Latent tuberculosis infection status of pregnant women in Uganda determined using QuantiFERON TB Gold-Plus

Felix Bongomin,1,2* MB ChB, MSc., Phillip Ssekatatte,3+ MSc., Gloria Nattabi,4+ BBLT, Ronald Olum2, Sandra Ninsiima,2 MB ChB, Andrew Peter Kyazze,3 MB ChB, Winnie Nabakka5, Rebecca Kukunda4, Stephen Cose,3 PhD, Davis Kibirige,4,5 MB ChB, MMED, Charles Batte,6 MB ChB, MPH, Mark Kaddumukasa,2 MD, PhD, Bruce J. Kirenga,2,6 MD, PhD, Annettee Nakimuli,7 MD, PhD, Joseph Baruch Baluku,8,9 MB ChB, MMED, Irene Andia-Biraro,2,5,10 MD, PhD

*These authors contributed equally to this work

Affiliations
1. Department of Medical Microbiology and Immunology, Faculty of Medicine, Gulu University, Gulu, Uganda
2. Department of Medicine, School of Medicine, Makerere University College of Health Sciences, Kampala, Uganda
3. Department of Immunology and Molecular Biology, School of Biomedical Sciences, Makerere University College of Health Sciences, Kampala, Uganda
4. Department of Medicine, Uganda Martyrs Hospital Lubaga, Kampala, Uganda
5. Medical Research Council/Uganda Virus Research Institute and London School of Hygiene and Tropical Medicine Uganda Research Unit, Entebbe, Uganda.
6. Lung Institute, Makerere University, Kampala, Uganda
7. Department of Obstetrics & Gynecology, School of Medicine, Makerere University College of Health Sciences, Kampala, Uganda
8. Division of Pulmonology, Kiruddu National Referral Hospital, Kampala, Uganda
9. Directorate of Programs, Mildmay Uganda, Wakiso, Uganda
10. Department of Clinical Research, Faculty of Infectious and Tropical Disease, London School of Hygiene and Tropical Medicine, London, UK

*Corresponding author
Dr. Felix Bongomin, MB ChB, MSc, FECMM
Department of Medical Microbiology and Immunology, Faculty of Medicine, Gulu University, Gulu, Uganda.
Email: drbongomin@gmail.com Phone: +256-784-523-395

© The Author(s) 2021. Published by Oxford University Press on behalf of Infectious Diseases Society of America.
This is an Open Access article distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs licence (http://creativecommons.org/licenses/by-nc-nd/4.0/), which permits non-commercial reproduction and distribution of the work, in any medium, provided the original work is not altered or transformed in any way, and that the work is properly cited. For commercial re-use, please contact journals.permissions@oup.com
Alternative corresponding author

Dr. Irene Andia-Biraro, MB ChB, MMED, PhD
Senior Lecturer
Department of Medicine, School of Medicine, Makerere University College of Health Sciences, Kampala, Uganda
Email: andiaodanga@yahoo.com Phone: +256 – 772-485-289

Key message: About one-third of pregnant women in Uganda had LTBI, especially those with HIV infection and those age 30 years or older. We recommend routine screening for LTBI and TB preventive therapy among eligible pregnant women.
Abstract

**Background:** The risk of progression of latent tuberculosis (TB) infection (LTBI) to active disease increases with pregnancy. This study determined the prevalence and risk factors associated with LTBI among pregnant women in Uganda.

**Methods:** We enrolled 261 pregnant women, irrespective of gestation age. Participants who had known or suspected active TB on the basis of clinical evaluation or who had recently received treatment for TB were excluded. LTBI was defined as an interferon- gamma (IFN-\(\gamma\)) concentration \(\geq 0.35\) IU/mL (calculated as either TB1 (eliciting CD4\(^+\) T-cell responses) or TB2 (eliciting CD8\(^+\) T-cell responses) antigen minus nil) using QuantiFERON TB Gold-Plus (QFT-plus) assay.

**Results:** LTBI prevalence was 37.9% (n=99) (95% Confidence Interval (CI): 32.3 - 44.0). However, 24 (9.2%) subjects had indeterminate QFT-plus results. Among participants with LTBI, TB1 and TB2 alone were positive in 11 (11.1%) and 18 (18.2%) participants, respectively. In multivariable analysis, HIV-infection (adjusted odds ratio (aOR): 4.4; 95% CI: 1.1 – 18.0; p=0.04) and age group 30-39 years (aOR: 4.0; 95% CI: 1.2 – 12.7; p=0.02) were independently associated with LTBI. Meanwhile, smoking status, alcohol use, nature of residence, crowding index, and TB contact were not associated with LTBI.

**Conclusion:** Our findings are in keeping with the evidence that HIV infection and advancing age are important risk factors for LTBI in pregnancy. In our setting, we recommend routine screening for LTBI and TB preventive therapy among eligible pregnant women.

**Key words:** Latent Tuberculosis Infection, CD4\(^+\) T-cell and CD8\(^+\) T-cell TB responses, risk factors, Pregnancy, Uganda
INTRODUCTION

The World Health Organization (WHO) estimates that close to 2 billion persons worldwide are latently infected with *Mycobacterium tuberculosis* complex [1,2]. Latent tuberculosis (TB) infection (LTBI) manifests as a specific immune response in the absence of clinical and radiological disease but with capacity to reactivate and cause clinical disease at a later time [1,2]. In fact, an estimated 5–10% of HIV negative individuals with LTBI progress to active disease at some point in their life, translating into approximately 10 million cases progressing to active TB cases and about 1.4 million deaths every year [3].

