     Prob > chi2 =    0.0000

Akaike's information criterion and Bayesian information criterion
```

Model	Obs	ll(null)	ll(model)	df	AIC	BIC
TB	451	-69.13483	-69.13483	1	140.2697	144.3811
TBfull	451	-69.13483	-54.62476	3	115.2495	127.5839

```
Note:  N=Obs used in calculating BIC; see [R] BIC note
```

Equation of final model

Log (p/1-p) = b0 + b1*hiv + b2*sym_sputum

P is the probability of having TB

Log (p/1-p) = -4.370805 + 2.679604*hiv + 3.197687*sym_sputum

Interpretation of final model

Variable	Coef	Odds Ratio	Std. error	P-value	95% CI	Z-test
hiv (HIV)	2.68	14.58	8.31	0.000	[1.56; 3.80]	4.70
sym_sputum (bloody sputum symptom)	3.20	24.48	25.74	0.002	[1.14; 5.26]	3.04
TB (constant)	-4.37	.0126	.4525	0.000	[-5.26 ; -3.48]	-9.66

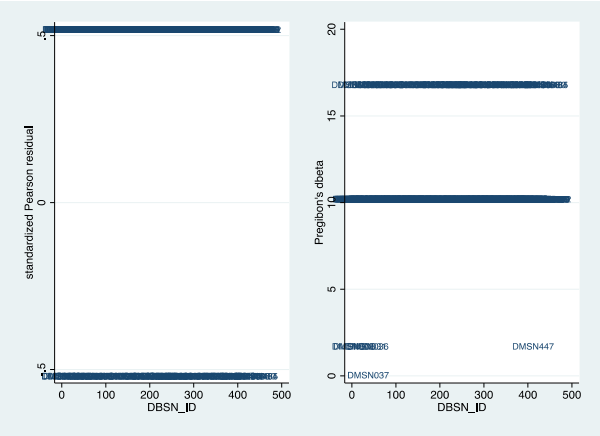
In a multivariate analysis HIV and bloody sputum symptom were strongly associated with TB among patients with DM. Holding all other variables constant, the odds of TB were 14.58 times larger for patients that were HIV positive [95% CI: 4.77 -44.57], compared to HIV negative patients. Holding all other variables constant, there was a 25.5-fold increase in odds of TB for those that reported a bloody sputum symptom [95% CI: 3.12 -192.26], compared to those that did not report the symptom.

MODEL VALIDATION

The Pearson goodness of fit test was done resulting in an X^2 of 0.27 (p= 0.6038). The p-value was large, so there was no significant difference between observed and modeled responses, therefore the model was a good approximation of the data and was considered a "good" fit.

Standardized Pearson residuals done to determine outlying observations. No outliers present as a result as residuals greater than +2 or less than -2 could not be observed. Influential observations however appeared to be present. Although DMSN037 appeared

to be influential it actually was not. This person was the only observation to be both positive for HIV and TB, and to have reported a bloody sputum symptom. DMSN037 also had an unusually small dbeta (.0579074) compared to rest of observations (10.21463) which had an unusually large dbeta. DMSN447 was an influential observation (1.718634), but this observation was unique in that the person was HIV negative, reported a bloody sputum symptom, but did not have TB.



D.3 APPENDIX 1: INFORMED CONSENT FORM (ENGLISH VERSION)

UNIVERSITY OF CAPE TOWN

ROOM S3.03

INSTITUTE OF INFECTIOUS DISEASES AND MOLECULAR MEDICINE

FACULTY OF HEALTH SCIENCES

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Epidemiology of Diabetes, TB and HIV co-infection in a high HIV/TB burden setting

INFORMED CONSENT and INFORMATION FORM

My name is _____

I wish to invite you to participate in a study that is trying to assess the interaction between tuberculosis (TB), HIV and Diabetes. This study is being run by the Institute of Infectious Diseases and Molecular Medicine at the University of Cape Town. Dr Tolullah Oni is the Principal Investigator.

Firstly, I wish to explain to you why this research is being done:

TB is a disease that is seen very commonly in Cape Town and, indeed, in most of South Africa. It is caused by bacteria that people breathe in and the infection results in cough, fevers and weight loss. The disease is more common, and can be more severe, in patients with HIV infection.

Diabetes is a disease caused by the body's inability to control sugar levels. This disease is becoming increasingly more common worldwide and in South Africa due to reduced exercise and increasing obesity. Previous studies have shown that like HIV, diabetes increases the risk of TB. But there are a few things we still do not know:

1. We do not know how much diabetes (and risk factors such as high cholesterol) there is in this setting.
2. We also do not know if the increasing levels of diabetes are contributing significantly to the TB epidemic, and
3. Although we know that HIV increases the risk of TB, we do not know if having HIV and diabetes further increases the TB risk. In other words, we do not know if a person with HIV and diabetes is at higher risk of TB than a person with HIV and no diabetes.

In addition, we know that low vitamin D levels increase the risk of TB. We also know that many people in Cape Town have very low levels of vitamin D. Therefore we also wish to measure your vitamin D level and see how this affects the risk of TB and recovery from TB in TB patients.

We request your participation in this study either because you are being investigated for TB, because you have been diagnosed with diabetes, or because you have recently undergone HIV counselling and testing (HCT).

As part of this study, we will check your blood for HIV and check your sputum (spit) for TB. We will also check you for Diabetes using the following tests:
We will ask you to come again before eating or drinking anything and check your sugar level that morning and 2 hours later after giving you a sugary drink. You must not eat or drink anything else during these 2 hours. We will also do an HbA1c test, a blood test for diabetes and check your blood cholesterol level. We will pay 30 Rand travel expenses for this purpose.

We will also take blood to measure your vitamin D level. If we diagnose HIV, we will give you a referral letter to take to any HIV clinic of your choice where baseline bloods will be done and your eligibility for antiretroviral therapy can be assessed. If TB is found, we will also give you a letter to take to your nearest TB clinic as it will be important to start TB treatment as soon as possible. If diabetes is diagnosed, we will write a referral letter for you and advise you of your nearest diabetes club where you can be started on appropriate treatment and receive regular advice on diet and

lifestyle. If high cholesterol is diagnosed, we will write a referral letter to your ARV clinic or day hospital for further management.
