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Effect of Diabetes Mellitus on Risk of Latent TB Infection after BCG Vaccination in A High TB Incidence Area: A Community-based Study in Taiwan

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Effect of Diabetes Mellitus on Risk of Latent TB Infection after BCG Vaccination in A High TB Incidence Area: A Community-based Study in Taiwan

Running title: Individuals with diabetes have a moderately increased risk of LTBI.

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ABSTRACT

Objectives: To investigate the latent tuberculosis infections (LTBIs) in patients with diabetes, especially in high TB incidence areas with a high coverage of Bacillus Calmette–Guérin (BCG) vaccination.

Design: prospective

Setting: thirteen secondary health care facilities

Participants: A total of 2948 patients with diabetes who were older than 40 years were recruited, and 453 non-diabetic participants from the community were enrolled.

Primary and secondary outcome measures: The interferon-gamma release assay (IGRA) and the tuberculin skin test were used to detect LTBI. The IGRA result was used as a surrogate of LTBI in logistic regression analysis.

Result: Diabetes was significantly associated with LTBI and age correlated positively with LTBI. Many subjects with diabetes also had additional risk factors including current smokers, comorbid chronic kidney disease, and prior history of TB. The presence of a BCG scar was protective, and the adjusted odds ratio for LTBI was 0.69 for those with 1 scar and 0.49 for those with 2 or more scars. BCG protection was not modified by the DM status and persisted in middle-aged adulthood until it waned in old age. Duration of diabetes and poor glycemic control were unrelated to the risk of LTBI.

Conclusion: BCG prevented acquisition of TB infection in diabetes population. There was a moderately increased risk of LTBI in diabetes patients from this high TB incidence area. This finding suggests incorporating other risk factors and comorbidities, in addition to diabetes, to better identify high-risk groups and improve the efficacy of targeted screening for LTBI.

Keywords: Diabetes Mellitus, Tuberculosis, Latent Infection, Bacillus Calmette–Guérin vaccination

Strengths and limitations of this study

1. A total of 2948 patients with diabetes who were older than 40 years were recruited.

2. The interferon-gamma release assay and the tuberculin skin test were used to detect latent tuberculosis infections.

3. Participants in this study might generally have better general health status because they were enrolled from the national diabetes disease management program, which may underestimate the risk of tuberculosis infection.

Abbreviations

Bacillus Calmette–Guérin (BCG)

Changhua Christian Healthcare System (CCHS)

Changhua Community-based Integrated Screening (CHCIS)

Chronic kidney disease (CKD)

Diabetes mellitus (DM)

Glycated hemoglobin (HBA1c)

Interferon-gamma release assay (IGRA)

Latent TB infections (LTBIS)

Tuberculin skin test (TST)

Tuberculosis (TB)

BACKGROUND

The co-occurrence of diabetes mellitus (DM) and tuberculosis (TB) cannot be overemphasized because of the high prevalence of each disease throughout the world.[1] Previous studies found that DM affects TB disease presentation, leads to poor treatment outcomes, and increases the risk for active TB.[1, 2] The recently published World Health Organization guideline recommends screening for active TB with DM ,[3] but it remains elusive whether screening and treatment of latent TB infections (LTBIs) should be prioritized to target individuals with DM.[1, 4-6]

There is only limited information on the LTBI status of patients with DM. Most of antecedent relevant studies were in low TB incidence countries [7] or based on selected population by identifying DM in high-risk populations, such as TB contacts, immunocompromised patients with comorbid DM, or crisis-affected people. [6] The results of these studies are also inconsistent, mainly due to the different methods used to ascertain DM status (*e.g.* self-report, medical records, or laboratory testing) and differences in the control of potentially important confounders.[1, 5-7]

In high TB incidence areas, where most of TB infection occurred at young age before the onset of DM, the temporal relationship between DM and TB infection is different from that in low incidence areas (Figure 1).[6, 8] This temporal characteristic may lead to non-differential misclassification of DM-related TB infection in cross-sectional studies and therefore bias the association toward the null (Figure 1c).[6] It is also worth noting that Bacillus Calmette–Guérin (BCG) vaccine is widely used in high burden TB countries for childhood immunization against TB. Recent studies found BCG vaccine has a protective effect against TB infection in children [9] and can even last into adulthood.[10] Although several researches have been devoted to assessment of DM-LTBI association, rather less attention has been paid to confounding from the protection conferred by BCG vaccination in settings where coverage of BCG vaccination in study subjects was high. Furthermore, while DM occurs most often in middle-aged and older people, few attempts have been made in this population to examine whether BCG protection is modified by the status of DM and whether the protection wanes with age in older adults.

In this study, using data from community-based screening programs in an area with a high incidence of TB and a high coverage of BCG vaccination, we assessed the overall risk of LTBI in people with and without DM by carefully controlling for potential confounders, including BCG vaccination, risk of infection with TB, comorbidities, important lifestyle factors and DM severity. We also investigated the effect modification of DM and age on the protective effect of BCG vaccination.

METHODS

Patient and Public Involvement

We conducted a community-based study that comprised DM and non-DM subjects by combining two community-based programs to investigate the effect of DM on risk of LTBI in Changhua, a county in Taiwan with a TB incidence of 58.7 per 100000 and a BCG vaccination coverage of around 99% in 2012.[11] The first program recruited the DM group from patients registered in the Changhua Diabetes Shared Care program [12] (CHDSC) to participate in the LTBI survey. The second recruited the community comparison (CC) group from participants of a community-based LTBI screening program. Due to the lack of a gold-standard test for LTBI, the tuberculin skin test (TST) was used in parallel with the interferon-gamma release assay (IGRA). All participants provided signed informed consent before enrollment. Both studies were approved by the Institutional Review Board of the Changhua Christian Hospital (numbers IRB:CCH-130102 and IRB:CCH-111012).

DM group

Nearly 60% of DM patients in Changhua were enrolled in the national diabetes disease management program (DDMP) and registered in the CHDSC.[12] The DM status of enrollees was ascertained by certified physicians according to national guidelines. We prospectively invited all registered cases who were more than 40 years-old, from April 1, 2013 to December 31, 2013, when they presented to diabetes outpatient clinics of the Changhua Christian Healthcare System (CCHS) or 13 nearby health centers in the surrounding townships. CCHS comprises one medical center and three branch hospitals, distributed evenly at different locations of the county, and covers urban and rural areas. Thus, the participants of this study were a representative sample of the total DM population.

All study subjects were screened for pulmonary TB based on respiratory symptoms and chest X-rays upon entry into the study. Suspected cases submitted sputum specimens for acid-fast bacilli smears and culture. The diagnosis of TB was confirmed by chest specialists. Subjects were excluded if they had active pulmonary TB, a life expectancy of less than 2 years, metastatic cancer, or organ failure (*e.g.* severe liver disease [Child-Pugh Class B or C]) except chronic renal failure. The included eligible DM cases then received a TST and IGRA for detection of LTBI.

Community comparison group

The Changhua Community-based Integrated Screening (CHCIS) program, which began in 2005, screens for neoplastic and non-neoplastic diseases (including DM and pulmonary TB).[13] The method used to screen for pulmonary TB was the same as that used in the DM group. We invited all consecutive attendees of the CHCIS program within the major catchment area of the CCHS to participate in LTBI screening in May 1, 2011. Participants with known DM or newly screened DM (*i.e.* fasting plasma glucose [FPG] $\geqq$ 126 mg/dL) were excluded.

Tests for LTBI

Venous blood was collected for the QuantiFERON-TB Gold In-Tube test (QFT-GIT; Cellestis, Carnegie, Australia), which was performed in 2 stages, according the manufacturer's instructions. The cutoff for a positive result was 0.35 IU/mL. The reaction of a nil control and a mitogen control were within the range provided by the manufacturer. After collection of blood samples for QFT-GIT, trained nurses administered the TST using 2 TU of PPD RT23 (Statens Serum Institut, Copenhagen, Denmark) by the Mantoux method, and inspected the presence of BCG scars. Tuberculin indurations were measured 48-72 h after injection using the palpation method, and a diameter of 10 mm or more was defined as positive. All TST procedures followed the national guidelines issued by the CDC of Taiwan.[14]

Data collection

We examined the DDMP database, and abstracted demographic data and information on DM care in the one year before each subject's entry. This included duration of DM, glycated hemoglobin (HbA1c), blood pressure, lipid profile, renal function, and other related cardiovascular disease risk factors. We also linked individual data with the TB registry at the local health authority to assess whether each study subject had a prior history of TB or contact with TB.

Statistical analysis

Although both TST and IGRA indicate a cellular immune response to Mycobacterium tuberculosis (MTB) and are useful for the diagnosis of LTBI, the two tests identified different population with distinct immunologic processes.[15] Tuberculin reactivity represents the cumulative effect of previous TB infection (caused by recent and/or remote infection) because after infection with TB, positive results often remain lifelong until old age. On the contrary, IGRA has a dose–response relationship with recent TB exposure but it wanes rapidly.[15, 16] Thus, for adjusting for the bias of DM-related TB infection resulting from the occurrence of TB before DM (Figure 1c) as explained earlier, we used the result of the QFT-GIT as a surrogate of LTBI status in the logistic regression models designed to identify risk factors for LTBI. The TST results were then included in the models for controlling the confounding of remote TB infection. We also tested for effect modification of BCG protection by age and DM by including interactions terms (ie.

Age×BCG scar and DM×BCG scar) in the regression analysis. All analyses were conducted using SAS version 9.3 (SAS Institute Inc., Cary, NC, USA).

RESULTS

Characteristics of Participants

We ultimately enrolled 2948 patients in the DM group and 453 non-DM subjects in CC group, and included all of these individuals in the final analysis (Figure 2). The mean duration of DM in the DM group was 9.0 years (standard deviation [SD]=6.6) and nearly half of these patients achieved good glycemic control (*i.e.* HbA1c < 7%) (Table 1). The DM group had a greater mean age, higher percentages of males and smokers, greater prevalences of obesity, chronic kidney disease (CKD), and prior history of TB. The DM group also had a greater prevalence of tuberculin reactivity (≧ 15mm) and of QFT-GIT positivity, and a higher proportion of indeterminate results (Table 1).

Table 1. Characteristics of the DM group and the community comparison (CC) group.

	DM group (n=2948)	CC group (n=453)	p
Age, years			
Mean (SD)	61.5 (9.3)	51.3 (10.5)	**<0.001**
<50	304 (10.3%)	209 (46.1%)	
50-59	918 (31.1%)	137 (30.2%)	**<0.001**
60-69	1123 (38.1%)	91 (20.1%)	
≥70	603 (20.5%)	16 (3.5%)	
Sex			
Male	1468 (49.8%)	109 (24.1%)	**<0.001**
Female	1480 (50.2%)	344 (75.9%)	
Prior history of TB			
Yes	61 (2.1%)	4 (0.9%)	0.0852
No	2887 (97.9%)	449 (99.1%)	
History of contact with TB			
Yes	115 (3.9%)	65 (14.3%)	
No	2283 (77.4%)	388 (85.7%)	**<0.001**
Unknown	550 (18.7%)	0 (0.0%)	
BCG scar			
Yes	2436 (82.6%)	440 (97.1%)	
1 scar	1481 (50.2%)		
2 scar	934 (31.7%)		**<0.001**
≧2 scars	21 (0.7%)		
No	502 (17.0%)	13 (2.9%)	
Unknown	10 (0.3%)		
BMI, kg/m^2			
Underweight (<18.5)	50 (1.7%)	7 (1.5%)	
Normal (18.5-24.9)	1175 (39.9%)	274 (60.5%)	**<0.001**
Overweight (25-29.9)	1265 (42.9%)	149 (32.9%)	
Obese (≧30)	458 (15.5%)	23 (5.1%)	
Smoking status			
Current	433 (14.7%)	31 (6.8%)	
Quit	434 (14.7%)	34 (7.5%)	**<0.001**
Never	2081 (70.6%)	388 (85.7%)	
Triglycerides, mg/dL			
<150 mg/dl	1962 (66.6%)	371 (81.9%)	**<0.001**
≧150	986 (33.4%)	82 (18.1%)	

HDL-C*			
Low	1336 (45.3%)	299 (66.0%)	**<0.001**
Ideal	1612 (54.7%)	154 (34.0%)	
CKD**			
Yes	948 (32.2%)	57 (12.6%)	
No	1941 (65.8%)	375 (82.8%)	**<0.001**
Unknown	59 (2.0%)	21 (4.6%)	
Duration of diabetes, years			
mean (SD)	9.0 (6.6)		
≦5	1077 (36.5%)		
>5	1871 (63.5%)		
HbA1c			
Mean (SD)	7.4 (1.5)		
<7%	1400 (47.5%)		
≧7%	1548 (52.5%)		
Unknown	6 (0.2%)		
TST positive			
≧5 mm	2280 (77.3%)	350 (77.3%)	0.9895
<5 mm	668 (22.7%)	103 (22.7%)	
≧10 mm	1665 (56.5%)	251 (55.4%)	0.6974
<10 mm	1283 (43.5%)	202 (44.6%)	
≧15 mm	890 (30.2%)	112 (24.7%)	**0.0211**
<15mm	2058 (69.8%)	341 (75.3%)	
QFT-GIT			
Positive	623 (21.1%)	44 (9.7%)	
Negative	2144 (72.7%)	406 (89.6%)	**<0.001**
Indeterminate	181 (6.1%)	3 (0.7%)	

Abbreviations: SD, standard deviation; HbA1c, glycated hemoglobin; BMI, body mass index; HDL-C, high density lipoprotein cholesterol; TST, tuberculin skin test; QFT-GIT, QuantiFERON-TB Gold In-Tube.

*Low HDL-C: <40 mg/dL (males) and <50 mg/dL (females).

**CKD (chronic kidney disease) was assessed by the Modification of Diet in Renal Disease (MDRD) study equation, using the estimated glomerular filtration rate (eGFR).

TST and QFT-GIT results

The presence of BCG scars had no effect on the tuberculin reactivity of the DM group (Figure 3a). TST positivity decreased with age, and was higher in the DM group than the CC group, except among the elderly. Figure 3b shows that QFT-GIT positivity of DM group was highest among those with no BCG scars, and that positivity declined dramatically in those more than 60 years-old. In contrast, the rate of QFT-GIT positivity in those with BCG scars gradually increased with age. For each age group, there was an inverse correlation between number of BCG scars and QFT-GIT positivity. Individuals in the CC group with BCG scars had the lowest QFT-GIT positivity, but elderly individuals from the CC group (age 70+) had the highest QFT-GIT positivity. Generally, the concordance between the TST and QFT-GIT was low (Supplementary Table 1).

Effect of DM and other factors on risk of LTBI

Multivariate regression analysis indicates DM significantly increased the risk of LTBI after controlling for major confounders (aOR=1.67; 95%CI, 1.18-2.38). This risk was similar after adjustment for tuberculin reactivity (aOR=1.59; 95% CI, 1.11-2.28). In addition, the presence of LTBIs increased with age. For age older than 70 years, TST adjustment had a marked effect and changed its aOR from 4.27 (95%

CI, 2.83-6.44) to 2.98 (95% CI, 2.00-4.44). Other variables, such as current smoking, CKD, and prior history of TB, were also significant risk factors for LTBI, and their effects were similar with or without adjustment for tuberculin reactivity. Notably, the presence of a BCG scar had a significantly protective effect on LTBI (aOR=0.66 [95%CI, 0.51-0.85]) (Table 2, Model 2); those with 1 scar had an aOR of 0.69 (95% CI, 0.52-0.90) and those with 2 or more scars had an aOR of 0.49 (95% CI, 0.36-0.67) (Table 2, Model 1).

Table 2. Logistic regression analysis of factors associated with LTBI in the DM group (model 1) and in the DM and CC groups combined (model 2), without and with adjustment for TST results.

	Model 1. DM group (n = 2767)				Model 2. DM and CC groups (n = 3217)		
Variable	Crude OR (95% CI)	aOR (95% CI)	aOR (95% CI) (TST adjustment)		Crude OR (95% CI)	aOR (95% CI)	aOR (95% CI) (TST adjustment)
				Age×BCG§			
TST 10+	3.85 (3.11-4.76)		4.77 (3.80-5.99)	4.75 (3.79-5.97)	3.66 (2.99-4.49)		4.56 (3.67-5.66)
DM					2.68 (1.94-3.71)	1.67 (1.18-2.38)	1.59 (1.11-2.28)
Age, years							
<50	Reference	Reference	Reference	Reference	Reference	Reference	Reference
50-59	1.63 (1.11-2.41)	1.85 (1.23-2.79)	2.20 (1.45-3.32)	1.33 (0.27-6.48)	1.98 (1.42-2.76)	1.86 (1.30-2.65)	2.12 (1.48-3.05)
60-69	2.12 (1.45-3.09)	2.28 (1.52-3.41)	2.96 (1.97-4.47)	0.73 (0.16-3.39)	2.52 (1.83-3.49)	2.25 (1.58-3.20)	2.77 (1.93-3.97)
≥70	3.19 (2.15-4.71)	2.84 (1.82-4.45)	4.35 (2.74-6.89)	1.10 (0.24-4.95)	4.07 (2.89-5.71)	2.98 (2.00-4.44)	4.27 (2.83-6.44)
Age×BCG§							
<50				0.16 (0.04-0.75)			
50-59				0.34 (0.08-1.52)			
60-69				0.54 (0.12-2.40)			
≥70				5.23 (1.08-25.40)			
Male	1.28 (1.07-1.54)	1.19 (0.94-1.50)	1.09 (0.85-1.38)	1.11 (0.87-1.41)	1.41 (1.19-1.68)	1.22 (0.98-1.52)	1.12 (0.89-1.41)

Smoking Never Current Quit	 Reference 1.53 (1.20-1.93) 0.93 (0.71-1.21)	 Reference 1.61 (1.2-2.15) 0.88 (0.64-1.2)	 Reference 1.36 (1.00-1.83) 0.82 (0.59-1.13)	 Reference 1.38 (1.02-1.86) 0.83 (0.60-1.15)	 Reference 1.54 (1.22-1.95) 1.00 (0.77-1.29)	 Reference 1.49 (1.13-1.97) 0.87 (0.64-1.17)	 Reference 1.28 (0.95-1.71) 0.82 (0.60-1.11)
CKD	1.46 (1.21-1.76)	1.20 (0.98-1.47)	1.30 (1.05-1.60)	1.29 (1.04-1.59)	1.56 (1.31-1.87)	1.17 (0.96-1.42)	1.26 (1.03-1.55)
DM 5+ yrs	0.95 (0.79-1.15)	0.84 (0.69-1.02)	0.79 (0.64-0.97)	0.78 (0.63-0.96)			
A1C 7+%	0.91 (0.76-1.08)	0.91 (0.75-1.10)	0.91 (0.75-1.11)	0.92 (0.76-1.12)			
BCG scar No Yes 1 scar 2+ scars	 Reference 0.63 (0.50-0.79) 0.43 (0.33-0.56)	 Reference 0.76 (0.59-0.99) 0.55 (0.41-0.74)	 Reference 0.69 (0.52-0.90) 0.49 (0.36-0.67)		 Reference 0.51 (0.41-0.63)	 Reference 0.73 (0.57-0.93)	 Reference 0.66 (0.51-0.85)
PHx TB	2.99 (1.78-5.03)	2.35 (1.36-4.05)	2.21 (1.25-3.91)	2.16 (1.22-3.83)	2.95 (1.78-4.89)	2.17 (1.28-3.68)	2.08 (1.19-3.63)
TB contact	0.82 (0.50-1.32)	0.83 (0.50-1.36)	0.75 (0.45-1.25)	0.73 (0.43-1.22)	0.75 (0.50-1.13)	0.92 (0.60-1.40)	0.86 (0.55-1.33)

Abbreviations: DM, the diabetes mellitus; CC, community comparison; OR, odds ratio; aOR, adjusted odds ratio; CI, confidence interval; TST 10+, results of tuberculin skin test ≧ 10mm; BMI, body mass index; CKD, chronic kidney disease; DM 5+ yrs, duration of diabetes mellitus ≧5 years; A1C, glycated hemoglobin. PHx TB, prior history of TB. § Measure of effect modification of the association between LTBI and BCG scar by age on multiplicative scale.

