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The impact of diabetes and pre-diabetes on prevalence of *M. tuberculosis* infection among household contacts of active tuberculosis cases in Ethiopia

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The impact of diabetes and pre-diabetes on prevalence of *M. tuberculosis* infection among household contacts of active tuberculosis cases in Ethiopia

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Abstract

The impact of diabetes and pre-diabetes on prevalence of *M. tuberculosis* infection among household contacts of active tuberculosis cases in Ethiopia

By Alison Grace Carswell Smith

Background: Whether diabetes affects the risk of developing latent tuberculosis (TB) infection following exposure to *Mycobacterium tuberculosis* is incompletely understood. We assessed the relationship of diabetes or pre-diabetes and latent TB infection among the close and household contacts (HHCs) of patients with active pulmonary TB disease in Addis Ababa, Ethiopia.

Methods: In this cross-sectional study we performed interferon-γ release assays, TB symptom screening, and point-of-care HbA1c testing among HHCs of active TB cases. Diabetes status was classified into diabetes (HbA1c ≥6.5% or self-reported diagnosis), pre-diabetes (5.7–6.4%), and euglycemia (≤5.6%). Multivariate logistic regression was used to determine the association of pre-diabetes and diabetes with latent TB infection.

Results: Among 597 study participants, 123 (20.6%) had diabetes (n=31) or pre-diabetes (n=92), and 423 (70.9%) were diagnosed with latent TB infection. Twelve (38.7%) of 31 HHCs with diabetes were previously undiagnosed. The prevalence of latent TB infection in HHCs with diabetes, pre-diabetes and euglycemia was 27/31 [87.1%], 67/92 [72.8%], and 329/474 [69.4%], respectively. Prevalence of latent TB infection was significantly higher among HHCs with diabetes compared to euglycemia (prevalence difference 17.7% [95% CI 5.2-30.2%], odds ratio 2.97 [95% CI 1.14-10.2]). In multivariable analysis, the increased risk of latent TB infection among HHCs with diabetes did not reach statistical significance (adjusted odds ratio 1.60, 95% CI 0.56-5.79).

Conclusion: We found high rates of latent TB infection among HHCs of active TB cases in Addis Ababa, and HHCs with diabetes were significantly more likely to have latent TB infection than those with euglycemia. Further investigation is needed to assess mechanisms by which DM may increase risk for latent TB infection after *M. tuberculosis* exposure.

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INTRODUCTION

Tuberculosis (TB) is an enormous global public health problem and was the leading cause of infectious disease mortality worldwide prior to the COVID-19 pandemic, with an estimated 10 million incident cases of TB and 1.5 million deaths due to TB in 2020.[1] The global prevalence of latent tuberculosis infection (LTBI) is estimated to be approximately 25%,[2] and an improved understanding of the risk factors associated with LTBI is fundamental to improving global TB control.[3] An association between diabetes mellitus (DM) and active TB disease has long been recognized.[4-7] DM increases the risk of active TB disease by approximately three-fold,[5] and an estimated 15-25% of active TB cases worldwide are attributable to DM.[8,9] The prevalence of DM is increasing rapidly in sub-Saharan Africa and other low- and middle-income countries (LMICs),[10] regions which also bear the highest burden of TB disease. The expanding epidemic of DM in LMICs is one of several challenges to meeting the goals of the End TB Strategy,[1,8,11,12] making improved understanding of the relationship between LTBI and DM a global health research priority.

While emerging data has provided insights into the relationship between DM and active TB, much less is understood about the association between dysglycemia and LTBI. Importantly, it is not known whether the presence of dysglycemia (DM or pre-DM) increases the risk of *Mycobacterium tuberculosis (Mtb)* infection and development of LTBI after exposure. Additionally, there are substantial gaps in knowledge of whether DM and pre-DM may impair an effective immune response to *Mtb*.[13,14] Although IFN-γ release assays (IGRAs) have become commonly used diagnostic tests to assess LTBI prevalence, the relationship between HbA1c and

the magnitude of the T cell IFN-γ release in IGRA assays is incompletely understood in patients with LTBI.[15]

The goals of the present study were to assess whether there is an increased prevalence of LTBI among HHCs of active TB cases with DM and pre-DM, and to determine whether quantitative IFN- γ responses to *Mtb* antigens are increased among those with DM and pre-diabetes. An ongoing NIH-supported Tuberculosis Research Unit (TBRU) study in Addis Ababa, Ethiopia enabled us to conduct a cross-sectional study among a cohort of the close and household contacts (HHCs) of active pulmonary TB cases in Ethiopia.

BACKGROUND

The relationship between DM, pre-DM and LTBI is not well understood. There are limited data on the relationship of DM and LTBI, and most studies conducted to date have methodologic limitations including reliance on self-reported DM diagnosis and limited geographic variability.[16] Notably, a 2017 meta-analysis of the association of DM and LTBI found a significantly increased odds ratio of LTBI among those with DM (OR 1.18, 95% CI 1.06-1.30), despite that only two of the included studies had yielded a significant association of DM and LTBI.[16] Two additional recent studies conducted in large population-representative samples in the United States provide strong evidence for a positive association and dose-response relationship between glycemic control and LTBI prevalence.[17] [18] However, it remains uncertain whether this association is generalizable to low- and middle-income countries with the highest burdens of TB disease and LTBI, which have markedly different demographic and population characteristics.

Importantly, there are no prior studies to our knowledge conducted in sub-Saharan Africa despite the high burden of TB in this region.[16] There is also very limited data describing the association of DM and LTBI among the close and household contacts (HHCs) of patients with active TB disease,[19,20] a highly exposed group that is at high risk of developing LTBI after exposure and of subsequent progression to active TB disease. Studying the close contacts of patients with TB provides a novel opportunity to assess the risk of LTBI among a high-risk group with recent known TB exposure.

As mentioned in the Introduction, this project leverages data from an ongoing NIH-supported TBRU study in Addis Ababa, Ethiopia. The goal of the TBRU is to further understanding of antigen-specific T cell responses and their relationships to the distinct outcomes

of Mtb infection: mycobacterial clearance, prolonged LTBI, or progression from LTBI to active TB disease. From 2018 to 2021, the TBRU recruited a cohort of the HHCs of active pulmonary TB cases identified from selected health facilities in the sub-cities of Addis Ababa, Ethiopia. Data collected on this HHC cohort at time of enrollment enabled us to conduct the first cross-sectional study of diabetes status and the risk of LTBI in sub-Saharan Africa.

METHODS

To accomplish the study goal, we investigated the following aims and associated hypotheses:

1. To determine the relationship between diabetes and pre-diabetes and LTBI among a cohort of household contacts of active TB cases in Ethiopia.

Hypothesis: Household contacts with diabetes and pre-diabetes will have a higher risk of latent TB infection compared to those with euglycemia.

2. To determine the correlation between HbA1c and quantitative IGRA (IFN-γ) response among household contacts with LTBI.

Hypothesis: There will be a positive correlation and dose-response relationship between HbA1c and magnitude of IFN-γ response to Mtb antigens.

