Prevalence of latent tuberculosis infection and its risk factors in schoolchildren and adolescents in Shanghai, China

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Objectives: As tuberculosis (TB) infection in childhood contributes to the pool of individuals with latent tuberculosis infection (LTBI) from which future TB cases will arise, this study aimed to determine the prevalence and risk factors associated with LTBI in schoolchildren and adolescents from Shanghai, China. Methods: In this cross-sectional study, we administered T-SPOT.TB and TB infection risk factor questionnaire to children and adolescents aged between 10 and 18 years in 2010 in Shanghai. LTBI cases were defined by positive T-SPOT.TB test results and X-ray confirmation. Results: A total of 1106 schoolchildren and adolescents were enrolled, of which 46.1% were male, and 91.8% were vaccinated with Bacille Calmette Guerin (BCG). Overall, 52 (4.7%) children had a positive T-SPOT.TB result, with significant increase in age distribution. However, none of the participants demonstrated TB-related abnormality on X-ray examination. Multivariate analysis showed that LTBI was associated with no BCG vaccination (odds ratio: 2.40; 95% confidence interval: 1.182–5.335) and a history of TB exposure (odds ratio: 6.89; 95% confidence interval: 3.095–15.35). For 46 children and adolescents with history of TB exposure, contact hours per week of TB cases were significantly associated with risk of LTBI. Conclusions: Prevalence of LTBI in schoolchildren and adolescents in Shanghai is relatively low compared with other high epidemic areas of TB. A higher risk of LTBI was observed among children with no BCG vaccination and those with a history of TB exposure, which suggests that the prevalence of LTBI among schoolchildren could be further reduced by strengthening BCG vaccination under the national immunization programme and enhancing contact investigation of active TB patients.

Introduction

Mycobacterium tuberculosis (Mtb) infection in childhood continues to be a concern and growing problem in countries with a medium or high prevalence of tuberculosis (TB). In resource-poor areas, children carry a large proportion of the overall burden, representing 15–40% of all cases and causing more than 10% of paediatric hospital admissions and deaths.1–3 Although childhood TB has a limited influence on TB epidemiology, children with latent tuberculosis infection (LTBI) could contribute to the pool of individuals with LTBI from which future TB cases will arise, and the occurrence of LTBI in children is an indicator for current and ongoing transmission.3 To prevent TB occurrence, U.S. and European countries provide preventive treatment for people with LTBI, especially HIV-infected children,4–6 persons with a weakened immune system5 and immigrants from high TB-burden countries.7

Additionally, to improve the detection of TB disease and LTBI in children in developing countries, there should be a simple rapid and non-invasive test that can be performed at the health centre level. T-cell-based blood tests have increasingly been used in recent years and are endorsed by U.S. and European countries. Currently, the in vitro T-SPOT.TB test is used and detects the interferon-γ (IFN-γ) released by activated T lymphocytes (T cells) in response to antigens [early secretory antigenic target-6 (ESAT-6) and culture filtrate-10 (CFP-10)]. These aforementioned antigens were expressed solely in Mtb but absent from Bacille Calmette Guerin (BCG) and majority of other environmental mycobacteria.8–10 This assay is highly specific for LTBI and is also proved to be more sensitive than the tuberculin skin test (TST) for detection of LTBI in schoolchildren in some high TB-burden areas.11 Therefore, the TSPOT.TB assay could be the most accurate method for identifying LTBI, especially for children in countries like China, where there is a high incidence of TB despite a high coverage with BCG vaccination.12

Thus, the aim of this study was to investigate the proportion of LTBI by T-SPOT.TB in schoolchildren and adolescents from the primary school to the high school, and to identify risk factors that were associated with LTBI, especially the risk of exposure to active TB cases in Shanghai, China. This information would improve our understanding of the biology of Mtb transmission and the burden of TB infection in children in urban areas of China, and to develop tailored strategies for control of TB infection in children.

