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

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S U M M A R Y

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 98.3%), 11.2% by auramine stain (sensitivity 56.3%, specificity 98.8%), and 17.8% were positive for PCR amplification of the IS6110 sequence (sensitivity 100%, specificity 98.8%).

Conclusions: It is concluded that whilst TST and IS6110 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.

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1. Introduction

The extent of tuberculosis (TB) in children has not been completely established. According to the World Health Organization (WHO), the incidence of TB in children is 10.4 million cases with 74000 deaths annually. 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 includes 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, as these yield Mycobacterium tuberculosis in about 50% of cases at best. Apart from microbiological culture, alternative methodological approaches have been recommended to overcome the limitations faced in the diagnosis of childhood TB. 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.

The TB burden in Sudan is high. In 2009, the prevalence per 100000 persons was 209, with an incidence of 50000 cases. 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). 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. 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.
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 meningeal disease. 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. 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. 

The aims 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 IS6110 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. 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.

Following the TST, specimens (gastric lavage or sputum) were collected from each enrolled patient and processed as per standard procedures. 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.

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. to amplify a target IS6110 fragment of 123 base pairs (bp). The primers used had the following sequences: CCTGGAGCG-TAGGCCGGC and CTCGTCCAGGCCTTGG.

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:

\[
\text{sensitivity} = \frac{\text{true-positives}}{\text{true-positives} + \text{false-negatives}} \times 100; \quad \text{specificity} = \frac{\text{true-negatives}}{\text{true-negatives} + \text{false-positives}} \times 100.
\]

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.

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 IS6110 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
Table 1
Demographic and vaccination data and the results of the different diagnostic tests performed for the 197 children with symptoms of tuberculosis in Khartoum State, Sudan; the results are stratified by age group and according to the NIH consensus classification