The interaction term DM×BCG scar added to the regression model did not show a significant effect. For assessing effect modification of the association between LTBI and BCG vaccination by age, the people aged younger than 50 years without BCG scar were taken as the reference group. The aORs of the interaction terms (age×BCG scar) were 0.2 (95%CI, 0.0-0.7) for ages < 50 years, 0.3 (95%CI, 0.1-1.5) for ages 50-60 years, 0.5 (95%CI, 0.1-2.4) for ages 60-70 years, and 5.2 (95%CI, 1.1-25.4) for ages 70+ years respectively. (Table 2, Model 1)

Comorbidities, such as hyperlipidemia, hypertension, and abnormal body mass index (BMI), had no significant associations with LTBI. We further investigated the effect of long duration DM and poor glycemic control by dividing the DM group into four sub-groups according to duration of DM and HbA1c level, and then compared the risk of LTBI of these different sub-groups, using the CC group as a reference. All of the DM sub-groups had similar risks for LTBI. This indicates that long duration of DM and poor glycemic control had no effect on the risk of LTBI (Supplementary Table 2). However, among the DM group, those people with DM history for 5+ years had an aOR of 0.78 (95%CI, 0.63-0.96) (Table 2, Model 1).

DISCUSSION

Very few studies have examined the effect of DM on the risk of LTBI in high TB incidence areas and simultaneously taken into account the protection of BCG against TB infection. To our best knowledge, this is the first study to describe the association between LTBI and BCG vaccination in diabetes population. In this community-based study, we found BCG had a protective effect on TB infection in adults with an aOR of 0.66 (95%CI, 0.51-0.85), which did not vary by the status of DM. BCG protection correlated positively with the number of BCG scars. This effect also persisted in middle-aged adulthood until it waned in old age. Meanwhile, by use of stringent diagnostic criteria and adjusting for major confounding variables in an effort to overcome the limitations of many previous studies, our multivariate analysis indicated that DM had a positive association with risk of LTBI (aOR, 1.59 [95%CI, 1.11-2.28]). A recently published population-based study in US, a country where BCG is not generally recommended for use, using similar diagnostic criteria as this study, showed DM was associated with LTBI with an aOR 1.5 (95% CI, 1.0–2.2),[7] very similar to our finding.

Until recently, there has been a scarcity of evidence on whether BCG can prevent acquisition of TB infection because conventional measurement of LTBI utilizing TST cannot distinguish if a positive response is due to MTB infection, or BCG vaccination, or non-tuberculous mycobacterial (NTM) infection.[9, 17] Unlike TST, the recently developed IGRAs use MTB specific antigens that do not cross react with BCG. Studies have been conducted to investigate BCG protection on LTBI measured as positive responses on IGRA. A recent meta-analysis of 14 studies and 3855 child or adolescent participants (< 16 years) suggested that BCG is associated with protection from TB infection (overall risk ratio, 0.81; 95% CI, 0.71 to 0.92).[9] Three additional studies in high burden TB countries, not included in the meta-analysis, revealed that the protection existed in young adults decades after BCG vaccination with aORs ranging from 0.6 (95%CI, 0.2–1.8) to 0.52 (95% CI, 0.32-0.85),[10, 18, 19] close to our estimate.

What is the duration of BCG protection against TB infection is a question not yet determined. A long-term follow-up of trial participants of a BCG vaccine in US Native-American populations reported good protective efficacy against TB disease that extended up to 60 years after vaccination.[21] By contrast, new research using a case–control study design in the UK found BCG protection lasted for 20 years.[22]

Nonetheless, since in our regression model, the aORs of interaction terms DM×BCG raised dramatically to 5.2 (95%CI, 1.1-25.4) in the age group of 70+ years (i.e. a cohort of school-aged BCG vaccination as described above), it is reasonable to assume that BCG protection against TB infection may last 60 years and then waned, an estimate in accordance with the US study.

Since DM is a progressive disease, longer diabetes duration was found to be a major predictor of DM-related complications and death, independent of glycemic control.[23] An earlier study also revealed the risk of developing TB disease increased among those with increasing diabetes severity.[2] Thus, in this study, we investigated the combined effect of longer duration of DM and poor glycemic control (Supplementary Table 2). We found they did not affect the risk of LTBI. Similarly, association between glycemic control and risk of TB was not observed in other studies targeting DM cases under routine medical care for years.[26, 27]

Many of the subjects with DM in the present study also smoked (14.7%), had abnormal BMIs (overweight and obese, 58.4%; underweight, 1.7%), CKD (32.2%), prior history of TB (2.1%), and advanced age (58.6% older than 60 years), and these may also be risk factors for TB.[30-32] We found that each factor alone had only a mild to moderate association with LTBI (Table 2) after adjustment of BCG protection. However, when an individual has all of these other factors as well as DM, there may be a particularly high-risk of LTBI, especially in those who are elderly. For example, male DM patients older than 60 years who smoke have an aOR of LTBI up to 6.7 (derived by summation of the estimated regression coefficients). Conventional targeted screening for LTBI mostly focuses on host variables and the environment, such as infectiousness of index cases and contact patterns, but seldom consider the effects of multiple factors simultaneously.[33, 34] Our results underscore the necessity of incorporating DM and related risk factors to develop a composite scoring system that improves the efficacy of LTBI screening programs.

There are some limitations in our study. First, although the *in vivo* TST and *ex vivo* IGRAs are the only two methods available for diagnosing LTBI, there is concern that immune dysfunction in DM may compromise the performance of these tests.[5] Second, all DM patients were enrolled from the DDMP, an intervention designed to facilitate lifestyle modification in patients with DM.[12] Thus, these study subjects may have better general health status, and hence lower risk of TB infection, than DM patients from the general population.[27]

The comorbidity of TB and DM is due to the interaction between DM-impaired immunity and the occurrence of active TB by endogenous reactivation (Figure 1c) or exogeneous new or reinfection (Figure 1a, 1b).[1, 5] This process is further complicated by the protection conferred from BCG vaccination, social environment, the co-existence of multiple non-communicable risk factors and other related comorbidities and complications.[5, 32, 40] We tried to control for all possible confounders, but this remains challenging due to the lack of a gold standard for diagnosis of LTBI and the presence of only limited tools to identify DM patients who have the greatest risk of progressing to active TB. Studies are therefore required to identify the predictive value for progression to active TB based on IGRA and/or TST results in patients with DM. There is an ongoing longitudinal study of the present study cohort.

CONCLUSION

In conclusion, our study demonstrated BCG had an apparent protective effect on TB infection in adults and there were a 1.59-fold increased risk of LTBI in patients with DM from a geographic area that has a high incidence of TB and a high coverage of BCG vaccination. This finding suggests that practitioners should incorporate other coexisting risk factors and comorbidities, in addition to DM, to better identify high-risk groups and enhance the efficacy of targeted screening for LTBI. Efficacy of new vaccines against TB infection should be a major focus of TB vaccine development.

DECLARATIONS

Authors' contributions:

1 Guarantor of integrity of the entire study: Ching-Hsiung Lin, Shu-Chen Kuo, Yen-Po Yeh, Shih-Te Tu

2 study concepts: Ching-Hsiung Lin, Shu-Chen Kuo, Ming-Chia Hsieh, Ih-Jen Su, Sheng-Hao Lin , Yen-Po Yeh, Shih-Te Tu

3 study design: Ching-Hsiung Lin, Shu-Chen Kuo, Ming-Chia Hsieh, Ih-Jen Su, Sheng-Hao Lin , Yen-Po Yeh, Shih-Te Tu

4 definition of intellectual content: Ching-Hsiung Lin, Shu-Chen Kuo, Ih-Jen Su, Yee-Chun Chen, Yen-Po Yeh, Shih-Te Tu

5 literature research: Shu-Chen Kuo, Ming-Chia Hsieh, Chia-Yu Chi, Yee-Chun Chen, Horng Yunn Dou

6 clinical studies: Ming-Chia Hsieh, Ching-Hsiung Lin, Shang-Yun Ho, Sheng-Hao Lin, Shih-Li Su, Chiung-Ying Liao, Shang-Ren Hsu, Yuan-Chun Huang, Shu Yi Wang, Shi-Dou Lin, Yen-Po Yeh, Shih-Te Tu

7 experimental studies: Ching-Hsiung Lin, Shu-Chen Kuo, Ming-Chia Hsieh, Ih-Jen Su, Chia-Yu Chi, Chiung-Ying Liao, Yuan-Chun Huang, Horng Yunn Dou Jen-Shiou Lin, Yen-Po Yeh, Shih-Te Tu

8 data acquisition: Ming-Chia Hsieh, Shang-Yun Ho, Sheng-Hao Lin, Shih-Li Su, Chiung-Ying Liao, Shang-Ren Hsu, Yuan-Chun Huang, Shu Yi Wang, Shi-Dou Lin

9 data analysis: Shu-Chen Kuo, Ming-Chia Hsieh, Shang-Yun Ho, Chia-Yu Chi, Chiung-Ying Liao, Yuan-Chun Huang, Fan-Chen Tseng, Yen-Po Yeh, Shih-Te Tu

10 statistical analysis: Ming-Chia Hsieh, Fan-Chen Tseng, Yen-Po Yeh,

11 manuscript preparation: Ching-Hsiung Lin, Shu-Chen Kuo, Sheng-Hao Lin, Yen-Po Yeh, Shih-Te Tu

12 manuscript editing: Ching-Hsiung Lin, Shu-Chen Kuo, Sheng-Hao Lin, Yen-Po Yeh, Shih-Te Tu

13 manuscript review: Ching-Hsiung Lin, Ming-Chia Hsieh, Sheng-Hao Lin , Yee-Chun Chen, Yen-Po Yeh, Shih-Te Tu

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Conflict of interest statements:

All authors declare that they have no conflict of interest.

Role of funding source:

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Ethics committee approval:

All participants provided signed informed consent before enrollment. Both studies were approved by the Institutional Review Board of the Changhua Christian Hospital (numbers IRB:CCH-130102 and IRB:CCH-111012).

Availability of data and material: Not applicable.

Consent for publication: Not applicable.

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FIGURE LEGENDS

Figure 1 Possible temporal relationships between the onset of DM and the occurrence of TB infection. Circled letters indicate times when DM could possibly affect the pathogenesis of TB (i, increased susceptibility to TB infection; p, accelerated progression from infection to clinical disease). (a) Onset of DM before the primary TB infection. (b) Onset of DM with a pre-existing latent TB infection (LTBI), but before re-infection. (c) Onset of DM with pre-existing LTBI.

Figure 2 Patient selection and enrolment in the DM group (left) and the CC group (right).

Abbreviations: TST, tuberculin skin test; IGRA, interferon-gamma release assay; CHCIS, Changhua Community-based Integrated Screening program; DM, diabetes mellitus.

Figure 3 Tuberculin skin test (TST) positivity (a) and interferon-gamma release assay (IGRA) positivity (b) in the DM group (DMG) and the CC group (CCG) according to age and BCG scar status. A tuberculin induration size of 10 mm was used as the cutoff point for both groups.

Abbreviations: TST, tuberculin skin test; QFT, QuantiFERON-TB Gold In-Tube.

Figure 1

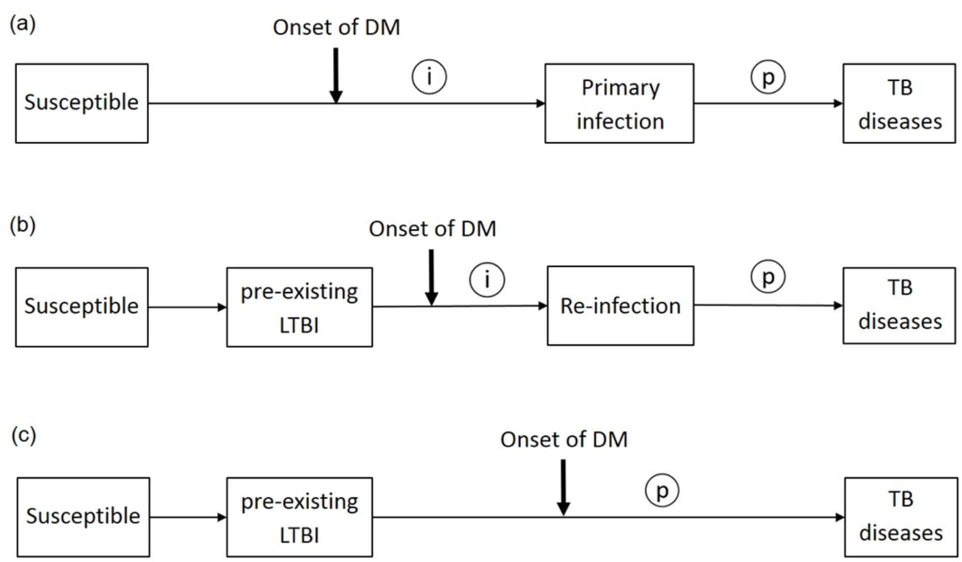

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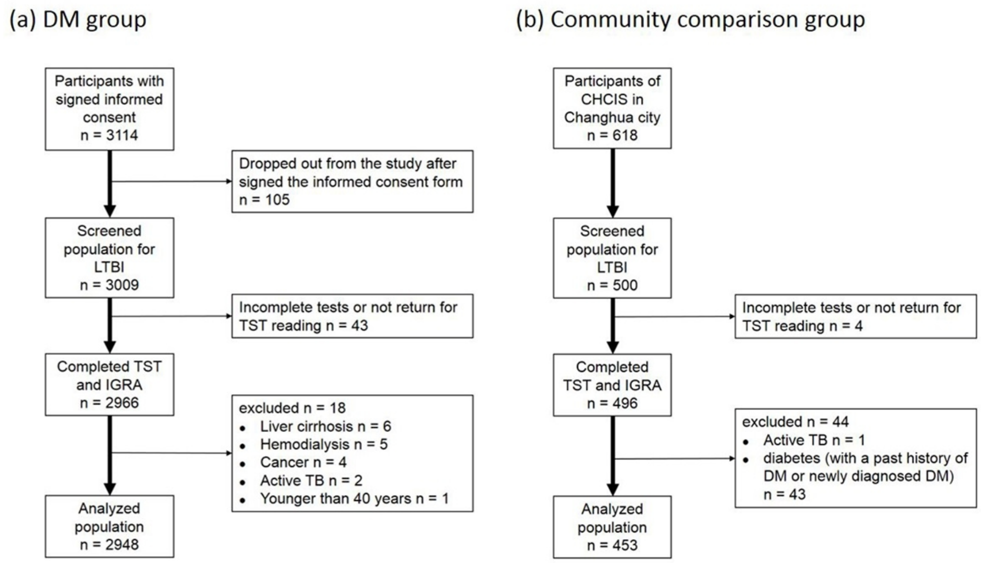
Figure 2

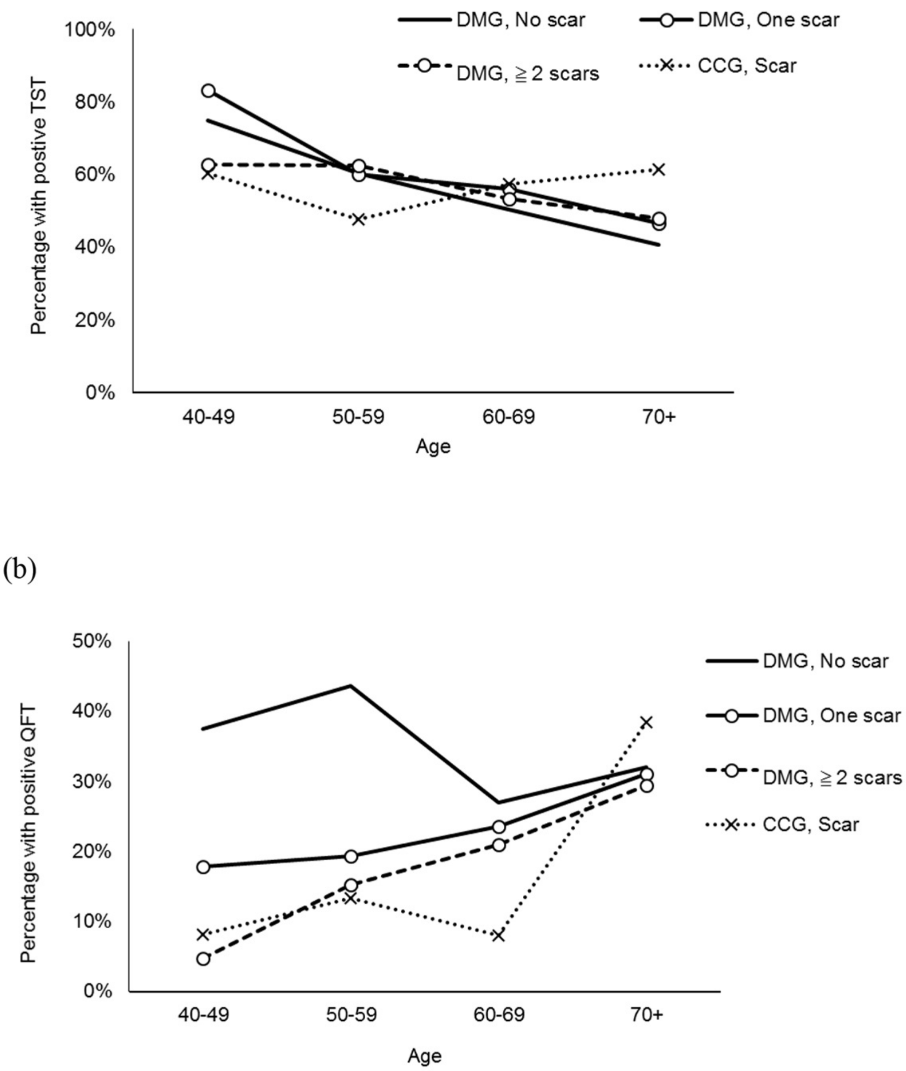


99x65mm (300 x 300 DPI)

Figure 3

(a)

(b)



99x127mm (300 x 300 DPI)

Supplementary Table 1. Agreement between TST and QFT-GIT

Results	Overall n = 3217			DM group n = 2767			CC group n = 450		
	TST cut point			TST cut point			TST cut point		
	5 mm	10 mm	15 mm	5 mm	10 mm	15 mm	5 mm	10 mm	15 mm
TST+/QFT-GIT+	613	528	363	37	32	23	576	496	340
TST-/QFT-GIT-	664	1252	1950	95	188	318	569	1064	1632
TST+/QFT-GIT-	1886	1298	600	311	218	88	1575	1080	512
TST-/QFT-GIT+	54	139	304	7	12	21	47	127	283
Agreement%	39.7%	55.3%	71.9%	29.3%	48.9%	75.8%	41.4%	56.4%	71.3%
Kappa (95% CI)	0.09 (0.74-0.10)	0.17 (0.15-0.20)	0.27 (0.23-0.30)	0.10 (0.08-0.12)	0.19 (0.16-0.22)	0.27 (0.23-0.31)	0.02 (-0.01-0.05)	0.06 (0.01-0.11)	0.18 (0.09-0.28)

Abbreviations: TST, tuberculin skin test; QFT-GIT, QuantiFERON-TB Gold In-Tube; CI, confidence interval.
Test results with an indeterminate QFT-GIT response are not included in the table.