Methods

Survey design and sample size

Shanghai is one of China’s most comprehensive industrial and commercial cities, having the largest population in urban China. The registration rate of TB cases was 39.4 per 10^5 in 2010, around 3.8% of which were from children aged between 11 and 18 years. Routinely, at the time of entry in primary school, secondary/high school and university, all the freshmen are interviewed for history of BCG vaccination, history of TB exposure and symptoms of TB. Chest radiographs are also routinely provided during the physical examination. Those suspected of having TB will be referred to specialized health facilities for further diagnosis. Additionally, TST is sometimes used to screen for LTBI in schoolchildren. However, due to comprehensive BCG vaccination under the expanded
immunization programme in China, the TST-based result is not as good an indicator for TB infection in schoolchildren and adolescents.

Between January and August 2010, four primary schools, two middle schools and two high/college schools located in seven districts of Shanghai were purposively selected as the study sites. The selection of the districts and participating schools was based on the comparable socioeconomics, demographics, willingness of the cooperation and capacity for carrying out questionnaire interview, specimen collection and transportation for LTBI investigation. As the screening was carried out together with the student routine physical examinations before entry into middle school and high school/college, the subjects were schoolchildren and adolescents in grade 5 in primary school, grade 8 in middle school and grade 12 in high school in the semester 2009/2010. Eligible participants were defined as not having a history of TB or previous anti-TB treatment. In each selected school, all the eligible students were invited for participation.

Our target sample size was 1000 children aged between 9 and 18 years. This target sample size was estimated based on the prevalence of TB infection reported by the fourth national TB survey in China. On the basis of an estimate that the prevalence of LTBI in the high-risk group would be 30% compared with 5% in the low-risk group, with a power of 80% and 95% confidence intervals (CIs), a sample size of 950 was calculated. This was increased to 1056 to account for an estimated 10% of children who did not have the T-SPOT.TB result or failed to be interviewed.

**Study procedures and data collection**

The written informed consent was obtained from parents or guardians of all participants, and this study was approved by the institutional review board from the School of Public Health, Fudan University. All children and adolescents were examined for TB during the routine physical examination, which included clinical consultation and chest radiographs. A T-SPOT.TB test was given at the same time for detecting LTBI. TB was diagnosed by X-ray examination in combination with sputum smear microscopy. The status of LTBI was reported to district centres for disease control (CDCs) and the participating school. The health staff in the school clinic informed the parents of their child’s status and educated them on TB prevention. Meanwhile, the students were asked to complete a questionnaire with their parents regarding risk factors related to TB infection. The questionnaire requested information on age, sex of the student, birthplace of the child and parents, length of their residence in Shanghai as well as the nutritional status. Parents were also asked about other TB risk factors, including their children’s exposure to people who had active TB. The BCG status was determined by the scar of vaccination by doctors in physical examination as well as the answer to the question on BCG vaccination history in the questionnaire. If the answer and the scar were consistent, the subject was judged as ‘BCG vaccinated’. If not, the BCG status was then primarily determined through doctor’s visualization of the scar. Body mass index (BMI) was measured in percentiles according to World Health Organization’s definition for schoolchildren and adolescents. Underweight was defined as the BMI less than the fifth percentile based on the U.S. CDC BMI-for-age growth charts.

The degree of exposure to TB was quantified on the basis of a history of contact with a diagnosed TB patient. Degree of contact was defined as ‘close’, if patients and children usually had a meal together and/or lived in the same room. Duration of contact was the reported period in a unit of hours per week.

**LTBI diagnosis**

T-SPOT.TB assay was performed according to the manufacturer’s instructions (Oxford Immunotec, Oxford, UK). Positive T-SPOT.TB results were reported when the number of spots in the test wells (against at least one of the two tested antigens) was six or more, if the negative control had less than five spots, or two or more times the number of spots in the negative control wells, if the negative control had more than six spots. This cutoff was predefined by the manufacturer’s instructions.