Study design

HHCs of newly diagnosed active pulmonary TB disease cases were identified through ongoing public health surveillance at selected community health facilities in the 10 sub-cities of Addis Ababa, Ethiopia. All index patients had active TB confirmed by positive acid-fast bacilli (AFB) smear or positive medium or high nucleic acid amplification test result (Xpert MTB/RIF, Cepheid; Sunnyvale, California, USA). The HHCs of active pulmonary TB cases identified through public health surveillance activities were referred to our study staff for potential study participation. After HHC participants provided written informed consent, demographic and medical history data were collected and participants had an IGRA, HIV serology, and HbA1c

screening performed at the time of study enrollment. The study was approved by the Institutional Review Board (IRB) at Emory University, the Armauer Hansen Research Institute, and the National Research Ethics Review Committee of Ethiopia.

Participants

All HHCs who had a valid IGRA result at time of study enrollment and an HbA1c result at time of enrollment or within 12 months of enrollment were included. HHCs of active TB cases were defined as 1) persons who shared the same home residence as the index case for ≥5 nights during the 30 days prior to the date of TB diagnosis in the index case; or 2) persons who shared the same indoor living or working space as the index case ≥5 hours per day for ≥5 days during the 30 days prior to the index case's TB diagnosis. Exclusion criteria included: HHCs < 15 years of age, hemoglobin level <7 g/dL, positive screen for active TB (detailed below), a history of TB disease or TB treatment, a history of isoniazid (INH) preventative therapy or other treatment for LTBI; pregnant at time of screening, or taking immunosuppressive medications (equivalent to prednisone ≥15 mg/day) within 30 days of screening. HHCs were screened for active TB by history of TB-related symptoms (cough, fever, night sweats and weight loss). Participants with symptoms consistent with active TB received a chest radiograph and had two sputum samples collected for AFB smear microscopy, AFB culture and TB PCR (Xpert MTB/RIF assay). Written informed consent to participate was obtained from all participants at time of enrollment.

Measures and Definitions

The primary exposure was DM status, determined by a point-of-care capillary HbA1c test (Siemens Vantage Analyzer; Malvern, Pennsylvania, USA) and classified according to the American Diabetes

Association guidelines: euglycemia (≤5.6%), pre-DM (5.7–6.4%), and DM (≥6.5%).[21] Participants self-reporting a history of DM diagnosis were also defined as having DM regardless of HbA1c result. Secondary definitions of DM status included new and previously known DM diagnosis, and classification of DM status by HbA1c result without inclusion of self-reported diagnoses.

The primary outcome was LTBI status at study enrollment, measured by an interferon-γ release assay (IGRA; QuantiFERON-TB Gold Plus [QFT] [Qiagen, Venlo, Netherlands]) performed according to the manufacturer's recommendations and as previously described.[22,23] Prevalent LTBI was defined as a positive IGRA and lack of symptoms suggestive of TB at time of study enrollment. Quantitative IGRA results were reported as IFN-γ response to TB-1 antigen minus nil (the negative control, adjusting for background IFN-γ expression) and IFN-γ response to TB-2 antigen minus nil, in units IU/ml IFN-γ.[24] Self-reported demographic and medical history information was collected and HIV rapid antibody testing (Chembio Diagnostic Systems; Hauppauge, New Nork, USA) was performed at the time of enrollment. Weight and height measurements at time of study enrollment were used to calculate body mass index (BMI), and participants were categorized as underweight (<18.5 kg/m^2), normal weight (18.5-25 kg/m^2), overweight (25-30 kg/m^2), and obese (>30 mg/m^2). All data were stored in an online REDCap database[25] managed by the TBRU-ASTRa Data Management Center at Emory University.

Analytic Plan

Analytic methods for each Aim were as follows:

1. To determine the relationship between diabetes and pre-diabetes and LTBI among the cohort of household contacts of active TB cases, we first assessed both the associations between participant characteristics and diabetes status and associations between participant

characteristics and the prevalence of LTBI. Chi-square or Fisher's exact tests were utilized for categorical variables and Wilcoxon or Kruskal-Wallis were utilized for non-normally distributed continuous variables. The prevalence difference of LTBI was defined as the difference between the prevalence of LTBI among participants in the comparison group for a given characteristic and the prevalence of LTBI among participants in the referent group for that characteristic.

Multivariable logistic models were used to estimate the association between participants' DM status (categorized into a three-level variable: no DM, pre-DM, and DM) and LTBI status, with adjustment for sex, BMI, HIV status and age as potential confounders. Wald 95% confidence intervals were used for all logistic regression models. Covariates included in the multivariable models were chosen based on observed bivariate associations with DM and LTBI (demonstrated in Table 1 and Table 3), directed acyclic graph theory (shown in Figure 2), and biologic plausibility. We also considered age an an effect modifier of the association between DM and LTBI, implementing alternative logistic regression models that include interaction terms between age (as a dichotomous variable, under vs. over 40 years of age) and DM, and between age and pre-DM. Likelihood ratio testing was used to determine the effects of interaction terms on model fit.

Additional multivariable models performed as sensitivity analyses included 1) treating DM as a two-category variable grouping together participants with pre-DM and euglycemia, and 2) utilizing only baseline HbA1c results to classify participants' DM status.

2. To determine the correlation between HbA1c and quantitative IGRA (IFN-γ) response among household contacts with LTBI, Wilcoxon rank-sum tests were used to compare the median IFN-γ

responses to TB1 and TB2 antigens in the QFT. Pearson's correlation coefficient was used to assess the association of HbA1c and quantitative IFN-γ responses. We also assessed the Pearson's correlation coefficient with log-transformed quantitative IFN-γ responses to address the limitation that quantitative IFN- γ responses in the QuantiFERON-Gold Plus are right-censored at 10.0 IU/ml.

For both aims, we considered an association significant if p-value was <0.05 or if the 95% CI excluded the null value (0 for prevalence ratios and 1 for odds ratios). All analyses were performed using R 4.0.2[26] and SAS version 9.4 (Cary, NC, USA).

RESULTS

Among the 2316 eligible household contacts of 1342 active pulmonary TB cases who were identified at the selected collaborating health centers in Addis Ababa, 857 were contacted and agreed to participate in pre-screening. Following pre-screening, 693 HHCs of active pulmonary TB cases were consented and enrolled in the study, and 597 HHCs met study inclusion criteria for our analysis (Figure 1). Among these 597 HHCs, 240 (40.2%) were male, the median age was 28.5 years (IQR 22.6-37.6 years), median BMI was 21.0 m/kg^2 (IQR 19.0-24.2 m/kg^2), and 20 (3.4%) were HIV-seropositive. Based on IGRA testing and lack of symptoms suggestive of TB, 423 (70.9%) of HHC participants had LTBI (Table 1). HHCs were highly exposed to active TB cases, with 168 (28.1%) sleeping in the same bed as their index case and an additional 217 (36.3%) sleeping in the same room (Table 3). Additional demographic information and LTBI risk factors are shown in Table 1 and Table 3.