It is entirely up to you to decide whether or not to take part in this study. If you do decide to take part, you will be asked to sign this consent form. We will then request you to provide us with an extra blood sample (15ml) that can be taken at the same time as your normal blood tests and may perform a Chest X Ray.

Although blood testing very rarely causes problems, if anything goes wrong the University provides insurance to cover this possibility. This study will also be monitored by the Research Ethics Committee of the University of Cape Town. Their job is to ensure your safety and protect you during the study.

The decision to participate is entirely your own. IF YOU DECIDE NOT TO PARTICIPATE, YOUR TREATMENT WILL NOT BE DIASDVANTAGED IN ANY WAY. In addition, at any point during the study you are free to withdraw without telling us why.

Throughout the study your privacy will be maintained and nobody other than the doctors and nurses looking after you will know that you are participating. Samples will be labelled with code numbers and hence the laboratory staff will not know your identity. When the results of the study become available, names of the participating patients will not be included.

Do you have any questions? During the study you may contact either the **Human Research Ethics Committee** (021 406 6492) or **Dr Tolullah Oni** (021 406 6079) if you have further questions. Please remember that Dr Oni will not be directly responsible for your medical care which will be conducted by your regular doctors and nurses.

Consent to participate in the study:

I have read the above / have had the above read to me. I have had the opportunity to discuss the study

with _____ and have also had the opportunity to ask any questions. I consent to

take part in this study:

Name _____

Signature _____

Date _____

Name of Staff member consenting _____

Signature _____

Date _____

Witness (if participant is illiterate)

Name _____

Signature _____ Date _____

D.4 APPENDIX 4: DATA COLLECTION TOOL- STEPS QUESTIONNAIRE

Participant Study Number: DBSN- _ _ _

Chronic Disease Risk Factor Surveillance

Survey Information

Date		Response	Code
1	Interviewer name		I3
2	Date of consent	dd mmm yyyy	I4

Participant Study Number: DBSN - _ _ _

Consent, Interview Language and Name		Response	Code
3	Consent has been read and obtained	Yes 1 No 2 **If No, END**	I5
4	Consent Language *(interview should be done in the same language as the consent)*	Xhosa 1 English 2	I6
5	Time of consent (24 hour clock)	: hrs mins	I7
6	Participant Surname		I8
7	Participant First Name		I9
8	Sex	Male 1 Female 2	C1
9	What is your date of birth? *Don't Know 77 777 7777*	dd mmm yyyy	C2
10	Have the TB screening tool questions and vital signs been recorded	Yes 1 No 2 **If No, obtain and record on TB screening tool**	I10
Additional Information that may be helpful			
11	Contact phone number(s) *(where possible, also give a second telephone number of friend/relative)*		I11
12	Home address		I12

Location and Date	Response	Code
Appointment date for completion of steps 1-3 and TB results (48 hours – 1 week later as per clinic protocol)	dd mmm yyyy	I13

Record and file identification information (I5 to I13) with consent form but separately from the rest of the completed questionnaire.

Participant Study Number: DBSN- _ _ _

Step 1 Demographic Information **(start on page 8)**			
Date		**Response**	**Code**
13	Interviewer name		I14
14	Date	dd mmm yyyy	I15
15	Baseline TB group assignment (as per log book) *TB Case: TB diagnosed on smear/Xpert/culture PLUS TB treatment to be commenced* *TB Control: Symptoms resolved PLUS TB investigations negative to date PLUS no TB treatment commenced* ***If excluded, do not proceed***	TB case 1 TB control 2	I16

EXPANDED: Demographic Information			
16	What is the **highest level of education** you have completed?	No formal schooling 1 Started primary school but did not complete 2 Primary school completed 3 Started high school but did not complete 4 High school completed 5 College/University completed 6 Post graduate degree 7 Refused 88	C5
17	What is your **marital status**?	Never married 1 Currently married 2 Separated 3 Divorced 4 Widowed 5 Co-habiting 6 Refused 88	C7
18	Which of the following best describes your **main work** status over the past 12 months?	Government employee 1 Non-government employee 2 Self-employed 3 Non-paid 4 Student 5 Homemaker 6 Retired 7 Unemployed (able to work) 8 Unemployed (unable to work) 9 Unemployed (receiving grants) 10 Refused 88	C8
19	How many people older than 18 years, including yourself, live in your household?	Number of people	C9
20	Taking the past year, can you tell me what the average income of the household (including grants) have been? **(RECORD ONLY ONE, NOT ALL 3)**	Per week |_|_|_|_|_|_|_|_|	C10a
		OR per month |_|_|_|_|_|_|_|_|	C10b
		OR per year |_|_|_|_|_|_|_|_|	C10c
		Refused 88	C10d

Participant Study Number: DBSN- _ _ _

Step 2 Behavioural Measurements

CORE: Tobacco Use

Now I am going to ask you some questions about various health behaviours. This includes things like smoking, drinking alcohol, eating fruits and vegetables and physical activity. Let's start with tobacco.

