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MINI-REVIEW

Molecular diagnosis of childhood tuberculosis and infection with Bacilli Calmette-Guerin in Taiwan

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Molecular techniques along with clinical evaluation have been demonstrated to be effective for differentiating childhood tuberculosis (TB), and for establishing an enhanced survey of adverse reactions of Bacilli Calmette-Guerin vaccination in Taiwan. Future development and evaluation of new diagnostics should be prioritized in strengthening the management of childhood TB.

Introduction

Tuberculosis (TB) causes 9 million cases annually, of which at least 15–20% cases occur in children.[1,2] In 2010, the TB incidence rate was 58 per million in the population and the absolute numbers of new TB cases was 13,237 in Taiwan. Of these new cases, 0.6% occur in children (0–14 years).[3] Clinical presentations of childhood TB are different from adult cases. They tend to have shorter and more frequent transition from primary infection to obvious disease and a higher proportion of cases develop noncavitary pulmonary TB or extrapulmonary TB. It is assumed that most children respond well to anti-TB treatment and have less side effects.[4] Children TB cases do not contribute to immediate transmission, however, they may later contribute to an epidemic. Detection of childhood TB is based on epidemiological, clinical and X-ray findings. Due to the difficulties in obtaining microbiological diagnosis and less infectiousness, children generally receive lower priority in a TB control program. Bacilli Calmette-Guerin (BCG) immunization is recommend to preventing serious miliary TB and disseminated TB in children.[5] In Taiwan, neonatal BCG vaccination has been included in the National Immunization Program since 1965.

Accurate diagnosis of childhood TB was hindered by improper specimen and inadequate diagnostics. In general, only a few children cases are confirmed using standard bacteriological laboratory tests since children are less likely to expectorate, and the bacterial load is lower in young children. To overcome the obstacle of laboratory diagnosis, the reference laboratory of mycobacteriology at Taiwan Centers for Disease Control (Taiwan CDC) has set up several

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streamlined algorithms using various molecular techniques for diagnosing suspected childhood TB cases and for confirming BCG-related adverse effects. Nevertheless, conventional bacteriological tests including acid-fast-stain microscopy, cultivation, nonmolecular-based identification and drug susceptibility testing (DST) are suggested. Taiwan CDC implemented policies regarding childhood TB cases without lung involvement in children aged under 5 years old since 2008, and in cases in the age group <15 from 2010 to 2011 whereby it is mandatory to submit their specimens for further identification.

Methods

Types of specimen for bacteriological diagnosis include expectorated sputum, induced sputum, nasopharyngeal aspirate, gastric washing, stool, string test, lymph node aspiration, pus, cerebrospinal fluid, bone and biopsy samples etc. Various molecular diagnostics including real-time polymerase chain reaction (PCR), multiplex PCR, line-probe assays, genotyping and gene sequencing have been applied in our routine services. A real-time PCR was designed using an IS*6110* probe for *Mycobacterium tuberculosis* complex detection.[6,7] Indeterminate real-time PCR results are further confirmed by IS*6110* nested PCR.[8,9] Multiplex PCR was performed for differentiating *M. tuberculosis* complex, *M. bovis* and *M. bovis*-BCG, and PCR products are further analyzed using sequencing.[10] Two line-probe assay kits (Hain Lifescience GmbH, Nehren, Germany) are used according to manufacturer's suggestions: (1) GenoType MTBC for differentiating BCG from other *M. tuberculosis* complex species; (2) GenoType MTBDR*plus* for detecting *M. tuberculosis* complex and its rifampicin and isoniazid resistance.[11] Besides, genotyping method, mainly spoligotyping, is adopted for differentiating *M. bovis*, *M. bovis*-BCG and *M. tuberculosis* complex.[12] DNA sequencing of 16S[13] or *pncA* gene[14] can be used for mycobacteria identification and *M. bovis* family differentiation, respectively.

Diagnosis of TB and BCG infection

Children with symptoms suggestive of pulmonary TB cases and those with no symptoms but with identifiable TB contact and with normal immune status are suggested to have rapid TB diagnosis and/or genotyping for identifying infectious sources. In 2007, we confirmed a mother–infant transmission case according to matched genotyping results of the mother's *M. tuberculosis* isolate and an intestinal specimen from the 90-day-old infant.[15] In 2009, Taiwan CDC implemented a rapid diagnosis policy on multidrug-resistant TB (MDR-TB) cases using the GenoType MTBDR*plus* assay directly on clinical sputa of high risk populations, such as retreated cases (relapse, treatment after failure and default) and contacts of MDR-TB cases. For example, we timely identified a family MDR-TB cluster within 3 working days, and two young girls were able to be promptly cared in our MDR-TB treatment consortium without waiting for results of lengthy bacterial culture and DST.

We have established the laboratory diagnosis program for identifying of BCG adverse effects since 2003. During 2005–2007, we received 19 clinical specimens from 19 childhood TB cases and found 15 (78.9%) cases were infected with *M. bovis*-BCG.[16] The estimated incidence of BCG osteitis/osteomyelitis was 12.9 per million vaccinations in Taiwan during 2005–2007 and that of world was 1–700.[17] In 2008, Taiwan CDC initiated a laboratory-based comprehensive BCG adverse events following immunization surveillance program to monitor local adverse events and severe complications. During 2008–2009, 53 childhood TB cases without lung involvement were notified, and specimens of 41 (77.4%) cases were submitted for differential diagnosis. Of the 41 cases, 24 (58.5%) were infected *M. bovis*-BCG (unpublished data). Therefore, contact investigation of the index TB case could be halted if rapid bacteriological clarification was applied. Nevertheless, the causes and risk factors of side effects are merited to further identification.

Summary

Rapid and differential diagnosis of childhood TB using modern molecular techniques is crucial for prompt treatment and appropriate management. Nevertheless, specimen collection remains a major obstacle in the diagnosis of children TB cases. Improved sputum and multiple anatomical site specimen collection can improve sensitivity and shorten the time of diagnosis.[18] Advances in the diagnosis of TB have included molecular methods including in-house PCR, integrated real-time PCR, sequencing and genotyping, etc. Along with clinical evaluation, we have successfully employed molecular techniques for differentiating childhood TB and for establishing an enhanced survey of adverse reactions of BCG. The development and evaluation of new diagnostics should be prioritized in strengthening laboratory diagnosis of childhood TB.

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