Characteristics of diabetes

Among 597 HHC participants, 31 (5.3%) had DM, 92 (15.4%) had pre-DM, and 474 (79.4%) were euglycemic at time of study enrollment. The median HbA1c measurements among those with pre-DM and DM were 5.8% (IQR 5.7-6.0%) and 7.0% (QR 6.6-8.4%), respectively (Table 1). Median BMI was higher among those with pre-DM (23.3 kg/m^2) and DM (26.1 kg/m^2) compared to those with euglycemia (20.5 kg/m^2, $p<0.01$, Table 1), and participants with pre-DM or DM were older than those without DM (value, $p<0.01$, Table 1). Among the 31 HHC participants with DM, 19 (61.3%) had previously been diagnosed with DM, while the remaining 12 (38.7%) were unaware of their diabetes status at the time of enrollment (Table 2). Twenty-

eight (90.3%) of 31 participants with DM had a HbA1c measurement of >6.5 and 3 participants with self-reported DM diagnoses had HbA1c < 6.5% at study enrollment and were categorized as DM based on self-report alone. Among all HHCs with DM, 11 (33.3%) were receiving metformin at time of study enrollment, 4 (13.3%) were receiving sulfonylurea, and 2 (10.5%) reported insulin use (Table 2).

Characteristics of latent TB infection

Four hundred twenty-three (70.9%) of the 598 HHC study participants had LTBI. The median age of participants with LTBI (30.2 years [IQR 23.6-38.8 years]) was older than the median age of those without LTBI (24.7 years [IQR 19.8-34.5 years, p-value < 0.01]). Median BMI was also higher among participants with LTBI (21.4 kg/m^2 [IQR 19.0-24.5 kg/m^2]) compared to those without LTBI (20.5 kg/m^2 [IQR 18.8-22.9 kg/m^2]). The prevalence of LTBI did not differ by sex, ethnic group, or HIV status (Table 3).

Aim 1: Association of diabetes and latent TB infection

The prevalence of LTBI was significantly higher among persons with DM compared to those with euglycemia; there was a prevalence difference of 17.7% (95% CI 5.2-30.2%, p <0.01) among participants with DM (87.1%) as compared to those with euglycemia (69.4%) (Table 3) (OR 2.97 [95% CI 1.14-10.2], p=0.04). Prevalence of LTBI did not significantly differ between those with pre-DM and euglycemia (prevalence difference 3.4% [95% CI -6.6-13.4%], p=0.50). The prevalence difference of LTBI in participants with DM compared to euglycemia was also assessed by strata of sex, age, and BMI category (Table 5). The prevalence difference of LTBI when comparing participants with DM to euglycemia was similar among women (18.3% [95%

CI 1.7%-35.0%], p=0.03) and men (17.8% [95% CI -0.0% - 35.8%, p=0.05]). When stratified by age, the prevalence difference of LTBI among DM as compared to euglycemia differed among participants >40 years old (prevalence difference 16.7% [95% CI 1.1-32.2%], p=0.04) and those <40 years old (prevalence difference 2.8% [95% CI -84.6 - 54.4%], p=0.87). For HHCs who were underweight the prevalence difference was 35.0% (95% CI 25.7-44.2%, p<0.01), and among HHCs who were overweight or obese the prevalence difference was 18.0% (95% CI 3.0-32.9%, p=0.02).

In multivariable analysis controlling for sex and HIV, those with DM had significantly higher odds of LTBI (aOR 3.01, 95% CI 1.03-8.79), and the increased odds of LTBI persisted but did not reach statistical significance when controlling for sex, HIV, and BMI grouping (aOR 2.33, 95% CI 0.77-7.08). When controlling for age in addition to sex, HIV, and BMI, the increased odds of LTBI also did not reach statistical significance (aOR 1.89, 95% CI 0.59-6.00)**.** In multivariate analysis of LTBI prevalence by per-unit change in HbA1c, the unadjusted odds of LTBI increased by 1.34 times (95% CI 0.99-1.82, p=0.05) per 1% increase in HbA1c, and the fully adjusted odds of LTBI increased by 1.11 times (95% CI 0.83-1.48, p=0.50) per 1% increase in HbA1c.

We also assessed age as an effect modifier of the association between DM and LTBI prevalence after determining that the prevalence difference of LTBI by DM status differed by age group. When including age as an interaction term, the odds of LTBI with DM among those ≥40 years of age were not significantly increased as compared to those without DM (aOR 1.14, 95% I 0.22 - 5.98). Among participants >40 years old, the odds of LTBI in those with DM were 3.67 times the odds of LTBI without DM, although the association did not reach statistical significance (aOR 3.67, 95% CI 0.77-17.47). Addition of age interaction terms to the crude model did not significantly

improve model fit (likelihood ratio test of models with vs. without interaction terms, p=0.55). Additional alternative multivariate models were carried out as shown in Table 4 and Table 6.

Aim 2: Quantitative IFN- γ responses among participants with latent TB infection

Among 423 participants with LTBI, the median quantitative IFN- γ responses to TB-1 antigen was 4.73 IU/ml (IQR 1.78-7.94; Table 7). The median TB-1 antigen response was non-significantly (p=0.51) higher among participants with DM (5.34 IU/ml; IQR 2.86-7.39) as compared to participants with euglycemia (4.59 IU/ml; IQR 1.57-7.90). The median IFN- γ response to TB-2 antigen among all HHCs with LTBI was 4.95 IU/ml (IQR 1.88-7.94 IU/ml) and antigen response among participants with DM (5.00 IU/ml; IQR 2.78-7.00 IU/ml) was not significantly elevated compared to TB-2 antigen response among those with euglycemia (4.87 IU/ml; IQR 1.68-7.95 IU/ml, p=0.22). We did not observe a meaningful correlation between HbA1c measurements at time of study enrollment and quantitative IFN- γ responses to TB-1 (r=-0.001, p=0.98) or TB-2 antigens (r=0.016, p=0.74).

Because IFN- γ responses in the QFT are censored at 10.0 IU/ml (such that measurements >10.0 IU/ml are reported as 10.0 IU/ml), we also assessed the correlation of HbA1c with log-transformed IFN-γ responses to TB-specific antigens. With log transformation, we also did not observe a meaningful correlation between HbA1c and quantitative IFN- γ response to the TB-1 antigen (r=0.005, p=0.96).

DISCUSSION

In this study of the HHCs of active TB cases in Addis Ababa, Ethiopia, we found a high prevalence of LTBI (>70%) and determined that HHCs with DM had a significantly higher prevalence of LTBI compared to those with euglycemia (prevalence difference of 17.7%). Our study is the first to our knowledge to examine an association of pre-DM and DM with LTBI in sub-Saharan Africa, and our results suggest an association of DM and LTBI in this highly exposed population. We also found a substantial proportion of study participants with DM (38%) were previously unaware of their DM diagnosis, highlighting the importance of globally accessible HbA1c screening.[27] Recently updated WHO LTBI guidelines emphasize the importance of finding and treating LTBI in high-risk populations worldwide including LMIC settings.[3] Our findings highlight how the use of public health surveillance data has the potential to focus future efforts on the treatment of LTBI among high-risk groups, in this case the HHCs of active TB cases. The data also underscore the urgent need to prioritize treatment of LTBI among high-risk groups including those with diabetes.