Question		Response	Code
21	Do you currently smoke any **tobacco products**, such as cigarettes, cigars or pipes?	Yes 1 No 2 *If No, go to q26*	T1
22	Do you currently smoke tobacco products **daily**?	Yes 1 No 2 *If No, go to q26*	T2
23	How old were you when you **first started** smoking daily?	Age (years) Don't know 77 ⌊_⌋⌊_⌋ *If Known, go to q25*	T3
24	Do you remember how long ago it was? ***(RECORD ONLY 1, NOT ALL 3)*** *Don't know 77*	In Years ⌊_⌋⌊_⌋	T4a
		OR in Months ⌊_⌋⌊_⌋	T4b
		OR in Weeks ⌊_⌋⌊_⌋	T4c
25	On average, **how many** of the following do you smoke each day? ***(RECORD FOR EACH TYPE)*** *Don't Know 77*	Manufactured cigarettes ⌊_⌋⌊_⌋	T5a
		Hand-rolled cigarettes ⌊_⌋⌊_⌋	T5b
		Pipes full of tobacco ⌊_⌋⌊_⌋	T5c
		Cigars ⌊_⌋⌊_⌋	T5d
		Other ⌊_⌋⌊_⌋	T5e
		Other (please specify):	T5other

EXPANDED: Tobacco Use

Question		Response	Code
26	In the past, did you ever smoke **daily**?	Yes 1 No 2 *If no, go to q29*	T6
27	How old were you when you **stopped** smoking **daily**?	Age (years) Don't Know 77 ⌊_⌋⌊_⌋ *If known, go to q29*	T7
28	How **long ago** did you stop smoking daily? *(RECORD ONLY 1, NOT ALL 3)* *Don't Know 77*	Years ago ⌊_⌋⌊_⌋	T8a
		OR Months ago ⌊_⌋⌊_⌋	T8b
		OR Weeks ago ⌊_⌋⌊_⌋	T8c

CORE: Alcohol Consumption			
The next questions ask about the consumption of alcohol. See bottom of page for definitions.			
Question		**Response**	**Code**
29	Have you ever consumed an alcoholic drink such as beer, wine, spirits, cider?	Yes 1 No 2 *If No, go to q37*	A1a
30	Have you consumed an alcoholic drink within the **past 12 months**?	Yes 1 No 2 *If No, go to q37*	A1b
31	During the past 12 months, **how frequently** have you had at least one alcoholic drink?	Daily 1 5-6 days per week 2 1-4 days per week 3 1-3 days per month 4 Less than once a month 5	A2
32	Have you consumed an alcoholic drink within the **past 30 days**?	Yes 1 No 2 *If No, go to q37*	A3
33	During the past 30 days, on how many **occasions** did you have at least one alcoholic drink?	Number Don't know 77 __|__	A4
34	During the past 30 days, when you drank alcohol, **on average**, how many **standard alcoholic drinks** did you have during one drinking occasion?	Number Don't know 77 __|__	A5
35	During the past 30 days, what was the **largest number** of standard alcoholic drinks you had on a single occasion, counting all types of alcoholic drinks together?	Largest number Don't Know 77 __|__	A6
36	During the past 30 days, how many times did you have for **men**: **five or more** for **women**: **four or more** standard alcoholic drinks in a single drinking occasion?	Number of times Don't Know 77 __|__	A7

Alcoholic drink definitions
1 drink = 1 glass of wine OR 1 small bottle of beer /cider OR 1 tot of spirit

1 litre bottle of wine = 5 drinks
1 litre bottle of spirits (e.g. vodka, whisky, brandy, gin) = 12 drinks
1 large bottle of beer or cider = 2 drinks

CORE: Physical Activity

Next I am going to ask you about the time you spend doing different types of physical activity in a typical week. Please answer these questions even if you do not consider yourself to be a physically active person.
Think first about the time you spend doing work. Think of work as the things that you have to do such as paid or unpaid work, study/training, household chores, harvesting food/crops, fishing or hunting for food, seeking employment. In answering the following questions 'vigorous-intensity activities' are activities that require hard physical effort and cause large increases in breathing or heart rate, 'moderate-intensity activities' are activities that require moderate physical effort and cause small increases in breathing or heart rate.

Question		Response	Code
Work			
43	Does your work involve vigorous-intensity activity that causes large increases in breathing or heart rate like *carrying or lifting heavy loads, digging or construction work* for at least 10 minutes continuously?	Yes 1 No 2 *If No, go to q46*	P1
44	In a typical week, on how many days do you do vigorous-intensity activities as part of your work?	Number of days ⌊_⌋⌊_⌋	P2
45	How much time do you spend doing vigorous-intensity activities at work on a typical day?	Hours : minutes ⌊_⌋⌊_⌋ : ⌊_⌋⌊_⌋ hrs mins	P3 (a-b)
46	Does your work involve moderate-intensity activity that causes small increases in breathing or heart rate such as *brisk walking or carrying light loads* for at least 10 minutes continuously?	Yes 1 No 2 *If No, go to q49*	P4
47	In a typical week, on how many days do you do moderate-intensity activities as part of your work?	Number of days	P5
48	How much time do you spend doing moderate-intensity activities at work on a typical day?	Hours : minutes ⌊_⌋⌊_⌋ : ⌊_⌋⌊_⌋ hrs mins	P6 (a-b)
Travel to and from places			
The next questions exclude the physical activities at work that you have already mentioned. Now I would like to ask you about the usual way you travel to and from places. For example to work, for shopping, to market, to place of worship.			
49	Do you walk or use a bicycle for at least 10 minutes continuously to get to and from places?	Yes 1 No 2 *If No, go to q52*	P7
50	In a typical week, on how many days do you walk or bicycle for at least 10 minutes continuously to get to and from places?	Number of days	P8
51	How much time do you spend walking or bicycling for travel on a typical day?	Hours : minutes ⌊_⌋⌊_⌋ : ⌊_⌋⌊_⌋ hrs mins	P9 (a-b)

Participant Study Number: DBSN- _ _ _

CORE: Diet			
The next questions ask about the fruits and vegetables that you usually eat. As you answer these questions please think of a typical week in the last year.			