Supplementary Table 2. Combined effects of glycemic control (HbA1c) and duration of DM on risk of latent tuberculosis infection without and with adjustment for TST results.

Variables	Crude OR (95% CI)	aOR* (95% CI)	aOR* (95% CI) (TST adjustment)
Community comparison group	Reference	Reference	Reference
A1C ≧7%, duration >5 years	2.49 (1.77-3.52)	1.42 (0.97-2.07)	1.33 (0.90-1.96)
A1C ≧7%, duration ≦5 years	2.71 (1.84-3.98)	1.87 (1.24-2.82)	1.80 (1.18-2.74)
A1C <7%, duration >5 years	2.84 (1.99-4.04)	1.67 (1.13-2.46)	1.54 (1.03-2.28)
A1C <7%, duration ≦5 years	2.81 (1.95-4.04)	1.84 (1.24-2.72)	1.82 (1.22-2.72)

Abbreviations: OR, odds ratio; aOR, adjusted odds ratio; CI, confidence interval; TST, tuberculin skin test; A1C, glycated hemoglobin.
*The multivariable model adjusted for the comorbidities indicated in Table 3, Model 2.

STROBE 2007 (v4) checklist of items to be included in reports of observational studies in epidemiology*

Checklist for cohort, case-control, and cross-sectional studies (combined)

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	4
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	4
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	8-9
Objectives	3	State specific objectives, including any pre-specified hypotheses	9
Methods			
Study design	4	Present key elements of study design early in the paper	10
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	10
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	10-12
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	11-13
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*11-12*
Bias	9	Describe any efforts to address potential sources of bias	-
Study size	10	Explain how the study size was arrived at	10-11
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	10-12
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	13-14
		(*b*) Describe any methods used to examine subgroups and interactions	13-14
		(*c*) Explain how missing data were addressed	-
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed	-

		Cross-sectional study—If applicable, describe analytical methods taking account of sampling strategy	
		(*e*) Describe any sensitivity analyses	-
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	14
		(b) Give reasons for non-participation at each stage	-
		(c) Consider use of a flow diagram	-
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	14-16
		(b) Indicate number of participants with missing data for each variable of interest	-
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	17-21
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	17-21
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	17-21
		Cross-sectional study—Report numbers of outcome events or summary measures	17-21
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	17-21
		(*b*) Report category boundaries when continuous variables were categorized	17-21
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	-
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	
Discussion			
Key results	18	Summarise key results with reference to study objectives	21-25
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	24
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	24-25
Generalisability	21	Discuss the generalisability (external validity) of the study results	24-25
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	28

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

Effect of Diabetes Mellitus on Risk of Latent TB Infection after BCG Vaccination in A High TB Incidence Area: A Community-based Study in Taiwan

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Effect of Diabetes Mellitus on Risk of Latent TB Infection after BCG Vaccination in A High TB Incidence Area: A Community-based Study in Taiwan

Running title: Individuals with diabetes have a moderately increased risk of LTBI.

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ABSTRACT

Objectives: To investigate latent tuberculosis infections (LTBIs) in patients with diabetes, especially in high TB incidence areas with a high coverage of BCG vaccination.

Design: Community-based comparison study

Setting: Outpatient diabetes clinics at 4 hospitals and 13 health centers in urban and rural townships. A community-based screening program was used to recruit non-diabetic participants.

Participants: A total of 2948 patients with diabetes aged older than 40 years were recruited, and 453 non-diabetic participants from the community were enrolled.

Primary and secondary outcome measures: The interferon-gamma release assay (IGRA) and the tuberculin skin test were used to detect LTBI. The IGRA result was used as a surrogate of LTBI in logistic regression analysis.

Result: Diabetes was significantly associated with LTBI ([aOR] = 1.59; 95% CI, 1.11-2.28) and age correlated positively with LTBI. Many subjects with diabetes also had additional risk factors (current smokers [aOR=1.28; 95% CI, 0.95-1.71], comorbid chronic kidney disease [aOR = 1.26; 95% CI, 1.03-1.55], and prior history of TB [aOR = 2.08; 95% CI, 1.19-3.63]). The presence of a BCG scar was protective, and the adjusted odds ratio for LTBI was 0.69 (95% CI, 0.52-0.90) for those with 1 scar and 0.49 (95% CI, 0.36-0.67) for those with 2 or more scars. BCG protection was not modified by the DM status and persisted in middle-aged adulthood until it waned in old age. Duration of diabetes and poor glycemic control were unrelated to the risk of LTBI.

Conclusion: BCG prevented acquisition of TB infection in diabetes population. There was a moderately increased risk of LTBI in diabetes patients from this high TB incidence area. This finding suggests incorporating BCG vaccination, comorbidities and

other risk factors, in addition to diabetes, to better identify high-risk groups and improve the efficacy of targeted screening for LTBI.

Keywords: Diabetes Mellitus, Tuberculosis, Latent Infection, Bacillus Calmette–Guérin vaccination, Interferon-gamma release assay, Tuberculin skin test

Strengths and limitations of this study

1. A total of 2948 patients with diabetes (DM) aged older than 40 years were recruited.

2. The interferon-gamma release assay and the tuberculin skin test (TST) were used to detect latent tuberculosis infections (LTBIs). TST results were included in the regression models for adjusting the effect of remote TB infection.

3. BCG vaccine has a protective effect against TB infection in DM population, whose confounding was controlled for assessing overall risk of LTBI in people with and without DM.

4. DM Participants in this study might generally have better general health status because they were enrolled from the national diabetes disease management program, which may underestimate the risk of LTBI.

Abbreviations

Bacillus Calmette–Guérin (BCG)

Changhua Christian Healthcare System (CCHS)

Changhua Community-based Integrated Screening (CHCIS)

Chronic kidney disease (CKD)

Diabetes mellitus (DM)

Glycated hemoglobin (HbA1c)

Interferon-gamma release assay (IGRA)

Latent TB infection (LTBI)

Tuberculin skin test (TST)

Tuberculosis (TB)

BACKGROUND

The co-occurrence of diabetes mellitus (DM) and tuberculosis (TB) cannot be overemphasized because of the high prevalence of each disease throughout the world [1]. Previous studies found that DM affects TB disease presentation, leads to poor treatment outcomes, and increases the risk for active TB [1, 2]. The recently published World Health Organization guideline recommends screening for active TB with DM [3], but it remains elusive whether screening and treatment of latent TB infections (LTBIs) should be prioritized to target individuals with DM [1, 4-6].

There is only limited information on the LTBI status of patients with DM. Most of antecedent relevant studies were in low TB incidence countries [7] or based on selected population by identifying DM in high-risk populations, such as TB contacts, immunocompromised patients with comorbid DM, or crisis-affected people [6]. The results of these studies are also inconsistent, mainly due to the different methods used to ascertain DM status (*e.g.* self-report, medical records, or laboratory testing) and differences in the control of potentially important confounders [1, 5-7].

In high TB incidence areas, where most of TB infection occurred at young age before the onset of DM [8], the temporal relationship between DM and TB infection is different from that in low incidence areas (Figure 1a-1b v.s. 1c) [6]. This temporal characteristic may lead to non-differential misclassification of DM-related TB infection in cross-sectional studies and therefore bias the association toward the null (Figure 1a) [6]. It is also worth noting that Bacillus Calmette–Guérin (BCG) vaccine is widely used in high burden TB countries for childhood immunization against TB. Recent studies found BCG vaccine has a protective effect against TB infection in children [9] and can even last into adulthood [10]. Although several researches have been devoted to assessment of DM-LTBI association, rather less attention has been paid to confounding

from the protection conferred by BCG vaccination in settings where coverage of BCG vaccination in study subjects was high. Furthermore, while DM occurs most often in middle-aged and older people, few attempts have been made in this population to examine whether BCG protection is modified by the status of DM and whether the protection wanes with age in older adults.

In this study, using data from community-based programs in an area with a high incidence of TB and a high coverage of BCG vaccination, we assessed the overall risk of LTBI in people with and without DM by carefully controlling for potential confounders, specifically including BCG vaccination, risk of remote TB infection, comorbidities, important lifestyle factors and DM severity. We also investigated the effect modification of DM and age on the protective effect of BCG vaccination.

METHODS

We conducted a community-based study that comprised DM and non-DM subjects by combining two community-based programs to investigate the effect of DM on risk of LTBI in Changhua, a county in Taiwan with a TB incidence of 58.7 per 100000 and a BCG vaccination coverage of around 99% in 2012 [11]. The first program recruited the DM group from patients registered in the Changhua Diabetes Shared Care program (CHDSC) [12] to participate in the LTBI survey. The second recruited the community comparison (CC) group from participants of a community-based LTBI screening program [13]. Due to the lack of a gold-standard test for LTBI, the tuberculin skin test (TST) was used in parallel with the interferon-gamma release assay (IGRA). All participants provided signed informed consent before enrollment. Both studies were approved by the Institutional Review Board of the Changhua Christian Hospital (numbers IRB:CCH-130102 and IRB:CCH-111012).

DM group

Nearly 60% of DM patients in Changhua were enrolled in the national diabetes disease management program (DDMP) and registered in the CHDSC [12]. These enrollees were suitable to be included as the study subjects, because their DM status had been ascertained by certified physicians who provided diabetes care according to national guidelines. We prospectively invited all those registered DM cases aged older than 40 years when they presented to outpatient diabetes clinics of the Changhua Christian Healthcare System (CCHS) or 13 nearby health centers in the surrounding townships from April 1, 2013 to December 31, 2013. CCHS comprises one medical center and three branch hospitals, distributed evenly at different locations of the county, and covers urban and rural areas. Thus, the participants of this study were a representative sample of the total DM population.

All study subjects were screened for pulmonary TB based on respiratory symptoms and chest X-rays upon entry into the study. Suspected cases submitted sputum specimens for acid-fast bacilli smears and culture. The diagnosis of TB was confirmed by chest specialists. Subjects were excluded if they had active pulmonary TB, a life expectancy of less than 2 years, metastatic cancer, or organ failure (*e.g.* severe liver disease [Child-Pugh Class B or C]) except chronic renal failure. The included eligible DM cases then received a TST and IGRA for detection of LTBI.

Community comparison group

The Changhua Community-based Integrated Screening (CHCIS) program, which began in 2005, screens for neoplastic and non-neoplastic diseases (including DM and pulmonary TB) [13]. The method used to screen for pulmonary TB was the same as that used in the DM group. We invited all consecutive attendees of the CHCIS program within the major catchment area of the CCHS to participate in LTBI screening in May 1, 2011. Participants with known DM or newly screened DM (*i.e.* fasting plasma glucose [FPG] ≧ 126 mg/dL) were excluded.

Tests for LTBI

Venous blood was collected for the QuantiFERON-TB Gold In-Tube test (QFT-GIT; Cellestis, Carnegie, Australia), which was performed in 2 stages, according the manufacturer's instructions. The cutoff for a positive result was 0.35 IU/mL. The reaction of a nil control and a mitogen control were within the range provided by the manufacturer. After collection of blood samples for QFT-GIT, trained nurses administered the TST using 2 TU of PPD RT23 (Statens Serum Institut, Copenhagen, Denmark) by the Mantoux method, and inspected the presence of BCG scars. Tuberculin indurations were measured 48-72 h after injection using the palpation method, and a diameter of 10 mm or more was defined as positive. All TST procedures followed the national guidelines issued by the CDC of Taiwan [14].

Data collection

We examined the DDMP database, and abstracted demographic data and information on DM care in the same year when each subject was recruited. This included duration of DM, glycated hemoglobin (HbA1c), blood pressure, lipid profile, renal function, and other related cardiovascular disease risk factors. We also linked individual data with the TB registry at the local health authority to assess whether each study subject had a prior history of TB or contact with TB.

Statistical analysis

Although both TST and IGRA indicate a cellular immune response to Mycobacterium tuberculosis (MTB) and are useful for the diagnosis of LTBI, the two tests identified different population with distinct immunologic processes [15]. Tuberculin reactivity represents the cumulative effect of previous TB infection (caused by recent and/or remote infection) because after infection with TB, positive results often remain lifelong until old age. On the contrary, IGRA has a dose–response relationship with recent TB exposure but it wanes rapidly [15, 16]. Thus, for adjusting for the bias of DM-related TB infection resulting from the occurrence of TB before DM (Figure 1a) as explained earlier, we used the result of the QFT-GIT as a surrogate of LTBI status in the logistic regression models designed to identify risk factors for LTBI. The TST results were then included in the models for controlling the confounding of remote TB infection. We also tested for effect modification of BCG protection by age and DM by including interactions terms (ie. Age×BCG scar and DM×BCG scar) in the regression analysis. All analyses were conducted using SAS version 9.3 (SAS Institute Inc., Cary, NC, USA).

Patient and Public Involvement

Patients and/or the public were not involved in this study. There are no plans to disseminate the results of the research to study participants.

RESULTS

Characteristics of Participants

We ultimately enrolled 2948 patients in the DM group and 453 non-DM subjects in CC group, and included all of these individuals in the final analysis (Figure 2). The mean duration of DM in the DM group was 9.0 years (standard deviation [SD]=6.6) and nearly half of these patients achieved good glycemic control (*i.e.* HbA1c < 7%) (Table 1). The DM group had a greater mean age, higher percentages of males and smokers, greater prevalences of obesity, chronic kidney disease (CKD), and prior history of TB. The DM group also had a greater prevalence of tuberculin reactivity (≧ 15mm) and of QFT-GIT positivity, and a higher proportion of indeterminate results (Table 1).

TST and QFT-GIT results

The presence of BCG scars had no effect on the tuberculin reactivity of the DM group (Figure 3a). TST positivity decreased with age, and was higher in the DM group than the CC group, except among the elderly (60+ years). Figure 3b shows that QFT-GIT positivity of DM group was highest among those with no BCG scars, and that positivity declined dramatically in those more than 60 years-old. In contrast, the rate of QFT-GIT positivity in those with BCG scars gradually increased with age. For each age group, there was an inverse correlation between number of BCG scars and QFT-GIT positivity. Individuals in the CC group with BCG scars had the lowest QFT-GIT positivity, but elderly individuals from the CC group (age 70+) had the highest QFT-GIT positivity. Generally, the concordance between the TST and QFT-GIT was low (Supplementary Table 1).

Effect of DM and other factors on risk of LTBI

Multivariate regression analysis indicates DM significantly increased the risk of LTBI after controlling for major confounders (aOR=1.67; 95%CI, 1.18-2.38). This risk was similar after adjustment for tuberculin reactivity (aOR=1.59; 95% CI, 1.11-2.28). In addition, the presence of LTBIs increased with age. For age older than 70 years, TST adjustment had a marked effect and changed its aOR from 4.27 (95% CI, 2.83-6.44) to 2.98 (95% CI, 2.00-4.44). Other variables, such as current smoking, CKD, and prior history of TB, were also significant risk factors for LTBI, and their effects were similar with or without adjustment for tuberculin reactivity. Notably, the presence of a BCG scar had a significantly protective effect on LTBI (aOR=0.66 [95%CI, 0.51-0.85]) (Table 2, Model 2); those with 1 scar had an aOR of 0.69 (95% CI, 0.52-0.90) and those with 2 or more scars had an aOR of 0.49 (95% CI, 0.36-0.67) (Table 2, Model 1).

The interaction term DM×BCG scar added to the regression model did not show a significant effect. For assessing effect modification of the association between LTBI and BCG vaccination by age, the people aged younger than 50 years without BCG scar were taken as the reference group. The aORs of the interaction terms (age×BCG scar) were 0.2 (95%CI, 0.0-0.7) for ages < 50 years, 0.3 (95%CI, 0.1-1.5) for ages 50-60 years, 0.5 (95%CI, 0.1-2.4) for ages 60-70 years, and 5.2 (95%CI, 1.1-25.4) for ages 70+ years respectively. (Table 2, Model 1)

Comorbidities, such as hyperlipidemia, hypertension, and abnormal body mass index (BMI), had no significant associations with LTBI. We further investigated the effect of long duration DM and poor glycemic control by dividing the DM group into four sub-groups according to duration of DM and HbA1c level, and then compared the risk of LTBI of these different sub-groups, using the CC group as a reference. All of the DM sub-groups had similar risks for LTBI. This indicates that long duration of DM and poor glycemic control had no effect on the risk of LTBI (Supplementary Table 2). However, among the DM group, those people with DM history for 5+ years had an aOR of 0.78 (95%CI, 0.63-0.96) (Table 2, Model 1).

DISCUSSION

Very few studies have examined the effect of DM on the risk of LTBI in high TB incidence areas and simultaneously taken into account confounding effects resulting from protection of BCG vaccination and remote TB infection. To our best knowledge, this is the first study to describe the association between LTBI and BCG vaccination in diabetes population. In this community-based study, we found BCG had a protective effect on TB infection in adults with an aOR of 0.66 (95%CI, 0.51-0.85), which did not vary by the status of DM. This effect also persisted in middle-aged adulthood until it waned in old age. Meanwhile, by use of stringent diagnostic criteria and adjusting for major confounding variables, our multivariate analysis indicated that DM had a positive association with risk of LTBI (aOR, 1.59 [95%CI, 1.11-2.28]). A recently published population-based study in US, a country where BCG is not generally recommended for use, using similar diagnostic criteria as this study, showed DM was associated with LTBI with an aOR 1.5 (95% CI, 1.0–2.2) [7], very similar to our finding.

Until recently, there has been a scarcity of evidence on whether BCG can prevent acquisition of TB infection because conventional measurement of LTBI utilizing TST cannot distinguish if a positive response is due to MTB infection, or BCG vaccination, or non-tuberculous mycobacterial (NTM) infection [9, 17]. Unlike TST, the recently developed IGRAs use MTB specific antigens that do not cross react with BCG. Studies have been conducted to investigate BCG protection on LTBI measured as positive responses on IGRA. A recent meta-analysis of 14 studies and 3855 child or adolescent participants (< 16 years) suggested that BCG is associated with protection from TB infection (overall risk ratio, 0.81; 95% CI, 0.71 to 0.92) [9]. Three additional studies in high burden TB countries, not included in the meta-analysis, revealed that the protection existed in young adults decades after BCG vaccination with aORs ranging from 0.6 (95%CI, 0.2–1.8) to 0.52 (95% CI, 0.32-0.85) [10, 18, 19], close to our estimate.

Additionally, since in our regression model, the aORs of interaction terms DM×BCG raised dramatically to 5.2 (95%CI, 1.1-25.4) in the age group of 70+ years, it is reasonable to assume that BCG protection against TB infection may last 60 years and then waned, an estimate in accordance with previous observations [18]. These findings raised the concern that researchers must be cautious in interpreting DM-LTBI association when confounding of BCG was not controlled, especially in settings with high BCG coverage. For example, the lowered OR of DM after adjustment (crude OR 2.68 *vs.* aOR 1.59) (Table 2) simply reflected that the CC group had higher proportion of subjects with BCG scars than the DM group (Table 1).

Since DM is a progressive disease, longer diabetes duration was found to be a major predictor of DM-related complications and death, independent of glycemic control [19]. An earlier study also revealed the risk of developing TB disease increased among those with increasing diabetes severity [2]. Thus, in this study, we investigated the combined effect of longer duration of DM and poor glycemic control (Supplementary Table 2). We found they did not affect the risk of LTBI. While association between glycemic control and risk of TB was not observed either in other researches targeting patients with long-established DM [20, 21], there have been several studies on cases of pre-diabetes or untreated early diabetes supported the hypothesis [1, 5, 7]. Diabetes patients with poor glycemic control and longer disease duration tend to have a smaller social network and less contact with their family members or friends [22, 23]. This may reduce the opportunity of social contact with TB cases and trumped the risk for recent TB infection.