One notable finding of our study was the high prevalence of LTBI in this highly exposed participant group. LTBI prevalence in this cohort was more than two-fold higher than previously published estimates of LTBI prevalence in Africa: in a recent meta-analysis of studies utilizing IGRA testing, the prevalence of LTBI was estimated as 33.6% (95% CI 24.4-42.9%).[28] Prevalence of LTBI in our cohort was also higher than documented in prior investigation of LTBI among HHCs in Kenya (55.7%).[29] The very high prevalence of LTBI in this HHC cohort is likely related to HHCs' intensive exposure to index cases, as nearly two-thirds (64.5%) of HHCs slept in the same bed or room as the index case. Taken together with recent evidence that HHCs are at high risk for

progression to active TB disease,[30] this finding highlights the urgent need for public health action to increase case detection and implement LTBI treatment among HHCs.

Although we found a significant association of DM and LTBI prevalence in crude analysis, the association of DM and LTBI did not reach statistical significance in multivariable analysis after adjustment for multiple potential confounders (sex, HIV status, BMI, and age group). These results indicate that age and other demographic variables may confound the observed univariate association of DM and LTBI, particularly as age may be indicative of a a longer history of exposure to active TB in this population. However, it is also highly likely that our study was under-powered to detect a true association of DM and LTBI in multivariate analysis due to the relatively low rate of DM (5.2%) and the notably high rate of LTBI (70.9%) in this HHC cohort.

When assessing age as an effect modifier of the association between DM and LTBI prevalence, we found a trend towards a stronger association of DM and LTBI prevalence among those >40 years old as compared to those ≥40 years old, suggesting that age may act as an effect modifier of the association between DM and LTBI prevalence. Based on our results, it is highly plausible that age may act as both a confounder and effect modifier of the association of DM and LTBI. However, definitive conclusions of how age impacts the association of DM and LTBI are somewhat limited by the young age distribution of the study population and further study is needed to determine how age may modify the observed association of DM and LTBI prevalence.

Our finding of higher prevalence of LTBI among participants with DM is consistent with emerging literature on the association of DM and LTBI in the general population. In a meta-analysis that pooled the adjusted odds ratios from 13 observational studies, a statistically significant increase in the odds of LTBI (OR 1.18, 95% CI 1.06-1.30) was found among patients with DM despite the fact that only two included studies yielded a significant increase in the adjusted odds of LTBI among

patients with DM.[16] A number of these prior studies may have not been adequately powered to detect a small but significant increased risk of LTBI in patients with DM, particularly when accounting for variation in LTBI prevalence by age, gender, and other key demographic characteristics. Most early studies of the association of LTBI and DM were also limited by a reliance on self-reported diabetes diagnosis, which is known to lead to substantial under-reporting.[16,27] Recent evidence from two large population-based studies in the United States also indicates that patients with DM may have increased susceptibility to tuberculosis infection and suggests a dose-response relationship between glycemic control and risk of TB infection.[17,18]

Importantly, our study's focus on the HHCs of active TB cases enables us to examine the effect of dysglycemia on LTBI risk following known recent exposure to *M. tuberculosis*. There is very limited evidence to date on the association of dysglycemia and LTBI among HHCs. One notable recent study conducted in Brazil found that the HHCs of persons with pulmonary TB and dysglycemia were more likely to have LTBI at baseline and 6-month follow-up than the contacts of pulmonary TB patients who were euglycemic.[20] However, this study measured HbA1c in the index pulmonary TB patients but did not investigate the association of dysglycemia in the close contacts with LTBI incidence in this cohort.[20] The only other study to examine the association of DM and LTBI in a highly exposed HHC population was conducted in Chennai and Pune, India, and found that the increased risk of LTBI among HHCs with DM did not reach statistical significance (prevalence ratio 1.4, 95% CI 0.8-2.5).[19] Our results provided needed data to further investigate the association of DM and LTBI among a highly exposed HHC cohort.

The mechanisms of immunocompromise in DM are poorly understood, and there are substantial gaps in our understanding of how DM and pre-DM may mediate host response to *M. tuberculosis*.[13,14] Our in-vivo measurement of IFN-γ release among participants with LTBI did not

show differences in IFN-γ responses to TB-specific antigens by participant diabetes status, suggesting that IFN-γ responses in IGRA testing may not accurately capture DM-mediated immune dysfunction. Further investigation is needed to elucidate the immune mechanisms by which dysglycemia may impact host susceptibility to *M. tuberculosis* infection. Future research is also needed to determine how demographic factors such as age and body composition may modify the relationship of dysglycemia and LTBI. Moreover, future studies should adopt a longitudinal design to assess whether patients with LTBI and dysglycemia are at increased risk for progression to active TB disease.

Our study is subject to several limitations. First, as this was a cross-sectional observational study leveraging data from a larger ongoing clinical research project, we were not able to control for all potential sources of confounding. Additionally, both the higher-than-expected rate of LTBI (>70%) and the lower-than-expected rate of DM (5.2%) may have limited the power of our study to detect the true association of DM and LTBI in the study population. Among the unmeasured potential sources of confounding are serum Vitamin D (25-OHD) level and the prevalence of hemoglobinopathies. Vitamin D deficiency is associated with both active TB disease and dysglycemia,[31,32] and may increase the risk of LTBI among patients with DM,[33] while hemoglobinopathies accelerated red blood cell turnover that decreases HbA1c, reducing the sensitivity of HbA1c as a screening tool for DM and pre-DM. However, we did exclude participants with severe anemia (hemoglobin < 7.0) which likely mitigated the effect of any confounding from hemoglobinopathies.

Conclusions

A high prevalence (>70%) of LTBI was seen among HHCs of active pulmonary TB cases in Addis Ababa, Ethiopia. HHCs had intensive exposure to index cases which may in part explain the high overall prevalence of LTBI. Those with DM were significantly more likely to have LTBI compared to HHCs with euglycemia (prevalence difference of 17.7%). The increased risk of LTBI among HHCs with DM did not reach statistical significance when adjusting for multiple confounding variables (age, sex, HIV and BMI). Many (38%) participants with diabetes were unaware of their diabetes diagnosis. Our results highlight opportunities for scaling up detection and treatment of LTBI according to the latest WHO guidelines for LTBI treatment[3] and support the inclusion of both HHCs and individuals with DM among high-risk groups prioritized for LTBI treatment in LMICs.