Question		**Response**	**Code**
37	In a typical week, on how many days do you **eat fruit**?	Number of days Don't Know 77 ⌊_⌋⌊_⌋ *If Zero days, go to q39*	D1
38	How many **servings** of fruit do you eat on **one** of those days?	Number of servings Don't Know 77 ⌊_⌋⌊_⌋	D2
39	In a typical week, on how many days do you **eat vegetables**?	Number of days Don't Know 77 ⌊_⌋⌊_⌋ *If Zero days, go to q41*	D3
40	How many **servings** of vegetables do you eat on one of those days?	Number of servings Don't know 77 ⌊_⌋⌊_⌋	D4

EXPANDED: Diet			
41	What type of **oil or fat is most often** used for meal preparation in your household?	Vegetable / fish / olive oil 1 Animal fats 2 Butter or ghee 3 Margarine 4 Other 5 *If Other, go to D5other* None in particular 6 None used 7 Don't know 77	D5
		Other	D5other
42	On average, how many meals per week do you eat that were not prepared at home? By meal, I mean breakfast, lunch and dinner.	Number Don't know 77 ⌊_⌋⌊_⌋	D6

Participant Study Number: DBSN- _ _ _

CORE: Physical Activity, Continued			
Question		**Response**	**Code**
Recreational activities			
The next questions exclude the work and transport activities that you have already mentioned. Now I would like to ask you about sports, fitness and recreational activities (leisure).			
52	Do you do any vigorous-intensity sports, fitness or recreational *(leisure)* activities that cause large increases in breathing or heart rate like *running or football* for at least 10 minutes continuously?	Yes 1 No 2 *If No, go to q55*	P10
53	In a typical week, on how many days do you do vigorous-intensity sports, fitness or recreational *(leisure)* activities?	Number of days __|__	P11
54	How much time do you spend doing vigorous-intensity sports, fitness or recreational activities on a typical day?	Hours : minutes __ : __ hrs mins	P12 (a-b)
55	Do you do any moderate-intensity sports, fitness or recreational *(leisure)* activities that cause a small increase in breathing or heart rate such as *brisk walking, cycling, swimming, softball* for at least 10 minutes continuously?	Yes 1 No 2 *If No, go to q58*	P13
56	In a typical week, on how many days do you do moderate-intensity sports, fitness or recreational *(leisure)* activities?	Number of days __|__	P14
57	How much time do you spend doing moderate-intensity sports, fitness or recreational *(leisure)* activities on a typical day?	Hours : minutes __ : __ hrs mins	P15 (a-b)

Sedentary behaviour			
The following question is about sitting or reclining at work, at home, getting to and from places, or with friends including time spent sitting at a desk, sitting with friends, traveling in car, bus, train, reading, playing cards or watching television, but do not include time spent sleeping.			
58	How much time do you usually spend sitting or reclining on a typical day?	Hours : minutes __ : __ hrs mins	P16 (a-b)

ANNEXURE: To be completed by staff member

DIABETES HISTORY			
Question		**Response**	**Code**
59	Any family history of diabetes?	Yes 1 No 2	R1
60	Have you ever had diabetes during pregnancy (gestational diabetes)?	Yes 1 No 2	R2

Participant Study Number: DBSN- _ _ _ _

Step 3 Biochemical Measurements

CORE: Blood Glucose and HbA1c			
Question		Response	Code
61	Blood pressure	_ _ _ /_ _ _	J1
62	During the past 8-12 hours have you had anything to eat or drink, other than water? *If yes, complete STEPS 1 and 2, and re-schedule appointment for blood tests:* *Date:* *dd mmm yyyy*	Yes 1 No 2	B1
63	Fasting blood glucose and HbA1c	Hours : minutes L_I_I_ L_I_I_ Fasting glucose mmol/l L_I_I_I_ L_I_ HbA1c % L_I_I_ L_I_I_	B5
64	Oral glucose tolerance test	Hours : minutes L_I_I_ L_I_I_ 2 hour glucose mmol/l L_I_I_I_ L_I_	B6
CORE: Blood Lipids			
65	Total cholesterol	mmol/l L_I_I_ L_I_I_	B8

EXPANDED: Triglycerides and HDL Cholesterol			
66	Triglycerides	mmol/l L_I_I_ L_I_I_	B10
67	HDL Cholesterol	mmol/l L_I_ L_I_I_	B11

Return Date		Response	Code
68	Appointment date for diabetes and cholesterol results (1 week)	dd mmm yyyy	B12
69	Appointment date for completion of step 4 (2 months)	dd mmm yyyy	B13

Participant Study Number: DBSN- _ _ _

Step 4 Repeat Biochemical Measurements

Date		Response	Code
70	Interviewer name		I17
71	Date	dd mmm yyyy	I18

RECENT DIABETES AND TUBERCULOSIS HISTORY			
Are you currently receiving any of the following treatments/advice for diabetes prescribed by a doctor or other health worker?			
72	Diabetes pills or insulin	Yes 1 No 2	H8a
Are you currently receiving treatment for tuberculosis?			
73	TB medication	Yes 1 No 2	H8b
Are TB culture results (from baseline TB screen) available?			
74	TB culture results	Positive 1 Negative 2 Pending 3 Not done 4	H8c
Have presenting TB symptoms resolved?			
75	TB symptoms resolved	Yes 1 No 2	H8d
FINAL TB GROUP ASSIGNMENT			
76	Final Study group assignment	TB case 1 TB control 2 Excluded 3	I19

CORE: HbA1c			
Question		**Response**	**Code**
77	Blood pressure	_ _ _ / _ _ _	J2
78	During the past 8-12 hours have you had anything to eat or drink, other than water? *If yes, do not proceed. Re-schedule appointment.* *Date:* *dd mmm yyyy*	Yes 1 No 2	B14
79	HbA1c *Participant to be recalled for results only if new diabetes diagnosis made*	Hours : minutes ⌊_⌋⌊_⌋ . ⌊_⌋⌊_⌋ HbA1c % ⌊_⌋⌊_⌋⌊_⌋ . ⌊_⌋	B15

D.5 APPENDIX 5: DATA COLLECTION TOOL-- TB SUSPECT

Ref: CT/TB 6/13 version 6

TB SUSPECT SCREENING TOOL

For TB-suspects, contacts, prophylaxis in HIV. To be used as part of PALSA Plus based screening.

