Prevalence of Latent Tuberculosis Infection Among Healthy Young Children and Adolescents and a Two-step Approach for the Diagnosis of Tuberculosis Infection in Chengdu, China

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Background: China has a high burden of tuberculosis and latent tuberculosis infection (LTBI). The aim of this study was to estimate the prevalence of LTBI among healthy young children and adolescents and test a 2-step approach to explore the threshold for the diagnosis of tuberculosis infection in Chengdu, China.

Methods: Healthy preschool children and school-going children in Chengdu, Sichuan Province, were screened for LTBI using the tuberculin skin test (TST). Preschool children with TST ≥ 5 mm also underwent interferon-γ release assay (IGRA) to explore the threshold of this 2-step approach.

Results: In total, 5667 healthy young children and adolescents completed TST test between July 2020 and January 2021 and were included in the present analysis. The age of the participants ranged from 2.4 to 18 years (median 7.25 ± 4.514 years), of which 2093 (36.9%) were younger than 5 years. The overall prevalence of LTBI was 6.37% and 6.64% in children younger than 5 years old. Fourteen of the 341 preschool children with TST ≥5 mm were interferon-γ release assay positive, of which 4 showed a TST result of 5–10 mm, and 6 preschool children received preventive treatment for LTBI.

Conclusions: Healthy young children and adolescents should also be considered as important target populations for LTBI screening. TST can be recommended for first-line screening as part of a 2-step approach for LTBI screening using a positive threshold of 5 mm.

Key Words: latent tuberculosis infection, tuberculin skin test, young children, adolescents

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Latent tuberculosis infection (LTBI) is a state of persistent immune response to *Mycobacterium tuberculosis* (MTB) antigens without any clinical symptoms of active tuberculosis (TB).[1] About a quarter of the global population has TB infection,[2] and a considerable proportion is in the LTBI state without symptoms and infectivity. However, the LTBI population is a large reservoir of MTB, and can progress to active and highly contagious TB. The risk of LTBI progression is higher in children and adolescents, who are also more likely to develop severe diseases such as miliary tuberculosis and tuberculosis meningitis, and also, have a shorter latency period.[3] However, there is no gold standard for the diagnosis of LTBI currently. Tuberculin skin test (TST) and interferon-γ release assay (IGRA) are both recommended to diagnose LTBI, which based on cellular immune responses to MTB antigens. TST has also been used as an epidemiologic tool to evaluate the prevalence of LTBI based on different cutoff values. Nevertheless, the optimal test of screening LTBI in young children is controversial and epidemiologic evidence is limited.

When considering the cost-effectiveness analyses and diagnosis accuracy of screening tests, a 2-step approach is adopted by many countries, including Britain, France, Germany, Switzerland, Ireland, Italy and Spain,[4–10] that is, using TST in the first-line and, if positive, using IGRA in the second step (TST-IGRA). A 2-step approach could reduce the cost of prophylactic treatment for more people detected by TST.[11] In addition, the confirmation of positive IGRA results might be conducive to encourage acceptance and adherence to treatment and management of latent tuberculosis infection in high tuberculosis burden countries.

World Health Organization reported that an estimated 10.0 million individually were diagnosed with TB in 2018 and childhood TB accounted for 11% of the total, China ranked among the top 30 countries with the highest TB burden with a TB incidence of 866,000 cases, including 99,000 cases of childhood TB.[12] Therefore, the timely detection and treatment of LTBI in children are essential and urgent to eliminate TB in China as well as other high-burden countries. However, little is known regarding the prevalence of LTBI among children and the most suitable test to be used to screen for LTBI in young children and the longitudinal follow-up about the populations.

The aim of the study is to estimate the prevalence of LTBI using TST among young children and adolescents and test a 2-step approach (TST-IGRA) to explore the threshold for tuberculosis infection in Chengdu, in Western China, to better understand the LTBI burden and improve its management for high TB burden countries.