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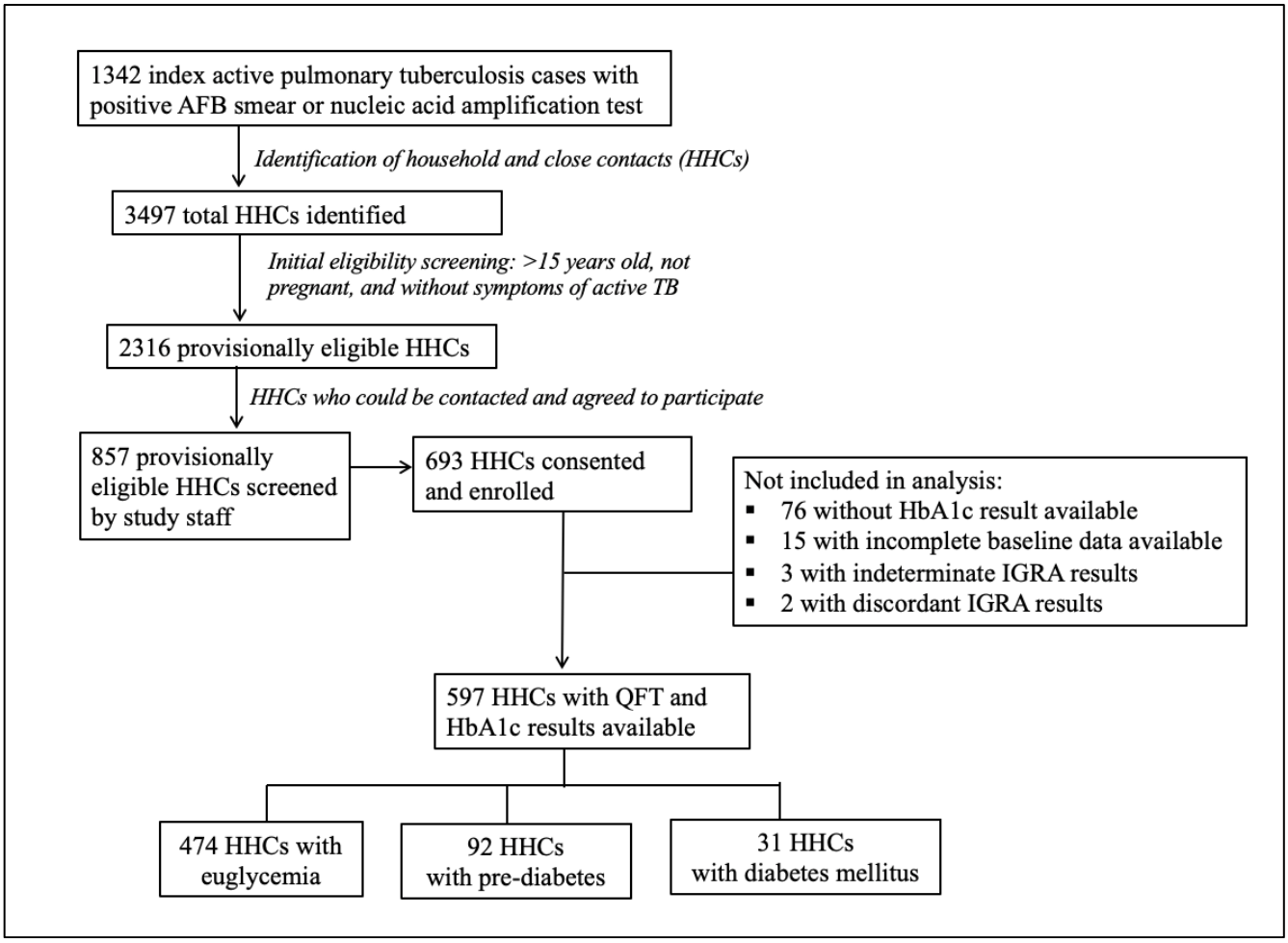
Figure 1. Study flow diagram illustrating selection of participants among the close and household contacts of active pulmonary TB cases in Addis Ababa, Ethiopia, 2018-2021



Abbreviations used: AFB: Acid-fast bacilli; DM: Diabetes mellitus; HbA1c: Glycated hemoglobin A1c; HHC: Household contact; IGRA: Interferon-γ release assay.

Figure 2. Directed acyclic graph model of the relationship of diabetes and latent tuberculosis infection among the close and household contacts of patients with active pulmonary tuberculosis.

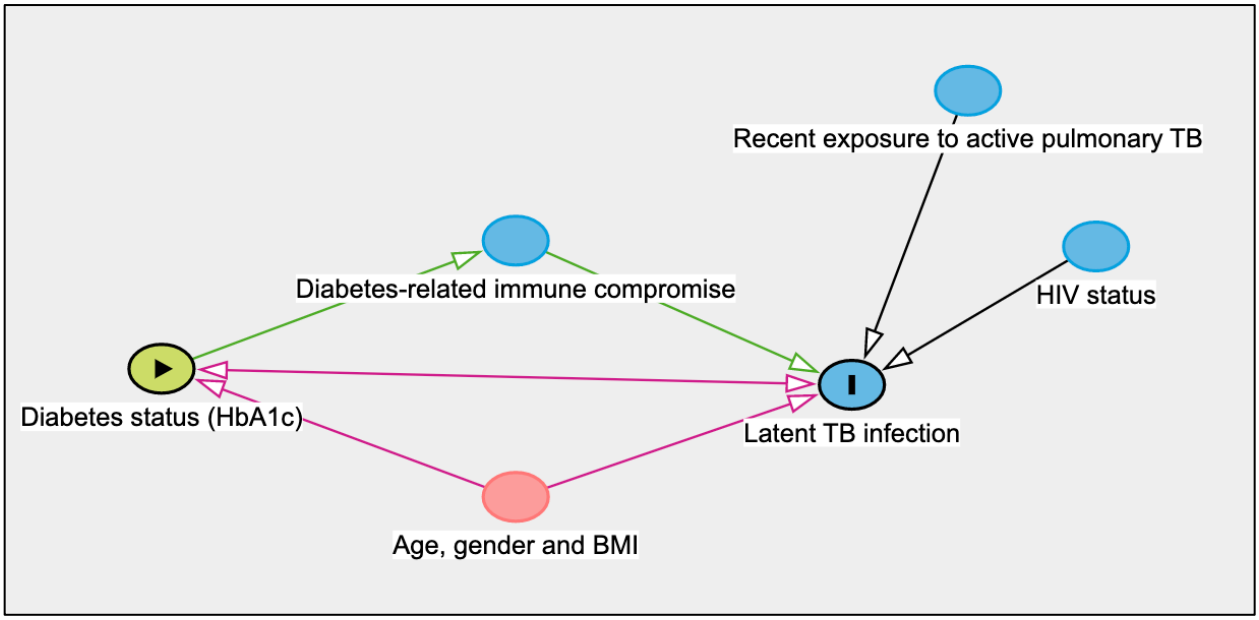


Table 1. Prevalence of diabetes and prediabetes among household contacts in Addis Ababa, Ethiopia, 2018-2021

