

## Interferon- $\gamma$ Release Assay among Tuberculin Skin Test Positive Students in Korean High Schools

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**Background:** There are several active tuberculosis (TB) cases in Korean high schools each school year. The risk of transmission in schools is extremely high due to the considerable time spent in closed classrooms. We evaluated the control of latent tuberculosis infection in Korean high schools.

**Methods:** When a student was identified with active TB, tuberculin skin testing was performed on their classmates and on students in their same school grade. When a student had a positive tuberculin skin tests (TST), they underwent follow-up testing with QuantiFERON-TB Gold In-Tube (QFT). The manufacturer recommended a cut-off of 0.35 IU/mL to determine QFT positivity was applied.

**Results:** A total of 131 pulmonary tuberculosis (TB) patients were included based on the criteria for screening TB contacts in the National Tuberculosis Control Program. Seventy-five (57.2%) students tested smear positive. TST were performed on 7,109 students who were classmates of, or in the same grade as, a TB patient. Of the contacts, 1,231 students (17.3%) were TST positive and they were screened with QFT. Six hundred-sixty-six (55.0%) of the tested students returned a positive QFT result and the rate of positivity was significantly associated with the increasing size of TST indurations ( $p < 0.0001$ ).

**Conclusion:** The use of QFT resulted in approximately 45% of TST positive students not being given chemoprophylaxis.

**Key Words:** Interferon-gamma Release Assay; Latent Tuberculosis Infection; Tuberculin Test; Students

### Introduction

Tuberculosis (TB) remains a major cause of death worldwide. World Health Organization (WHO) estimates that 9.27 million new cases of TB occurred in 2007, compared with 8.8 million new cases in 2005<sup>1</sup>. In Korea TB is a major public health concern; with a total of 34,710 (71.6/100,000) new TB patients notified and 2,376 deaths attributable to TB being reported in

2007<sup>2</sup>. As is typical for TB distribution by age group in developing countries, two peaks were observed in Korean patients, those in their 20's (101/100,000) and those over 70 years of age (283.8/100,000)<sup>2</sup>.

TB infection in adolescents is a key element of TB control in most developing countries. Many active TB cases occur in Korean high schools every year, where the risk of transmission is extremely high due to the considerable time spent in closed classrooms. The possibility of large TB outbreaks in schools was highlighted in 2006 when in one Korean high school over 60 children developed TB within a 6 month period. The *IS6110* RFLP patterns of *Mycobacterium tuberculosis* isolates from students developing TB in the school were identical with those from the index case (data not published).

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This incident provided the motive to increase screening for latent TB infection (LTBI) in high school TB contact investigations. Additionally, chemoprophylaxis for those detected with LTBI is an important strategy if we are to reduce the incidence of TB patients in younger generations. This approach is supported by the National Tuberculosis Control Program in Korea that recently amended their recommendations to include testing for LTBI as well as active TB in control activities<sup>3</sup>.

Screening for LTBI has traditionally been performed using the tuberculin skin test (TST). However, the utility of the TST is hampered by that test's poor specificity in the BCG vaccinated Korean population<sup>4,5</sup>. Recently, interferon- $\gamma$  release assays (IGRAs) that measure a cellular immune response to *M. tuberculosis*-specific antigens have been developed. The antigens used in IGRAs, ESAT-6, CFP-10, and TB7.7 are absent from all BCG strains and from most non-tuberculous mycobacteria<sup>6</sup>. Two commercial IGRA test kits, QuantiFERON<sup>®</sup>-TB Gold In Tube (QFT; Cellestis Ltd., Carnegie, Victoria, Australia) and T-SPOT<sup>™</sup>.TB (Oxford Immunotec, Oxford, UK) are being used worldwide. In contact investigations, IGRAs results have been demonstrated as more closely associated with the presence of known risk factors for TB exposure, whereas TST results are strongly associated a prior history of BCG vaccination<sup>7,8</sup>. Given the high rate of BCG vaccination in Korea, we therefore sought to examine the use of an IGRA in contact investigations in Korean high schools.

We chose to use QFT for screening for LTBI, primarily because QFT is convenient for processing large numbers of samples and the test's very high specificity, even in BCG-vaccinated populations<sup>9</sup>. Our study design was to initially screen contacts using the TST, followed by QFT for those testing TST positive. This study is the first to report IGRA data collected nationwide from Korean high schools.