Many of the subjects with DM in the present study also smoked (14.7%), had abnormal BMIs (overweight and obese, 58.4%; underweight, 1.7%), CKD (32.2%), prior history of TB (2.1%), and advanced age (58.6% older than 60 years), and these may also be risk factors for TB [24-26]. We found that each factor alone had only a mild to moderate association with LTBI (Table 2) after adjustment of BCG protection. However, when an individual has all of these other factors as well as DM, there may be a particularly high-risk of LTBI, especially in those who are elderly. For example, male DM patients older than 60 years who smoke have an aOR of LTBI up to 6.7 (derived by summation of the estimated regression coefficients). Conventional targeted screening for LTBI mostly focuses on host variables and the environment, such as infectiousness of index cases and contact patterns, but seldom consider the effects of multiple factors simultaneously [27, 28]. Our results underscore the necessity of incorporating DM, BCG, TST results and related risk factors to develop a composite scoring system that improves the efficacy of LTBI screening programs.

There are some limitations in our study. First, although the *in vivo* TST and *ex vivo* IGRAs are the only two methods available for diagnosing LTBI, there is concern that immune dysfunction in DM may compromise the performance of these tests [5]. Reduced sensitivity of the QFT-GIT and TST in elderly diabetics (Figure 3a, 3b) may lead to false negatives, and therefore underestimate the effect of DM. Second, all DM patients were enrolled from the DDMP, an intervention designed to facilitate lifestyle modification in patients with DM [12]. Thus, these study subjects may have better general health status, and hence lower risk of TB infection, than DM patients from the general population [21].

The comorbidity of TB and DM is due to the interaction between DM-impaired immunity and the occurrence of active TB by endogenous reactivation (Figure 1a) or exogeneous new or reinfection (Figure 1b, 1c) [1, 5]. This process is further complicated by the protection conferred from BCG vaccination, remote TB infection, social environment, the co-existence of multiple non-communicable risk factors and other related comorbidities and complications [5, 26, 29]. We tried to control for all possible confounders, but this remains challenging due to the lack of a gold standard for diagnosis of LTBI and the presence of only limited tools to identify DM patients who have the greatest risk of progressing to active TB. More studies are therefore required to identify the predictive value for progression to active TB based on IGRA and/or TST results in patients with DM. There is an ongoing longitudinal study of the present study cohort.

CONCLUSION

In conclusion, our study demonstrated BCG had an apparent protective effect on TB infection in adults and there were a 1.59-fold increased risk of LTBI in patients with DM from a geographic area that has a high incidence of TB and a high coverage of BCG vaccination. This finding suggests that practitioners should incorporate BCG vaccination, comorbidities and other coexisting risk factors, in addition to DM, to better identify high-risk groups and enhance the efficacy of targeted screening for LTBI. Efficacy of new vaccines against TB infection should be a major focus of TB vaccine development.

DECLARATIONS

Authors' contributions:

1 Guarantor of integrity of the entire study: Ching-Hsiung Lin, Shu-Chen Kuo, Yen-Po Yeh, Shih-Te Tu

2 Study concepts: Ching-Hsiung Lin, Shu-Chen Kuo, Ming-Chia Hsieh, Ih-Jen Su, Sheng-Hao Lin , Yen-Po Yeh, Shih-Te Tu

3 Study design: Ching-Hsiung Lin, Shu-Chen Kuo, Ming-Chia Hsieh, Ih-Jen Su, Sheng-Hao Lin , Yen-Po Yeh, Shih-Te Tu

4 Definition of intellectual content: Ching-Hsiung Lin, Shu-Chen Kuo, Ih-Jen Su, Yee-Chun Chen, Yen-Po Yeh, Shih-Te Tu

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13 Manuscript review: Ching-Hsiung Lin, Ming-Chia Hsieh, Sheng-Hao Lin , Yee-Chun Chen, Yen-Po Yeh, Shih-Te Tu

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Conflict of interest statements:

All authors declare that they have no conflict of interest.

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Ethics committee approval:

All participants provided signed informed consent before enrollment. Both studies were approved by the Institutional Review Board of the Changhua Christian Hospital (numbers IRB:CCH-130102 and IRB:CCH-111012).

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Consent for publication: Not applicable.

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TABLES

Table 1. Characteristics of the DM group and the community comparison (CC) group.

	DM group (n=2948)	CC group (n=453)	p
Age, years			
Mean (SD)	61.5 (9.3)	51.3 (10.5)	**<0.001**
<50	304 (10.3%)	209 (46.1%)	
50-59	918 (31.1%)	137 (30.2%)	**<0.001**
60-69	1123 (38.1%)	91 (20.1%)	
≥70	603 (20.5%)	16 (3.5%)	
Sex			
Male	1468 (49.8%)	109 (24.1%)	**<0.001**
Female	1480 (50.2%)	344 (75.9%)	
Prior history of TB			
Yes	61 (2.1%)	4 (0.9%)	0.0852
No	2887 (97.9%)	449 (99.1%)	
History of contact with TB			
Yes	115 (3.9%)	65 (14.3%)	**<0.001**
No	2283 (77.4%)	388 (85.7%)	
Unknown	550 (18.7%)	0 (0.0%)	
BCG scar			
Yes	2436 (82.6%)	440 (97.1%)	
1 scar	1481 (50.2%)		
2 scar	934 (31.7%)		**<0.001**
≧2 scars	21 (0.7%)		
No	502 (17.0%)	13 (2.9%)	
Unknown	10 (0.3%)		
BMI, kg/m^2			
Underweight (<18.5)	50 (1.7%)	7 (1.5%)	**<0.001**
Normal (18.5-24.9)	1175 (39.9%)	274 (60.5%)	
Overweight (25-29.9)	1265 (42.9%)	149 (32.9%)	
Obese (≧30)	458 (15.5%)	23 (5.1%)	
Smoking status			
Current	433 (14.7%)	31 (6.8%)	**<0.001**
Quit	434 (14.7%)	34 (7.5%)	
Never	2081 (70.6%)	388 (85.7%)	
Triglycerides, mg/dL			
<150 mg/dl	1962 (66.6%)	371 (81.9%)	**<0.001**
≧150	986 (33.4%)	82 (18.1%)	
HDL-C*			**<0.001**
Low	1336 (45.3%)	299 (66.0%)	
Ideal	1612 (54.7%)	154 (34.0%)	

CKD**			
Yes	948 (32.2%)	57 (12.6%)	**<0.001**
No	1941 (65.8%)	375 (82.8%)	
Unknown	59 (2.0%)	21 (4.6%)	
Duration of diabetes, years			
mean (SD)	9.0 (6.6)		
≦5	1077 (36.5%)		
>5	1871 (63.5%)		
HbA1c			
Mean (SD)	7.4 (1.5)		
<7%	1400 (47.5%)		
≧7%	1548 (52.5%)		
Unknown	6 (0.2%)		
TST positive			
≧5 mm	2280 (77.3%)	350 (77.3%)	0.9895
<5 mm	668 (22.7%)	103 (22.7%)	
≧10 mm	1665 (56.5%)	251 (55.4%)	0.6974
<10 mm	1283 (43.5%)	202 (44.6%)	
≧15 mm	890 (30.2%)	112 (24.7%)	**0.0211**
<15mm	2058 (69.8%)	341 (75.3%)	
QFT-GIT			
Positive	623 (21.1%)	44 (9.7%)	**<0.001**
Negative	2144 (72.7%)	406 (89.6%)	
Indeterminate	181 (6.1%)	3 (0.7%)	

Abbreviations: SD, standard deviation; HbA1c, glycated hemoglobin; BMI, body mass index; HDL-C, high density lipoprotein cholesterol; TST, tuberculin skin test; QFT-GIT, QuantiFERON-TB Gold In-Tube.
*Low HDL-C: <40 mg/dL (males) and <50 mg/dL (females).
**CKD (chronic kidney disease) was assessed by the Modification of Diet in Renal Disease (MDRD) study equation, using the estimated glomerular filtration rate (eGFR).

Table 2. Logistic regression analysis of factors associated with LTBI in the DM group (model 1) and in the DM and CC groups combined (model 2), without and with adjustment for TST results.

Variable	Model 1. DM group (n = 2767)				Model 2. DM and CC groups (n = 3217)		
	Crude OR (95% CI)	aOR (95% CI)	aOR (95% CI) (TST adjustment)		Crude OR (95% CI)	aOR (95% CI)	aOR (95% CI) (TST adjustment)
				Age×BCG[§]			
DM					2.68 (1.94-3.71)	1.67 (1.18-2.38)	1.59 (1.11-2.28)
BCG scar							
No	*Reference*	*Reference*	*Reference*		*Reference*	*Reference*	*Reference*
Yes					0.51 (0.41-0.63)	0.73 (0.57-0.93)	0.66 (0.51-0.85)
1 scar	0.63 (0.50-0.79)	0.76 (0.59-0.99)	0.69 (0.52-0.90)				
2+ scars	0.43 (0.33-0.56)	0.55 (0.41-0.74)	0.49 (0.36-0.67)				
TST 10+	3.85 (3.11-4.76)		4.77 (3.80-5.99)	4.75 (3.79-5.97)	3.66 (2.99-4.49)		4.56 (3.67-5.66)
Age, years							
<50	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
50-59	1.63 (1.11-2.41)	1.85 (1.23-2.79)	2.20 (1.45-3.32)	1.33 (0.27-6.48)	1.98 (1.42-2.76)	1.86 (1.30-2.65)	2.12 (1.48-3.05)
60-69	2.12 (1.45-3.09)	2.28 (1.52-3.41)	2.96 (1.97-4.47)	0.73 (0.16-3.39)	2.52 (1.83-3.49)	2.25 (1.58-3.20)	2.77 (1.93-3.97)
≥70	3.19 (2.15-4.71)	2.84 (1.82-4.45)	4.35 (2.74-6.89)	1.10 (0.24-4.95)	4.07 (2.89-5.71)	2.98 (2.00-4.44)	4.27 (2.83-6.44)
Male	1.28 (1.07-1.54)	1.19 (0.94-1.50)	1.09 (0.85-1.38)	1.11 (0.87-1.41)	1.41 (1.19-1.68)	1.22 (0.98-1.52)	1.12 (0.89-1.41)
Smoking							
Never	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
Current	1.53 (1.20-1.93)	1.61 (1.2-2.15)	1.36 (1.00-1.83)	1.38 (1.02-1.86)	1.54 (1.22-1.95)	1.49 (1.13-1.97)	1.28 (0.95-1.71)
Quit	0.93 (0.71-1.21)	0.88 (0.64-1.2)	0.82 (0.59-1.13)	0.83 (0.60-1.15)	1.00 (0.77-1.29)	0.87 (0.64-1.17)	0.82 (0.60-1.11)
CKD	1.46 (1.21-1.76)	1.20 (0.98-1.47)	1.30 (1.05-1.60)	1.29 (1.04-1.59)	1.56 (1.31-1.87)	1.17 (0.96-1.42)	1.26 (1.03-1.55)
PHx TB	2.99 (1.78-5.03)	2.35 (1.36-4.05)	2.21 (1.25-3.91)	2.16 (1.22-3.83)	2.95 (1.78-4.89)	2.17 (1.28-3.68)	2.08 (1.19-3.63)
TB contact	0.82 (0.50-1.32)	0.83 (0.50-1.36)	0.75 (0.45-1.25)	0.73 (0.43-1.22)	0.75 (0.50-1.13)	0.92 (0.60-1.40)	0.86 (0.55-1.33)

DM 5+ yrs	0.95 (0.79-1.15)	0.84 (0.69-1.02)	0.79 (0.64-0.97)	0.78 (0.63-0.96)
A1C 7+%	0.91 (0.76-1.08)	0.91 (0.75-1.10)	0.91 (0.75-1.11)	0.92 (0.76-1.12)
Age×BCG§				
<50				0.16 (0.04-0.75)
50-59				0.34 (0.08-1.52)
60-69				0.54 (0.12-2.40)
≥70				5.23 (1.08-25.40)

Abbreviations: DM, the diabetes mellitus; CC, community comparison; OR, odds ratio; aOR, adjusted odds ratio; CI, confidence interval; TST 10+, results of tuberculin skin test ≧ 10mm; BMI, body mass index; CKD, chronic kidney disease; DM 5+ yrs, duration of diabetes mellitus ≧5 years; A1C, glycated hemoglobin. PHx TB, prior history of TB. § Measure of effect modification of the association between LTBI and BCG scar by age on multiplicative scale.

FIGURE LEGENDS

Figure 1 Possible temporal relationships between the onset of DM and the occurrence of TB infection. Circled letters indicate times when DM could possibly affect the pathogenesis of TB (i, increased susceptibility to TB infection; p, accelerated progression from infection to clinical disease). (a) Onset of DM with a pre-existing latent TB infection (LTBI). (b) Onset of DM with a pre-existing LTBI, but before re-infection. (c) Onset of DM before the primary TB infection.

Figure 2 Patient selection and enrolment in the DM group (left) and the CC group (right).

Abbreviations: TST, tuberculin skin test; IGRA, interferon-gamma release assay; CHCIS, Changhua Community-based Integrated Screening program; DM, diabetes mellitus.

Figure 3 Tuberculin skin test (TST) positivity (a) and interferon-gamma release assay (IGRA) positivity (b) in the DM group (DMG) and the CC group (CCG) according to age and BCG scar status. A tuberculin induration size of 10 mm was used as the cutoff point for both groups.

Abbreviations: TST, tuberculin skin test; QFT, QuantiFERON-TB Gold In-Tube.

Figure 1

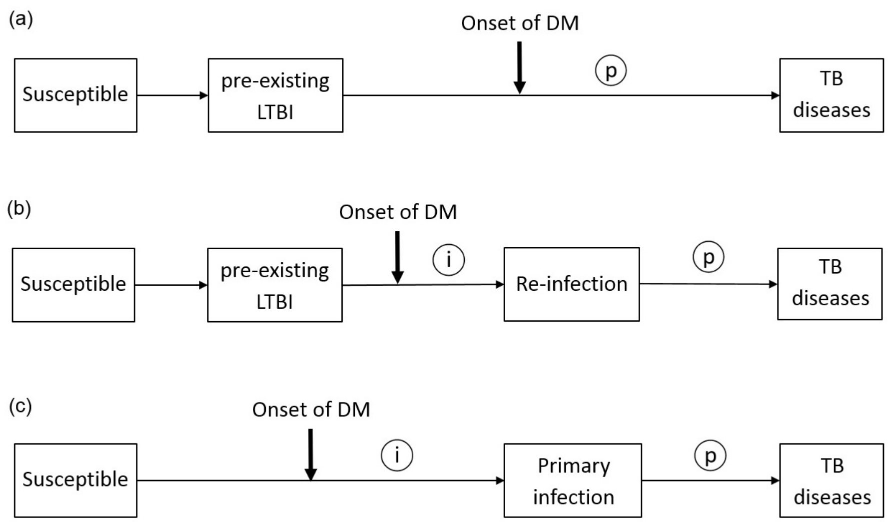

Figure 1 Possible temporal relationships between the onset of DM and the occurrence of TB infection. Circled letters indicate times when DM could possibly affect the pathogenesis of TB (i, increased susceptibility to TB infection; p, accelerated progression from infection to clinical disease). (a) Onset of DM with a pre-existing latent TB infection (LTBI). (b) Onset of DM with a pre-existing LTBI, but before re-infection. (c) Onset of DM before the primary TB infection.

294x192mm (120 x 120 DPI)

Figure 2

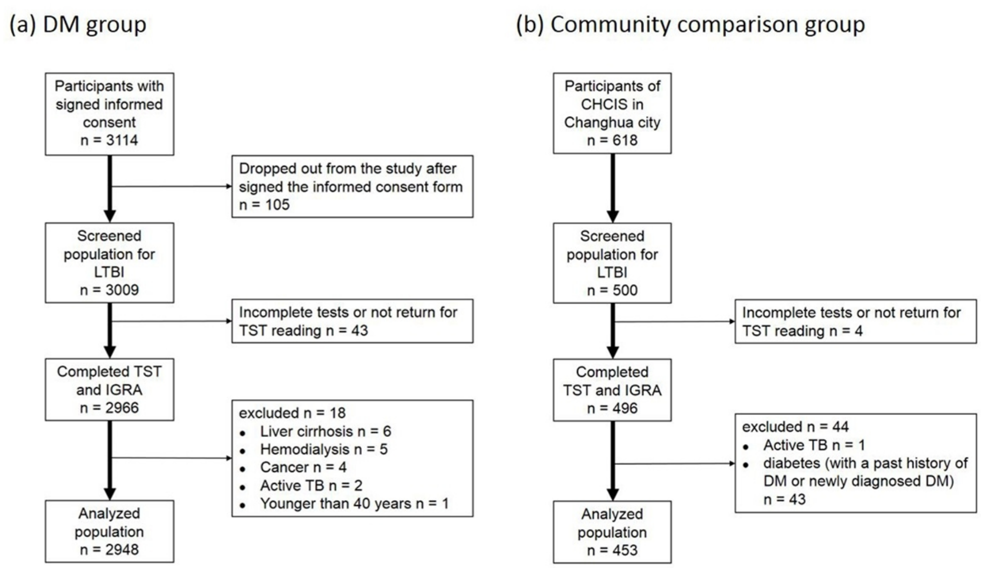

Figure 2 Patient selection and enrolment in the DM group (left) and the CC group (right). Abbreviations: TST, tuberculin skin test; IGRA, interferon-gamma release assay; CHCIS, Changhua Community-based Integrated Screening program; DM, diabetes mellitus.

99x65mm (300 x 300 DPI)

Figure 3

(a)

(b)

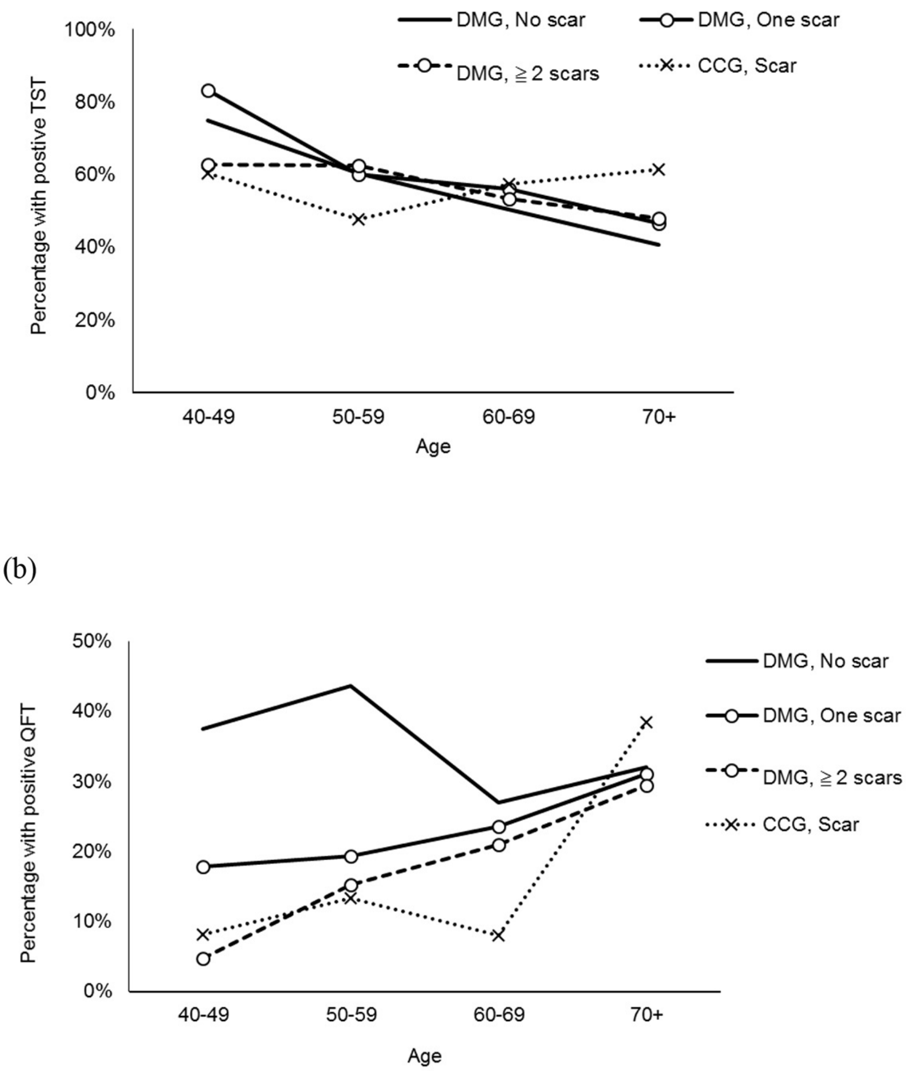

Figure 3 Tuberculin skin test (TST) positivity (a) and interferon-gamma release assay (IGRA) positivity (b) in the DM group (DMG) and the CC group (CCG) according to age and BCG scar status. A tuberculin induration size of 10 mm was used as the cutoff point for both groups.
Abbreviations: TST, tuberculin skin test; QFT, QuantiFERON-TB Gold In-Tube.