Characteristic	**Overall,** N=597	**Euglycemic,** N = 474 (79.4%)	**Pre-diabetes,** N = 92 (15.4%)	**Diabetes,** N = 31 (5.2%)	**Pre-diabetes vs. euglycemic:** p-value[1]	**Diabetes vs. euglycemic:** p-value[1]
Age (years), Median (IQR)	28.5 (22.6, 37.6)	26.0 (22.2, 34.2)	37.7 (27.0, 48.1)	44.6 (40.3, 50.4)	**<0.01***	**<0.01***
Sex (male), n (%)	240 (40.2%)	201 (83.8%)	28 (11.7%)	11 (4.6%)	**0.04***	0.657
Ethnic group, n (%)[2]						
Oromo	115 (19.3%)	93 (80.9%)	18 (15.7%)	4 (3.5%)	0.67	0.39
Amhara	193 (32.3%)	154 (79.8%)	28 (14.5%)	11 (5.7%)		
Tigraway	22 (3.7%)	15 (68.1%)	5 (22.7%)	2 (9.1%)		
Sidama	10 (1.8%)	10 (100.0%)	0 (0.0%)	0 (0.0%)		
HIV positive	20 (3.4%)	16 (80.0%)	1 (5.0%)	3 (15.0%)	0.33	0.10
HbA1c, Median (IQR)	5.4 (5.1-5.6)	5.3 (5.1-5.4)	5.8 (5.7-6.0)	7.0 (6.6-8.4)	**<0.01***	**<0.01***
BMI, Median (IQR)	21.0 (19.0-24.2)	20.5 (18.8-23.0)	23.3 (19.9-26.9)	26.1 (23.7-30.4)	**<0.01***	**<0.01***
BMI category, n (%)						
Underweight (<18.5 m/kg^2)	119 (19.9%)	103 (86.6%)	15 (12.6%)	1 (0.8%)	**<0.01***	**<0.01***
Normal weight (18.5-25 m/kg^2)	367 (61.5%)	315 (85.8%)	41 (11.2%)	11 (3.0%)		
Overweight (25-30 m/kg^2)	85 (14.2%)	47 (55.3%)	27 (31.8%)	11 (12.9%)		
Obese (>30 m/kg^2)	26 (4.4%)	9 (34.6%)	9 (34.6%)	8 (30.8%)		
Tobacco smoker, n (%)	41 (6.9%)	30 (73.2%)	8 (19.5%)	3 (7.3%)	0.55	0.45
Ever been in prison, n (%)	29 (4.9%)	25 (86.2%)	4 (13.8%)	0 (0.0%)	>0.99	0.39
Alcohol consumption in past 6 months, n (%)	234 (39.2%)	191 (80.3%)	33 (14.1%)	10 (4.3%)	0.50	0.49
Received the BCG vaccine	279 (46.7%)	224 (79.5%)	39 (14.0%)	16 (4.2%)	0.43	0.66
Ever a healthcare worker, n (%)	22 (3.7%)	13 (59.1%)	7 (31.8%)	2 (9.1%)	**0.03***	0.23
Latent tuberculosis infection[3]	423 (70.9%)	329 (77.8%)	67 (15.8%)	27 (6.4%)	0.60	0.06

[1] Statistical tests performed: Wilcoxon rank-sum test of medians, and chi-square or Fisher's exact for categorical comparisons.
[2] Ethnicity information not available for 257 (43.0%) of participants; 209 (35.0%) reported their ethnicity as 'Other' and 48 (8.0%) declined to answer the question.
[3]Latent tuberculosis infection defined as positive IGRA (QuantiFERON Gold+) assay and negative symptom screen for active tuberculosis infection.
* Indicates statistical significance ($p < 0.05$)
Abbreviations used: BCG: Bacille Calmette-Guérin; CI: Confidence interval; BMI: Body mass index; HbA1c: Glycated hemoglobin A1c; HIV: Human immunodeficiency virus; IFN: Interferon; IQR: Interquartile range; TB: Tuberculosis.

Table 2. Characteristics of diabetes management among participants with diabetes

Characteristic	***N = 31***
Age (years), Median (IQR)	44.6 (40.3, 50.4)
Sex (male), n (%)	11 (35.5%)
BMI, Median (IQR)	26.1 (23.7, 30.4)
Previously diagnosed with diabetes, n (%)	19 (61.3%)
Type of diabetes, if previously diagnosed (n=19), n (%)	
Gestational	1 (5.3%)
Type I	2 (10.5%)
Type II	11 (57.9%)
Don't know/missing	5 (26.3%)
Ever prescribed metformin, n (%)	11 (33.3%)
Ever prescribed sulfonylurea, n (%)	4 (12.9%)
Ever prescribed insulin, n (%)	2 (10.5%)
HbA1c test result (percent), Median (IQR)	**7.0 (6.6, 8.4)**
Acronyms: BMI: Body mass index; HbA1c: Glycated hemoglobin A1c. IQR: Interquartile range	

Table 3. Summary of household contact characteristics stratified by IGRA result

Characteristic	**Overall**, N = 597	**Without LTBI,** N = 174 (29.1%)	**With LTBI,** N = 423 (70.9%)	**Prevalence Difference (95% CI)**[1,2]
Diabetes status[3]				
Euglycemic	476 (79.4%)	145 (30.6%)	329 (69.4%)	*Ref.*
Pre-diabetes	92 (19.4%)	24 (26.1%)	64 (69.6%)	3.4% (-6.6 - 13.4%)
Diabetes[3]	31 (5.2%)	4 (13.3%)	26 (86.7%)	**17.7% (5.2 - 30.2%)***
HbA1c, Median (IQR)	5.4 (5.1, 5.6)	5.3 (5.1, 5.5)	5.4 (5.1, 5.6)	0.37
Age (years), Median (IQR)	28.5 (22.6-37.6)	24.7 (19.8-34.5)	30.2 (23.6-38.8)	**<0.01**
Sex, n (%)				
Female	357 (59.8%)	113 (31.7%)	244 (68.3%)	*Ref.*
Male	240 (40.2%)	61 (25.4%)	179 (76.4%)	6.2% (-1.1 - 13.6%)
Ethnic group, n (%)[4]				
Amhara	193 (32.3%)	59 (30.6%)	134 (68.3%)	*Ref.*
Oromo	115 (19.3%)	33 (28.7%)	82 (71.3%)	1.9% (-8.6 - 12.4%)
Tigraway	22 (3.7%)	4 (18.2%)	18 (81.8%)	12.4% (-5.0 - 29.8%)
Sidama	10 (1.7%)	2 (20.0%)	8 (80.0%)	10.6% (-15.1 - 36.2%)
HIV test result, n (%)				
Negative	577 (96.6%)	169 (29.3%)	408 (70.3%)	*Ref.*
Positive	20 (3.3%)	5 (25.0%)	15 (75.0%)	4.3% (-15.0 - 23.6%)
BMI, Median (IQR)	21.0 (19.0-24.2)	20.5 (18.6-22.9)	21.4 (19.0-24.5)	**0.008**
BMI category, n (%)				
Normal weight (18.5-25 m/kg^2)	367 (61.5%)	109 (29.7%)	258 (70.3%)	*Ref.*
Underweight (<18.5 m/kg^2)	119 (19.9%)	43 (36.1%)	76 (63.9%)	-6.4% (-16.3 - 3.4%)
Overweight (25-30 m/kg^2)	85 (14.2%)	17 (20.0%)	68 (80.0%)	9.7% (0.0 - 19.4%)
Obese (>30 m/kg^2)	26 (4.4%)	5 (19.2%)	21 (80.8%)	10.5% (-5.4 - 26.3%)
Tobacco smoker, n (%)				
No	556 (93.3%)	164 (28.9%)	392 (71.1%)	*Ref.*
Yes	41 (6.9%)	10 (24.4%)	31 (75.6%)	5.1% (-8.6 - 18.8%)
Ever been in prison, n (%)				
No	568 (95.1%)	164 (31.0%)	404 (71.1%)	*Ref.*
Yes	29 (4.9%)	10 (34.5%)	19 (65.5%)	-5.6% (-23.3 - 12.1%)
Alcohol consumption (past 6 months), n (%)				
No	363 (60.8%)	111 (30.6%)	252 (69.4%)	*Ref.*
Yes	234 (39.2%)	63 (26.9%)	171 (73.1%)	3.7% (-3.7 - 11.1%)
Received the BCG vaccine				
No or unsure	318 (53.3%)	99 (31.1%)	219 (68.0%)	*Ref.*
Yes	279 (46.7%)	75 (26.9%)	204 (73.1%)	4.3% (-3.0 - 11.6%)
Ever a healthcare worker, n (%)				
No	575 (96.3%)	170 (29.6%)	405 (70.4%)	*Ref.*
Yes	22 (3.7%)	4 (18.2%)	18 (81.8%)	11.4% (-5.2 - 27.9%)
Extent of HHC participant's exposure to active TB index cases				
Household members with active TB within past 3 months				

