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Challenges in diagnosing tuberculosis in children: a comparative study from Sudan

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SUMMARY

Objectives: The diagnosis of tuberculosis (TB) in children is challenging due to insufficient specimen material and the scarcity of bacilli in specimens. This study aimed to evaluate methods for diagnosing TB in children in Sudan.
Methods: Patients ($N = 197$) were subjected to the tuberculin skin test (TST). Gastric lavage or sputum specimens were then collected, processed, and cultured as per standard procedures.
Results: Culture on Löwenstein–Jensen medium, the reference standard, revealed growth in 16.2% of the specimens. Comparative analysis showed that 43.7% were positive for the TST (sensitivity 100%, specificity 67.3%), 8.1% were positive by Ziehl–Neelsen stain (sensitivity 43.8%, specificity 98.8%), 11.2% by auramine stain (sensitivity 56.3%, specificity 98.8%), and 17.8% were positive for PCR amplification of the IS*6110* sequence (sensitivity 100%, specificity 98.8%).
Conclusions: It is concluded that whilst TST and IS*6110* achieved 100% sensitivity based on the reference standard of culture, the latter was more specific. The TST is recommended for routine diagnosis and the use of PCR for particular cases, depending on the facilities and the urgency.

1. Introduction

The extent of tuberculosis (TB) in children has not been completely established. According to the World Health Organization (WHO), the incidence of childhood TB is half a million cases with 74 000 deaths annually.[1] It has been argued that due to the challenges faced in the diagnosis of TB in childhood, the real burden of TB in children is higher. These challenges include the definition of a TB case as per the WHO (which requires a positive sputum smear), the varied and vague clinical presentation of TB in this age group, and the dereliction of national tuberculosis control programs in reporting child TB cases. Children commonly have a poor bacillary count, and many are negative on culture,[2] as these yield *Mycobacterium tuberculosis* in about 50% of cases at best.[3,4] Apart from microbiological culture, alternative methodological approaches have been recommended to overcome the limitations faced in the diagnosis of childhood TB.[5,6] The risk of progression of infection with *M. tuberculosis* to active disease is 5–10% for immune-competent older children and adults and 40–50% for children in their first 2 years of life.[7]

The TB burden in Sudan is high. In 2009, the prevalence per 100 000 persons was 209, with an incidence of 50 000 cases.[8] Knowledge of many aspects of TB, especially childhood TB, is still lacking. A report in the recent literature has indicated that latent TB infection could be better diagnosed in household contacts and community controls using interferon-gamma release assays than with the tuberculin skin test (TST).[9] In a cross-sectional study in Gezira, Sudan, it was found that the risk factors for being a patient with low access to pulmonary TB care included poverty, urbanization, a low level of education, and/or being idle.[10] Drug resistance in *M. tuberculosis* was found to be high (30%) in Kassala State and was found to be due predominantly to mutations in the *rpoB* gene.[11]

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TB in children manifests with severe dissemination and clinical presentations. In this age group, hematogenous and lymphatic spread of primary infection cause extrapulmonary symptoms such as miliary and meningitic disease.[12] Overall, disseminated TB occurs in 40% of active TB cases in children aged less than 1 year and in less than 1% of adults with active TB.[13] Young children with severe and complicated disease have a much higher mortality rate than older children and adults. Some studies have reported a mortality rate exceeding 50% in children less than 1 year of age who have not received anti-TB medication.[14]

The aim of the present study was to evaluate different approaches to the diagnosis of TB in children by comparing the results of TST, conventional culture and microscopy methods, and the molecular analysis of IS*6110* insertion sequences.

2. Methods

2.1. Study type, population, and sampling

This was a cross-sectional study. Children (n = 197) less than 15 years old suspected of having TB, who attended five TB centers in Khartoum State, were included in this study after providing informed consent. The children presented with persistent cough for more than 2 weeks, weight loss, failure to thrive, and prolonged fatigue.[15] Basic data were collected using a standard data questionnaire. The study population was then categorized according to the US National Institutes of Health (NIH) consensus classification.[16]

Following the TST, specimens (gastric lavage or sputum) were collected from each enrolled patient and processed as per standard procedures.[17] Early morning gastric lavage samples were collected from young children (less than 7 years of age), while sputum samples were collected from children over 7 years of age. Of the 197 samples collected, 89 (45.2%) were gastric aspirate samples, while 108 (54.8%) were sputum samples.