Chest radiography was provided to all students in the physical examination. None of the schoolchildren and adolescents was reported for active pulmonary TB. After getting the results of T-SPOT.TB, the students with a T-SPOT.TB positive result were reinterviewed for TB symptoms, and sputum samples were requested if applicable. The radiograph reports of these schoolchildren and adolescents were reviewed for excluding TB. The diagnosis of active TB among children in Shanghai follows the national guideline, which is mainly based on the smear microscopy and chest X-ray examination.

LTBI was defined as a positive T-SPOT.TB in the absence of TB disease. TB disease was suspected if the interviewees reported chronic cough (longer than 3 weeks), weight loss, night sweats and fever, or if signs of extrapolmonary TB were observed.

**Statistical analyses**

Data were entered in Epidata freeware (http://www.epidata.dk). All records were cross-checked with the original datasheets. The analysis was carried out with SPSS package for Windows (version 11, Chicago, IL). The $\chi^2$ test was used to compare the proportions of LTBI according to children and adolescent population with different sociodemographic and clinical features. Fisher’s exact test was used to determine the association between LTBI and the degree of exposure with expected count less than 5. The $\chi^2$ test for trend was also applied to further assess trends of associations between ordinal exposures and LTBI. The following risk factors of LTBI were evaluated in multivariate analyses through logistic regression model: sex, age, residential area, BCG vaccination, history of respiratory disease, history of TB exposure, etc. $P<0.05$ was considered as statistically significant.

**Results**

**Survey participation**

A total of 1136 children and adolescents aged between 10 and 18 years participated in this study. Blood samples were collected from 1120 children and adolescents for T-SPOT.TB test. Of the tested subjects, 14 (1.3%) children and adolescents did not have T-SPOT.TB results due to technical errors during sampling or processing (incomplete set of blood specimen, >16 hours delay in transportation, incubation at inappropriate temperature and high background IFN-γ level) and were also excluded.

Of the 1106 children and adolescents included in the present analysis, 471 (42.6%) were from primary schools and aged between 11 and 12 years, 197 (17.8%) were from middle high schools and aged between 14 and 15 years and 438 (39.6%) were from high schools and aged between 17 and 18 years. Five hundred ten (46.1%) children and adolescents were male; 1055 (95.4%) were born in Shanghai. Despite the widespread use of BCG, 91 (8.2%) children and adolescents were not vaccinated with BCG (Table 1). According to the U.S. CDC growth chart, 86 (7.8%) schoolchildren and adolescents belonged to the low 5% BMI category and were defined as underweight. Additionally, 46 (4.2%) children and adolescents reported having a history of TB exposure (Table 1).

**LTBI identification**

T-SPOT.TB was positive in 52 children (4.7%), whereas it was negative in 1054 (95.3%). The number of spots to ESAT-6 and the number of spots to CFP-10 did not differ significantly between age groups and groups with and without BCG scar (Figure 1). All of the schoolchildren and adolescents with a positive T-SPOT.TB were
identified as LTBI by the absence of TB-related signs on the chest radiography.

**Trend of LTBI infection by age**
The prevalence of LTBI in the sampled children and adolescents increased with age ($\chi^2$ test for trend, $P=0.0001$). This trend was observed both in boys and girls (Figure 2).

**Risk factors related to LTBI**
Adjusting for age and sex, it was found that BCG vaccination and a history of TB exposure were independently associated with the risk of LTBI. The schoolchildren and adolescents with BCG vaccination had an adjusted odds ratio (OR) of 2.40 (95% CI: 1.182–5.335) to have LTBI compared with those unvaccinated with BCG. Previous exposures to persons with TB significantly increased the risk of LTBI in children and adolescents (OR: 6.89, 95%CI: 3.095–15.35). However, none of the other factors assessed was associated with risk of LTBI (Table 2).