99x127mm (300 x 300 DPI)

1

Supplementary Table 1. Agreement between TST and QFT-GIT

Results	Overall n = 3217			DM group n = 2767			CC group n = 450		
	TST cut point			TST cut point			TST cut point		
	5 mm	10 mm	15 mm	5 mm	10 mm	15 mm	5 mm	10 mm	15 mm
TST+/QFT-GIT+	613	528	363	37	32	23	576	496	340
TST-/QFT-GIT-	664	1252	1950	95	188	318	569	1064	1632
TST+/QFT-GIT-	1886	1298	600	311	218	88	1575	1080	512
TST-/QFT-GIT+	54	139	304	7	12	21	47	127	283
Agreement%	39.7%	55.3%	71.9%	29.3%	48.9%	75.8%	41.4%	56.4%	71.3%
Kappa (95% CI)	0.09 (0.74-0.10)	0.17 (0.15-0.20)	0.27 (0.23-0.30)	0.10 (0.08-0.12)	0.19 (0.16-0.22)	0.27 (0.23-0.31)	0.02 (-0.01-0.05)	0.06 (0.01-0.11)	0.18 (0.09-0.28)

Abbreviations: TST, tuberculin skin test; QFT-GIT, QuantiFERON-TB Gold In-Tube; CI, confidence interval.
Test results with an indeterminate QFT-GIT response are not included in the table.

2

Supplementary Table 2. Combined effects of glycemic control (HbA1c) and duration of DM on risk of latent tuberculosis infection without and with adjustment for TST results.

Variables	Crude OR (95% CI)	aOR* (95% CI)	aOR* (95% CI) (TST adjustment)
Community comparison group	Reference	Reference	Reference
A1C ≧7%, duration >5 years	2.49 (1.77-3.52)	1.42 (0.97-2.07)	1.33 (0.90-1.96)
A1C ≧7%, duration ≦5 years	2.71 (1.84-3.98)	1.87 (1.24-2.82)	1.80 (1.18-2.74)
A1C <7%, duration >5 years	2.84 (1.99-4.04)	1.67 (1.13-2.46)	1.54 (1.03-2.28)
A1C <7%, duration ≦5 years	2.81 (1.95-4.04)	1.84 (1.24-2.72)	1.82 (1.22-2.72)

Abbreviations: OR, odds ratio; aOR, adjusted odds ratio; CI, confidence interval; TST, tuberculin skin test; A1C, glycated hemoglobin.

*The multivariable model adjusted for the comorbidities indicated in Table 3, Model 2.

STROBE 2007 (v4) checklist of items to be included in reports of observational studies in epidemiology*

Checklist for cohort, case-control, and cross-sectional studies (combined)

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	4
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	4
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	8-9
Objectives	3	State specific objectives, including any pre-specified hypotheses	9
Methods			
Study design	4	Present key elements of study design early in the paper	10
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	10
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	10-12
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	11-13
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*11-12*
Bias	9	Describe any efforts to address potential sources of bias	-
Study size	10	Explain how the study size was arrived at	10-11
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	10-12
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	13-14
		(*b*) Describe any methods used to examine subgroups and interactions	13-14
		(*c*) Explain how missing data were addressed	-
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed	-

		Cross-sectional study—If applicable, describe analytical methods taking account of sampling strategy	
		(*e*) Describe any sensitivity analyses	-
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	14
		(b) Give reasons for non-participation at each stage	-
		(c) Consider use of a flow diagram	-
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	14-16
		(b) Indicate number of participants with missing data for each variable of interest	-
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	17-21
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	17-21
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	17-21
		Cross-sectional study—Report numbers of outcome events or summary measures	17-21
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	17-21
		(*b*) Report category boundaries when continuous variables were categorized	17-21
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	-
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	
Discussion			
Key results	18	Summarise key results with reference to study objectives	21-25
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	24
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	24-25
Generalisability	21	Discuss the generalisability (external validity) of the study results	24-25
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	28

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

Effect of Diabetes Mellitus on Risk of Latent TB Infection in A High TB Incidence Area: A Community-based Study in Taiwan

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	Medicine Yeh, Yen-Po; National Taiwan University, Innovation and Policy Center for Population Health and Sustainable Environment; Changhua Public Health Bureau
Primary Subject Heading:	Infectious diseases
Secondary Subject Heading:	Diabetes and endocrinology, Epidemiology, Public health, Immunology (including allergy), Respiratory medicine
Keywords:	Diabetes Mellitus, Tuberculosis < INFECTIOUS DISEASES, Latent Infection, Bacillus Calmette–Guérin vaccination, Interferon gamma release essay, Tuberculin skin test

SCHOLARONE™ Manuscripts

Effect of Diabetes Mellitus on Risk of Latent TB Infection in A High TB Incidence Area: A Community-based Study in Taiwan

Running title: Individuals with diabetes have a moderately increased risk of latent TB infection.

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ABSTRACT

Objective: To investigate the association between diabetes and latent tuberculosis infections (LTBIs) in high TB incidence areas

Design: Community-based comparison study

Setting: Outpatient diabetes clinics at 4 hospitals and 13 health centers in urban and rural townships. A community-based screening program was used to recruit non-diabetic participants.

Participants: A total of 2948 patients with diabetes aged older than 40 years were recruited, and 453 non-diabetic participants from the community were enrolled.

Primary and secondary outcome measures: The interferon-gamma release assay (IGRA) and the tuberculin skin test were used to detect LTBI. The IGRA result was used as a surrogate of LTBI in logistic regression analysis.

Results: Diabetes was significantly associated with LTBI ([aOR] = 1.59; 95% CI, 1.11-2.28) and age correlated positively with LTBI. Many subjects with diabetes also had additional risk factors (current smokers [aOR=1.28; 95% CI, 0.95-1.71], comorbid chronic kidney disease [aOR = 1.26; 95% CI, 1.03-1.55], and prior history of TB [aOR = 2.08; 95% CI, 1.19-3.63]). The presence of BCG scar was protective (aOR = 0.66 ; 95% CI, 0.51-0.85). Duration of diabetes and poor glycemic control were unrelated to the risk of LTBI.

Conclusion: There was a moderately increased risk of LTBI in diabetes patients from this high TB incidence area. This finding suggests LTBI screening for the diabetics be combined with other risk factors and comorbidities of TB to better identify high-risk groups and improve the efficacy of targeted screening for LTBI.

Keywords: Diabetes Mellitus, Tuberculosis, Latent Infection, Bacillus Calmette–Guérin

vaccination, Interferon-gamma release assay, Tuberculin skin test

Strengths and limitations of this study

1. The strengths of this study are the adoption of stringent diagnostic criteria for DM and comprehensiveness of information obtained from community-based programs.

2. Protection of BCG vaccination, remote TB infection and other important potential confounding variables were controlled for assessing the DM-LTBI association.

3. The study limitations are the reduced sensitivity of the QFT-GIT and TST in elderly diabetics and better general health status among the DM group.

Abbreviations

Bacillus Calmette–Guérin (BCG)

Changhua Christian Healthcare System (CCHS)

Changhua Community-based Integrated Screening (CHCIS)

Chronic kidney disease (CKD)

Diabetes mellitus (DM)

Glycated hemoglobin (HbA1c)

Interferon-gamma release assay (IGRA)

Latent TB infection (LTBI)

Tuberculin skin test (TST)

Tuberculosis (TB)

BACKGROUND

The co-occurrence of diabetes mellitus (DM) and tuberculosis (TB) cannot be overemphasized because of the high prevalence of each disease throughout the world [1]. Previous studies found that DM affects TB disease presentation, leads to poor treatment outcomes, and increases the risk for active TB [1, 2]. The recently published World Health Organization guideline recommends screening for active TB with DM [3], but it remains elusive whether screening and treatment of latent TB infections (LTBIs) should be prioritized to target individuals with DM [1, 4-6].

There is only limited information on the LTBI status of patients with DM. Most of antecedent relevant studies were in low TB incidence countries [7] or based on selected population by identifying DM in high-risk populations, such as TB contacts, immunocompromised patients with comorbid DM, or crisis-affected people [6]. The results of these studies are also inconsistent, mainly due to the different methods used to ascertain DM status (*e.g.* self-report, medical records, or laboratory testing) and differences in the control of potentially important confounders [1, 5-7].

In high TB incidence areas, where most of TB infection occurred at young age before the onset of DM [8], the temporal relationship between DM and TB infection is different from that in low incidence areas (Figure 1a-1b v.s. 1c) [6]. This temporal characteristic may lead to non-differential misclassification of DM-related TB infection in cross-sectional studies and therefore bias the association toward the null (Figure 1a) [6]. It is also worth noting that Bacillus Calmette–Guérin (BCG) vaccine is widely used in high burden TB countries for childhood immunization against TB. Recent studies found BCG vaccine has a protective effect against TB infection in children [9] and can even last into adulthood [10]. Although several researches have been devoted to assessment of DM-LTBI association, rather less attention has been paid to confounding

from the protection conferred by BCG vaccination in settings where coverage of BCG vaccination in study subjects was high.

In this study, using data from community-based programs in an area with a high incidence of TB and a high coverage of BCG vaccination, we assessed the overall risk of LTBI in people with and without DM by carefully controlling for potential confounders, specifically including BCG vaccination, risk of remote TB infection, comorbidities, important lifestyle factors and DM severity.

METHODS

We conducted a community-based study that comprised DM and non-DM subjects by combining two community-based programs to investigate the effect of DM on risk of LTBI in Changhua, a county in Taiwan with a TB incidence of 58.7 per 100000 and a BCG vaccination coverage of around 99% in 2012 [11]. The first program recruited the DM group from patients registered in the Changhua Diabetes Shared Care program (CHDSC) [12] to participate in the LTBI survey. The second recruited the community comparison (CC) group from participants of a community-based LTBI screening program [13]. Due to the lack of a gold-standard test for LTBI, the tuberculin skin test (TST) was used in parallel with the interferon-gamma release assay (IGRA). All participants provided signed informed consent before enrollment. Both studies were approved by the Institutional Review Board of the Changhua Christian Hospital (numbers IRB:CCH-130102 and IRB:CCH-111012).

DM group

Nearly 60% of DM patients in Changhua were enrolled in the national diabetes disease management program (DDMP) and registered in the CHDSC [12]. These enrollees were suitable to be included as the study subjects, because their DM status had been ascertained by certified physicians who provided diabetes care according to national guidelines. We prospectively invited all those registered DM cases aged older than 40 years when they presented to outpatient diabetes clinics of the Changhua Christian Healthcare System (CCHS) or 13 nearby health centers in the surrounding townships from April 1, 2013 to December 31, 2013. CCHS comprises one medical center and three branch hospitals, distributed evenly at different locations of the county, and covers urban and rural areas. Thus, the participants of this study were a representative sample of the total DM population.

All study subjects were screened for pulmonary TB based on respiratory symptoms and chest X-rays upon entry into the study. Suspected cases submitted sputum specimens for acid-fast bacilli smears and culture. The diagnosis of TB was confirmed by chest specialists. Subjects were excluded if they had active pulmonary TB, a life expectancy of less than 2 years, metastatic cancer, or organ failure (*e.g.* severe liver disease [Child-Pugh Class B or C]) except chronic renal failure. The included eligible DM cases then received a TST and IGRA for detection of LTBI.

Community comparison group

The Changhua Community-based Integrated Screening (CHCIS) program, which began in 2005, screens for neoplastic and non-neoplastic diseases (including DM and pulmonary TB) [13]. The method used to screen for pulmonary TB was the same as that used in the DM group. We invited all consecutive attendees of the CHCIS program within the major catchment area of the CCHS to participate in LTBI screening in May 1, 2011. Participants with known DM or newly screened DM (*i.e.* fasting plasma glucose [FPG] ≧ 126 mg/dL) were excluded.

Tests for LTBI

Venous blood was collected for the QuantiFERON-TB Gold In-Tube test (QFT-GIT; Cellestis, Carnegie, Australia), which was performed in 2 stages, according the manufacturer's instructions. The cutoff for a positive result was 0.35 IU/mL. The reaction of a nil control and a mitogen control were within the range provided by the manufacturer. After collection of blood samples for QFT-GIT, trained nurses administered the TST using 2 TU of PPD RT23 (Statens Serum Institut, Copenhagen, Denmark) by the Mantoux method, and inspected the presence of BCG scars. Tuberculin indurations were measured 48-72 h after injection using the palpation method, and a diameter of 10 mm or more was defined as positive. All TST procedures followed the national guidelines issued by the CDC of Taiwan [14].

Data collection

We examined the DDMP database, and abstracted demographic data and information on DM care in the same year when each subject was recruited. This included duration of DM, glycated hemoglobin (HbA1c), blood pressure, lipid profile, renal function, and other related cardiovascular disease risk factors. We also linked individual data with the TB registry at the local health authority to assess whether each study subject had a prior history of TB or contact with TB.

Statistical analysis

Although both TST and IGRA indicate a cellular immune response to Mycobacterium tuberculosis (MTB) and are useful for the diagnosis of LTBI, the two tests identified different population with distinct immunologic processes [15].

Substantial discordance of TST and IGRA has been found in previous studies [15, 16]. Nevertheless, IGRA has a dose–response relationship with recent TB exposure and it wanes rapidly [15, 16]. It may be better than TST at detecting recent rather than remote TB infection. Thus, we used the result of the QFT-GIT as a surrogate of LTBI status to estimate univariate odds ratios (ORs) and 95% confidence intervals (CIs) using a logistic regression model. Variables that met p-values less than 0.2 at univariable analysis were retained for the multivariable model, which also incorporated standard sociodemographic variables such as age and gender. The multivariable model was fitted to generate adjusted odds ratios (aOR) of the association between LTBI and DM by comparing the DM group with the CC group and adjusting for other independent variables including age, gender, BCG scar, smoking, prior history of TB, contact with TB and comorbid chronic kidney disease etc.

Since tuberculin reactivity was known to represent the cumulative effect of previous TB infection, we further included the TST results in the models in attempt to control the confounding of remote TB infection (i.e. TB infection acquired at young age before the onset of DM [Figure 1a]). All analyses were conducted using SAS version 9.3 (SAS Institute Inc., Cary, NC, USA).

Patient and Public Involvement

Patients and/or the public were not involved in this study. There are no plans to disseminate the results of the research to study participants.

RESULTS

Characteristics of Participants

We ultimately enrolled 2948 patients in the DM group and 453 non-DM subjects in

CC group, and included all of these individuals in the final analysis (Figure 2). The mean duration of DM in the DM group was 9.0 years (standard deviation [SD]=6.6) and nearly half of these patients achieved good glycemic control (*i.e.* HbA1c < 7%) (Table 1). The DM group had a greater mean age, higher percentages of males and smokers, greater prevalences of obesity, chronic kidney disease (CKD), and prior history of TB. The DM group also had a greater prevalence of tuberculin reactivity (≧ 15mm) and of QFT-GIT positivity, and a higher proportion of indeterminate results (Table 1).

TST and QFT-GIT results

The presence of BCG scars had no effect on the tuberculin reactivity of the DM group (Figure 3a). TST positivity decreased with age, and was higher in the DM group than the CC group, except among the elderly (60+ years). Figure 3b shows that QFT-GIT positivity of DM group was highest among those with no BCG scars, and that positivity declined dramatically in those more than 60 years-old. In contrast, the rate of QFT-GIT positivity in those with BCG scars gradually increased with age. For each age group, there was an inverse correlation between number of BCG scars and QFT-GIT positivity. Individuals in the CC group with BCG scars had the lowest QFT-GIT positivity, but elderly individuals from the CC group (age 70+) had the highest QFT-GIT positivity. Generally, the concordance between the TST and QFT-GIT was low (Supplementary Table 1).

Effect of DM and other factors on risk of LTBI

Multivariate regression analysis indicates DM significantly increased the risk of LTBI after controlling for major confounders (aOR=1.67; 95%CI, 1.18-2.38). This risk was similar after adjustment for tuberculin reactivity (aOR=1.59; 95% CI, 1.11-2.28). In addition, the presence of LTBIs increased with age. For age older than 70 years, TST adjustment had a marked effect and changed its aOR from 4.27 (95% CI, 2.83-6.44) to 2.98 (95% CI, 2.00-4.44). Other variables, such as current smoking, CKD, and prior history of TB, were also significant risk factors for LTBI, and their effects were similar with or without adjustment for tuberculin reactivity. Notably, the presence of a BCG scar had a significantly protective effect on LTBI (aOR=0.66 [95%CI, 0.51-0.85]) (Table 2).

Comorbidities, such as hyperlipidemia, hypertension, and abnormal body mass index (BMI), had no significant associations with LTBI. We further investigated the effect of long duration DM and poor glycemic control by dividing the DM group into four sub-groups according to duration of DM and HbA1c level, and then compared the risk of LTBI of these different sub-groups, using the CC group as a reference. All of the DM sub-groups had similar risks for LTBI. This indicates that long duration of DM and poor glycemic control had no effect on the risk of LTBI (Supplementary Table 2).

DISCUSSION

Very few studies have examined the effect of DM on the risk of LTBI in high TB incidence areas and simultaneously taken into account confounding effects resulting from protection of BCG vaccination and remote TB infection. To our best knowledge, this is the first study of this kind. The key strengths of this study included adoption of stringent diagnostic criteria for DM and comprehensiveness of information obtained from community-based programs, which enabled thorough adjustment for important potential confounding variables. Our multivariate analysis indicated that DM had a positive association with risk of LTBI (aOR, 1.59 [95%CI, 1.11-2.28]). This finding has profound implications.

A recent systematic review concluded the pooled odds ratio estimate for DM on risk of LTBI was 1.18 (95% CI, 1.06–1.30) [6]. Reasons for the different results from our study could be related to differences in the populations studied, methods for pooling results from distinct measurements for LTBI (i.e. TST versus IGRA), ascertainment of diabetes status by self-reports or medical records, and lack of control for several major confounders. By contrast, a recently published population-based study in US, a country where BCG is not generally recommended for use, using similar diagnostic criteria as this study, showed DM was associated with LTBI with an aOR 1.5 (95% CI, 1.0–2.2) [7], very similar to our finding.