After decontamination, aliquots of each collected sample were used for culture, microscopy (Ziehl–Neelsen and auramine fluorescence stains), and PCR.

2.2. Tuberculin skin test

The TST was performed on all children using the Mantoux test (Statens Serum Institut, Denmark; tuberculin RT23) by injecting 100 μl of the antigen intradermally. Results were recorded as the diameter of the palpable induration at 48–72 h post injection.

2.3. Ziehl–Neelsen and auramine fluorescence stains

One aliquot of the decontaminated sediment was used to prepare two slides, one for Ziehl–Neelsen staining and the other for auramine fluorescence staining.

2.4. Culture on Löwenstein–Jensen (LJ) medium

Commercially obtained LJ medium base (Hi Media) was prepared as per the manufacturer's instructions. One aliquot of the decontaminated sediment was used to inoculate two LJ slants. Slants were incubated aerobically at 37 °C and growth was monitored daily during the first week for bacteria other than tuberculosis (MOTT) and every week up to the eighth week for *M. tuberculosis*. Grown isolates were first identified according to the methods described by Kent and Kubica.[17]

2.5. PCR amplification and gel electrophoresis of the IS6110 sequence

DNA was extracted by phenol–chloroform method. The primers used and the PCR methodology were those described by Eisenach et al.[18] to amplify a target IS*6110* fragment of 123 base pairs (bp). The primers used had the following sequences: CCTGCGAGCG-TAGGCGTCGG and CTCGTCCAGCGCCGCTTCGG.

The amplified DNA target (123 bp) was visualized by electrophoresis on 1.8% agarose gel stained with ethidium bromide and observed under UV light.

2.6. Analysis

Data were analyzed using SPSS version 16 software (SPSS Inc., Chicago, IL, USA). Sensitivity and specificity were calculated using the following formulas:

% sensitivity = true-positives/(true-positives + false-negatives) × 100; % specificity = true-negatives/(true-negatives + false-positives) × 100.

3. Results

3.1. Epidemiological findings and risk factors

Seventy-one (36%) of the study subjects were female and 126 (64%) were male, giving an average sex ratio of 1:1.8. The patients were categorized into three age groups. The sex distribution, vaccination status, and diagnostic test results, as well as the clinical classification of each group based on the NIH consensus, are shown in Table 1. The children's history of previous contact with adults with TB stratified by age group is summarized in Figure 1. Table 2 shows the clinical classification of the patients into categories of confirmed TB, probable TB, possible TB, and TB unlikely depending on the diagnostic method.[16]

Contact with an adult with TB was reported for 61 children (31.0%) (Figure 1), and 173 children (87.8%) had a cough lasting for more than 2 weeks. Weight was recorded as being less than 40% of the expected weight-for-age according to the standards of the Ministry of Health (Primary Health Care) for 166 children (84.3%). Of the 197 children studied, 174 (86.6%) were suffering from fever. No HIV-positive cases were recorded among the participating children.

3.2. Isolation and identification of M. tuberculosis

Following culture on LJ slants, 32 (16.2%) showed slow growth, out of which 30 organisms (93.8%) were isolated from sputum samples and two (6.2%) were isolated from gastric aspirates. Two slants (1.0%) showed rapidly growing mycobacteria and were considered negative for TB. *M. tuberculosis*-like colonies were confirmed by conventional methods. All of the 32 isolates were positive for nitrate reduction and negative for catalase test at 68 °C. The remaining cultures (n = 163, 83.8%) showed no growth.

Of the 197 specimens directly subjected to PCR, 35/197 (17.8%) showed a band typical in size (123 bp) to the target gene (IS*6110* insertion sequence), as indicated by the standard DNA marker (Figure 2); four (11.4%) were yielded from gastric aspirate samples, while 31 (88.6%) were yielded from sputum samples.

Routine diagnosis requires that the child start anti-TB therapy immediately following a positive test. Regarding the study methodology, the three patients with positive PCR results who were negative by culture started early anti-TB therapy on the basis of the PCR results, which were sent immediately to the physicians.

3.3. Performance of different diagnostic tests

The performances of the different diagnostic tests done on the samples from the 197 pediatric patients with symptoms of TB in