**Association between BCG vaccination and LTBI**
The association between BCG vaccination and LTBI was further analysed by stratifying the subjects in terms of sex, age, health status and a history of TB exposure (Table 3). The risk of LTBI was significantly higher in girls vaccinated with BCG compared with those unvaccinated with BCG (14.6% vs. 3.8%; OR: 4.35; 95% CI: 1.389–14.93), whereas the association became less strong in the age-groups of 14–15 years and 17–18 years. The BCG vaccination was associated with LTBI regardless of a history of TB exposure.

**LTBI among schoolchildren with a history of TB exposure**
Of the 46 schoolchildren and adolescents with a history of exposure to TB patients, further analysis on the extent of exposure was conducted on 10 subjects with LTBI and 36 without LTBI. There was increasing likelihood for LTBI with longer exposure to TB patient (duration of contact: 46.9 ± 9.36 vs. 29.9 ± 13.36 hours per week, $P=0.001$) and close contact to TB patients (80.0% vs. 38.9%, $P=0.025$) (Table 4). A significantly high proportion of LTBI subjects had a history of contact with smear-positive TB patients (90% vs. 50%, $P=0.031$). But no statistically significant difference in prevalence of LTBI was found between children and adolescents having household contacts and other contacts.

**Discussion**
Knowledge of LTBI among children is essential in understanding the long-term trend of TB in specific countries and areas, and remains a key indicator in reflecting the severity of TB transmission in population. However, there is still no gold standard for the diagnosis of LTBI. TST was routinely used as the first step in the diagnosis of LTBI. TST was routinely used as the first step in the diagnosis of TB infection. But as previous studies showed, TST does not accurately reflect the risk of LTBI in a setting with extensive BCG vaccination coverage.$^{13,14}$ In contrast, the IFN-γ-based assay is not influenced by the BCG vaccination and other environmental mycobacteria and therefore is certified as the standard diagnosis.

### Table 1 General characteristics of studied schoolchildren by ages

<table>
<thead>
<tr>
<th>Variables</th>
<th>11–12 (years)</th>
<th>14–15 (years)</th>
<th>17–18 (years)</th>
<th>$P^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$n$</td>
<td>471</td>
<td>197</td>
<td>438</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>216 (45.9%)</td>
<td>91 (46.2%)</td>
<td>203 (46.3%)</td>
<td>0.989</td>
</tr>
<tr>
<td>BCG vaccinated</td>
<td>436 (92.6%)</td>
<td>186 (94.4%)</td>
<td>393 (89.7%)</td>
<td>0.098</td>
</tr>
<tr>
<td>Born in Shanghai</td>
<td>453 (96.2%)</td>
<td>188 (95.4%)</td>
<td>414 (94.5%)</td>
<td>0.492</td>
</tr>
<tr>
<td>Having exposure to TB</td>
<td>10 (2.1%)</td>
<td>8 (4.1%)</td>
<td>28 (6.4%)</td>
<td>0.006**</td>
</tr>
<tr>
<td>Underweight</td>
<td>30 (6.4%)</td>
<td>18 (8.1%)</td>
<td>40 (9.1%)</td>
<td>0.293</td>
</tr>
</tbody>
</table>

*P value is calculated from $\chi^2$ test.

**Figure 1** The spot numbers of ESAT-6 and CFP-10 in different age-groups (left) and group with and without BCG scar (right). The distribution of spot number was presented in the box-plot in terms of percentiles, using the median of a group as the midpoint (50th percentile). The box surrounding the median shows the 25th (lower) and 75th (higher) percentiles. The upper and lower boundaries of the box-plot denoted the maximum value and minimum value, respectively.

**Figure 2** Age-specific trend of LTBI in schoolchildren stratified by sex.
method of LTBI in Europe and the USA.15 This study is the first to report results when using T-SPOT.TB for screening for LTBI among schoolchildren and adolescents in Shanghai, China, where incidence of TB declined slowly and BCG vaccination has been provided to all newborns for more than half a century.