<table>
<thead>
<tr>
<th>Age group, years</th>
<th>NIH consensus classification</th>
<th>Sex, n (%) Male: Female</th>
<th>Vaccination, n (%) Yes: No</th>
<th>Culture, n (%) Positive: Negative</th>
<th>TST, n (%) Positive: Negative</th>
<th>ZN, n (%) Positive: Negative</th>
<th>Auramine, n (%) Positive: Negative</th>
<th>PCR, n (%) Positive: Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤6</td>
<td>Total</td>
<td>49 (56.9): 75 (87.2)</td>
<td>3 (3.5): 32 (37.2)</td>
<td>0 (0.0): 86 (100.0)</td>
<td>0 (0.0): 86 (100.0)</td>
<td>3 (100.0): 3 (100.0)</td>
<td>0 (0.0): 4 (100.0)</td>
<td>0 (0.0): 4 (100.0)</td>
</tr>
<tr>
<td>n = 3</td>
<td>3 (100.0)</td>
<td>1 (33.3): 0 (0.0)</td>
<td>0 (0.0): 3 (100.0)</td>
<td>0 (0.0): 3 (100.0)</td>
<td>0 (0.0): 3 (100.0)</td>
<td>0 (0.0): 3 (100.0)</td>
<td>0 (0.0): 3 (100.0)</td>
<td>0 (0.0): 3 (100.0)</td>
</tr>
<tr>
<td>n = 6</td>
<td>37 (43.1)</td>
<td>11 (12.8): 83 (96.5)</td>
<td>54 (62.8): 86 (100.0)</td>
<td>86 (100.0): 1 (3.4)</td>
<td>86 (100.0): 1 (3.4)</td>
<td>86 (100.0): 1 (3.4)</td>
<td>86 (100.0): 1 (3.4)</td>
<td>86 (100.0): 1 (3.4)</td>
</tr>
<tr>
<td>n = 9</td>
<td>17 (58.6)</td>
<td>25 (86.2): 0 (0.0)</td>
<td>14 (48.3): 0 (0.0)</td>
<td>0 (0.0): 0 (0.0)</td>
<td>0 (0.0): 0 (0.0)</td>
<td>0 (0.0): 0 (0.0)</td>
<td>0 (0.0): 0 (0.0)</td>
<td>0 (0.0): 0 (0.0)</td>
</tr>
<tr>
<td>n = 12</td>
<td>12 (41.4)</td>
<td>4 (13.8): 29 (100.0)</td>
<td>15 (51.7): 29 (100.0)</td>
<td>29 (100.0): 15 (51.7)</td>
<td>29 (100.0): 15 (51.7)</td>
<td>29 (100.0): 15 (51.7)</td>
<td>29 (100.0): 15 (51.7)</td>
<td>29 (100.0): 15 (51.7)</td>
</tr>
<tr>
<td>n = 15</td>
<td>7 (46.7)</td>
<td>11 (73.3): 0 (0.0)</td>
<td>15 (100.0): 0 (0.0)</td>
<td>0 (0.0): 0 (0.0)</td>
<td>0 (0.0): 0 (0.0)</td>
<td>0 (0.0): 0 (0.0)</td>
<td>0 (0.0): 0 (0.0)</td>
<td>0 (0.0): 0 (0.0)</td>
</tr>
<tr>
<td>n = 23</td>
<td>25 (64.1)</td>
<td>37 (94.9): 0 (0.0)</td>
<td>0 (0.0): 0 (0.0)</td>
<td>0 (0.0): 0 (0.0)</td>
<td>0 (0.0): 0 (0.0)</td>
<td>0 (0.0): 0 (0.0)</td>
<td>0 (0.0): 0 (0.0)</td>
<td>0 (0.0): 0 (0.0)</td>
</tr>
<tr>
<td>n = 39</td>
<td>14 (35.9)</td>
<td>2 (5.1): 39 (100.0)</td>
<td>39 (100.0): 39 (100.0)</td>
<td>39 (100.0): 39 (100.0)</td>
<td>39 (100.0): 39 (100.0)</td>
<td>39 (100.0): 39 (100.0)</td>
<td>39 (100.0): 39 (100.0)</td>
<td>39 (100.0): 39 (100.0)</td>
</tr>
<tr>
<td>n = 63</td>
<td>21 (3.3)</td>
<td>3 (4.8): 53 (84.1)</td>
<td>33 (52.4): 55 (87.3)</td>
<td>54 (85.7): 51 (81.0)</td>
<td>51 (81.0): 54 (85.7)</td>
<td>54 (85.7): 51 (81.0)</td>
<td>54 (85.7): 51 (81.0)</td>
<td>54 (85.7): 51 (81.0)</td>
</tr>
<tr>
<td>n = 17</td>
<td>6 (35.3)</td>
<td>1 (5.9): 17 (100.0)</td>
<td>10 (58.8): 17 (100.0)</td>
<td>17 (100.0): 17 (100.0)</td>
<td>17 (100.0): 17 (100.0)</td>
<td>17 (100.0): 17 (100.0)</td>
<td>17 (100.0): 17 (100.0)</td>
<td>17 (100.0): 17 (100.0)</td>
</tr>
<tr>
<td>n = 23</td>
<td>6 (46.2)</td>
<td>11 (84.6): 0 (0.0)</td>
<td>13 (100.0): 0 (0.0)</td>
<td>0 (0.0): 2 (15.4)</td>
<td>2 (15.4): 6 (46.2)</td>
<td>6 (46.2): 2 (15.4)</td>
<td>6 (46.2): 2 (15.4)</td>
<td>6 (46.2): 2 (15.4)</td>
</tr>
<tr>
<td>n = 39</td>
<td>7 (53.8)</td>
<td>2 (15.4): 13 (100.0)</td>
<td>0 (0.0): 13 (100.0)</td>
<td>13 (100.0): 11 (84.6)</td>
<td>11 (84.6): 7 (53.8)</td>
<td>7 (53.8): 11 (84.6)</td>
<td>7 (53.8): 11 (84.6)</td>
<td>7 (53.8): 11 (84.6)</td>
</tr>
<tr>
<td>13–18</td>
<td>Total</td>
<td>35 (72.9): 43 (89.6)</td>
<td>19 (39.6): 24 (50.0)</td>
<td>24 (50.0): 16 (33.3)</td>
<td>16 (33.3): 24 (50.0)</td>
<td>24 (50.0): 16 (33.3)</td>
<td>24 (50.0): 16 (33.3)</td>
<td>24 (50.0): 16 (33.3)</td>
</tr>
<tr>
<td>n = 48</td>
<td>13 (27.1)</td>
<td>5 (10.4): 29 (60.4)</td>
<td>24 (50.0): 40 (83.3)</td>
<td>40 (83.3): 29 (60.4)</td>
<td>29 (60.4): 40 (83.3)</td>
<td>29 (60.4): 40 (83.3)</td>
<td>29 (60.4): 40 (83.3)</td>
<td>29 (60.4): 40 (83.3)</td>
</tr>
<tr>
<td>n = 72</td>
<td>25 (34.7)</td>
<td>2 (2.8): 72 (100.0)</td>
<td>72 (100.0): 72 (100.0)</td>
<td>72 (100.0): 72 (100.0)</td>
<td>72 (100.0): 72 (100.0)</td>
<td>72 (100.0): 72 (100.0)</td>
<td>72 (100.0): 72 (100.0)</td>
<td>72 (100.0): 72 (100.0)</td>
</tr>
</tbody>
</table>