Women in their reproductive years, during pregnancy or early postpartum period are disproportionately affected by TB and TB remains an important cause of death in this group [3]. In 2019 alone, 450,000 of the estimated 3.2 million women who felt ill with TB died of the disease [3]. The WHO has no specific report on active TB in pregnancy. However, a study estimated that over 200,000 annual cases of active TB disease occur during pregnancy globally [4].

TB in pregnancy poses a substantial risk of morbidity to both the pregnant woman and the fetus if not diagnosed and treated in a timely manner [5]. Immune dysregulation in pregnancy is associated with a more insidious onset of active TB, increased risk of LTBI and progression of LTBI to active TB disease [4,6]. Globally, an estimated 900 million women have a latent LTBI [4,6]. These women have a considerably increased risk of re-activation to active disease during pregnancy or in puerperium [4,6].

In a recent systematic review, among pregnant women in the USA, the prevalence of LTBI ranged from 14-48% [6]. In this study, the diagnostic performance of both tuberculin skin test (TST) and Interferon-gamma (IFN-Ɣ) release assays (IGRA) was comparable and was unaffected by pregnancy [6]. However, there is a limited data and understanding of factors associated with LTBI among pregnant women in Africa, a region with a high TB and HIV burden. One study from Tanzania, found
a prevalence of LTBI among pregnant women as high as 37.4% [7]. Two studies from Ethiopia showed a prevalence of LTBI of 31.9% [8] and 33% [9] among pregnant women using IGRA.

IGRA, such as the QuantiFERON TB Gold-Plus (QFT-plus) assay have very high sensitivities and are not influenced by prior BCG vaccination [10]. QFT-plus, has TB antigens that elicit CD4+ T-cell IFN-Ɣ response reflecting a recall from re-exposure and antigens that elicit CD8+ T-cell IFN-Ɣ induced from viable or recent exposure [11].

Uganda is a high TB burden country with over 30% of reported TB cases affecting women [12]. It was estimated that 1400 – 4400 pregnant women in Uganda had active TB in 2011 [4] but the TB epidemiology has changed considerably since Uganda achieved the 2015 TB-related millennium development goals. The burden of both active TB and LTBI among pregnant women is largely unknown. Therefore, we aimed at determining the prevalence of and risk factors for LTBI among pregnant women in Uganda.

METHODS

Study design

This was a single-center, antenatal care-based, cross-sectional study conducted among pregnant women attending routine antenatal care clinic at Kawempe National Referral Hospital (KNRH), Kampala, Uganda. The study was conducted between September 2020 and December 2020.

Study setting

KNRH is an Obstetrics and Gynecology hospital, and a Teaching and Research Centre affiliated to Makerere University College of Health Science. It is a 170-bed national referral hospital located along the Kampala-Gulu Highway that receives referrals mainly from lower health centers in Kampala and
neighboring districts. The antenatal care clinic at KNRH runs on Tuesday through Thursday every week, offering antenatal care services to about 50-60 new mothers every clinic day.

Study population

We enrolled pregnant women who were willing and competent to provide informed written consent, regardless of gestational age or gravidity. We excluded from the study patients who had known or suspected active TB based on the Uganda National Intensified TB Case Finding Guide [13] followed by a routine physical examination or who had recently (past 6 months) received treatment for TB. Trained study nurses consecutively enrolled eligible participants from daily antenatal care attendance register until the sample size was reached.

Sample size

Using formula for a single population, we calculated a sample size of 260 participants based on an estimated prevalence of LTBI of 16.1% as reported in a previous study in the general population in Uganda [14], a margin of error of 5%, 20% incomplete data or withdrawal of consent, and a z-statistics at 95% confidence interval (95% CI).

Demographic and obstetric data

A study assistant administered a semi-structured study questionnaire through a face-to-face interview to collect information on maternal characteristics such as age, gravidity, education level, occupation, marital status, HIV status and anti-retroviral therapy, TB contact, gestational age, history of abortion, smoking and alcohol usage and the number of antenatal care visits in the current pregnancy. Gestation age was estimated using the date of the last normal menstrual period.

Anthropometric data

Body mass index (weight [kg]/(height [m])²) and waist-hip ratio (waist circumference (cm)/hip circumference (cm)) were calculated following anthropometric measurements. Specifically, weight
was measured with minimal clothing and without shoes using a digital bathroom weighing scale 
(SECA-Germany) while height measured used a stadiometer (Fazzini S208 height rod). The weighing 
scale was calibrated on a daily basis. The waist and hip circumferences were measured using a 
tailor’s measuring tape. The brachial blood pressure (BP) was measured on both arms using 
MEDQUIP® arm-type fully automatic digital blood pressure monitor (Model: BP-2400) with an 
appropriate adult cuff size and the participant seated upright in a comfortable position after resting 
for had rested. BPs were taken 5 minutes apart and the average of the two measurements was 
considered as the participant’s BP.