A striking observation was that 3.4% of the studied 11–12-year-old children, 4.5% of 14–15-year-old adolescents, and 6.2% of 17–18-year-old adolescents were identified as LTBI, with a significant increasing trend of prevalence of LTBI by age-groups. The association of age and LTBI might reflect the increase in cumulative exposure to Mtb with age or the increase in interferon production in response to mycobacterial antigens in whole blood.16–18 However, in present study, the variation of cell-mediated response with age was not observed, as there was no significant difference in the number of ESAT-6 and CFP-10 between different age-groups. On the other hand, the association between age and LTBI was in accordance with having a history of TB exposure. Therefore, the likeliest explanation for the strong association is that age is a marker of TB exposure within a high TB-burden community. It is also known that continued exposure to TB has contributed to the risk of LTBI among the schoolchildren and adolescents, as documented in other settings.19

This study found that the prevalence of LTBI in schoolchildren varied between 3.4% and 6.2%, which was much lower than reported in other studies in low- and middle-income countries using T-SPOT.TB and/or the TST induration diameter of 10 mm (Bangkok, 47.8%20; Chang Rai, 55.6%21; Philippines, 69.2%21). But the prevalence of LTBI in this study was still a little higher than in high-income countries like the USA and Canada. Several factors might be responsible for these differences: 1) T-SPOT.TB might be a more sensitive test than the traditional TST indicator and reduced the false positivity due to BCG vaccination among schoolchildren and adolescents22; 2) a relatively low prevalence of LTBI might result from the success of TB control activities in Shanghai, but compared with high-income countries, the prevalence still
remained higher due to the historically high TB burden and the domestic migrant population from inland areas where TB is still epidemic. These observations highlight the necessity of developing strategies for infection prevention among children in local TB control programmes.

Different from other reports, which used the TST, T-SPOT.TB applied in this present study allowed for differentiation between Mtb infection and previous effective BCG vaccination. This made it possible to assess whether effective BCG vaccination affects the risk of infection in schoolchildren. Contrary to the prevailing theory that BCG vaccination protects only against TB disease, our results, however, suggested that at least part of BCG’s protective effect might be attributable to protection against infection in children. Preliminary evidence for such a mechanism of BCG has come from the bovine and mouse model of TB. Because our marker of infection was the presence of Mtb-antigen-specific T cells, these data suggest that the mechanism of protection induced by BCG vaccination may act at an early stage in the process of infection, before detectable numbers of these T cells have appeared in peripheral blood. This mechanism would be consistent with a pivotal role for alveolar macrophage intracellular effector mechanisms that might destroy the bacillus before processing and presentation of antigens to T cells in regional lymph nodes. Since 1998, the booster of BCG has been stopped in Shanghai. The BCG vaccination is routinely given to newborns in maternity hospital under the national Expanded Programme on Immunization. For children younger than 7 years, BCG will be provided if they have never been vaccinated. National and municipal CDCs monitor the quality of vaccination with a standard protocol for implementation and evaluation. Findings from this study suggested that the protection from BCG vaccination against TB infection might still have important practical consequences for public health in Shanghai. Successful BCG vaccination programmes may have lessened the burden of LTBI in children as well as reduced the incidence of active TB. Thus, the BCG vaccination strategy should be implemented continuously and expanded to unvaccinated children who have emigrated from high TB-burden areas of China. This could help to further reduce the transmission of Mtb infection in Shanghai and accomplish the global target of TB elimination.