MATERIALS AND METHODS

Study Setting and Population

We conducted our study in Chenghua District, Chengdu. Chengdu is the capital of Sichuan Province, which is located in Western China and has a relatively higher TB burden compared with the

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rest of the country. The mean reported incidence rate of pulmonary TB in Chengdu is 75.11/100,000 in all age groups and 8.05/100,000 in children 0–14 years of age,[13] which necessitates mandatory Bacille Calmette-Guérin (BCG) vaccination at birth. During our survey period ranged from July 2020 to January 2021, the COVID-19 pandemic in China had been initially under control and schools were open for students. Sichuan Province reported a number of <900 COVID-19 cases until the survey was over. Epidemiologic studies revealed that the infection of COVID-19 in children was less frequent and less severe than in adults.[14] Consistent with the suggestions in a study,[15] children and adolescents under 18 years are not the prior group for vaccination in China, and the vaccination campaign was not initiated for priority groups until December 2020, so all the participants in the study did not receive COVID-19 vaccine, what's important, none of the participants infected COVID-19; therefore, the performance of TST would not be affected during this period and the pandemic of COVID-19 did not substantially affect our findings.

In this study, a multistage stratified cluster sampling method was used to conduct the survey in the schools and kindergartens in Chenghua District, Chengdu. A sample size of 700 was calculated based on the prevalence of LTBI among children (9%, TST ≥ 6 mm) as per the fourth National Tuberculosis Epidemiology survey conducted in 2000,[16] with alpha of 0.05. The sample size was increased to 770 to account for an estimated 10% of children who failed to be interviewed for TST results. In the first stage, Chenghua District was selected from 12 urban districts of Chengdu on the basis of population size and per capita income. In the second stage, each school and kindergarten was used as the unit in Chenghua District and the stratified cluster sampling method was used to select the study sites. And 10 kindergartens and 6 schools were randomly selected in the kindergarten and school strata, respectively. In the third stage, according to the calculated sample size, 225 and 545 participants were randomly selected from the selected 10 kindergartens and 6 schools, respectively. Due to the high intention of parental acceptance of the tuberculosis investigation, we obtained a larger sample size than expected with a number of 5798 preschool children and school-going children. Overall, 131 questionnaires were excluded if with personal information that confirmed to our exclusion criteria or incomplete or with disagreement. Then, 5667 participants were enrolled in the study and accomplished TST test and were included in the present analysis.

We defined the inclusion criterion as provision of written informed consent by parents or guardians, and the ability to complete the questionnaire. Exclusion criteria include individuals with TST contraindications, diagnosis of active TB, history of TB, malignancy, HIV infection, immunosuppressive therapies, severe malnutrition and other immunocompromised conditions.

The study protocol was approved by the ethics committee of the Institute of West China Second University Hospital, Sichuan University. Written informed consent was obtained from parents or guardians.

PROJECT DESIGN AND PROCEDURE

Staff Selection and Study Procedure

The research group comprised of doctors and nurses form the Department of Pediatrics, West China Second University Hospital, Sichuan University and all investigators received standard training for operating procedures, questionnaire assessment and TST result analysis. A presurvey was also conducted to ensure quality control. IGRA quality control was maintained using reagents with the same batch number. All participants were required to complete a standard questionnaire with their parents for the following information: name, age, sex, history of TB, history of TB exposure, time of BCG vaccination and BCG scar and length of residence in Chengdu. The BCG scars and TST results of preschool children were checked by the same 2 investigators to ensure quality control.

Trained nurses performed TST using the Mantoux method by intradermally injecting 0.1 mL of 5 tuberculin units of purified protein derivative (Xiangrui, Beijing, China) into the left forearm as per patient preference.[17] The transversal diameter of induration (mm) was measured 72 hours later. IGRA was performed with the T-SPOT.TB kit according to the manufacturer's recommendations (Oxford Immunotec Ltd, Oxford, UK) using 2–3 mL venous blood from school children who volunteered for the test, which can be referred to the article of Sun L et al.[18] Preschool children with TST ≥ 5 mm also underwent IGRA subsequently.