Interferon-gamma release assay

A study nurse drew blood for IGRA directly into the IGRA tubes. The IGRA assay, QuantiFERON-TB 
Gold-plus (QIAGEN, Hilden, Germany) was performed according to the manufacturer’s instructions 
[11]. All samples were processed and ran in a clinical laboratory at the Department of Immunology 
and Molecular Biology, School of Biomedical Sciences, Makerere University, which is 30 minutes 
away from KNRH. Briefly, 1 mL of blood was drawn directly into four separate heparinized tubes: the 
nil control (containing only heparin), the mitogen control (containing phytohemagglutinin), TB1 
(containing M. tuberculosis-specific antigens ESAT-6, CFP-10 modified for eliciting CD4⁺ T-cell 
responses), and TB2 (containing M. tuberculosis-specific antigens ESAT-6, CFP-10 modified for 
eliciting CD8⁺ T-cell responses). Immediately after filling each tube, the tube was inverted at least 10 
times to allow the blood to coat the entire wall. Within 2 hours of venipuncture, the tubes were 
remixed by inverting them again 10 times before immediately being placed in an incubator set at 
37°C. After 24 hours of incubation, the tubes were centrifuged at 3000 xg and the plasma was 
collected using single wrapped 3ml Pasteur pipettes. The amount of IFN-γ in the plasma was 
measured by enzyme-linked immunosorbent assay (ELISA) with the reagents included in the test kit 
according to the manufacturer’s recommendations. Briefly, 50 µl of working-strength conjugate 
were added to each well of the QFT-Plus ELISA plate, followed by 50 µl of the plasma and standards
to the appropriate wells. The plate was incubated for 2 hours in the biosafety cabinet at room
temperature. The plates were washed at least 6 times and an allowance of 5 seconds of soak time
with 400 µl of 1X wash buffer using an ELISA washer. After, 100 µl of the substrate solution were
added and the plate incubated at room temperature for 30 minutes. Lastly, 50 µl of substrate
solution were then added and the plate was immediately read using an ELISA reader at 450 nm with
a reference wavelength of 620 nm to obtain optical densities. Results were calculated using QFT-Plus
analysis software version 2.71.2 [11].

Definitions

LTBI was defined as IFN-γ concentration ≥0.35 IU/mL (calculated as either TB1 or TB2 antigen minus
nil) per the manufacturer's guideline [11]. If antigen-nil was <0.35 IU/mL or <25% of the nil value,
when the mitogen was ≥0.5 IU/mL, the result was considered negative. If (1) nil was >8 IU/mL or (2)
antigen-nil ≥0.35 IU/mL and <25% of the nil value when the nil was ≤8.0 IU/mL and the mitogen was
<0.5 IU/mL, the results were considered indeterminate.

Statistical Methods

We used STATA version 16 (StataCorp, College Station, Texas, USA) to perform all statistical analyses.
Categorical variables were expressed as frequencies and percentages. For all numerical variables,
Shapiro-Wilk normality test was completed to select an appropriate test. Normally distributed data
were summarized as mean and standard deviations (mean ± SD) and non-normally distributed data
as median and interquartile range (IQR). Chi-square or Fischer’s exact tests were used to assess for
associations between LTBI and categorical variables while Mann-Whitney U/student t-tests and
Wilcoxon-signed rank/Analysis of Variance (ANOVA) were used to assess for associations between
LTBI and continuous variables (age, blood pressure, weight, age, gestational age, height, waist and
hip circumferences). All variables with p<0.2 in the bivariate analyses were fitted into a multivariate
logistic regression model to adjust for potential confounders such as age, parity, gestational age and
HIV status. Multivariable logistic regression model was used to assess for independent predictors of LTBI among the study participants. Results were presented as odds ratios (OR) and 95% confidence intervals (95% CI). All analyses were two-tailed and $P<0.05$ was considered significant at a 95% CI. Graphs were prepared using GraphPad Prism version 8.0.2 (GraphPad Software, La Jolla, CA).

**Patient Consent Statement**

All participants provided informed written consents after the study procedure, risks and benefits were explained to them. The study protocol was approved by the Makerere University School of Medicine Ethics and Research Committee (SOMREC) (reference number #REC REF 2020-113). All principles of research involving human subjects outlined in the Declaration of Helsinki were adhered to.

**Results**

**Baseline characteristics**

A total of 261 pregnant women met the inclusion criteria. Table 1 summarizes the characteristics the recruited participants. The median age of the participants was 26 (IQR: 23.0 – 30.0) years. Majority of the mothers were married (86.2%), and had reported for their first antenatal care visit (75.9%). Up to 99% (n=258) of the participants were in the second trimester with a median gestational age at enrolment of 26 (IQR: 20 – 31) weeks. About 11.1% had history of TB contact with family members. Thirteen (5%) participants reported that they were HIV positive. All HIV positive women were on antiretroviral therapy and had not received TB preventive therapy.
QuantiFERON-TB Gold-plus results

Overall, 99 (37.9%) participants had a positive QFT-plus. Of these, TB1 and TB2 alone were positive in 11 (11.1%) and 18 (18.2%) participants, respectively (Figure 1). Twenty-four (9.2%) and 138 (52.9%) participants had indeterminate and negative QFT-plus results, respectively. Considering indeterminate results as negative, the overall prevalence of LTBI prevalence was 37.9% (n=99) (95% CI: 32.3% - 44.0%).