In present study, LTBI was associated with a history of exposure to active TB cases. This result is in keeping with the findings of Hill et al. Mtb infection is almost exclusively transmitted through the air from patients with pulmonary disease. Therefore, proximity and persistence of contact are major determinants of the risk of transmission of infection. Although previous studies have generally shown a grading in the indicators of transmission (active TB and LTBI) by closeness and duration of contact with the infectious source, two main risk factors were found to be significantly associated with the increased risk of LTBI in this study, i.e., the infective status of source TB patients as well as the length and degree of contact. The longer the duration of contact indicates the greater likelihood in Mtb transmission. Additionally, contact with infectious TB patients has undoubtedly contributed to the maintenance of TB transmission in population and has also increased the risk of infection among children. Based on our observations, children in contact with adult TB patients are at the highest risk of getting infection, which suggests the importance of detecting TB early and reducing the spread of transmission. Contact tracing and health education for patients, family members and their contacts have been effective prevention measures in high TB-burden countries. Screening for LTBI in high-risk populations including TB contacts could also be considered on the premise of cost-effectiveness.

There were several limitations to this study. First, we did not do the secondary detection test for TB infection, considering that the traditional TST test might not be suitable in the Chinese setting due to the extensive BCG vaccination programme. Second, the T-SPOT.TB assay was based on the individual activated T cell. The results therefore might be influenced by the individual immunological response to the Mtb. Thus, to help distinguish between true-positive and false-positive results, it is recommended that the participating schoolchildren with a positive T-SPOT.TB test should be followed-up long-term. In addition, using BCG scar as the major indicator of vaccination might underestimate the rate of BCG vaccination in this study; however, the influence from this bias is most likely limited.

Conclusions

Prevalence of LTBI in schoolchildren and adolescents in Shanghai is relatively low compared with other high epidemic areas of TB. BCG vaccination still remained effective in protection against Mtb infection in childhood. Continuous implementation of BCG vaccination in newborns had a great impact in the prevention of TB transmission in endemic regions before the launch of new TB vaccines. Moreover, the prevalence of LTBI in Shanghai could be further reduced by strengthening health education on TB prevention, improving awareness of TB symptoms in the population, enhancing contact investigation of all index patients and establishing contacts’ surveillance for early TB detection.

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Conflicts of interest: None declared.

Key points

- The present study reported the data from the first population-based survey of LTBI in schoolchildren in China.
- The prevalence of LTBI in schoolchildren was 3.4–6.2%, which was much lower than that reported by other studies in middle/low-income countries.
- The exposure to TB is an independent contributor to latent TB infection in schoolchildren in Shanghai.
- The BCG vaccination has proven its effectiveness in protecting against the acquisition of Mtb infection in childhood.
- Successful BCG vaccination programmes and interventions against household contacts contributed toward lessening the burden of LTBI in children.

References

Unhealthy drinking in the Belgian elderly population: prevalence and associated characteristics

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Background: Knowledge about alcohol consumption patterns and alcohol problems among the Belgian elderly population is scarce. The aims of this study were to explore alcohol consumption patterns and alcohol problems among the Belgian elderly population aged ≥65 years living at home, and to determine their association with socio-demographic characteristics, health status and socio-economic status. Methods: In this cross-sectional study based on a representative sample of 4825 non-institutionalized Belgian elderly people (≥65 years) in the Belgian Health Interview Surveys 2001 and 2004, alcohol consumption patterns and alcohol problems were estimated according to age, gender, survey year, living situation, frequency of social contacts, smoking status, and socio-economic status. Results: In all, 50.4% of the sample were non- or occasional drinkers, 29.1% were moderate drinkers, 10.4% at-risk drinkers, 4.6% heavy drinkers and 5.5% problematic drinkers. In total, 20.5% of the Belgian elderly population drank in excess of the National Institute on Alcohol Abuse and Alcoholism guidelines, and 4.7% had an alcohol problem according to the CAGE. In addition, 81.3% of the elderly people who consume alcohol used prescribed medications in the past 2 weeks. After adjustment for risk factors we found that, compared with moderate drinking, unhealthy drinking was significantly associated with age, gender, frequency of social contacts, health status and socio-economic status. Conclusions: Belgian health policy should be aware of the high level of at-risk drinkers in the elderly population and the underdetection and misdiagnosis of alcohol problems in this age group. An increased attention in public health initiatives among the Belgian elderly population is needed.