History

(This section can be completed by administrative support staff)

PATIENT PERSONAL DETAILS (add patient sticker)	Name	Folder number
	Surname	Clinic
	Address	Date of Birth
		Contact No

TB HISTORY	Previous TB	Y	N	Number of previous TB episodes		Year of last episode		Number of months on TB treatment at last episode		
CHRONIC DISEASE HISTORY AND CURRENT MEDICATION	HIV	Y	N	Antiretroviral therapy	Y	N	Depression / Anxiety	Y	N	
	Diabetes	Y	N	Diabetes Meds	Y	N	Depression / Anxiety Meds	Y	N	
	High blood pressure	Y	N	Blood pressure Meds	Y	N	Epilepsy	Y	N	
	High cholesterol	Y	N	Cholesterol Meds	Y	N	Epilepsy Meds	Y	N	
	Asthma / COPD	Y	N	Asthma / COPD Meds	Y	N				

HISTORY OF TB CONTACT	Known contact with confirmed TB patient		Y	N				
	MDR/XDR contact		Y	N	Name		Clinic	

EXPOSURE RISK	Health worker		Y	N	Mines / Quarry / Sandblasting…		Y	N
	Prisoner		Y	N	Other		Y	N
RISK FACTORS	Current cigarette smoker		Y	N	Previous cigarette smoker		Y	N
	Number smoked per day			per day	Number smoked per day			per day
	Number of years smoking			years	Number of alcoholic drinks consumed **in the past week**			

TB SYMPTOMS	**Adults**			**Children < 8 years**		
	Cough > 2 weeks	Y	N	Cough/wheeze > 2 weeks	Y	N
	Fever ≥ 2 weeks	Y	N	Fever ≥ 2 weeks	Y	N
	Weight loss	Y	N	Weight loss	Y	N
	Fatigue	Y	N	Fatigue (child does not play)	Y	N
	Blood stained sputum	Y	N	Not gaining weight (failure to thrive)	Y	N
	Drenching night sweats	Y	N			
	Chest pain on breathing	Y	N			

DIABETES SYMPTOMS	Polyuria	Y	N	Nocturia	Y	N	Polydipsia (excessive thirst)	Y	N

Action

(This section to be completed by Clinician (PN and /or MO))

HCT	HIV	Pos	Neg	Refused	CD4 result		ART start date	

OBSERVATIONS	Weight		Kg	Height		cm
	Temperature		C	Waist circumferenoe		cm
	Respiratory rate		/min	Neck stiffness	Y	N
	BP		mmHg	Visible masses neck/axilla/groin	Y	N
	Pulse		/min	Failure to thrive (check growth curve in RTH Card)	Y	N

TB SKIN TEST	Date performed:		Date read:		Result		mm

BACTERIOLOGY	Type of specimen Eg sputum, aspirate etc	Type of test	Date	Lab no	Result	Drug Sensitivity Testing Rif (S/R)	INH (S/R)
	1.	Xpert / Direct / Culture					
	2.	Xpert / Direct / Culture					
	3.						
	4.						
ANTIBIOTIC PRESCRIPTION	Name of antibiotic		Date antibiotic prescribed:				

TB DM study	Referred to study	Y	N	Consent given	Y	N	Study number	DBSN	___ ___
NAME & SIGNATURE (PN/MO)				Today's visit date:				Follow up date:	

D.6 APPENDIX 6: WESTERN CAPE DEPARTMENT OF HEALTH-PROVINCIAL LETTER OF APPROVAL

Western Cape Government
Health

STRATEGY & HEALTH SUPPORT
healthres@pgwc.gov.za
tel: +27 21 483 9907; fax: +27 21 483 9895
1st Floor, Norton Rose House., 8 Riebeek Street, Cape Town, 8001
www.capegateway.gov.za

REFERENCE: RP 116/2011
ENQUIRIES: Dr V Appiah-Baiden

Room 3.03 Wolfson Pavillion
Institute of Infectious Disease and Molecular Medicine
Faculty of Health Sciences,
University of Cape Town
Observatory
7925

For attention: Dr Tolu Oni, Professor Robert Wilkinson

Re: Epidemiology of Diabetes, TB and HIV co-infection in a high HIV/TB burden setting

Thank you for submitting your proposal to undertake the above-mentioned study. We are pleased to inform you that the department has granted you approval for your research.
Please contact the following people to assist you with any further enquiries.

Khayelitsha Site B **Ms Notshe** **(021) 361 4835**

Kindly ensure that the following are adhered to:

1. Arrangements can be made with managers, providing that normal activities at requested facilities are not interrupted.
2. Researchers, in accessing provincial health facilities, are expressing consent to provide the department with an electronic copy of the final report within six months of completion of research. This can be submitted to the provincial Research Co-ordinator (healthres@pgwc.gov.za).
3. The reference number above should be quoted in all future correspondence.

We look forward to hearing from you.

Yours sincerely

Signed

DR T NALEDI
DIRECTOR: HEALTH IMPACT ASSESSMENT
DATE: 07/03/2012

CC **DR G PEREZ** **DIRECTOR: EASTERN/KHAYELITSHA**

D.7 APPENDIX 7: PROTOCOL SUMMARY OF PARENT STUDY

OVERAL RESEARCH OBJECTIVE

The main aim of this study is to investigate the incremental yield and best screening algorithms for diabetes mellitus (DM) and tuberculosis (TB) among a) newly diagnosed TB patients and b) patients attending diabetic clinics, respectively.