Overall, the inter-reliability of QFT-Plus assay for TB1 versus TB2 was moderate (agreement=88.9%, kappa=0.75, p<0.0001). In sub-analysis, there was also moderate inter-reliability agreement between TB1 and TB2 among HIV-negative pregnant mothers (agreement= 89.5%, kappa=0.75, p<0.0001), but this was weak for HIV+ clients (agreement=76.9%, kappa=0.49, p=0.036).

There was a strong positive correlation between IFN-γ value in TB1 and TB2 (Pearson’s coefficient = 0.88, p<0.0001), Figure 2.

The quantitative IFN-γ value in TB2 was similar to that in TB1 irrespective of HIV status (TB1: 2.7±3.4 (HIV +, n=13) vs. 1.4±2.7 IU/mL (HIV -, n=248), p=0.2; TB2: 1.9±2.5 (HIV +, n=13) vs.1.3±2.5 IU/mL (HIV -, n=248), p=0.4) (Figure 3A). However, participants with BCG scars had higher values compared to those without (TB1: 1.4±2.7 (BCG scar, n=177) vs. 0.7±1.9 IU/mL (No BCG scar, n=84), p=0.003; TB2: 1.3±2.4 (BCG scar, n=177) vs.0.6±1.7 IU/mL (No BCG scar, n=84), p=0.002) (Figure 3B). There was no statistically significant difference between mitogen and nil levels across HIV status; mitogen (9.4 IU/mL (HIV +) vs. 7.3 IU/mL (HIV -), p=0.85), and nil (0.24 IU/mL (HIV +) vs. 0.15 IU/mL (HIV -), p=0.26).

Risk factors for latent tuberculosis infection among the participants

At bivariate analysis (Table 2), LTBI was associated with advancing age (p<0.001), and HIV status (p=0.006). BCG scar (p=0.259), smoking status (p=0.433), nature of residence (p=0.707), crowding index (p=0.433), and TB contact (p=0.302) were not significantly associated with LTBI status.
At multivariable logistic regression (Table 3), pregnant women in their third decade of life (30 – 39 years of age), were 4 times more likely to have LTBI compared to teenagers (adjusted odds ratio (aOR): 3.96, 95% CI: 1.23 – 12.73, P=0.021). In addition, participants with HIV were also 4 times more likely to have LTBI (aOR: 4.37, 95% CI: 1.06 – 18.04, p=0.041) than HIV negative women.

**DISCUSSION**

Routine antenatal care visit provides a unique opportunity to identify pregnant women with LTBI and facilitate further evaluation and follow up as needed. In the present study, we found that about 38% of pregnant women attending antenatal care at a tertiary hospital in Uganda had LTBI, using IGRA. Our findings suggest that pregnant women who had HIV-infection and those aged 30 years or older were four-fold more likely to have LTBI. Our finding is congruent with similar studies from Tanzania [7] and Ethiopia [9] which found a prevalence of LTBI of 37.4% and 33% among pregnant women, respectively. In two systematic reviews, LTBI was reported in about 33% of women in the Middle-East and North Africa [15] and among 14 - 48% of pregnant women in the USA [6].

Interesting, we showed that participants with BCG scars had much higher IFN-Ɣ concentration, reflecting the immunomodulatory effect of BCG in LTBI. BCG re-vaccination may be further investigated in this population.

A few prior studies looked into LTBI prevalence in the general and special populations in Uganda. Two observational studies conducted in Uganda found the prevalence of LTBI to be 16.1% among adolescents and 49.0% among general adult population [14,16]. Both studies used TST to demonstrate LTBI status which has a relatively lower diagnostic accuracy than IGRA in populations which have high BCG vaccination coverage [10]. Another study investigated household contacts of TB patients in Uganda [17]. The prevalence of LTBI in this study was found to be as high as 65%.

Among persons living with HIV in Western Uganda who are engaged in hazardous alcohol use, the prevalence of LTBI based on TST positivity was reported at 35% [18]. In this study, the prevalence of LTBI among women was 33.9%.
Pregnancy is characterized by immunological changes that may predispose or increase TB reactivation [6]. Immune dysregulation in pregnancy, mainly driven by a surge in serum progesterone levels is marked by an anti-inflammatory milieu characterized by a quantitative and qualitative defects in circulating CD4+ and CD8+ T-cells with a corresponding increase in the number of circulating regulatory T-cells [19]. This dysregulation dampens the pro-inflammatory immune response, often by producing IL-10. Furthermore, progesterone also induces the placenta to produce IL-10, [20] which suppresses cell-mediated Th1 cytokines (IFN-γ, IL-2, TNF-α) [21]. Consequently, pregnant women are at higher risk of acquiring both primary TB disease (from recent TB infection) and progression of LTBI to active disease compared to non-pregnant women [22].

Active TB is more likely during pregnancy and early postpartum period than any other time in a woman’s life [23]. Therefore, women of reproductive age are suffering disproportionate morbidity and mortality due to TB. TB in pregnancy is associated with poor outcomes, including increased mortality in both the neonate and the pregnant woman [4]. In our study, HIV infection was found in 13 participants of which 10 (77%) had LTBI and HIV infection was independently associated with LTBI. This finding is consistent with published case series, which shows that the risk of TB in HIV-infected pregnant women appears to be higher, with 1–11% developing active TB during pregnancy or the early postpartum period [22,24]. However, recent work has shown that despite the high TB incidence among HIV positive individuals, detection of LTBI among these individuals is lower [8]. In Africa, WHO estimates revealed that TB rates are up to 10 times higher in pregnant women living with HIV than in pregnant women without HIV infection [3].