One with active TB	588 (98.5%)	172 (29.3%)	416 (70.7%)	*Ref.*
Two with active TB	2 (0.3%)	0 (0.0%)	2 (100.0%)	**29.3% (25.6- 32.9%)****
None (exposure at workplace)	7 (1.2%)	2 (28.6%)	5 (71.4%)	0.6% (-33.0 - 34.3%)
Sleeping arrangement with the index case[5]				
Same bed	168 (28.1%)	45 (26.8%)	123 (73.2%)	*Ref.*
Other bed in same room	217 (36.3%)	58 (26.7%)	159 (73.3%)	-0.0% (-8.9 - 9.0%)
Other room in same building	156 (26.1%)	57 (36.5%)	99 (63.5%)	**-10.2% (-20.2 - -0.0%)**[#]
Other building in household	40 (6.7%)	12 (30.0%)	28 (70.0%)	-3.2% (-18.9 - 12.5%)
Ever workplace exposure to active TB				
No	586 (98.0%)	170 (29.4%)	416 (70.6%)	*Ref.*
Yes	11 (1.8%)	4 (36.4%)	7 (63.6%)	-7.4% (-36.0 - 21.3%)

[1] Prevalence difference is defined as the difference between the prevalence of LTBI among participants in the comparison group for a characteristic and the prevalence of LTBI among participants in the referent group for that characteristic.

Bold font indicates statistical significance ($p < 0.05$); *: p=0.006; **: p<0.001; #: p=0.048

[2] Statistical tests performed for comparison of medians: Kruskal-Wallis test, Wilcoxon rank-sum test.

[3] Three participants were determined to have DM based solely on self-reported diagnosis (with HbA1c < 6.5), while the remainder had HbA1c > 6.5 at time of study enrollment.

[4] Ethnicity information not available for 257 (43.0%) of participants; 209 (35.0%) reported their ethnicity as 'Other' and 48 (8.0%) declined to answer the question.

[5] "During the 30 days before the index case started treatment for TB, how close did you sleep with the index case?"

Abbreviations used: BCG: Bacille Calmette-Guérin; BMI: Body mass index; CI: Confidence Interval; HbA1c: Glycated hemoglobin A1c; HIV: Human immunodeficiency virus; IFN: Interferon; IQR: Interquartile range; LTBI: Latent tuberculosis infection.

Table 4. Multivariable model for odds of latent tuberculosis infection by diabetes group among the close and household contacts of active pulmonary TB patients in Addis Ababa, Ethiopia

	Unadjusted odds ratio (95% CI*)*	**Adjusted for sex and HIV only** (95% CI*)*	**Adjusted for sex, HIV and BMI** (95% CI*)*	**Adjusted for sex, HIV, BMI and age** (95% CI)
Model 1:				
Euglycemic *(n=474)*	*Ref.*	*Ref.*	*Ref.*	*Ref.*
Pre-diabetes *(n=92)*	1.18 (0.72 - 1.95)	1.24 (0.75 - 2.05)	1.08 (0.64 - 1.82)	0.96 (0.55 - 1.66)
Diabetes *(n=31)*[2]	**2.97 (1.02 - 8.67)**	**3.01 (1.03 - 8.79)**	2.33 (0.77 - 7.08)	1.89 (0.59 - 6.00)
Model 2:				
Euglycemic/pre-diabetes *(n=566)*	*Ref.*	*Ref.*	*Ref.*	*Ref.*
Diabetes *(n=31)*	2.90 (0.99 - 8.41)	2.91 (1.00 - 8.48)	2.28 (0.76 - 6.88)	1.66 (0.54 - 5.11)
Model 3: HbA1c[3] (%)				
< 5.7 *(n=475)*	*Ref.*	*Ref.*	*Ref.*	*Ref.*
5.7 - 6.4 *(n= 94)*	1.21 (0.74-1.99)	1.27 (0.77 - 2.10)	1.10 (0.65 - 1.85)	0.91 (0.53 - 1.56)
> 6.5 *(n=28)*	2.64 (0.90 - 7.74)	2.69 (0.91 - 7.93)	2.09 (0.69 - 6.35)	1.44 (0.46 - 4.50)
Model 4: *HBA1c as continuous outcome*				
per 1% increase in HbA1c	1.34 (0.99 - 1.82)	1.34 (0.99 - 1.83)	1.23 (0.91 - 1.66)	1.11 (0.83 - 1.48)

***** Indicates statistical significance ($p < 0.05$)

Models adjusted for age, sex, HIV test result, and BMI (as a categorical variable, with normal weight as reference group). Age was considered as a categorical variable with four groupings for each age quartile, as follows: 1st quartile: < 22.6 years; 2nd quartile; 22.6-28.5 years; 3rd quartile: 28.1-37.6 years; 4th quartile: >37.6 years of age).

[2] Diabetes status determined by self-report (answered ‘‘yes” to having been told by a doctor or health professional that he/she had diabetes) and according to American Diabetes Association guidelines;[21] participants who self-reported diabetes were classified as having diabetes regardless of HbA1c.