NIH, National Institutes of Health; TST, tuberculin skin test; ZN, Ziehl–Neelsen stain; TB, tuberculosis.

Figure 1. Age groups and history of contact with adults with tuberculosis among the 197 children suspected of having tuberculosis in hospitals in Khartoum State, Sudan.

Khartoum State, Sudan, are shown in Figure 3. The sensitivity and specificity of each test when using culture results as the reference standard are shown along with the performance of each test (Figure 3).

3.4. Statistical analysis
The positive predictive value (PPV) was 37.23% for the TST, 86.36% for auramine stain, 87.50% for Ziehl–Neelsen stain, and
the diagnosis showed 4.

Figure 91.43%.

Clinical discussion tuberculosis; unlikely specimens.

confirmation national institutes of health; tst, tuberculin skin test.

Table 2
Clinical classification of the 197 children with suspected TB according to the NIH consensus classification.

<table>
<thead>
<tr>
<th>Clinical diagnostic group</th>
<th>Number (%) of children</th>
<th>Definition</th>
<th>Vaccinated n (%)</th>
<th>Non-vaccinated n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Confirmed TB</td>
<td>32 (16.2)</td>
<td>Cough &gt;2 weeks + culture-positive</td>
<td>28 (87.5)</td>
<td>4 (12.5)</td>
</tr>
<tr>
<td>Probable TB</td>
<td>56 (28.4)</td>
<td>Cough &gt;2 weeks + chest radiography + contact with household TB patient</td>
<td>49 (87.5)</td>
<td>7 (12.5)</td>
</tr>
<tr>
<td>Possible TB</td>
<td>37 (18.8)</td>
<td>Cough &gt;2 weeks + contact with household TB patient + TST-positive</td>
<td>29 (78.4)</td>
<td>8 (21.6)</td>
</tr>
<tr>
<td>TB unlikely</td>
<td>72 (36.5)</td>
<td>Symptomatic but no laboratory evidence of TB and no alternative diagnosis is suggested</td>
<td>72 (100)</td>
<td>0 (0.0)</td>
</tr>
</tbody>
</table>

TB, tuberculosis; NIH, National Institutes of Health; TST, tuberculin skin test.

91.43% for PCR. The negative predictive value (NPV) was 100%, 92.57%, 90.06%, and 100%, respectively.