Definitions

TST result was read after 72 hours, absent or <5 mm TST induration was considered negative, and indurations ≥5 mm were considered positive. LTBI was diagnosed when the TST induration was ≥10 mm after 72 hours, without any clinical symptoms or radiologic evidence related to the disease in the BCG vaccination setting.[1] A positive T-SPOT.TB result was defined as ≥6 spots in an ESAT-6 cell or a CFP-10 cell after subtraction of the number of spots observed in the negative control cell where the negative control had 0–5 spots. If the negative control had ≥6 spots, the ESAT-6 or CFP-10 panel had to contain at least twice as many spots compared with the negative panel for the result to be considered positive. Results were considered negative if the spot number did not meet the above criteria, and the positive control performed normally. Results were considered undetermined if the spot amounts in the positive control were <20 or >10 in the negative control.[19]

Data Analysis

Data analysis was performed using SPSS (version 23). All records were cross-checked with the original datasheets. Continuous variables were presented as mean ± SD and categorical variables as frequencies and percentages. The Chi-squared test was used to compare proportions. All tests were two-tailed and $P < 0.05$ was considered statistically significant.

RESULTS

Study Population

Between July 2020 and January 2021, a total of 5667 healthy young children and adolescents 2.4–18 years of age (median age 7.25 ± 4.514 years), including 3865 (68.2%) 2.4–7.25 years old preschool children and 1802 (32.8%) 5–18 years old school-going children, completed the TST and were included in the present analysis. Of all participants, 2902 (51.2%) were male and 2765 (48.8%) were female. In terms of age, 2093 were younger than 5 years (36.9%), 1871 were 5–10 years old, 867 were 10–15 years old and 836 were 15–18 years old. Furthermore, 4717 (83.2%) subjects had an induration <5 mm, 589 (10.4%) 5–10 mm, 307 (5.4%) 10–15 mm and 54 (1%) ≥15 mm. Overall, 950 (16.76%) subjects were diagnosed as TST positive based on the induration measuring at least 5 mm. The sex and TST induration distribution differed significantly between preschool children and school children (Table 1).

LTBI Identification

A total of 361 subjects presented an induration measuring ≥10 mm, and the overall prevalence of LTBI was 6.37%. The prevalence rate among preschool children was 6.33% (245/3865), and 6.44% (116/1802) for school children. Furthermore, the prevalence of LTBI was 6.64% (139/2093) for subjects younger than 5 years and 6.21% (222/3574) for those older than 5 years. There were no significant differences in the prevalence of LTBI in terms of the sex and age (Table 2).

TABLE 1. General Characteristics of the Study Population

Variables	Total (N = 5667) No. (%)	Preschool Children (N = 3865) No. (%)	School Children (N = 1802) No.(%)	χ^2	*P*
Sex					
Male	2902 (51.2)	1923 (49.8)	979 (54.3)	10.292	0.001
Female	2765 (48.8)	1942 (50.2)	823 (45.7)		
Age (year)					
<5	2093 (36.9)	2093 (54.2)	0		
5–10	1871 (33.0)	1772 (45.8)	99 (5.5)		
10–15	867 (15.3)	0	867 (48.1)		
≥15	836 (14.8)	0	836 (46.4)		
TST induration (mm)					
<5	4717 (83.2)	3193 (82.6)	1524 (84.6)	16.978	0.000
5–10	589 (10.4)	427 (11.0)	162 (9.0)		
10–15	307 (5.4)	219 (5.7)	88 (4.9)		
≥15	54 (1.0)	26 (0.7)	28 (1.6)		
Age (year)					
<5	2093 (36.9)	2093 (54.2)	0		
≥5	3574 (63.07)	1772 (45.8)	1802 (100%)		