[3] Glycated hemoglobin (HbA1c) categories determined according to American Diabetes Association guidelines.[21]

Abbreviations used: BMI: Body mass index; CI: Confidence interval; HbA1c: Glycated hemoglobin A1c; HIV: Human immunodeficiency virus.

Table 5. Prevalence differences of LTBI among participants with diabetes as compared to euglycemia or pre-diabetes, stratified by sex, age, and body mass index

Characteristic	**Diabetes status**	**LTBI** N/Total (%)	**Prevalence difference** (95%CI)
Sex			
Male	Euglycemic	147/201 (73.1%)	*Ref.*
	Pre-diabetes	22/28 (78.6%)	5.6% (-10.8 - 22.1%)
	Diabetes	10/11 (90.9%)	17.8% (0.0% - 35.8%)
Female	Euglycemic	182/273 (66.7%)	*Ref.*
	Pre-diabetes	45/64 (70.3%)	3.6% (-8.9 - 16.2%)
	Diabetes	17/20 (85.0%)	**18.3% (1.7% - 35.0%)***
Age			
Age ≤ 40 years	Euglycemic	284/414 (68.6%)	*Ref.*
	Pre-diabetes	38/53 (71.7%)	3.1% (-9.8 - 16.0%)
	Diabetes	5/7 (71.4%)	2.8% (-30.9 - 36.6%)
Age > 40 years	Euglycemic	45/60 (75.0%)	*Ref.*
	Pre-diabetes	29/39 (74.4%)	-0.6% (-18.2 - 16.9%)
	Diabetes	22/24 (91.7%)	**16.7% (1.1 - 32.2%)***
BMI			
Underweight *(<18.5 m/kg^2)*	Euglycemic	67/103 (65.0%)	*Ref.*
	Pre-diabetes	8/15 (53.3%)	-11.7% (-38.6 - 15.2%)
	Diabetes	1/1 (100.0%)	**35.0% (25.7 - 44.2%)***
Normal weight *(18.5-25 m/kg^2)*	Euglycemic	219/315 (69.6%)	*Ref.*
	Pre-diabetes	31/41 (75.6%)	6.1% (-8.0 - 20.2%)
	Diabetes	8/11 (76.8%)	3.2% (-23.6% - 30.0%)
BMI overweight/obese *(>25 m/kg^2)*	Euglycemic	43/56 (76.8%)	Ref.
	Pre-diabetes	28/36 (77.8%)	1.0% (-16.45 - 18.5%)
	Diabetes	18/19 (94.7%)	**18.0% (3.0 - 32.9%)***

[1] Prevalence difference defined as the difference between the prevalence of LTBI in participants with DM and the prevalence of LTBI in participants with either euglycemia or pre-diabetes.
* Indicates statistical significance ($p < 0.05$)

Abbreviations used: BMI: Body mass index; CI: Confidence Interval; LTBI: Latent tuberculosis infection.

Table 6. Multivariable model for odds of latent tuberculosis infection by diabetes group, with age interaction terms, among the close and household contacts of active pulmonary TB patients in Addis Ababa, Ethiopia

	LTBI prevalence (n/total, %)	**Model 1 (crude)** (95% CI)	**Model 2 (adjusted)** (95% CI)	**Model 3 (adjusted)** (95% CI)
With three diabetes categories				
Age < 40 years				
Euglycemic *(n=414)*	284/414 (68.6%)	*Ref.*	*Ref.*	*Ref.*
Pre-diabetes *(n=53)*	38/53 (71.7%)	1.16 (0.62 - 2.18)	1.22 (0.65 - 2.32)	1.11 (0.58 - 2.13)
Diabetes *(n=7)*[2]	5/7 (71.4%)	1.14 (0.22 - 5.98)	1.15 (0.22 - 6.05)	0.93 (0.17 - 5.14)
Age >=40 years				
Euglycemic *(n=60)*	45/60 (75.0%)	*Ref.*	*Ref.*	*Ref.*
Pre-diabetes *(n=39)*	29/39 (74.4%)	0.97 (0.38 - 2.44)	0.98 (0.39 - 2.49)	0.86 (0.33 - 2.21)
Diabetes *(n=24)*[2]	22/24 (91.7%)	3.67 (0.77 - 17.47)	3.68 (0.77 - 17.56)	2.95 (0.60 - 14.43)
With two diabetes categories				
Age < 40 years				
No diabetes (*n=467)*	322/467 (69.0%)	*Ref.*	*Ref.*	*Ref.*
Diabetes *(n=7)*	5/7 (71.4%)	1.13 (0.22 - 5.87)	1.13 (0.22 - 5.90)	0.98 (0.17 - 5.03)
Age >=40 years				
No diabetes *(n=99)*	74/99 (74.7%)	*Ref.*	*Ref.*	*Ref.*
Diabetes *(n=24)*	22/24 (91.7%)	3.72 (0.82 - 16.94)	3.71 (0.81 - 16.94)	3.12 (0.67 - 14.54)

Notes:
Model 1: Diabetes status as predictor, with interaction terms between diabetes status and age (age included as a dichotomous variable, under vs. over 40 years of age).
Model 2: Diabetes status as predictor, with sex and HIV status as covariates and interaction terms between diabetes status and age (age included as a dichotomous variable, under vs. over 40 years of age).
Model 3: Diabetes status as predictor, with sex, HIV status and body mass index (BMI) as covariates and interaction terms between diabetes status and age. Age was considered as a dichotomous variable, under vs. over 40 years of age), and BMI was considered as a four-level variable (underweight, normal weight, overweight and obese) with normal weight as the referent category.

Table 7. Quantitative IFN- γ responses in QuantiFERON-TB Gold Plus testing of household contacts with latent tuberculosis infection

	IFN response (IU/ml)	**p-value**
TB1-Nil[1] among participants with LTBI, Median (IQR)		
Overall (*n=423)*	4.73 (1.78-7.94)	
Euglycemic, (*n= 329)*	4.59 (1.57-7.90)	*Ref.*
Pre-Diabetes (*n=67)*	4.94 (2.05-8.37)	0.22
Diabetes (*n=27)*	5.34 (2.86-7.39)	0.51
TB2-Nil[1] among participants with LTBI, Median (IQR)		
Overall (*n=423)*	4.95 (1.88-7.94)	
Euglycemic, (*n= 329)*	4.87 (1.68-7.95)	*Ref.*
Pre-Diabetes (*n=67)*	5.17 (1.91-7.99)	0.29
Diabetes (*n=27)*	5.00 (2.78-7.00)	0.40

[1]*Description of antigens included in QuantiFERON-TB Gold Plus IGRA testing:*
TB1: Contains mycobacterial peptides ESAT-6 and CFP-10, designed to elicit cell-mediated immune responses from CD4+ T-helper lymphocytes; **TB2**: Contains additional peptides targeted to elicit cell-mediated immune responses from CD8+ cytotoxic T lymphocytes.

Abbreviations used: IFN: Interferon; IQR: Interquartile range; IU: International units; LTBI: Latent tuberculosis infection; TB: Tuberculosis