Similar to our study, Sheriff and others [7] in Tanzania reported, parity, BMI, and gestational age, and HIV sero-status were not associated with LTBI among pregnant women. However, in contrast, we found a significant association between age and HIV infection with LTBI among our participants. Notably, Sheriff and others used TST for the diagnosis of LTBI and 3 different cut offs were considered in classifying participants as having LTBI meanwhile we used IGRA. Additionally, almost
30% of the study participants (significantly younger women) were lost to follow up. This could explain the differences with our study.

Currently, there is no gold-standard diagnostic tool for the diagnosis of LTBI. TST or IGRA, both of which screen for immunological memory are the most common screening tools in clinical practice [25]. IGRA is specific for \textit{M. tuberculosis} while there is cross-reactivity with non-tuberculous mycobacterial infections when using TST or in those who previously got BCG vaccine, hence a false positive [25]. QFT-plus, an IGRA platform used in the present study has \textit{M. tuberculosis}-specific antigen which trigger both CD4$^{+}$ and CD8$^{+}$ T-cell response [11]. Whereas positivity of both TB1-nil and TB2-nil indicates LTBI, TB2 tube response can be associated with active or subclinical TB disease and/or recent TB infection [26]. In our study, up to 18.2% of the participants had an isolated positive response to TB2 antigens, suggestive of possible acquisition of \textit{M. tuberculosis} infection during pregnancy or a subclinical TB disease.

Our study has limitations. Participants were derived from a single center thus our findings may not be generalizable. Secondly, we had a high number of patients who had indeterminate QFT-plus, and it is likely we may have underestimated the true burden of LTBI in this population. Moreover, 21/24 (88%) of the indeterminate results were due to low mitogen- indicating dampened immune response. Thirdly, the cross-sectional design did not allow us to prospectively look for whether CD8$^{+}$ women were more likely to develop active TB disease (which would support subclinical disease) and we were unable assess for prior exposure (which would support recent exposure). Fourthly, we were unable to obtain a complete detail of antiretroviral treatment regimen; duration, current viral load and CD4$^{+}$ T-cell count for HIV positive participants. However, we used QFT-plus assay, which is a more specific test for \textit{M. tuberculosis} infection than the TST. To the best of our knowledge, this is the first study to report on the prevalence of LTBI among pregnant women in Uganda. We recommend future studies to prospectively evaluate maternal and fetal outcomes in a population of LTBI vs. non-LTBI mothers. Also, studies looking at determinants of other important risk factors for LTBI
progression such as diabetes mellitus, and chronic kidney disease among pregnant women are welcome. Lastly but not least, the high burden of LTBI and TB2 in this population merits active TB investigation and TB preventive therapy.

In conclusion, we report a high prevalence of LTBI among pregnant women in Uganda, especially those with HIV infection and those with advanced age. These findings suggest a need for strategies to scale up LTBI screening among high-risk pregnant women and TB preventive treatment in this population to advance progress towards TB elimination.
Author contributions

IAB, JBB and FB designed the study. GN, PS, SN, APK, WN, RK, SC, DK, CB, MK, BJK and FB collected the data. OR performed the statistical analyses with oversight from FB, IAA and JBB. FB, RO, PS, GN, JBB, MK, and IAB wrote the manuscript with input from all the authors. All the authors reviewed the manuscript and approved the final version.

Data availability: Data are available upon request from the corresponding author

Funding

Research reported in this publication was supported by the Fogarty International Centre of the National Institutes of Health under Award Number D43 TW011401. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. Also, his work was supported by the Crick African Network which receives its funding from the UK’s Global Challenges Research Fund (MR/P028071/1)

Acknowledgement

None

Potential Conflicts of Interest

The authors declare no potential conflicts of interest.
References

[1] World Health Organization. Latent tuberculosis infection. Updated and consolidated guidelines for programatic management. Geneva, Switzerland: 2018.

[2] Nuermberger E, Bishai WR, Grosset JH. Latent Tuberculosis Infection. Semin Respir Crit Care Med 2004;25:317–36. https://doi.org/10.1055/s-2004-829504.

[3] World Health Organization. Global Tuberculosis Report 2020. Geneva, Switzerland: 2020.

[4] Sugarman J, Colvin C, Moran AC, Oxlade O. Tuberculosis in pregnancy: an estimate of the global burden of disease. Lancet Glob Heal 2014;2:e710–6. https://doi.org/10.1016/S2214-109X(14)70330-4.

[5] Miele K, Bamrah Morris S, Tepper NK. Tuberculosis in Pregnancy. Obstet Gynecol 2020;135:1444–53. https://doi.org/10.1097/AOG.0000000000003890.

[6] Malhamé I, Cormier M, Sugarman J, Schwartzman K. Latent Tuberculosis in Pregnancy: A Systematic Review. PLoS One 2016;11:e0154825. https://doi.org/10.1371/journal.pone.0154825.