## Materials and Methods

### 1. Study population

Contacts were recruited into this study following the

Guidelines of the National TB Control Program for TB outbreaks in schools in Korea. All contacts involved in this study were normal healthy students at annual chest radiography screening to detect TB patients for the high-schools in Korea. When one smear positive or two active TB patients through chest radiography screening were identified in a school, TST screening was performed for all classmates. If more than two smear positive or more than three active TB patients were identified in the one class, TST screening was performed for all students in the same grade in the school. The case of only one smear negative patient in school is excluded for Screening TB Contacts in National Tuberculosis control Program. This work was followed by the National TB Control Program Guideline, and Public Health Centers obtained the informed consents signed from all subjects.

We analyzed retrospectively the data enrolled from April to December in 2008 for this study.

This study protocol was approved by the Institutional Review Board of Korean Institute of Tuberculosis.

### 2. TST

A standard dose of 2 Tuberculin units (0.1 mL) of Statens Serum Institute (SSI) tuberculin RT23 was injected intradermally into the volar aspect of the forearm and results read 48 to 72 hours later. The reaction was read by measuring the diameter of induration across the forearm in millimeters. If there was no induration, the result was recorded as 0 mm. The TST is interpreted as positive for infection in Korea when the induration diameter is  $\geq 10$  mm.

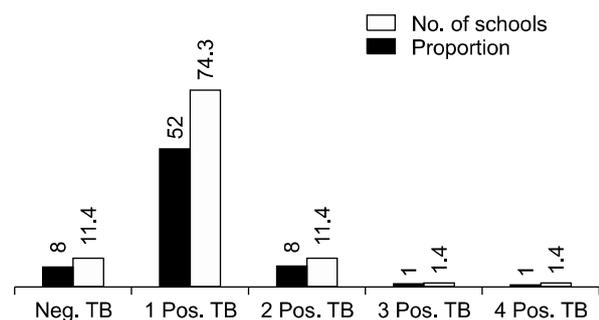
### 3. QFT

QFT assays were performed on the day of reading the TST for students who had an induration diameter  $\geq 10$  mm. QFT was performed according to the manufacturer's instructions (Cellestis Ltd.). Results were positive if the ESAT-6 and CFP-10, and TB7.7(p4) stimulated plasma samples contained more than 0.35 IU/mL of IFN- $\gamma$  above that of the saline control. If a person's response was less than 0.35 IU/mL for TB specific anti-

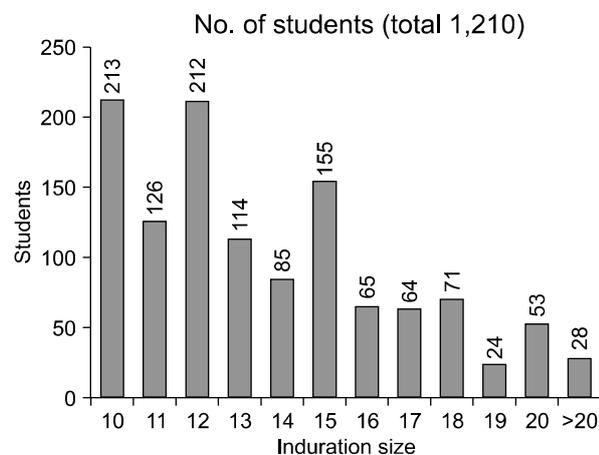
gens and their response to the mitogen positive control was above 0.5 IU/mL, they were considered to be negative. If the response to mitogen was less than 0.5 IU/mL the results was considered indeterminate, and the test was repeated until a valid QFT result was obtained.

#### 4. Statistical analysis

Statistical analyses were performed using SAS version 9.1.3 (SAS Institute, Cary, NC, USA). The relationship between QFT positivity and TST induration size was evaluated using multivariate logistic regression analysis, adjusting for gender and index case number. The relationship between QFT positive results and other variables were analyzed using the Z test. Statistical significance was assumed at a p value <0.05.



**Figure 1.** Number of schools according to the tuberculosis (TB) patients of smear microscopy results.



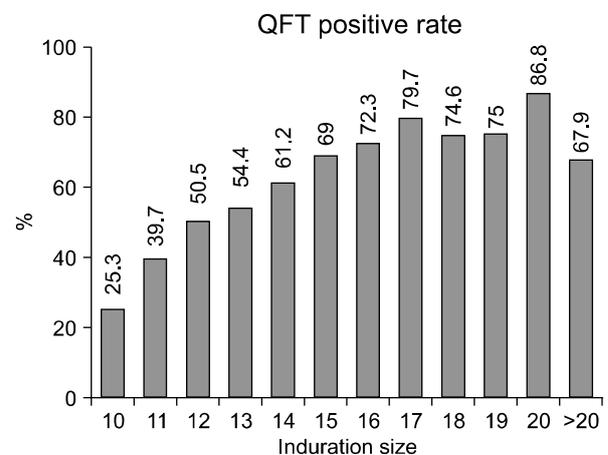
**Figure 2.** Number of students according to tuberculin skin test induration size (mm).