TABLE 2. Prevalence of LTBI Among Healthy Young Children and Adolescents

Variables	Total (N = 5667) No. (%)	LTBI (N = 361) No. (%)	χ^2	*P*
Sex				
Male	2902 (51.2)	169 (5.82)	2.980	0.084
Female	2765 (48.8)	192 (6.94)		
Age (year)			2.162	0.540
<5	2093 (36.9)	139 (6.64)		
5–10	1871 (33.0)	112 (5.99)		
10–15	867 (15.3)	50 (5.77)		
≥15	836 (14.8)	60 (7.18)		
Group			0.020	0.888
Preschool children	3865 (68.2)	245 (6.33)		
School children	1802 (32.8)	116 (6.44)		
Age (year)				
<5	2093 (36.9)	139 (6.64)	0.409	0.523
≥5	3574 (63.07)	222 (6.21)		

Distribution of LTBI by Age and Sex

LTBI prevalence showed an age-dependent decrease in females. The 15- to 18-year age group showed the highest prevalence in the entire cohort and also among males. In contrast, LTBI was most prevalent among those younger than 5 years in the female subgroup (Fig. 1).

BCG Scar and LTBI

The data of BCG vaccination, scar and age of vaccination were available for 2520 preschool children, including 1278 boys (50.71%) and 1242 girls (49.29%). A total of 2499 had received the BCG vaccine (99.17% vaccination rate), of which 1332 (53.3%) were vaccinated at birth, 469 (18.8%) within 1 month after birth, 222 (8.9%) at the age of 1–2 months, 106 (4.2%) at the age of 2–3 months and 370 (14.8%) after 3 months. BCG scars were noted among 1874 (74.99%) subjects, while 625 (25.01%) lacked the scars. The 21 unvaccinated children were not studied further due to the small sample size.

A Two-step Approach in Preschool Children

The T-SPOT assay was performed for 341 preschool children with TST diameter ≥5 mm, and the interval time ranged from 4 to 74 days. Fourteen subjects tested positive, of which 4 had TST induration 5–10 mm, seven had 10–15 mm and three had ≥15 mm (Table 3). None of the subjects manifested any clinical symptoms of TB or had a history of close contact with TB patients. Nine T-SPOT positive children were further examined in a specialized hospital to distinguish between LTBI and TB, of which 6 (age range: 2 years and 5 months to 6 years and 2 months) were diagnosed with LTBI and 3 with TB (microbiologically negative). Preventive therapy was given to the former and the latter received anti-TB treatment. The follow-up is ongoing for the children with LTBI.

DISCUSSION

We analyzed the prevalence of LTBI among healthy young children and adolescents in Chengdu, Sichuan Province, China. Under the BCG vaccination setting, TST induration ≥10 mm was defined as the cutoff value,[1] and the overall prevalence of LTBI was 6.37% in this demographic group, which is lower compared with that reported for children by the fourth National Tuberculosis Epidemiology survey in 2000 (9%, TST ≥ 6 mm)[16] and that among Chinese adolescents in rural districts in 2013 (9.3%, TST ≥ 10 mm).[20] However, the LTBI rate in our study is higher than that reported for school children and adolescents in Shanghai (4.7%, T-SPOT),[21] and comparable with the rate observed for preschool children 3–6 years of age in Jiangsu Province (6.47%, TST ≥ 10 mm).[22] Finally, our results indicate a lower prevalence of LTBI compared with that reported in children and adolescents in the Tibetan schools in India (18%, TST ≥ 10 mm)[23]and in South Africa (50,9%, QFT, 34.3%, TST ≥ 5 and ≥10 mm, 23%, QFT-TB Gold-plus),[24–26] which are also countries with high TB burdens. Although there was a high degree of heterogeneity in our study, compared with previous studies, in terms of background TB prevalence rates, age of the participants and methodologic differences (varying TST cutoff values for TST and IGRA), our findings provide epidemiologic data that show that screening should be conducted on healthy groups and not merely those at a high-risk. The findings of our study also suggest that stronger measures are needed to decrease the prevalence of LTBI among young children and adolescents.