[7] Sheriff FG, Manji KP, Manji MP, Chagani MM, Mpembeni RM, Jusabani AM, et al. Latent tuberculosis among pregnant mothers in a resource poor setting in Northern Tanzania: A cross-sectional study. BMC Infect Dis 2010;10. https://doi.org/10.1186/1471-2334-10-52.

[8] Birku M, Desalegn G, Kassa G, Tegbaru B, Howe R, Tsegaye A, et al. Pregnancy suppresses Mycobacterium tuberculosis-specific Th1, but not Th2, cell-mediated functional immune responses during HIV/latent TB coinfection. Clin Immunol 2020;218:108523. https://doi.org/10.1016/j.clim.2020.108523.

[9] Walles JK, Tesfaye F, Jansson M, Balcha TT, Winqvist N, Kefeni M, et al. Performance of QuantiFERON-TB gold plus for detection of latent tuberculosis infection in pregnant women living in a tuberculosis- and HIV-endemic setting. PLoS One 2018;13:1–15. https://doi.org/10.1371/journal.pone.0193589.

[10] Diel R, Goletti D, Ferrara G, Bothamley G, Cirillo D, Kampmann B, et al. Interferon-γ release assays for the diagnosis of latent Mycobacterium tuberculosis infection: A systematic review and meta-analysis. Eur Respir
[11] QIAGEN. QuantiFERON®-TB Gold Plus (QFT®-Plus) ELISA Package Insert. Hilden, Germany: 2017.

[12] World Health Organization. Uganda Tuberculosis Profile. 2018.

[13] Ministry of Health Uganda. The Uganda National Tuberculosis Prevalence Survey, 2014-2015 Survey Report. MOHU 2017 2016. http://health.go.ug/sites/default/files/Uganda National TB Prevalence Survey 2014-2015_final 23rd Aug17.pdf (accessed March 2, 2021).

[14] Mumpe-Mwanja D, Verver S, Yeka A, Etwom A, Waako J, Ssengooba W, et al. Prevalence and risk factors of latent tuberculosis among adolescents in rural eastern uganda. Afr Health Sci 2015;15:851–60. https://doi.org/10.4314/ahs.v15i3.20.

[15] Barry M. Prevalence of Latent Tuberculosis Infection in the Middle East and North Africa: A Systematic Review. Pulm Med 2021;2021:1–12. https://doi.org/10.1155/2021/6680651.

[16] Kizza FN, List J, Nkwata AK, Okwera A, Ezeamama AE, Whalen CC, et al. Prevalence of latent tuberculosis infection and associated risk factors in an urban African setting. BMC Infect Dis 2015;15:1–8. https://doi.org/10.1186/s12879-015-0904-1.

[17] Biraro IA, Egesa M, Toulza F, Levin J, Cose S, Joloba M, et al. Impact of co-infections and BCG immunisation on immune responses Among household contacts of tuberculosis patients in a uganadan cohort. PLoS One 2014;9. https://doi.org/10.1371/journal.pone.0111517.

[18] Puryear SB, Fatch R, Beesiga B, Kekibiina A, Lodi S, Marson K, et al. Higher Levels of Alcohol Use Are Associated With Latent Tuberculosis Infection in Adults Living With Human Immunodeficiency Virus. Clin Infect Dis 2020;72:865–8. https://doi.org/10.1093/cid/ciaa527.

[19] Robinson DP, Klein SL. Pregnancy and pregnancy-associated hormones alter immune responses and disease pathogenesis. Horm Behav 2012;62:263–71. https://doi.org/10.1016/j.yhbeh.2012.02.023.

[20] Polanczyk MJ, Carson BD, Subramanian S, Afentoulis M, Vandenbark AA, Ziegler SF, et al. Cutting edge: estrogen drives expansion of the CD4+CD25+ regulatory T cell compartment. J Immunol 2004;173:2227–30. https://doi.org/10.4049/jimmunol.173.4.2227.

[21] Piccinni MP, Scaletti C, Maggi E, Romagnani S. Role of hormone-
controlled Th1- and Th2-type cytokines in successful pregnancy. J Neuroimmunol 2000;109:30–3. https://doi.org/10.1016/s0165-5728(00)00299-x.

[22] Mathad JS, Gupta A. Tuberculosis in pregnant and postpartum women: epidemiology, management, and research gaps. Clin Infect Dis 2012;55:1532–49. https://doi.org/10.1093/cid/cis732.

[23] Zenner D, Kruijshaar ME, Andrews N, Abubakar I. Risk of tuberculosis in pregnancy: a national, primary care-based cohort and self-controlled case series study. Am J Respir Crit Care Med 2012;185:779–84. https://doi.org/10.1164/rccm.201106-1083OC.

[24] Gounder CR, Wada NI, Kensler C, Violari A, McIntyre J, Chaisson RE, et al. Active tuberculosis case-finding among pregnant women presenting to antenatal clinics in Soweto, South Africa. J Acquir Immune Defic Syndr 2011;57:e77-84. https://doi.org/10.1097/QAI.0b013e31821ac9c1.

[25] Pai M, Zwerling A, Menzies D. Systematic review: T-cell-based assays for the diagnosis of latent tuberculosis infection: an update. Ann Intern Med 2008;149:177–84.