Specific aims:

- To assess the prevalence of DM among Cases
- To evaluate the best performing algorithms to diagnose DM in Cases
- To measure the prevalence of TB among diabetes patients

BACKGROUND

TB remains a leading cause of death globally, with an estimated 8.8 million new cases reported every year, threatening the goal of global TB elimination by year 2050 (1). Tackling this challenge will require not only improvements in diagnostic and treatment services, but identification and reduction of risk factors that increase

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Specific aims:

- To assess the prevalence of DM among TB cases
- To evaluate the best performing algorithms to diagnose DM in TB Cases
- To measure the prevalence of TB among diabetes patients

BACKGROUND

TB remains a leading cause of death globally, with an estimated 8.8 million new cases reported every year, threatening the goal of global TB elimination by year 2050 (1). Tackling this challenge will require not only improvements in diagnostic and treatment services, but identification and reduction of risk factors that increase susceptibility for TB. Medical conditions that impair immune function, such as malnutrition, alcoholism or HIV co-susceptibility for TB. Medical conditions that impair immune function, such as malnutrition, alcoholism or HIV co-infection, can increase the likelihood of infection or reactivation of latent TB. Increasing evidence suggests that DM is also a significant risk factor for TB. In a recent systematic review, the relative risk for TB in diabetic patients was 3.1 (2). The strength of this link was influenced by geographic/ethnic differences, and

young people were at particularly high risk; in India, DM is thought to be associated with nearly 15% of pulmonary Cases (3). However, these studies had a number of limitations. In particular, very few were carried out in low-income countries, with none in Africa, raising uncertainty about the strength of DM-TB association and benefit of bi-directional screening for DM and TB in these settings with high TB/HIV prevalence and AN increasing burden of DM. Practical guidance on when to suspect, and how best to diagnose, diabetes in TB patients, and how to confirm or exclude it are lacking. The World Health Organisation recommends HbA1c as a diagnostic test for diabetes with a cut-off value of 6.5% (4). However, less clear is the diagnostic value of results below the WHO cut-off (there is an argument for population-specific cut-off values). A study conducted on a population of mixed-ancestry in Cape Town showed that this cut-off value was sub-optimal (erasmus). Furthermore, it is not known how the diagnostic performance of the cut-off is affected by acute illness, such as TB. A point of care HbA1c test could make diabetes screening more effective and potentially more affordable. A study comparing POC devices found that only Afinion and DCA Vantage met the diagnostic performance criteria (6). There are also insufficient data on which to base TB screening guidelines for diabetic patients.

The growing epidemic of diabetes as a threat to TB control

While 95% of patients with TB live in the low- and middle-income countries, 70% of patients with DM also live in these same countries. It is estimated that, worldwide in 2010, there were 285 million people with DM, and the number predicted to rise to 438 million by 2030, making DM one of the most common NCDs globally (7). The majority of this increase will occur in developing countries where TB remains endemic. Diabetes on this scale is likely to pose another threat for global TB control. The potential public health and clinical importance of this seems to have been largely ignored until recently. Recent joint consultations by International Union Against Tuberculosis and Lung Disease, the World Diabetes Foundation and the World Health Organization emphasised the urgent need for research in this area (10). There is also a paucity of data on whether DM is associated with a high prevalence of subclinical TB among patients attending diabetic clinics, as observed in HIV-1 co-infected persons (13). This has major importance when considering TB screening strategies, with potential for amplification by transmission within diabetic clinics and also potentially serious consequences of false-negative TB screening. Research is therefore required to develop appropriate and evidence-based guidelines for TB screening among persons with DM.

Diabetes, HIV and TB in South Africa and the Western Cape

South Africa is among the 22 high TB burden countries globally, and also has the highest urban: rural ratio in sub-Saharan Africa, with 62% of the population being urban dwellers (17). Urbanisation, in addition to rapid epidemiological and demographic transition has resulted in a rising burden of NCDs. The global burden of disease study demonstrated that in Southern Africa, while HIV and TB rank first and 4th in the top ten causes of morbidity, 50% of the causes of morbidity are non-communicable; diabetes is ranked 7th. A community survey conducted in 2008/9 from Cape Town townships showed a DM prevalence of 13.1% (95% CI: 11.0-15.1) (26).

Mortality rates from NCDs are also increasing; diabetes was the 6th leading causes of mortality in South Africa in 2010. In the Western Cape, the province within which Cape Town is located, TB and DM are the top 2 causes of mortality. Against this background, the prevalence of HIV, the strongest known risk factor for TB, remains high, with 70% of TB cases being HIV-infected in Khayelitsha

PLAN OF INVESTIGATION

Setting and study population

The study will be conducted in Khayelitsha, reputed to be the fastest growing township in South Africa. Khayelitsha is located 30km from Cape Town with a population of over 500,000 predominantly black Africans, HIV antenatal prevalence of 28% and a TB case-notification rate of >1600/100,000 (27). The largest TB clinic in Khayelitsha is Ubuntu clinic. A survey by the Department of Health reported 1187 newly registered TB cases were diagnosed at Ubuntu clinic in 2011.

Study design

DM Screen in TB patients

We will recruit consecutive TB cases at Ubuntu clinic, prior to initiation of TB treatment. We will record demographic, medical and drug history, HIV status and (if positive) date of initiation of ART, regimen, and CD4 count, as well as clinical, microscopic and radiological characteristics of TB at diagnosis. In addition we will document other risk factors for diabetes, such as family history, body mass index (BMI), and waist circumference. Before starting TB chemotherapy blood samples will be collected to measure fasting blood glucose and oral glucose tolerance test, and HbA1c performed using Afinion, a validated HbA1c Point of care test. Patients will be considered to be diabetic if 1) the diagnosis is known at presentation, 2) they are taking oral hypoglycaemic therapy and/or insulin or 3) as per case definitions. Previous studies have suggested that it may be more reliable to screen for diabetes later in the course of TB treatment rather than at the start (11), because inflammation/cytokine stimulation associated with active TB may elevate blood glucose levels resulting in false positive diabetes diagnoses if tests are performed too early. We will, therefore, repeat fasting blood glucose and HbA1c tests after 2 months of TB treatment. Patients with a new diagnosis will be referred to the DM clinic for standard care. HIV testing is offered routinely to all patients with TB.

TB screen in diabetic patients

We will recruit consecutive diabetes patients presenting to the primary care diabetes clinic at Khayelitsha day hospital. A TB screen will be performed as per case definition. We will also record demographic, medical and drug history, previous TB and TB contact history, as well as HbA1c to measure glycaemic control at the time of recruitment.