Although there is abundant evidence on the positive association of DM and active TB, it is uncertain whether DM increases the susceptibility to TB infection or accelerates the progression from infection to clinical disease (Figure 1) [1, 5]. More recent studies found DM increased the risk of active TB disease with aORs ranging from 1.3-2.6, or no significant effect at all [17-19] . The strength of the association of DM and LTBI in our study was comparable to these estimates, and was particularly close to the results of Pealing et al.; like our study, Pealing et al. also examined DM patients under chronic disease management [19]. The observations above provide indirect evidence that increased susceptibility to TB infection might play a major contributory role in the occurrence of active TB in the diabetics. However, we must be cautious in this interpretation, because most new infections among LTBI subjects are attributable to reinfection in high TB incidence areas (Figure 1b). In such cases, LTBI is associated with a significantly lower risk of progressive TB relative to primary infection (incidence rate ratio, 0.21) [20]. Consequently, the effect of DM on TB still depends on the extent to which the negative impact of DM on the immune response overrides the presumably immuno-protective effect provided by preexisting LTBI.

Since DM is a progressive disease, longer diabetes duration was found to be a major predictor of DM-related complications and death, independent of glycemic control [21]. An earlier study also revealed the risk of developing TB disease increased among those with increasing diabetes severity [2]. Thus, in this study, we investigated the combined effect of longer duration of DM and poor glycemic control (Supplementary Table 2). We found they did not affect the risk of LTBI. While association between glycemic control and risk of TB was not observed either in other researches targeting patients with long-established DM [17, 19], there have been several studies on cases of pre-diabetes or untreated early diabetes supported the hypothesis [1, 5, 7]. Diabetes patients with poor glycemic control and longer disease duration tend to have a smaller social network and less contact with their family members or friends [22, 23]. This may reduce the opportunity of social contact with TB cases and trumped the risk for recent TB infection.

Many of the subjects with DM in the present study also smoked (14.7%), had abnormal BMIs (overweight and obese, 58.4%; underweight, 1.7%), CKD (32.2%), prior history of TB (2.1%), and advanced age (58.6% older than 60 years), and these may also be risk factors for TB [24-26]. We found that each factor alone had only a mild to moderate association with LTBI (Table 2) after adjustment of BCG protection. However, when an individual has all of these other factors as well as DM, there may be a particularly high-risk of LTBI, especially in those who are elderly. For example, male DM patients older than 60 years who smoke have an aOR of LTBI up to 6.7 (derived by summation of the estimated regression coefficients). Conventional targeted screening for LTBI mostly focuses on host variables and the environment, such as infectiousness of index cases and contact patterns, but seldom consider the effects of multiple factors simultaneously [27, 28]. Our results underscore the necessity of incorporating DM, BCG, TST results, related risk factors and comorbidities to develop a composite scoring system that improves the efficacy of LTBI screening programs.

There are some limitations in our study. First, although the *in vivo* TST and *ex vivo* IGRAs are the only two methods available for diagnosing LTBI, there is concern that immune dysfunction in DM may compromise the performance of these tests [5]. Reduced sensitivity of the QFT-GIT and TST in elderly diabetics (Figure 3a, 3b) may lead to false negatives, and therefore underestimate the effect of DM. Second, all DM patients were enrolled from the DDMP, an intervention designed to facilitate lifestyle modification in patients with DM [12]. Thus, these study subjects may have better general health status, and hence lower risk of TB infection, than DM patients from the general population [19]. Third, the differences in the characteristics of the DM group and the community comparison group suggested selection bias existed between the two groups. Some unmeasured confounders, such as the exposure of TB related to social environment and socioeconomic status, may have biased our estimation of effect of DM on risk of LTBI.

The comorbidity of TB and DM is due to the interaction between DM-impaired immunity and the occurrence of active TB by endogenous reactivation (Figure 1a) or exogeneous new or reinfection (Figure 1b, 1c) [1, 5]. This process is further complicated by the protection conferred from BCG vaccination, remote TB infection, social environment, the co-existence of multiple non-communicable risk factors and other related comorbidities and complications [5, 26, 29]. We tried to control for all possible confounders, but this remains challenging due to the lack of a gold standard for diagnosis of LTBI and the presence of only limited tools to identify DM patients who have the greatest risk of progressing to active TB. More studies are therefore required to identify the predictive value for progression to active TB based on IGRA and/or TST results in patients with DM. There is an ongoing longitudinal study of the present study cohort.

CONCLUSION

In conclusion, our study demonstrated a 1.59-fold increased risk of LTBI in patients with DM from a geographic area that has a high incidence of TB. This finding suggests that practitioners should incorporate BCG vaccination, comorbidities and other coexisting risk factors, in addition to DM, to better identify high-risk groups and enhance the efficacy of targeted screening for LTBI.

DECLARATIONS

Authors' contributions:

1 Guarantor of integrity of the entire study: Ching-Hsiung Lin, Shu-Chen Kuo, Yen-Po Yeh, Shih-Te Tu

2 Study concepts: Ching-Hsiung Lin, Shu-Chen Kuo, Ming-Chia Hsieh, Ih-Jen Su, Sheng-Hao Lin , Yen-Po Yeh, Shih-Te Tu

3 Study design: Ching-Hsiung Lin, Shu-Chen Kuo, Ming-Chia Hsieh, Ih-Jen Su, Sheng-Hao Lin , Yen-Po Yeh, Shih-Te Tu

4 Definition of intellectual content: Ching-Hsiung Lin, Shu-Chen Kuo, Ih-Jen Su, Yee-Chun Chen, Yen-Po Yeh, Shih-Te Tu

5 Literature research: Shu-Chen Kuo, Ming-Chia Hsieh, Chia-Yu Chi, Yee-Chun Chen, Horng Yunn Dou

6 Clinical studies: Ming-Chia Hsieh, Ching-Hsiung Lin, Shang-Yun Ho, Sheng-Hao Lin, Shih-Li Su, Chiung-Ying Liao, Shang-Ren Hsu, Yuan-Chun Huang, Shu Yi Wang, Shi-Dou Lin, Yen-Po Yeh, Shih-Te Tu

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13 Manuscript review: Ching-Hsiung Lin, Ming-Chia Hsieh, Sheng-Hao Lin , Yee-Chun Chen, Yen-Po Yeh, Shih-Te Tu

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Conflict of interest statements:

All authors declare that they have no conflict of interest.

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Ethics committee approval:

All participants provided signed informed consent before enrollment. Both studies were approved by the Institutional Review Board of the Changhua Christian Hospital (numbers IRB:CCH-130102 and IRB:CCH-111012).

Availability of data and material: All data relevant to the study are included in the article or uploaded as supplementary information

Consent for publication: Not applicable.

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TABLES

Table 1. Characteristics of the DM group and the community comparison (CC) group.

	DM group (n=2948)	CC group (n=453)	p
Age, years			
Mean (SD)	61.5 (9.3)	51.3 (10.5)	**<0.001**
<50	304 (10.3%)	209 (46.1%)	
50-59	918 (31.1%)	137 (30.2%)	**<0.001**
60-69	1123 (38.1%)	91 (20.1%)	
≥70	603 (20.5%)	16 (3.5%)	
Sex			
Male	1468 (49.8%)	109 (24.1%)	**<0.001**
Female	1480 (50.2%)	344 (75.9%)	
Prior history of TB			
Yes	61 (2.1%)	4 (0.9%)	0.0852
No	2887 (97.9%)	449 (99.1%)	
History of contact with TB			
Yes	115 (3.9%)	65 (14.3%)	**<0.001**
No	2283 (77.4%)	388 (85.7%)	
Unknown	550 (18.7%)	0 (0.0%)	
BCG scar		440 (97.1%)	
Yes	2436 (82.6%)		
1 scar	1481 (50.2%)		
2 scar	934 (31.7%)		**<0.001**
≧2 scars	21 (0.7%)	13 (2.9%)	
No	502 (17.0%)		
Unknown	10 (0.3%)		
BMI, kg/m^2			
Underweight (<18.5)	50 (1.7%)	7 (1.5%)	
Normal (18.5-24.9)	1175 (39.9%)	274 (60.5%)	**<0.001**
Overweight (25-29.9)	1265 (42.9%)	149 (32.9%)	
Obese (≧30)	458 (15.5%)	23 (5.1%)	
Smoking status			
Current	433 (14.7%)	31 (6.8%)	**<0.001**
Quit	434 (14.7%)	34 (7.5%)	
Never	2081 (70.6%)	388 (85.7%)	
Triglycerides, mg/dL			
<150 mg/dl	1962 (66.6%)	371 (81.9%)	**<0.001**
≧150	986 (33.4%)	82 (18.1%)	
HDL-C*			**<0.001**
Low	1336 (45.3%)	299 (66.0%)	
Ideal	1612 (54.7%)	154 (34.0%)	

CKD**			
Yes	948 (32.2%)	57 (12.6%)	**<0.001**
No	1941 (65.8%)	375 (82.8%)	
Unknown	59 (2.0%)	21 (4.6%)	
Duration of diabetes, years			
mean (SD)	9.0 (6.6)		
≦5	1077 (36.5%)		
>5	1871 (63.5%)		
HbA1c			
Mean (SD)	7.4 (1.5)		
<7%	1400 (47.5%)		
≧7%	1548 (52.5%)		
Unknown	6 (0.2%)		
TST positive			
≧5 mm	2280 (77.3%)	350 (77.3%)	0.9895
<5 mm	668 (22.7%)	103 (22.7%)	
≧10 mm	1665 (56.5%)	251 (55.4%)	0.6974
<10 mm	1283 (43.5%)	202 (44.6%)	
≧15 mm	890 (30.2%)	112 (24.7%)	**0.0211**
<15mm	2058 (69.8%)	341 (75.3%)	
QFT-GIT			
Positive	623 (21.1%)	44 (9.7%)	**<0.001**
Negative	2144 (72.7%)	406 (89.6%)	
Indeterminate	181 (6.1%)	3 (0.7%)	

Abbreviations: SD, standard deviation; HbA1c, glycated hemoglobin; BMI, body mass index; HDL-C, high density lipoprotein cholesterol; TST, tuberculin skin test; QFT-GIT, QuantiFERON-TB Gold In-Tube.

*Low HDL-C: <40 mg/dL (males) and <50 mg/dL (females).

**CKD (chronic kidney disease) was assessed by the Modification of Diet in Renal Disease (MDRD) study equation, using the estimated glomerular filtration rate (eGFR).

Table 2. Multivariable logistic regression analysis of factors associated with LTBI by comparing the DM group with the community comparison group (n = 3217)

Variable	Crude OR (95% CI)	aOR1 (95% CI)	aOR2 (95% CI)
DM	2.68 (1.94-3.71)	1.67 (1.18-2.38)	1.59 (1.11-2.28)
TST 10+	3.66 (2.99-4.49)		4.56 (3.67-5.66)
BCG scar			
No	*Reference*	*Reference*	*Reference*
Yes	0.51 (0.41-0.63)	0.73 (0.57-0.93)	0.66 (0.51-0.85)
Age, years			
<50	*Reference*	*Reference*	*Reference*
50-59	1.98 (1.42-2.76)	1.86 (1.30-2.65)	2.12 (1.48-3.05)
60-69	2.52 (1.83-3.49)	2.25 (1.58-3.20)	2.77 (1.93-3.97)
≥70	4.07 (2.89-5.71)	2.98 (2.00-4.44)	4.27 (2.83-6.44)
Male	1.41 (1.19-1.68)	1.22 (0.98-1.52)	1.12 (0.89-1.41)
Smoking			
Never	*Reference*	*Reference*	*Reference*
Current	1.54 (1.22-1.95)	1.49 (1.13-1.97)	1.28 (0.95-1.71)
Quit	1.00 (0.77-1.29)	0.87 (0.64-1.17)	0.82 (0.60-1.11)
Chronic kidney disease	1.56 (1.31-1.87)	1.17 (0.96-1.42)	1.26 (1.03-1.55)
Prior history of TB	2.95 (1.78-4.89)	2.17 (1.28-3.68)	2.08 (1.19-3.63)
TB contact	0.75 (0.50-1.13)	0.92 (0.60-1.40)	0.86 (0.55-1.33)

Abbreviations: DM, the diabetes mellitus; TST 10+, results of tuberculin skin test ≧ 10mm; OR, odds ratio; aOR, adjusted odds ratio; CI, confidence interval; aOR1: adjusted odds ratio without adjustment of TST results; aOR2: adjusted odds ratio with adjustment of TST results.

FIGURE LEGENDS

Figure 1 Possible temporal relationships between the onset of DM and the occurrence of TB infection. Circled letters indicate times when DM could possibly affect the pathogenesis of TB (i, increased susceptibility to TB infection; p, accelerated progression from infection to clinical disease). (a) Onset of DM with a pre-existing latent TB infection (LTBI). (b) Onset of DM with a pre-existing LTBI, but before re-infection. (c) Onset of DM before the primary TB infection.

Figure 2 Patient selection and enrolment in the DM group (left) and the CC group (right).

Abbreviations: TST, tuberculin skin test; IGRA, interferon-gamma release assay; CHCIS, Changhua Community-based Integrated Screening program; DM, diabetes mellitus.

Figure 3 Tuberculin skin test (TST) positivity (a) and interferon-gamma release assay (IGRA) positivity (b) in the DM group (DMG) and the CC group (CCG) according to age and BCG scar status. A tuberculin induration size of 10 mm was used as the cutoff point for both groups.

Abbreviations: TST, tuberculin skin test; QFT, QuantiFERON-TB Gold In-Tube.

Figure 1

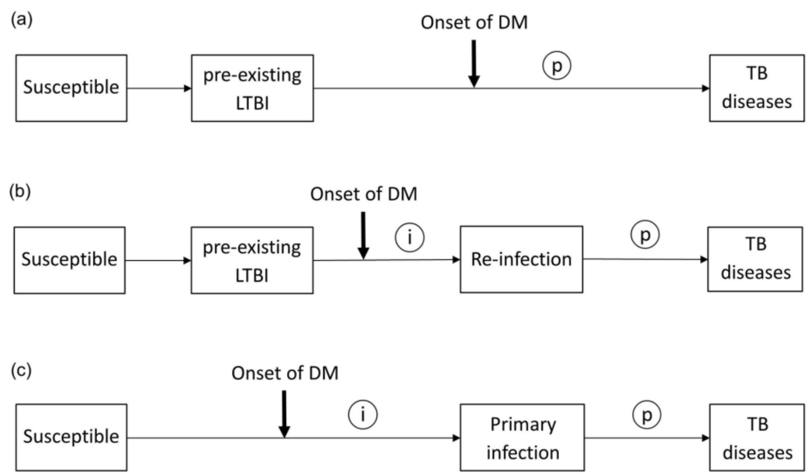


Figure 1 Possible temporal relationships between the onset of DM and the occurrence of TB infection. Circled letters indicate times when DM could possibly affect the pathogenesis of TB (i, increased susceptibility to TB infection; p, accelerated progression from infection to clinical disease). (a) Onset of DM with a pre-existing latent TB infection (LTBI). (b) Onset of DM with a pre-existing LTBI, but before re-infection. (c) Onset of DM before the primary TB infection.

89x89mm (600 x 600 DPI)

Figure 2

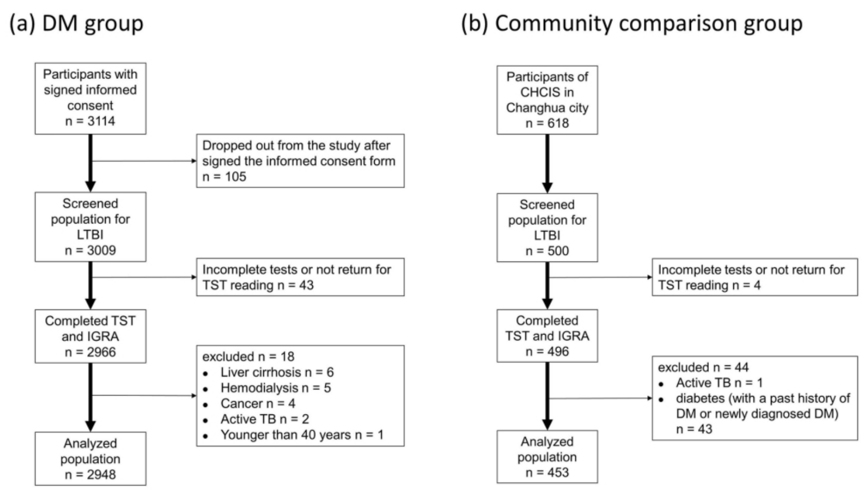
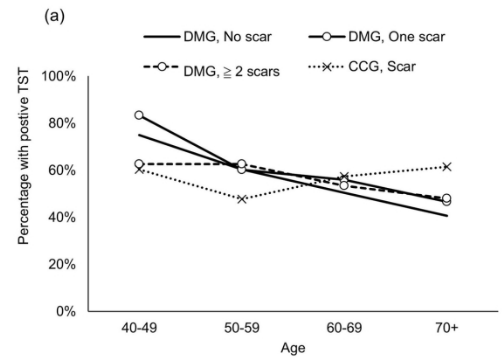
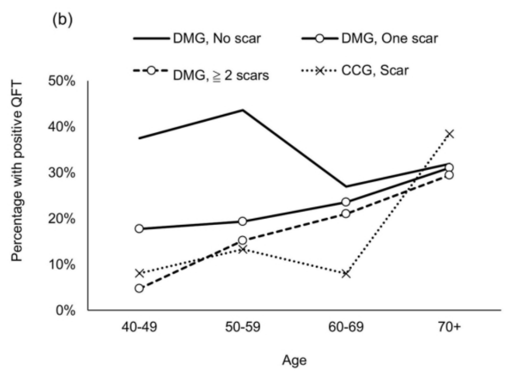


Figure 2 Patient selection and enrolment in the DM group (left) and the CC group (right). Abbreviations: TST, tuberculin skin test; IGRA, interferon-gamma release assay; CHCIS, Changhua Community-based Integrated Screening program; DM, diabetes mellitus.

90x90mm (600 x 600 DPI)

Figure 3





Tuberculin skin test (TST) positivity (a) and interferon-gamma release assay (IGRA) positivity (b) in the DM group (DMG) and the CC group (CCG) according to age and BCG scar status. A tuberculin induration size of 10 mm was used as the cutoff point for both groups.
Abbreviations: TST, tuberculin skin test; QFT, QuantiFERON-TB Gold In-Tube.

90x90mm (600 x 600 DPI)

Supplementary Table 1. Agreement between TST and QFT-GIT

Results	Overall n = 3217			DM group n = 2767			CC group n = 450		
	TST cut point			TST cut point			TST cut point		
	5 mm	10 mm	15 mm	5 mm	10 mm	15 mm	5 mm	10 mm	15 mm
TST+/QFT-GIT+	613	528	363	37	32	23	576	496	340
TST-/QFT-GIT-	664	1252	1950	95	188	318	569	1064	1632
TST+/QFT-GIT-	1886	1298	600	311	218	88	1575	1080	512
TST-/QFT-GIT+	54	139	304	7	12	21	47	127	283
Agreement%	39.7%	55.3%	71.9%	29.3%	48.9%	75.8%	41.4%	56.4%	71.3%
Kappa (95% CI)	0.09 (0.74-0.10)	0.17 (0.15-0.20)	0.27 (0.23-0.30)	0.10 (0.08-0.12)	0.19 (0.16-0.22)	0.27 (0.23-0.31)	0.02 (-0.01-0.05)	0.06 (0.01-0.11)	0.18 (0.09-0.28)

Abbreviations: TST, tuberculin skin test; QFT-GIT, QuantiFERON-TB Gold In-Tube; CI, confidence interval.
Test results with an indeterminate QFT-GIT response are not included in the table.