[26] Sauzullo I, Mengoni F, Mascia C, Pavone P, Savelloni G, Massetti AP, et al. Diagnostic performance in active TB of QFT-Plus assay and co-expression of CD25/CD134 in response to new antigens of Mycobacterium tuberculosis. Med Microbiol Immunol 2019;208:171–83. https://doi.org/10.1007/s00430-018-00576-4.
Table 1. Baseline characteristics of participants.

| Participant Characteristics (N=261) | Frequency (%) or Median (IQR) |
|-------------------------------------|-------------------------------|
| **Antenatal visit at enrolment**    |                               |
| First                               | 198 (75.9)                    |
| Second                              | 36 (13.8)                     |
| Third                               | 7 (2.7)                       |
| Fourth and more                     | 20 (7.7)                      |
| **Age: median (IQR), years**       | 26 (23.0 - 30.0)              |
| 15 - 19                             | 20 (7.7)                      |
| 20 - 29                             | 161 (61.7)                    |
| 30 - 39                             | 77 (29.5)                     |
| 40 - 49                             | 3 (1.2)                       |
| **Marital status**                  |                               |
| Married                             | 225 (86.2)                    |
| Single                              | 36 (13.8)                     |
| **Education level**                 |                               |
| Informal                            | 4 (1.5)                       |
| Primary                             | 59 (22.6)                     |
| Secondary                           | 151 (57.9)                    |
| Tertiary                            | 47 (18.0)                     |
| **Occupational status**             |                               |
| Unemployed                          | 119 (45.6)                    |
| Business                            | 80 (30.7)                     |
| Professional                        | 34 (13.0)                     |
| Skilled worker                      | 24 (9.2)                      |
| Unskilled worker                    | 4 (1.5)                       |
| **Smoking status**                  |                               |
| Former                              | 1 (0.4)                       |
| Never                               | 260 (99.6)                    |
| **Alcohol usage**                   |                               |
| Current                             | 29 (11.1)                     |
| Former                              | 38 (14.6)                     |
| Never                               | 194 (74.3)                    |
| **Family history of Diabetes**      |                               |
| No                                  | 218 (83.5)                    |
| Yes                                 | 43 (16.5)                     |
| **Residence**                       |                               |
| Urban                               | 217 (84.4)                    |
| Rural                               | 40 (15.6)                     |
| **HIV status**                      |                               |
| Negative                            | 248 (95.0)                    |
| Positive                            | 13 (5.0)                      |
| **BCG scar**                        |                               |
|                                |      |
|--------------------------------|------|
| **Yes**                        | 177  |
| **No**                         | 84   |
| **Family history of tuberculosis** |      |
| **No**                         | 232  |
| **Yes**                        | 29   |
| **Tuberculosis contact**        |      |
| **No**                         | 244  |
| **Yes**                        | 17   |
| **Crowding index**             |      |
| ≤4 household occupants         | 196  |
| ≥5 household occupants         | 65   |
| **Gravidity**                  |      |
| Primigravida                   | 89   |
| Multigravida                   | 154  |
| Grand multigravida             | 18   |
| **Previous abortion**          |      |
| **No**                         | 217  |
| **Yes**                        | 44   |
| **Gestation age at enrolment (weeks)** | 26 (20 – 31) |
| **Trimester at enrolment**     |      |
| 1                              | 1 (0.4) |
| 2                              | 258 (98.9) |
| 3                              | 2 (0.8) |
| **Anthropometry**              |      |
| Body mass index (kg/m2)        | 27.2 (23.7 - 31.4) |
| Waist-hip ratio: median (IQR)  | 0.91 (0.86 - 0.96) |
| **Blood pressure at enrolment** |      |
| SBP (mmHg)                     | 122.5 (114.0 - 131.0) |
| DBP (mmHg)                     | 76.5 (71.5 - 84.0) |
Table 2. Factors associated with latent tuberculosis infection among the study participants

| Participant Characteristics       | LTBI n (%) | No LTBI n (%) | P-value   |
|----------------------------------|------------|---------------|-----------|
|                                  |            |               |           |
| Antenatal care visit at enrolment|            |               |           |
| First                            | 74 (74.8)  | 124 (76.5)    | 0.919     |
| Second                           | 15 (15.2)  | 21 (13)       |           |
| Third                            | 2 (2)      | 5 (3.1)       |           |
| Fourth and more                  | 8 (8.1)    | 12 (7.4)      |           |
| Age                              |            |               | <0.001    |
| 15 - 19                          | 5 (5.1)    | 15 (9.3)      |           |
| 20 - 29                          | 49 (49.5)  | 112 (69.1)    |           |
| 30 - 39                          | 42 (42.4)  | 35 (21.6)     |           |
| 40 - 49                          | 3 (3)      | 0 (0)         |           |
| Marital status                   |            |               | 0.808     |
| Married                          | 86 (86.9)  | 139 (85.8)    |           |
| Single                           | 13 (13.1)  | 23 (14.2)     |           |
| Education level                  |            |               | 0.164     |
| Informal                         | 3 (3)      | 1 (0.6)       |           |
| Primary                          | 27 (27.3)  | 32 (19.8)     |           |
| Secondary                        | 51 (51.5)  | 100 (61.7)    |           |
| Tertiary                         | 18 (18.2)  | 29 (17.9)     |           |
| Occupational status              |            |               | 0.335     |
| Unemployed                       | 39 (39.4)  | 80 (49.4)     |           |
| Business                         | 34 (34.3)  | 46 (28.4)     |           |
| Professional                     | 13 (13.1)  | 21 (13)       |           |
| Skilled worker                   | 10 (10.1)  | 14 (8.6)      |           |
| Unskilled worker                 | 3 (3)      | 1 (0.6)       |           |
| Smoking status                   |            |               | 0.433     |
| Former                           | 0 (0)      | 1 (0.6)       |           |
| Never                            | 99 (100)   | 161 (99.4)    |           |
| Alcohol usage                    |            |               | 0.113     |
| Current                          | 9 (9.1)    | 20 (12.4)     |           |
| Former                           | 20 (20.2)  | 18 (11.1)     |           |
| Never                            | 70 (70.7)  | 124 (76.5)    |           |
| Residence                        |            |               | 0.707     |
| Rural                            | 16 (16.7)  | 24 (14.9)     |           |
| Urban                            | 80 (83.3)  | 137 (85.1)    |           |
| HIV status                       |            |               | 0.006     |
| Negative                         | 89 (89.9)  | 159 (98.2)    |           |
| Positive                         | 10 (10.1)  | 3 (1.9)       |           |
| BCG scar                         |            |               | 0.259     |
| No                               | 36 (36.4)  | 48 (29.6)     |           |
| Yes                              | 63 (63.6)  | 114 (70.4)    |           |
### Family history of TB