Diabetes screen in TB patients *(Baseline and 2 months after TB diagnosis)*	• Diabetes symptoms (nocturia, polyuria, polydipsia) • Random glucose measures on finger prick sample • Fasting glucose • Oral glucose tolerance test • HbA1c • Urine dipstick
TB screen in diabetics	• TB symptoms (cough, duration of cough, night sweats, fever, loss of weight or appetite, haemoptysis, pleuritic chest pain) • Spontaneous (or induced if necessary) sputum sent for smear microscopy and culture • Chest radiography • IGRA / TST
Definition of TB disease	• Smear microscopy positive for acid-fast bacilli • Isolation/identification of *Mycobacterium tuberculosis* by culture or Xpert MTB/RIF • In addition to these definitions, an exploratory endpoint will include patients without a definitive TB diagnosis but with compatible clinical symptoms with symptom resolution after TB therapy
Subclinical TB	• TB culture positivity in absence of the currently recommended TB symptom screen (cough of any duration, fever, weight loss, night sweats)
Diabetes / Impaired glucose tolerance (IGT)	• -Fasting plasma glucose 7.0mmol/l (IGT 5.6-6.9 mmol/l) • -Glucose tolerance test >=11.1mmol/l (IGT 7.8-11.0 mmol/l) • -HbA1c >6.5% (IGT 5.7-6.4%)

Table 1: Case definitions

Subclinical TB disease Smear microscopy positive for acid fast bacilli
Isolation

Outcome measures

Primary outcome

- The best performing screening algorithm to diagnose DM in TB patients

Secondary outcomes

- Accuracy of established intensified TB case-finding strategies among diabetics
- Prevalence of diabetes in TB patients
- Prevalence of TB in diabetics, including subclinical TB disease
- Effect of diabetes on TB clinical presentation (number and duration of pulmonary TB symptoms, TB sputum smear and microscopy results, CXR findings, non-pulmonary manifestation of disease) and/or treatment outcomes of TB (2 month TB sputum clearance, completion and cure rates)

Sample size and power calculations

Assuming a DM prevalence of 24% in TB patients (based on a TB/DM risk ratio of 2 and DM prevalence in the general population of 12%), in order to have 80% statistical power and a screening tool sensitivity of 60%, a sample size of 1537 TB cases is required in Malawi and South Africa respectively. This sample size will provide sufficient power to define and investigate different potential DM screening algorithms in TB patients.

For the TB prevalence in DM patients study, a sample size of 457 diabetic patients is required, assuming TB prevalence of 5% and at 2% precision.

Statistical analysis plan

The prevalence of diabetes and TB will be based on the respective gold standard diagnostic tests. We will calculate the sensitivity, specificity, positive and negative predictive values of the screening tools to diagnose

DM in TB cases, and TB in DM patients, using OGTT and TB culture gold standards, respectively. Among TB cases, risk factors associated with DM will be analysed using logistic regression. The model will be built manually with nested models compared using the likelihood ratio test. The Akaike's Information Criterion (AIC) will be used to compare non-nested models with a significantly lower AIC (>10%) indicating an improved model. In addition, outlying and influential observations will be identified and potential effect modification assessed using interaction variables.

To develop and evaluate a DM screening algorithm in TB cases, based on symptom screening, HIV status, ART, age, sex, BMI, waist circumference, and DM family history. Multivariable logistic regression analysis will then be performed to develop diagnostic models for DM using OGTT findings as the gold standard. A reduced clinical model without additional investigations will first be derived. These tests will then be added singly to the reduced clinical model and then simultaneously to explore the added predictive value of a single test and of combined tests, respectively. The ability of a multivariable model to discriminate persons with DM from those without will be assessed using Receiver Operator Characteristic curve and Area Under the Curve analysis. Significance testing will be done using a combination of two-sided p-values ($p<0.05$) and 95% confidence intervals. All data will be analysed using STATA 12.0 (StataCorp, College Station, TX, USA)

Ethical considerations

This study has received ethics approval from the University of Cape Town Human Ethics committee (HREC Ref: 403/2011). A written consent will be obtained from all participants who will be given detailed explanation of the study. An information sheet will be available both in English and Xhosa.

Study timeline

Task	Dec 2013	2014				2015			
Preparatory phase • Staff recruitment & training • Questionnaire development									
Study recruitment and follow-up • Identification and assessment of participants • Sample collection • Follow-up of participants									
Sample and Data analysis									
Report writing • Preparation for presentation and publication • Submission of report									

Anticipated overall outputs and impacts

This study performed at such an important time, with respect to the emerging diabetes epidemic in sub-Saharan Africa, will provide an invaluable set of data to document the strength of association between diabetes and TB and its clinical/public health impact. Data of this quality does not exist in the current literature on the link between TB and diabetes in sub-Sahara Africa; hence we expect this study to produce a series of high quality publications. Additionally, the study is designed to raise further research questions and provide some of the ground work needed, for example to determine 1) optimal screening models 2) whether diabetes influences recurrence of TB or anti-tuberculosis drug resistance 3) the need to develop randomised controlled trials of efficacy of interventions, such as TB chemoprophylaxis in people with diabetes, to improve outcomes of both diseases.

Contingency plans

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D.8 APPENDIX 8- ETHICS COMMITTEE APPROVAL FOR THIS STUDY

UNIVERSITY OF CAPE TOWN
Faculty of Health Sciences
Human Research Ethics Committee

Room E52-24 Old Main Building
Groote Schuur Hospital
Observatory 7925
Telephone [021] 406 6338 • **Facsimile** [021] 406 6411
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Website: www.health.uct.ac.za/fhs/research/humanethics/forms

23 July 2015

HREC REF: 377/2015

Dr T Oni
Public Health & Family Medicine
Room 2.24, Entrance 5
Falmouth Building

Dear Dr Oni

PROJECT TITLE: THE PREVALENCE AND DETERMINANTS OF ACTIVE TUBERCULOSIS AMONG DIABETES PATIENTS ATTENDING A PRIMARY HEALTH CARE CLINIC IN CAPE TOWN, SOUTH AFRICA (Masters Candidate-A Okorie) Sub-study linked to 403/2011

Thank you for your response to the Faculty of Health Sciences Human Research Ethics Committee dated 22 July 2015.