2

Supplementary Table 2. Combined effects of glycemic control (HbA1c) and duration of DM on risk of latent tuberculosis infection without and with adjustment of TST results.

Variables	Crude OR (95% CI)	aOR1 (95% CI)	aOR2 (95% CI)
Community comparison group	Reference	Reference	Reference
A1C ≧7%, duration >5 years	2.49 (1.77-3.52)	1.42 (0.97-2.07)	1.33 (0.90-1.96)
A1C ≧7%, duration ≦5 years	2.71 (1.84-3.98)	1.87 (1.24-2.82)	1.80 (1.18-2.74)
A1C <7%, duration >5 years	2.84 (1.99-4.04)	1.67 (1.13-2.46)	1.54 (1.03-2.28)
A1C <7%, duration ≦5 years	2.81 (1.95-4.04)	1.84 (1.24-2.72)	1.82 (1.22-2.72)

Abbreviations: OR, odds ratio; aOR, adjusted odds ratio; CI, confidence interval; TST, tuberculin skin test; A1C, glycated hemoglobin. aOR1: adjusted odds ratio without adjustment of TST results; aOR2: adjusted odds ratio with adjustment of TST results.

*The multivariable model adjusted for the comorbidities indicated in Table 2.

STROBE 2007 (v4) checklist of items to be included in reports of observational studies in epidemiology*

Checklist for cohort, case-control, and cross-sectional studies (combined)

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	4
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	4
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	8-9
Objectives	3	State specific objectives, including any pre-specified hypotheses	9
Methods			
Study design	4	Present key elements of study design early in the paper	10
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	10
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	10-12
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	11-13
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*11-12*
Bias	9	Describe any efforts to address potential sources of bias	-
Study size	10	Explain how the study size was arrived at	10-11
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	10-12
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	13-14
		(*b*) Describe any methods used to examine subgroups and interactions	13-14
		(*c*) Explain how missing data were addressed	-
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed	-

		Cross-sectional study—If applicable, describe analytical methods taking account of sampling strategy	
		(*e*) Describe any sensitivity analyses	-
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	14
		(b) Give reasons for non-participation at each stage	-
		(c) Consider use of a flow diagram	-
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	14-16
		(b) Indicate number of participants with missing data for each variable of interest	-
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	17-21
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	17-21
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	17-21
		Cross-sectional study—Report numbers of outcome events or summary measures	17-21
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	17-21
		(*b*) Report category boundaries when continuous variables were categorized	17-21
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	-
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	
Discussion			
Key results	18	Summarise key results with reference to study objectives	21-25
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	24
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	24-25
Generalisability	21	Discuss the generalisability (external validity) of the study results	24-25
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	28

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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Effect of Diabetes Mellitus on Risk of Latent TB Infection in A High TB Incidence Area: A Community-based Study in Taiwan

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Secondary Subject Heading:	Diabetes and endocrinology, Epidemiology, Public health, Immunology (including allergy), Respiratory medicine
Keywords:	Diabetes Mellitus, Tuberculosis < INFECTIOUS DISEASES, Latent Infection, Bacillus Calmette–Guérin vaccination, Interferon gamma release essay, Tuberculin skin test

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Effect of Diabetes Mellitus on Risk of Latent TB Infection in A High TB Incidence Area: A Community-based Study in Taiwan

Running title: Individuals with diabetes have a moderately increased risk of latent TB infection.

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ABSTRACT

Objective: To investigate the association between diabetes and latent tuberculosis infections (LTBIs) in high TB incidence areas

Design: Community-based comparison study

Setting: Outpatient diabetes clinics at 4 hospitals and 13 health centers in urban and rural townships. A community-based screening program was used to recruit non-diabetic participants.

Participants: A total of 2948 patients with diabetes aged older than 40 years were recruited, and 453 non-diabetic participants from the community were enrolled.

Primary and secondary outcome measures: The interferon-gamma release assay (IGRA) and the tuberculin skin test were used to detect LTBI. The IGRA result was used as a surrogate of LTBI in logistic regression analysis.

Results: Diabetes was significantly associated with LTBI ([aOR] = 1.59; 95% CI, 1.11-2.28) and age correlated positively with LTBI. Many subjects with diabetes also had additional risk factors (current smokers [aOR=1.28; 95% CI, 0.95-1.71], comorbid chronic kidney disease [aOR = 1.26; 95% CI, 1.03-1.55], and prior history of TB [aOR = 2.08; 95% CI, 1.19-3.63]). The presence of BCG scar was protective (aOR = 0.66 ; 95% CI, 0.51-0.85). Duration of diabetes and poor glycemic control were unrelated to the risk of LTBI.

Conclusion: There was a moderately increased risk of LTBI in diabetes patients from this high TB incidence area. This finding suggests LTBI screening for the diabetics be combined with other risk factors and comorbidities of TB to better identify high-risk groups and improve the efficacy of targeted screening for LTBI.

Keywords: Diabetes Mellitus, Tuberculosis, Latent Infection, Bacillus Calmette–Guérin

vaccination, Interferon-gamma release assay, Tuberculin skin test

Strengths and limitations of this study

1. The strengths of this study are the adoption of stringent diagnostic criteria for DM and comprehensiveness of information obtained from community-based programs.

2. Protection of BCG vaccination, remote TB infection and other important potential confounding variables were controlled for assessing the DM-LTBI association.

3. The study limitations are the reduced sensitivity of the QFT-GIT and TST in elderly diabetics and better general health status among the DM group.

Abbreviations

Bacillus Calmette–Guérin (BCG)

Changhua Christian Healthcare System (CCHS)

Changhua Community-based Integrated Screening (CHCIS)

Chronic kidney disease (CKD)

Diabetes mellitus (DM)

Glycated hemoglobin (HbA1c)

Interferon-gamma release assay (IGRA)

Latent TB infection (LTBI)

Tuberculin skin test (TST)

Tuberculosis (TB)

BACKGROUND

The co-occurrence of diabetes mellitus (DM) and tuberculosis (TB) cannot be overemphasized because of the high prevalence of each disease throughout the world [1]. Previous studies found that DM affects TB disease presentation, leads to poor treatment outcomes, and increases the risk for active TB [1, 2]. The recently published World Health Organization guideline recommends screening for active TB with DM [3], but it remains elusive whether screening and treatment of latent TB infections (LTBIs) should be prioritized to target individuals with DM [1, 4-6].

There is only limited information on the LTBI status of patients with DM. Most of antecedent relevant studies were in low TB incidence countries [7] or based on selected population by identifying DM in high-risk populations, such as TB contacts, immunocompromised patients with comorbid DM, or crisis-affected people [6]. The results of these studies are also inconsistent, mainly due to the different methods used to ascertain DM status (*e.g.* self-report, medical records, or laboratory testing) and differences in the control of potentially important confounders [1, 5-7].

In high TB incidence areas, where most of TB infection occurred at young age before the onset of DM [8], the temporal relationship between DM and TB infection is different from that in low incidence areas (Figure 1a-1b v.s. 1c) [6]. This temporal characteristic may lead to non-differential misclassification of DM-related TB infection in cross-sectional studies and therefore bias the association toward the null (Figure 1a) [6]. It is also worth noting that Bacillus Calmette–Guérin (BCG) vaccine is widely used in high burden TB countries for childhood immunization against TB. Recent studies found BCG vaccine has a protective effect against TB infection in children [9] and can even last into adulthood [10]. Although several researches have been devoted to assessment of DM-LTBI association, rather less attention has been paid to confounding

from the protection conferred by BCG vaccination in settings where coverage of BCG vaccination in study subjects was high.

In this study, using data from community-based programs in an area with a high incidence of TB and a high coverage of BCG vaccination, we assessed the overall risk of LTBI in people with and without DM by carefully controlling for potential confounders, specifically including BCG vaccination, risk of remote TB infection, comorbidities, important lifestyle factors and DM severity.

METHODS

We conducted a community-based study that comprised DM and non-DM subjects by combining two community-based programs to investigate the effect of DM on risk of LTBI in Changhua, a county in Taiwan with a TB incidence of 58.7 per 100000 and a BCG vaccination coverage of around 99% in 2012 [11]. The first program recruited the DM group from patients registered in the Changhua Diabetes Shared Care program (CHDSC) [12] to participate in the LTBI survey. The second recruited the community comparison (CC) group from participants of a community-based LTBI screening program [13]. Due to the lack of a gold-standard test for LTBI, the tuberculin skin test (TST) was used in parallel with the interferon-gamma release assay (IGRA). All participants provided signed informed consent before enrollment. Both studies were approved by the Institutional Review Board of the Changhua Christian Hospital (numbers IRB:CCH-130102 and IRB:CCH-111012).

DM group

Nearly 60% of DM patients in Changhua were enrolled in the national diabetes disease management program (DDMP) and registered in the CHDSC [12]. These enrollees were suitable to be included as the study subjects, because their DM status had been ascertained by certified physicians who provided diabetes care according to national guidelines. We prospectively invited all those registered DM cases aged older than 40 years when they presented to outpatient diabetes clinics of the Changhua Christian Healthcare System (CCHS) or 13 nearby health centers in the surrounding townships from April 1, 2013 to December 31, 2013. CCHS comprises one medical center and three branch hospitals, distributed evenly at different locations of the county, and covers urban and rural areas. Thus, the participants of this study were a representative sample of the total DM population.

All study subjects were screened for pulmonary TB based on respiratory symptoms and chest X-rays upon entry into the study. Suspected cases submitted sputum specimens for acid-fast bacilli smears and culture. The diagnosis of TB was confirmed by chest specialists. Subjects were excluded if they had active pulmonary TB, a life expectancy of less than 2 years, metastatic cancer, or organ failure (*e.g.* severe liver disease [Child-Pugh Class B or C]) except chronic renal failure. The included eligible DM cases then received a TST and IGRA for detection of LTBI.

Community comparison group

The Changhua Community-based Integrated Screening (CHCIS) program, which began in 2005, screens for neoplastic and non-neoplastic diseases (including DM and pulmonary TB) [13]. The method used to screen for pulmonary TB was the same as that used in the DM group. We invited all consecutive attendees of the CHCIS program within the major catchment area of the CCHS to participate in LTBI screening in May 1, 2011. Participants with known DM or newly screened DM (*i.e.* fasting plasma glucose [FPG] ≧ 126 mg/dL) were excluded.

Tests for LTBI

Venous blood was collected for the QuantiFERON-TB Gold In-Tube test (QFT-GIT; Cellestis, Carnegie, Australia), which was performed in 2 stages, according the manufacturer's instructions. The cutoff for a positive result was 0.35 IU/mL. The reaction of a nil control and a mitogen control were within the range provided by the manufacturer. After collection of blood samples for QFT-GIT, trained nurses administered the TST using 2 TU of PPD RT23 (Statens Serum Institut, Copenhagen, Denmark) by the Mantoux method, and inspected the presence of BCG scars. Tuberculin indurations were measured 48-72 h after injection using the palpation method, and a diameter of 10 mm or more was defined as positive. All TST procedures followed the national guidelines issued by the CDC of Taiwan [14].

Data collection

We examined the DDMP database, and abstracted demographic data and information on DM care in the same year when each subject was recruited. This included duration of DM, glycated hemoglobin (HbA1c), blood pressure, lipid profile, renal function, and other related cardiovascular disease risk factors. We also linked individual data with the TB registry at the local health authority to assess whether each study subject had a prior history of TB or contact with TB.

Statistical analysis

Although both TST and IGRA indicate a cellular immune response to Mycobacterium tuberculosis (MTB) and are useful for the diagnosis of LTBI, the two tests identified different population with distinct immunologic processes [15].

Substantial discordance of TST and IGRA has been found in previous studies [15, 16]. Nevertheless, IGRA has a dose–response relationship with recent TB exposure and it wanes rapidly [15, 16]. It may be better than TST at detecting recent rather than remote TB infection. Thus, we used the result of the QFT-GIT as a surrogate of LTBI status to estimate univariate odds ratios (ORs) and 95% confidence intervals (CIs) using a logistic regression model. Variables that met p-values less than 0.2 at univariable analysis were retained for the multivariable model, which also incorporated standard sociodemographic variables such as age and gender. The multivariable model was fitted to generate adjusted odds ratios (aOR) of the association between LTBI and DM by comparing the DM group with the CC group and adjusting for other independent variables including age, gender, BCG scar, smoking, prior history of TB, contact with TB and comorbid chronic kidney disease etc.

Since tuberculin reactivity was known to represent the cumulative effect of previous TB infection, we further included the TST results in the models in attempt to control the confounding of remote TB infection (i.e. TB infection acquired at young age before the onset of DM [Figure 1a]). All analyses were conducted using SAS version 9.3 (SAS Institute Inc., Cary, NC, USA).

Patient and Public Involvement

Patients and/or the public were not involved in this study. There are no plans to disseminate the results of the research to study participants.

RESULTS

Characteristics of Participants

We ultimately enrolled 2948 patients in the DM group and 453 non-DM subjects in

CC group, and included all of these individuals in the final analysis (Figure 2). The mean duration of DM in the DM group was 9.0 years (standard deviation [SD]=6.6) and nearly half of these patients achieved good glycemic control (*i.e.* HbA1c < 7%) (Table 1). The DM group had a greater mean age, higher percentages of males and smokers, greater prevalences of obesity, chronic kidney disease (CKD), and prior history of TB. The DM group also had a greater prevalence of tuberculin reactivity (≧ 15mm) and of QFT-GIT positivity, and a higher proportion of indeterminate results (Table 1).

TST and QFT-GIT results

The presence of BCG scars had no effect on the tuberculin reactivity of the DM group (Figure 3a). TST positivity decreased with age, and was higher in the DM group than the CC group, except among the elderly (60+ years). Figure 3b shows that QFT-GIT positivity of DM group was highest among those with no BCG scars, and that positivity declined dramatically in those more than 60 years-old. In contrast, the rate of QFT-GIT positivity in those with BCG scars gradually increased with age. For each age group, there was an inverse correlation between number of BCG scars and QFT-GIT positivity. Individuals in the CC group with BCG scars had the lowest QFT-GIT positivity, but elderly individuals from the CC group (age 70+) had the highest QFT-GIT positivity. Generally, the concordance between the TST and QFT-GIT was low (Supplementary Table 1).

Effect of DM and other factors on risk of LTBI

Multivariate regression analysis indicates DM significantly increased the risk of LTBI after controlling for major confounders (aOR=1.67; 95%CI, 1.18-2.38). This risk was similar after adjustment for tuberculin reactivity (aOR=1.59; 95% CI, 1.11-2.28). In addition, the presence of LTBIs increased with age. For age older than 70 years, TST adjustment had a marked effect and changed its aOR from 4.27 (95% CI, 2.83-6.44) to 2.98 (95% CI, 2.00-4.44). Other variables, such as current smoking, CKD, and prior history of TB, were also significant risk factors for LTBI, and their effects were similar with or without adjustment for tuberculin reactivity. Notably, the presence of a BCG scar had a significantly protective effect on LTBI (aOR=0.66 [95%CI, 0.51-0.85]) (Table 2).

Comorbidities, such as hyperlipidemia, hypertension, and abnormal body mass index (BMI), had no significant associations with LTBI. We further investigated the effect of long duration DM and poor glycemic control by dividing the DM group into four sub-groups according to duration of DM and HbA1c level, and then compared the risk of LTBI of these different sub-groups, using the CC group as a reference. All of the DM sub-groups had similar risks for LTBI. This indicates that long duration of DM and poor glycemic control had no effect on the risk of LTBI (Supplementary Table 2).

DISCUSSION

Very few studies have examined the effect of DM on the risk of LTBI in high TB incidence areas and simultaneously taken into account confounding effects resulting from protection of BCG vaccination and remote TB infection. To our best knowledge, this is the first study of this kind. The key strengths of this study included adoption of stringent diagnostic criteria for DM and comprehensiveness of information obtained from community-based programs, which enabled thorough adjustment for important potential confounding variables. Our multivariate analysis indicated that DM had a positive association with risk of LTBI (aOR, 1.59 [95%CI, 1.11-2.28]). This finding has profound implications.

A recent systematic review concluded the pooled odds ratio estimate for DM on risk of LTBI was 1.18 (95% CI, 1.06–1.30) [6]. Reasons for the different results from our study could be related to differences in the populations studied, methods for pooling results from distinct measurements for LTBI (i.e. TST versus IGRA), ascertainment of diabetes status by self-reports or medical records, and lack of control for several major confounders. By contrast, a recently published population-based study in US, a country where BCG is not generally recommended for use, using similar diagnostic criteria as this study, showed DM was associated with LTBI with an aOR 1.5 (95% CI, 1.0–2.2) [7], very similar to our finding.

Although there is abundant evidence on the positive association of DM and active TB, it is uncertain whether DM increases the susceptibility to TB infection or accelerates the progression from infection to clinical disease (Figure 1) [1, 5]. More recent studies found DM increased the risk of active TB disease with aORs ranging from 1.3-2.6, or no significant effect at all [17-19] . The strength of the association of DM and LTBI in our study was comparable to these estimates, and was particularly close to the results of Pealing et al.; like our study, Pealing et al. also examined DM patients under chronic disease management [19]. The observations above provide indirect evidence that increased susceptibility to TB infection might play a major contributory role in the occurrence of active TB in the diabetics. However, we must be cautious in this interpretation, because most new infections among LTBI subjects are attributable to reinfection in high TB incidence areas (Figure 1b). In such cases, LTBI is associated with a significantly lower risk of progressive TB relative to primary infection (incidence rate ratio, 0.21) [20]. Consequently, the effect of DM on TB still depends on the extent to which the negative impact of DM on the immune response overrides the presumably immuno-protective effect provided by preexisting LTBI.

Since DM is a progressive disease, longer diabetes duration was found to be a major predictor of DM-related complications and death, independent of glycemic control [21]. An earlier study also revealed the risk of developing TB disease increased among those with increasing diabetes severity [2]. Thus, in this study, we investigated the combined effect of longer duration of DM and poor glycemic control (Supplementary Table 2). We found they did not affect the risk of LTBI. While association between glycemic control and risk of TB was not observed either in other researches targeting patients with long-established DM [17, 19], there have been several studies on cases of pre-diabetes or untreated early diabetes supported the hypothesis [1, 5, 7]. Diabetes patients with poor glycemic control and longer disease duration tend to have a smaller social network and less contact with their family members or friends [22, 23]. This may reduce the opportunity of social contact with TB cases and trumped the risk for recent TB infection.

Many of the subjects with DM in the present study also smoked (14.7%), had abnormal BMIs (overweight and obese, 58.4%; underweight, 1.7%), CKD (32.2%), prior history of TB (2.1%), and advanced age (58.6% older than 60 years), and these may also be risk factors for TB [24-26]. We found that each factor alone had only a mild to moderate association with LTBI (Table 2) after adjustment of BCG protection. However, when an individual has all of these other factors as well as DM, there may be a particularly high-risk of LTBI, especially in those who are elderly. For example, male DM patients older than 60 years who smoke have an aOR of LTBI up to 6.7 (derived by summation of the estimated regression coefficients). Conventional targeted screening for LTBI mostly focuses on host variables and the environment, such as infectiousness of index cases and contact patterns, but seldom consider the effects of multiple factors simultaneously [27, 28]. Our results underscore the necessity of incorporating DM, BCG, TST results, related risk factors and comorbidities to develop a composite scoring system that improves the efficacy of LTBI screening programs.