|       | No     | Yes    | p     |
|-------|--------|--------|-------|
|       | 90 (90.9) | 9 (9.1) | 0.417 |

### TB contact

|       | No     | Yes    | p     |
|-------|--------|--------|-------|
|       | 95 (96) | 4 (4)  | 0.302 |

### Crowding index

|       | No     | Yes    | p     |
|-------|--------|--------|-------|
| ≤4 household members | 77 (77.8) | 22 (22.2) | 0.433 |
| ≥5 household members | 119 (73.5) | 43 (26.5) |    |

### Gravidity

|       | No     | Yes    | p     |
|-------|--------|--------|-------|
| Primigravida | 39 (39.4) | 50 (30.9) | 0.193 |
| Multigravida | 56 (56.6) | 98 (60.5) |    |
| Grand multigravida | 4 (4) | 14 (8.6) |    |

### Previous abortion

|       | No     | Yes    | p     |
|-------|--------|--------|-------|
|       | 86 (86.9) | 13 (13.1) | 0.209 |

### Gestation age at enrolment (weeks)

|       | No     | Yes    | p     |
|-------|--------|--------|-------|
|       | 26 (20 - 32) | 25.9 (19.7 - 30.6) | 0.962 |

### Trimester at enrolment

|       | No     | Yes    | p     |
|-------|--------|--------|-------|
| 1     | 1 (1)  | 0 (0)  | 0.054 |
| 2     | 96 (97) | 162 (100)|    |
| 3     | 2 (2)  | 0 (0)  |    |

### Anthropometry

|       | No     | Yes    | p     |
|-------|--------|--------|-------|
| BMI (kg/m2) | 27.1 (23.2 - 30.9) | 27.5 (24.0 - 31.8) | 0.174 |
| Waist - Hip ratio | 0.9 (0.9 - 1.0) | 0.9 (0.9 - 1.0) | 0.238 |

### Blood pressure at enrolment

|       | No     | Yes    | p     |
|-------|--------|--------|-------|
| SBP (mmHg), average | 123 (114 - 131) | 122 (115 - 131) | 0.589 |
| DBP (mmHg), average | 77 (70.5 - 84.5) | 76 (72 - 84) | 0.896 |
Table 3. A multivariable logistic regression model showing factors associated with latent tuberculosis among study participants.

| Variables         | Adjusted Odds Ratio | 95% Confidence Interval | P-value |
|-------------------|---------------------|-------------------------|---------|
| **Age category**  |                     |                         |         |
| 15 - 19           | Reference           |                         |         |
| 20 - 29           | 1.4                 | 0.5 - 4.1               | 0.580   |
| 30 - 39           | 4.0                 | 1.2 - 12.7              | **0.021** |
| **Alcohol usage** |                     |                         |         |
| Never             | Reference           |                         |         |
| Former            | 1.0                 | 0.5 - 2.3               | 0.928   |
| Current           | 0.8                 | 0.3 - 1.9               | 0.595   |
| **HIV status**    |                     |                         |         |
| Negative          | Reference           |                         |         |
| Positive          | 4.4                 | 1.1 - 18.0              | **0.041** |
| **Gravidity**     |                     |                         |         |
| Primigravida      | Reference           |                         |         |
| Multigravida      | 0.9                 | 0.47 - 1.57             | 0.632   |
| Grand multigravida| 0.4                 | 0.11 - 1.5              | 0.173   |
Figure legends

Figure 1. QuantiFERON-TB Gold-plus results of the participants

Figure 2: Correlation of quantitative interferon-gamma values in TB1 and TB2 tubes

Figure 3: Quantitative interferon-gamma value in TB1 and TB2 tubes stratified by HIV status (A) and presence or absence of a BCG scar (B)
Figure 2

A scatter plot showing the relationship between TB1 (IU/mL) and TB2 (IU/mL). The correlation coefficient, \( r = 0.88 \), indicates a strong positive correlation between the two variables.