4. Discussion

The institution of consistent methods for the early and precise diagnosis of childhood TB is still needed. This represents a vital element in the control of TB in children. Taking into consideration the difficulties in gaining a sufficient specimen and the scarcity of bacilli within these, the gastric lavage and sputum specimens in the present study were no exception. Culture on LJ medium showed positive growth for 32 of the 197 specimens (16.2%), while 163 specimens showed no growth or were excluded as non-M. tuberculosis cultures (83.8%). Depending on how the child was clinically suspected to have TB, this rate shows a low positivity and detection rate using culture on the egg-based LJ medium as the reference standard. Improvements in growth and an acceleration of the detection rate can be achieved using automated systems such as the BACTEC MGIT 960 Mycobacterial Detection System (BD Becton, Dickinson and Company, New Jersey, USA), or other procedures.15

The present study showed that the microscopic techniques – Ziehl–Neelsen and auramine stains – although having high specificity (98.8% and 98.2%, respectively), clearly show low sensitivity (43.8% and 56.3%, respectively). This level of performance is not sufficient to detect childhood TB either reasonably or accurately. Although microscopic techniques are applied widely in the diagnosis of adult TB, especially in developing countries and in laboratories with less extensive facilities,1,8 this would not be of much help in childhood TB. Such findings and conclusions have been noted in previous studies.2,5,13

In the present study, the detection rate of childhood TB using the TST was 100%, which means that all culture-positive cases reacted positively in the TST. This finding shows the TST to be a relatively easy and rapid test to diagnose TB in children. However, the specificity of this test was found to be very low (43.7%; Figure 3). The TST was misleadingly positive for 18 of the 163 cases that did not reveal growth of M. tuberculosis. This means that 11% of culture-negative cases could be reported to the consulting room as positive if the system relies on this test alone. Although the TST is not of great benefit in diagnosing new cases or in prevention, it can still be used for screening to monitor new infections.20 Furthermore, neither chest X-ray nor the TST are recommended for TB screening in health service employees.21 Moreover, nontuberculous mycobacteria may be responsible for a positive reading of the TST result. Vaccination with bacille Calmette–Guérin (BCG) is another explanation for the high TST positivity in children; the results may remain positive for many years after vaccination.2,21 In Sudan, BCG vaccination is very frequent and is considered a basic part of the national vaccination program. Thus the positive TST
results in the present study may have been due to vaccination rather than infection, especially given the fact that 178 (90.4%) children in this study had been vaccinated.

In a previous study involving children in Peru, pediatric TB was diagnosed by auramine smear microscopy, broth culture by microscopic observation drug susceptibility (MODS) technique, standard culture on LJ medium, and hemi-nested IS6110 PCR.22 The results recommended MODS culture as the best available diagnostic test; PCR detected only half of the culture-positive cases, even when samples were assayed in duplicate.19 However, another study concluded that PCR improves the diagnosis of pediatric TB because of its rapidity, sensitivity, and specificity.23 Adult TB has also been diagnosed rapidly through a GeneXpert system that is highly specific and as sensitive as culture, with the additional characteristic of detecting M. tuberculosis directly from sputum, thereby allowing an earlier start of therapy.24 Detjen et al. concluded that Gene Xpert offers better sensitivity over microscopy for the diagnosis of pulmonary TB in children, although its sensitivity remains suboptimal compared with culture, regardless of the rapid results.23 However, they found that a negative Xpert result does not rule out TB and stated that better clinical expertise is still needed.16

In conclusion, the TST can be used for screening as a quick and easy-to-perform test, as supported by previous studies.25 However, caution is still needed when considering the inability to differentiate between infection and disease. In this regard, the submission of a specimen to PCR analysis using the IS6110 sequence for confirmation of the case may be useful. This study revealed a disappointing performance of the microscopic techniques in diagnosing childhood TB. Although they had high specificity, sensitivity was markedly low (43.8–56.2%). Symptom-based screening is strongly recommended as it reduces the number of children who require further investigation, thereby facilitating the delivery of preventive chemotherapy to asymptomatic high-risk contacts, particularly in resource-limited settings.26

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