It is a pleasure to inform you that the HREC has **formally approved** the above-mentioned study.

Approval is granted for one year until the 30th July 2016.

Please submit a progress form, using the standardised Annual Report Form if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.
(Forms can be found on our website: www.health.uct.ac.za/fhs/research/humanethics/forms)

Please quote the HREC REF in all your correspondence.

We acknowledge that the student, Adaeze Okorie will also be involved in this study.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Yours sincerely

Signed
PROFESSOR M BLOCKMAN
CHAIRPERSON, FHS HUMAN RESEARCH ETHICS COMMITTEE
Federal Wide Assurance Number: FWA00001637.
Institutional Review Board (IRB) number: IRB00001938
This serves to confirm that the University of Cape Town Human Research Ethics Committee complies to the Ethics Standards for Clinical Research with a new drug in patients, based on the Medical Research Council (MRC-SA), Food and Drug Administration (FDA-USA), International Convention on Harmonisation Good Clinical Practice (ICH GCP), South African Good Clinical Practice Guidelines (DoH

D.9 APPENDIX 9 –INSTRUCTIONS FOR AUTHORS FROM THE LANCET DIABETES & ENDOCRINOLOGY

Types of article and manuscript requirements

Please ensure that anything you submit to The Lancet Diabetes & Endocrinology follows the guidelines provided for each article type. For instruction on how to format the text of your paper, including tables, figures, panels, and references, please see our Formatting guidelines.

Red section (Articles)

Articles

- From Jan 1, 2015, all research papers (apart from meta-analyses) submitted to any journal in *The Lancet* family must include a panel putting their research into context with previous work, with an enhanced structure and subheadings compared with papers submitted before this date (see Lancet 2014; 384: 2176–77, and panel below for guidance). Editors will use this information at the first assessment stage and peer reviewers will be specifically asked to check the content and accuracy

- The Lancet Diabetes & Endocrinology prioritises reports of original research that are likely to change clinical practice or thinking
- We invite submission of all clinical trials, whether phase 1, 2, 3, or 4. For phase 1 trials, we consider those of a novel substance for a novel indication, if there is a strong or unexpected beneficial or adverse response, or a novel mechanism of action

- We require the registration of all interventional trials, whether early or late phase, in a primary register that participates in WHO's International Clinical Trial Registry Platform (see Lancet 2007; 369: 1909–11). We also encourage full public disclosure of the minimum 20-item trial registration dataset at the time of registration and before recruitment of the first participant (see Lancet 2006; 367: 1631–35). The registry must be independent of for-profit interest
- Reports of trials must conform to CONSORT 2010 guidelines and should be submitted with their protocols
- All reports of randomised trials should include a section entitled Randomisation and masking, within the Methods section. Please refer to *The Lancet*'s formatting guidelines for randomised trials
- Cluster-randomised trials must be reported according to CONSORT extended guidelines
- Randomised trials that report harms must be described according to extended CONSORT guidelines
- Studies of diagnostic accuracy must be reported according to STARD guidelines
- Observational studies (cohort, case-control, or cross-sectional designs) must be reported according to the STROBE statement, and should be submitted with their protocols
- We encourage the registration of all observational studies on a WHO-compliant registry (see Lancet 2010; 375: 348)
- Genetic association studies must be reported according to STREGA guidelines
- Systematic reviews and meta-analyses must be reported according to PRISMA guidelines
- To find reporting guidelines see: http://www.equator-network.org

All Articles should, as relevant:

- Be up to 3000 words (4500 for randomised controlled trials) with 30 references (the word count is for the manuscript text only)
- Include an abstract (semistructured summary), with five paragraphs (Background, Methods, Findings, Interpretation, and Funding), not exceeding 250 words. Our electronic submission system will ask you to copy and paste this section at the "Submit Abstract" stage
- For randomised trials, the abstract should adhere to CONSORT extensions: abstracts (see Lancet 2008; 371: 281–83)
- For intervention studies, the abstract should include the primary outcome expressed as the difference between groups with a confidence interval on that difference (absolute differences are more useful than relative ones). Important secondary outcomes can be included as long as they are clearly marked as secondary
- Use the SI system of units and the recommended international non-proprietary name (rINN) for drug names. Ensure that the dose, route, and frequency of administration of any drug you mention are correct
- Use gene names approved by the Human Gene Organisation. Novel gene sequences should be deposited in a public database (GenBank, EMBL, or DDBJ), and the accession number provided. Authors of microarray papers should include in their submission the information recommended by the MIAME guidelines. Authors should also submit their experimental details to one of the publicly available databases: ArrayExpress or GEO
- Include any necessary additional data as part of your EES submission
- All accepted Articles should include a link to the full study protocol published on the authors' institutional website (see Lancet 2009; 373: 992 and Lancet 2010; 375: 348)

Putting research into context

- From Jan 1, 2015, all research papers submitted to any journal in *The Lancet* family must include a panel putting their research into context with previous work, with an enhanced structure and subheadings compared with papers submitted before this date (see *Lancet* 2014; 384: 2176–77, and panel below for guidance). This panel should not contain references. Editors will use this information at the first assessment stage and peer reviewers will be specifically asked to check the content and accuracy
- The Discussion section should contain a full description and discussion of the context. Authors are also invited to either report their own, up-to-date systematic review or cite a recent systematic review of other trials, putting their trial into context of the review

Research in context

Evidence before this study

This section should include a description of all the evidence that the authors considered before undertaking this study. Authors should briefly state: the sources (databases, journal or book reference lists, etc) searched; the criteria used to include or exclude studies (including the exact start and end dates of the search), which should not be limited to English language publications; the search terms used; the quality (risk of bias) of that evidence; and the pooled estimate derived from meta-analysis of the evidence, if appropriate.

Added value of this study

Authors should describe here how their findings add value to the existing evidence.

Implications of all the available evidence

Authors should state the implications for practice or policy and future research of their study combined with existing evidence.

Research in context panels should not contain references; key studies mentioned here should be referenced in the main text.