## Results

A total of 131 students with TB in 70 Korean high schools were included at the criteria for Screening TB Contacts within National Tuberculosis control Program in 2008; 75 (57.2%) of these were smear positive. Sixty-two (88.6%) of the 70 high schools had at least one smear positive patient; 52 had one smear positive patient, 8 schools had 2, and the other two schools had 3 and 4 patients respectively (Figure 1).

Initial TST screening was performed for 7,109 contacts who were classmates, or from the same school grade, of the index TB students (ranging from 19 to 501 contacts per school). Of the contacts, 1,231 (17.3%) were TST positive with induration sizes ranging from 10 mm to 28 mm. Among those TST positive, 690 (56%) were male. QFT was performed for all 1,231 contacts on the day of measurement of the TST and 680 (55.2%) of them returned a positive result. Four students (0.3%) initially returned an indeterminate QFT result, but determinate results were obtained from a repeat test.

Of the 1,231 TST positive contacts, 1,210 were available for analysis excluded 12 students from one school were recorded as “TST positive” without precise induration and 9 teachers. The distribution of TST induration sizes was shown in Figure 2. The most of TST positives were distributed within induration size 15 mm



**Figure 3.** QuantiFERON-TB Gold In-Tube (QFT) positive rate (%) by tuberculin skin test induration size (mm).

(905 students, 74.8% of 1,210 TST positives).

For QFT, 666 (55.0%) of the 1,210 TST positive students were positive. The rate of QFT positivity increased significantly with increasing size of TST induration ( $p < 0.0001$ ) when adjusted gender and index case number. QFT was positive for 25.3% of those with induration size of 10 mm, 69.0% at 15 mm, and 86.8% at 20 mm (Figure 3). There were no differences in the QFT positive rate by gender, with 385 (56.6%) of 680 males and 281 (53.0%) of 530 females positive ( $p = 0.1059$ ).

QFT was also performed for 22 TST negative student volunteers with TST induration size from 0 mm to 9 mm. Two of these students were QFT GIT positive; one 0.44 IU/mL of IFN- $\gamma$  with TST induration of 0 mm, and the other 15.55 IU/mL of IFN- $\gamma$  with TST induration of 9 mm. Although there were only three students with a 9 mm TST response tested by QFT, the positive rate (33.3%) was similar to that for students with induration of 10 mm, suggesting that use of a 10 mm TST cut-off may result in missed cases of *M. tuberculosis* infection.

The average QFT positive rate for the 52 schools with one smear positive index case was 52.9%, ranging from 0% to 100%, while that of schools with two smear positive index cases was 56.2%, and 57.89% for schools with no smear positive students. There was no association with the QFT positivity rate and smear microscopy results of the student index cases in this study.

There was no significant trend for the TST positive rate of the students screened to increase with the number of TB patients identified in the schools ( $p > 0.5$ ), although in the one school with 13 TB patients, 32% of the contacts were TST positive, compared an average of less than 20% for schools with 1 to 5 index cases. Similarly, there was no association between the rate of QFT positivity of the TST positive contacts and the number of TB patients identified in the schools.

## Discussion

Screening for LTBI and chemoprophylaxis of those positive is an important component of TB control pro-

grams in many developed countries. TST has been used globally as an immunological tool for detection of active TB or LTBI from more than 100 years. However, the TST has a number of limitations including poor specificity, especially in BCG vaccinated populations, as the ingredients of tuberculin are shared with the BCG vaccine and other mycobacteria<sup>10,11</sup>. Although we did not record the BCG vaccination status of the students screened in our study, the vaccination rate in Korean is very high (over 90%)<sup>12</sup>. As we suspect that many TST reactions in Korean are falsely positive due to BCG vaccination, we evaluated the use of the QFT assay (which is unaffected by BCG vaccination) as a secondary screening test for those initially found TST positive.

To the best of our knowledge, this study was the first data large scale use of an IGRA as a confirmatory test for detection latent TB infection in TST positive contacts. From a large cohort of 7,109 contacts exposed at school, we found 17.3% (1,231) were TST positive at a cut-off of 10 mm of induration. QFT testing of TST positive students confirmed *M. tuberculosis* infection in 55.2% (680) of the contacts and these were considered for isoniazid (INH) chemoprophylaxis. The *M. tuberculosis* isolated from the index cases were all INH susceptible strains. Importantly, use of QFT resulted in 551 students not being offered INH therapy, but who would likely have been treated if only the TST was used. This large reduction in the number of students to be offered INH carries not only health benefits (as INH therapy has notable side effects), but substantial economic savings.