Contradictory to some studies in high TB burden areas in India and South Africa,[23,24] the prevalence rate of LTBI was not significantly different between the various age groups in our study, but was consistent with the results reported in rural China.[20] One possible explanation is that the burden of LTBI in our study population was lower than that in cohorts from high-burden areas.[20,26] Furthermore, given the high proportion of children younger than 5 (36.9%)

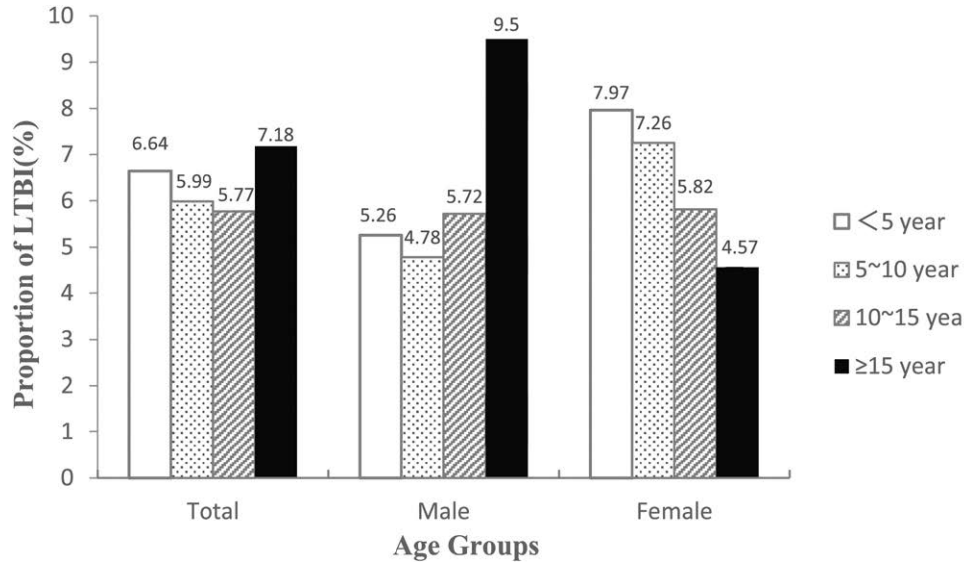

FIGURE 1. Age-specific trend of LTBI in healthy young children and adolescents stratified by sex. full color online

TABLE 3. Distribution of T-SPOT Positive Preschool Children With Different TST Indurations

TST Induration (mm)	No. Children Performed TST	No. T-SPOT Positive
5–10	203	4
10–15	124	7
≥15	14	3

and 10 years (69.9%) of age, it was not possible to determine the results of longer cumulative exposure to those with infectious TB.

Though children <5 years of age are listed on the high-risk populations, the LTBI data for them are limited. Two thousand ninety-three healthy children under 5 years of age from various kindergartens were recruited in the study, and the prevalence of LTBI using TST was 6.64%, which was higher than the prevalence reported for the same age group in Jiangsu, China (1.47%, TST ≥ 10 mm),[22] which is an economically developed area with low TB burden. Thus, regional differences, economic and healthcare status and environment factors should be taken into account when comparing the prevalence of LTBI across different areas, even in the same country. The Code of Practice for Prevention and Control of Tuberculosis in Schools recommends screening students for TB by TST, which has played an important role in controlling the TB epidemic in schools since 2017 in China. The Guidelines for Prevention and Control of Tuberculosis in School in China (Edition 2020) underscores TB infection screening for preschoolers to further improve LTBI management among the target population. Children younger than 5 years account for a high proportion in kindergartens, and their nascent immune system puts them at a higher risk of progressing to active TB after infection. Due to lack of clinical symptoms or radiologic evidence of the disease, latent infection is easily missed in some children, further leads to delay in establishing the diagnosis of active TB. Our study provides forceful epidemiologic evidence for screening this vulnerable group for LTBI.