There are some limitations in our study. First, although the *in vivo* TST and *ex vivo* IGRAs are the only two methods available for diagnosing LTBI, there is concern that immune dysfunction in DM may compromise the performance of these tests [5]. Reduced sensitivity of the QFT-GIT and TST in elderly diabetics (Figure 3a, 3b) may lead to false negatives, and therefore underestimate the effect of DM. Second, all DM patients were enrolled from the DDMP, an intervention designed to facilitate lifestyle modification in patients with DM [12]. Thus, these study subjects may have better general health status, and hence lower risk of TB infection, than DM patients from the general population [19]. Third, the differences in the characteristics of the DM group and the community comparison group suggested selection bias existed between the two groups. Some unmeasured confounders, such as the exposure of TB related to social environment and socioeconomic status, may have biased our estimation of effect of DM on risk of LTBI.

The comorbidity of TB and DM is due to the interaction between DM-impaired immunity and the occurrence of active TB by endogenous reactivation (Figure 1a) or exogeneous new or reinfection (Figure 1b, 1c) [1, 5]. This process is further complicated by the protection conferred from BCG vaccination, remote TB infection, social environment, the co-existence of multiple non-communicable risk factors and other related comorbidities and complications [5, 26, 29]. We tried to control for all possible confounders, but this remains challenging due to the lack of a gold standard for diagnosis of LTBI and the presence of only limited tools to identify DM patients who have the greatest risk of progressing to active TB. More studies are therefore required to identify the predictive value for progression to active TB based on IGRA and/or TST results in patients with DM. There is an ongoing longitudinal study of the present study cohort.

CONCLUSION

In conclusion, our study demonstrated a 1.59-fold increased risk of LTBI in patients with DM from a geographic area that has a high incidence of TB. This finding suggests that practitioners should incorporate BCG vaccination, comorbidities and other coexisting risk factors, in addition to DM, to better identify high-risk groups and enhance the efficacy of targeted screening for LTBI.

DECLARATIONS

Authors' contributions:

1 Guarantor of integrity of the entire study: Ching-Hsiung Lin, Shu-Chen Kuo, Yen-Po Yeh, Shih-Te Tu

2 Study concepts: Ching-Hsiung Lin, Shu-Chen Kuo, Ming-Chia Hsieh, Ih-Jen Su, Sheng-Hao Lin , Yen-Po Yeh, Shih-Te Tu

3 Study design: Ching-Hsiung Lin, Shu-Chen Kuo, Ming-Chia Hsieh, Ih-Jen Su, Sheng-Hao Lin , Yen-Po Yeh, Shih-Te Tu

4 Definition of intellectual content: Ching-Hsiung Lin, Shu-Chen Kuo, Ih-Jen Su, Yee-Chun Chen, Yen-Po Yeh, Shih-Te Tu

5 Literature research: Shu-Chen Kuo, Ming-Chia Hsieh, Chia-Yu Chi, Yee-Chun Chen, Horng Yunn Dou

6 Clinical studies: Ming-Chia Hsieh, Ching-Hsiung Lin, Shang-Yun Ho, Sheng-Hao Lin, Shih-Li Su, Chiung-Ying Liao, Shang-Ren Hsu, Yuan-Chun Huang, Shu Yi Wang, Shi-Dou Lin, Yen-Po Yeh, Shih-Te Tu

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Conflict of interest statements:

All authors declare that they have no conflict of interest.

Role of funding source:

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Ethics committee approval:

All participants provided signed informed consent before enrollment. Both studies were approved by the Institutional Review Board of the Changhua Christian Hospital (numbers IRB:CCH-130102 and IRB:CCH-111012).

Availability of data and material: All data relevant to the study are included in the article or uploaded as supplementary information

Consent for publication: Not applicable.

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TABLES

Table 1. Characteristics of the DM group and the community comparison (CC) group.

	DM group (n=2948)	CC group (n=453)	p
Age, years			
Mean (SD)	61.5 (9.3)	51.3 (10.5)	**<0.001**
<50	304 (10.3%)	209 (46.1%)	
50-59	918 (31.1%)	137 (30.2%)	**<0.001**
60-69	1123 (38.1%)	91 (20.1%)	
≥70	603 (20.5%)	16 (3.5%)	
Sex			
Male	1468 (49.8%)	109 (24.1%)	**<0.001**
Female	1480 (50.2%)	344 (75.9%)	
Prior history of TB			
Yes	61 (2.1%)	4 (0.9%)	0.0852
No	2887 (97.9%)	449 (99.1%)	
History of contact with TB			
Yes	115 (3.9%)	65 (14.3%)	**<0.001**
No	2283 (77.4%)	388 (85.7%)	
Unknown	550 (18.7%)	0 (0.0%)	
BCG scar		440 (97.1%)	
Yes	2436 (82.6%)		
1 scar	1481 (50.2%)		
2 scar	934 (31.7%)		**<0.001**
≧2 scars	21 (0.7%)	13 (2.9%)	
No	502 (17.0%)		
Unknown	10 (0.3%)		
BMI, kg/m^2			
Underweight (<18.5)	50 (1.7%)	7 (1.5%)	**<0.001**
Normal (18.5-24.9)	1175 (39.9%)	274 (60.5%)	
Overweight (25-29.9)	1265 (42.9%)	149 (32.9%)	
Obese (≧30)	458 (15.5%)	23 (5.1%)	
Smoking status			
Current	433 (14.7%)	31 (6.8%)	**<0.001**
Quit	434 (14.7%)	34 (7.5%)	
Never	2081 (70.6%)	388 (85.7%)	
Triglycerides, mg/dL			
<150 mg/dl	1962 (66.6%)	371 (81.9%)	**<0.001**
≧150	986 (33.4%)	82 (18.1%)	
HDL-C*			**<0.001**
Low	1336 (45.3%)	299 (66.0%)	
Ideal	1612 (54.7%)	154 (34.0%)	

CKD**			
Yes	948 (32.2%)	57 (12.6%)	**<0.001**
No	1941 (65.8%)	375 (82.8%)	
Unknown	59 (2.0%)	21 (4.6%)	
Duration of diabetes, years			
mean (SD)	9.0 (6.6)		
≦5	1077 (36.5%)		
>5	1871 (63.5%)		
HbA1c			
Mean (SD)	7.4 (1.5)		
<7%	1400 (47.5%)		
≧7%	1548 (52.5%)		
Unknown	6 (0.2%)		
TST positive			
≧5 mm	2280 (77.3%)	350 (77.3%)	0.9895
<5 mm	668 (22.7%)	103 (22.7%)	
≧10 mm	1665 (56.5%)	251 (55.4%)	0.6974
<10 mm	1283 (43.5%)	202 (44.6%)	
≧15 mm	890 (30.2%)	112 (24.7%)	**0.0211**
<15mm	2058 (69.8%)	341 (75.3%)	
QFT-GIT			
Positive	623 (21.1%)	44 (9.7%)	**<0.001**
Negative	2144 (72.7%)	406 (89.6%)	
Indeterminate	181 (6.1%)	3 (0.7%)	

Abbreviations: SD, standard deviation; HbA1c, glycated hemoglobin; BMI, body mass index; HDL-C, high density lipoprotein cholesterol; TST, tuberculin skin test; QFT-GIT, QuantiFERON-TB Gold In-Tube.

*Low HDL-C: <40 mg/dL (males) and <50 mg/dL (females).

**CKD (chronic kidney disease) was assessed by the Modification of Diet in Renal Disease (MDRD) study equation, using the estimated glomerular filtration rate (eGFR).

Table 2. Multivariable logistic regression analysis of factors associated with LTBI by comparing the DM group with the community comparison group (n = 3217)

Variable	Crude OR (95% CI)	aOR1 (95% CI)	aOR2 (95% CI)
DM	2.68 (1.94-3.71)	1.67 (1.18-2.38)	1.59 (1.11-2.28)
TST 10+	3.66 (2.99-4.49)		4.56 (3.67-5.66)
BCG scar			
No	*Reference*	*Reference*	*Reference*
Yes	0.51 (0.41-0.63)	0.73 (0.57-0.93)	0.66 (0.51-0.85)
Age, years			
<50	*Reference*	*Reference*	*Reference*
50-59	1.98 (1.42-2.76)	1.86 (1.30-2.65)	2.12 (1.48-3.05)
60-69	2.52 (1.83-3.49)	2.25 (1.58-3.20)	2.77 (1.93-3.97)
≥70	4.07 (2.89-5.71)	2.98 (2.00-4.44)	4.27 (2.83-6.44)
Male	1.41 (1.19-1.68)	1.22 (0.98-1.52)	1.12 (0.89-1.41)
Smoking status			
Never	*Reference*	*Reference*	*Reference*
Current	1.54 (1.22-1.95)	1.49 (1.13-1.97)	1.28 (0.95-1.71)
Quit	1.00 (0.77-1.29)	0.87 (0.64-1.17)	0.82 (0.60-1.11)
Chronic kidney disease	1.56 (1.31-1.87)	1.17 (0.96-1.42)	1.26 (1.03-1.55)
Prior history of TB	2.95 (1.78-4.89)	2.17 (1.28-3.68)	2.08 (1.19-3.63)
TB contact	0.75 (0.50-1.13)	0.92 (0.60-1.40)	0.86 (0.55-1.33)

Abbreviations: DM, the diabetes mellitus; TST 10+, results of tuberculin skin test ≧ 10mm; OR, odds ratio; aOR, adjusted odds ratio; CI, confidence interval. The aOR of DM has been adjusted for age, gender, smoking status, chronic kidney disease, prior history of TB and TB contact in this multivariable logistic regression model. aOR1: adjusted odds ratio without adjustment of TST results; aOR2: adjusted odds ratio with adjustment of TST results.

FIGURE LEGENDS

Figure 1 Possible temporal relationships between the onset of DM and the occurrence of TB infection. Circled letters indicate times when DM could possibly affect the pathogenesis of TB (i, increased susceptibility to TB infection; p, accelerated progression from infection to clinical disease). (a) Onset of DM with a pre-existing latent TB infection (LTBI). (b) Onset of DM with a pre-existing LTBI, but before re-infection. (c) Onset of DM before the primary TB infection.

Figure 2 Patient selection and enrolment in the DM group (left) and the CC group (right).

Abbreviations: TST, tuberculin skin test; IGRA, interferon-gamma release assay; CHCIS, Changhua Community-based Integrated Screening program; DM, diabetes mellitus.

Figure 3 Tuberculin skin test (TST) positivity (a) and interferon-gamma release assay (IGRA) positivity (b) in the DM group (DMG) and the CC group (CCG) according to age and BCG scar status. A tuberculin induration size of 10 mm was used as the cutoff point for both groups.

Abbreviations: TST, tuberculin skin test; QFT, QuantiFERON-TB Gold In-Tube.

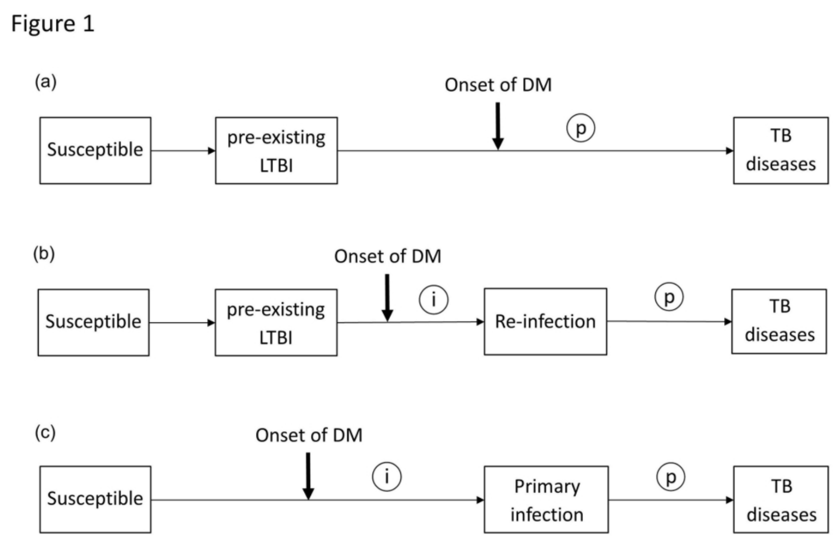


Figure 1 Possible temporal relationships between the onset of DM and the occurrence of TB infection. Circled letters indicate times when DM could possibly affect the pathogenesis of TB (i, increased susceptibility to TB infection; p, accelerated progression from infection to clinical disease). (a) Onset of DM with a pre-existing latent TB infection (LTBI). (b) Onset of DM with a pre-existing LTBI, but before re-infection. (c) Onset of DM before the primary TB infection.

89x89mm (600 x 600 DPI)

Figure 2

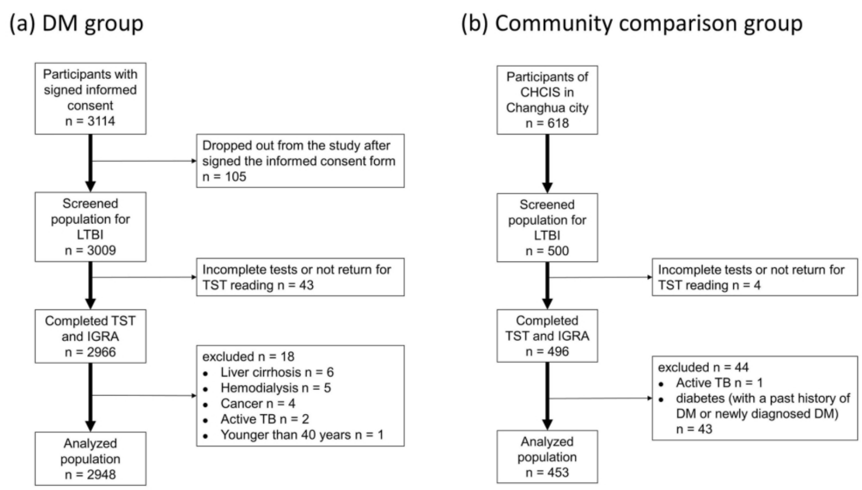
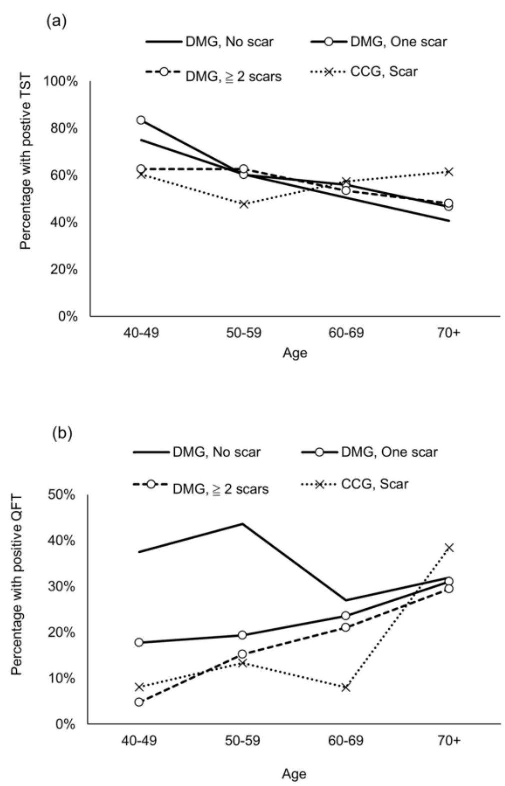


Figure 2 Patient selection and enrolment in the DM group (left) and the CC group (right). Abbreviations: TST, tuberculin skin test; IGRA, interferon-gamma release assay; CHCIS, Changhua Community-based Integrated Screening program; DM, diabetes mellitus.

90x90mm (600 x 600 DPI)

Figure 3



Tuberculin skin test (TST) positivity (a) and interferon-gamma release assay (IGRA) positivity (b) in the DM group (DMG) and the CC group (CCG) according to age and BCG scar status. A tuberculin induration size of 10 mm was used as the cutoff point for both groups.
Abbreviations: TST, tuberculin skin test; QFT, QuantiFERON-TB Gold In-Tube.

90x90mm (600 x 600 DPI)

1

Supplementary Table 1. Agreement between TST and QFT-GIT

Results	Overall n = 3217			DM group n = 2767			CC group n = 450		
	TST cut point			TST cut point			TST cut point		
	5 mm	10 mm	15 mm	5 mm	10 mm	15 mm	5 mm	10 mm	15 mm
TST+/QFT-GIT+	613	528	363	37	32	23	576	496	340
TST-/QFT-GIT-	664	1252	1950	95	188	318	569	1064	1632
TST+/QFT-GIT-	1886	1298	600	311	218	88	1575	1080	512
TST-/QFT-GIT+	54	139	304	7	12	21	47	127	283
Agreement%	39.7%	55.3%	71.9%	29.3%	48.9%	75.8%	41.4%	56.4%	71.3%
Kappa (95% CI)	0.09 (0.74-0.10)	0.17 (0.15-0.20)	0.27 (0.23-0.30)	0.10 (0.08-0.12)	0.19 (0.16-0.22)	0.27 (0.23-0.31)	0.02 (-0.01-0.05)	0.06 (0.01-0.11)	0.18 (0.09-0.28)

Abbreviations: TST, tuberculin skin test; QFT-GIT, QuantiFERON-TB Gold In-Tube; CI, confidence interval.
Test results with an indeterminate QFT-GIT response are not included in the table.

Supplementary Table 2. Combined effects of glycemic control (HbA1c) and duration of DM on risk of latent tuberculosis infection without and with adjustment of TST results.

Variables	Crude OR (95% CI)	aOR1 (95% CI)	aOR2 (95% CI)
Community comparison group	Reference	Reference	Reference
A1C ≧7%, duration >5 years	2.49 (1.77-3.52)	1.42 (0.97-2.07)	1.33 (0.90-1.96)
A1C ≧7%, duration ≦5 years	2.71 (1.84-3.98)	1.87 (1.24-2.82)	1.80 (1.18-2.74)
A1C <7%, duration >5 years	2.84 (1.99-4.04)	1.67 (1.13-2.46)	1.54 (1.03-2.28)
A1C <7%, duration ≦5 years	2.81 (1.95-4.04)	1.84 (1.24-2.72)	1.82 (1.22-2.72)

Abbreviations: OR, odds ratio; aOR, adjusted odds ratio; CI, confidence interval; TST, tuberculin skin test; A1C, glycated hemoglobin. aOR1: adjusted odds ratio without adjustment of TST results; aOR2: adjusted odds ratio with adjustment of TST results.

*The multivariable model adjusted for the comorbidities indicated in Table 2.

STROBE 2007 (v4) checklist of items to be included in reports of observational studies in epidemiology*

Checklist for cohort, case-control, and cross-sectional studies (combined)

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	4
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	4
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	8-9
Objectives	3	State specific objectives, including any pre-specified hypotheses	9
Methods			
Study design	4	Present key elements of study design early in the paper	10
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	10
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	10-12
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	11-13
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*11-12*
Bias	9	Describe any efforts to address potential sources of bias	-
Study size	10	Explain how the study size was arrived at	10-11
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	10-12
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	13-14
		(*b*) Describe any methods used to examine subgroups and interactions	13-14
		(*c*) Explain how missing data were addressed	-
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed	-

		Cross-sectional study—If applicable, describe analytical methods taking account of sampling strategy	
		(*e*) Describe any sensitivity analyses	-
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	14
		(b) Give reasons for non-participation at each stage	-
		(c) Consider use of a flow diagram	-
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	14-16
		(b) Indicate number of participants with missing data for each variable of interest	-
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	17-21
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	17-21
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	17-21
		Cross-sectional study—Report numbers of outcome events or summary measures	17-21
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	17-21
		(*b*) Report category boundaries when continuous variables were categorized	17-21
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	-
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	
Discussion			
Key results	18	Summarise key results with reference to study objectives	21-25
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	24
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	24-25
Generalisability	21	Discuss the generalisability (external validity) of the study results	24-25
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	28

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.