We decided to perform QFT as a confirmatory test for those TST positive students, instead of testing all contacts with QFT, as we thought this would be the most cost-effective strategy<sup>13</sup>. Although, it was not an aim of our study to directly evaluate cost effectiveness of the different testing strategies, there is a large differential between the reagent costs for QFT per person (US \$20 to \$30) and those for TST (approximately US \$5). Thus, purely on reagent costs, screening our 7,000 contacts would have cost between US \$140,000 and \$210,000 using QFT alone, compared with US \$35,000 for the TST + US \$36,900 ( $1,231 \times \$30$ ) for QFT of those TST pos-

itive, or a total of US \$71,900.

However, these calculations do not take into account the labor costs associated with each testing strategy, the cost of clinical examinations of those testing positive, or costs associated with INH chemoprophylaxis and potential side-effects. Additionally, our data demonstrated that a proportion of contacts who were TST negative would have been QFT positive, indicating that by initially screening with TST some infected individuals would have been undetected. Two students who were TST negative but QFT positive were not provided INH for chemoprophylaxis. Further studies directly evaluating the overall program costs for the different screening strategies are required to determine what strategy is the best from both a health and a cost perspective.

Our results are in accord with previous studies of QFT in TB contacts in Korea. Chun et al.<sup>14</sup> reported that 50% (7/14) of TST positive children from a close contact group were QFT positive, while 2 (25%) of 8 TST positive children in a casual contact group were QFT positive, which is similar to the 55% of TST positive close contacts found QFT positive in this study. Similarly, another study conducted in Korea found a commensurate QFT positive rate (13/25, 52%) among a high-risk group<sup>15</sup>.

In contrast, in a Japanese high school study of 349 students 95 (27.2%) had positive TST responses, but only four (4.5%) of the 88 TST-positive students tested with QFT-G were positive by this test<sup>16</sup>. In our study, we found a lower rate (17.3%) of TST positive reactions in the 7,109 students, and a much higher proportion (55.2%) of them were QFT positive. These differences are probably due to a number of factors. The BCG vaccination policy in Japan has, until recently, to re-vaccinate if TST negative at entry to primary school and again in secondary school. Thus, the false-positive TST rate in Japan is extremely high<sup>17</sup>. The TST reading method between Japan and Korea is different, Japan use erythema size while we use induration size. The prevalence of TB in the general population is higher in Korea (126/100,000 vs. 28/100,000 in Japan)<sup>1</sup>, which may affect a higher rate of LTBI in the student population of

Korea. Additionally, we used QFT In-Tube version, which is more sensitive than the earlier liquid antigen QFT-Gold version used in the Japanese study<sup>9</sup>.

The QFT positive rate for the 52 schools with one smear positive index case was variable from 0% to 100%. The index cases were found not from notified TB patients, but from annual chest X-ray screening performed in high schools. Therefore we could not determine the interval of exposed time to the contacts from index cases in each school. Early assessment for LTBI of contacts might be a cause of lower QFT positive rate in some schools. Misclassification of LTBI positive rate is also possible due to false reading of TST results or smear microscopy results. To improve implementation of the screening protocols in the National TB Programme, we need more concrete cooperation between several public sectors, such as laboratories, public health centers, and schools.

Our study has some limitations. Students who were TST positive but QFT negative may have been infected and conversely, it is likely that a number of students (who would have been QFT-positive) were truly infected were not detected due to a negative TST at the initial screening. Without a gold standard for LTBI infection, these limitations are difficult to address without long-term prospective studies.

We did not collect information of the proximity of extent of exposure of the contacts to their respective index cases, making evaluation of such exposure parameters with test results impossible.

Our study demonstrated that use of the QFT assay as a confirmatory test for screening contacts of active TB cases in Korean schools resulted in almost 50% fewer children being identified for chemoprophylaxis than if the TST alone had been used. This finding is consistent with the specificity of the TST being poor in our BCG vaccinated population. Use of a combined TST/QFT dual testing strategy appeared to be cost effective compared with using QFT alone, but this method may result in some people with LTBI - for example, those TST negative but QFT GIT positive - being misdiagnosed as uninfected. Further studies are required to ac-

accumulate more data, and prospectively follow student contacts for the development of active TB, whether treated by chemoprophylaxis or not. Data from the present study, along with further evaluation of the QFT test in school TB investigations and comprehensive cost-effectiveness analysis, should result in an improved National TB Control Guideline in Korea.

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