The guidelines for the use of TST and/or IGRA as a screening tool for young children are controversial, and there is limited evidence regarding the optimal diagnostic approach for LTBI. The American Academy of Pediatrics recommendations in 2018 recommended IGRA as the preferred test for LTBI among BCG-vaccinated children 2 years and older, and equally acceptable as TST in non-BCG-vaccinated children.[27] Studies included children with different population features also support the use of IGRAs for children.[28–30] On the contrary, the Canadian Thoracic Society suspected that IGRA performed worse in young children than in adults.[31] However, studies of meta-analysis confirmed that IGRA was more specific in low-risk, BCG-vaccinated individuals.[32,33] As a consequence, a 2-step approach (TST-IGRA) has been followed in many countries in terms of cost-effectiveness and diagnostic accuracy, but the TST cutoff value and target group population in the guidelines varies in different countries.[6,34,35] As a high TB burden country, it is also not feasible to screen tuberculosis infection using both methods simultaneously in China instantly. The latest expert consensus on LTBI screening and preventive treatment for children in China in 2020 supports using IGRA and TST for diagnosing LTBI, with IGRA given preference in economically developed areas.[2] The 2-step approach for LTBI screening to improve diagnostic accuracy was also recommended in Chinese students recently, although the accurate TST cutoff value was not well-defined.[36] Furthermore, an earlier study conducted in China found that the 2-step approach was suitable for adolescents.[1] In the context, we used TST ≥5 mm as the cutoff value, and 341 preschool children with TST ≥5 mm underwent T-SPOT. Four children younger than 5 years of age were T-SPOT positive with TST induration 5–10 mm, and did not exhibit any clinical symptoms of TB nor had any history of close contact with TB patients. Thus, TST ≥5 mm is an indication for a IGRA confirmation test, which can detect TB infection early and reduce the cost of subsequent examinations. Similarly, Gao et al[36] found that 9 of 406 school children 5–15 years of age with TST induration 5–15 mm were also T-SPOT positive, indicating that the 5-mm threshold was also suitable for older children. Therefore, more studies are needed to validate the TST positive threshold for children in the 2-step approach to develop an optimal testing strategy for Chinese children of different age groups, the implementation of this 2-step approach will significantly reduce the incidence of false positives and the burden of preventive treatment measures.

The incubation period of TB is typically several months to 2 years.[37] A 5-year follow-up study in China revealed that disease progression in the first 2 years was higher than in the latter 3,[38]

which was consistent with the report of WHO[37] and Behr et al.[39] Treatment of LTBI reduces the risk of disease progression, which is especially critical for young children. However, the absence of standard guidelines makes implementation difficult. Six preschool children in our study have been started on a prophylactic 3-month regimen of isoniazid and rifampicin, and the follow-up is ongoing to record any adverse events.

There are several limitations of this study that must be considered. TST results are affected by a complex array of factors, including BCG vaccination, and may result in an overestimation of LTBI prevalence in a high BCG vaccination setting. The BCG vaccination data of most young children and adolescents included in the study was not available and we assumed that all participants were successfully vaccinated based on the BCG vaccination rate of 2520 preschool children (99.17%). However, the only gold standard that may be valid for the detection of LTBI is the possibility of developing active TB at a later stage, which can be confirmed through longitudinal monitoring of individuals considered to be at risk. Therefore, it hints us that all participants diagnosed with LTBI showed be followed up in the future to confirm a true-positive or false-positive TST. Furthermore, 341 preschool children did not receive both tests on the same day or TST followed by IGRA within 3 days, but a positive diagnosis of LTBI was still observed. This was inconsistent with the guidelines in some countries, which claim that TST can subsequently boost IGRA results.[40,41] However, there is limited evidence to support this claim. Therefore, studies that can confirm and analyze this paradox in Chinese populations with different genetic backgrounds are needed.

In summary, healthy young children and adolescents should be considered as important target populations for LTBI screening. This study also suggests that stronger measures are still needed to decrease the prevalence of LTBI among these populations. Therefore, we recommend that a 2-step approach be used for LTBI screening of young children and adolescents, including the use of TST with a positive threshold of 5 mm as the first-line of screening. Identification and treatment of LTBI in younger children is crucial to minimize the risk of LTBI progression. Despite limitations, our study provides novel epidemiologic insights into LTBI burden among healthy young children and adolescents, and tests the efficiency of 2-step approach to improve the management of LTBI, which can be used for reference by other high TB burden countries.

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