Section: Pediatrics

Original Research Article

TUBERCULIN TEST REACTIVITY WITH 5TU VERSUS 2TU AMONG CHILDREN WITH SUSPECTED TUBERCULOSIS - A DIAGNOSTIC TEST STUDY

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ABSTRACT

Background: Tuberculosis in children is difficult to diagnose because bacteriological confirmation is often unavailable, and clinical findings are nonspecific. The Mantoux test is widely used to detect tuberculosis infection; however, its response may be affected by immune status, nutritional condition, and the strength of the tuberculin antigen used. This study aimed to compare the tuberculin reactivity of 2 TU and 5 TU PPD RT23 in children with suspected tuberculosis. **Materials and Methods:** This diagnostic study was conducted over two years in a pediatric tuberculosis clinic and inpatient wards of a tertiary care children's hospital in South India. Children aged 3 months to 12 years with suspected pulmonary or extra-pulmonary tuberculosis received both 2 TU and 5 TU Mantoux tests on the opposite forearms. Induration was measured after 48–72 hours, and ≥10 mm was considered positive. **Result:** Of the 450 children who were enrolled, 431 completed the evaluation. The mean age was 60 months, and 237 (55%) were boys. Tuberculosis disease was diagnosed in 58 children (13.4%), while 127 (30%) had tuberculosis infection. Among children with tuberculosis disease, Mantoux positivity occurred in 47 (81%) with 2 TU and 49 (84.5%) with 5 TU. The sensitivity and specificity of 2 TU were 81.03% and 64.88%, respectively, compared with 84.48% and 56.57% for 5 TU. The mean induration size was larger with 5 TU (8.6 ± 7.724 mm) than with 2 TU (6.75 ± 6.826 mm; p = 0.000). In children with severe acute malnutrition (n = 37), the induration remained higher with 5 TU (7.27 ± 8.164 mm) than with 2 TU (5.46 ± 6.772 mm; p = 0.000). **Conclusion:** The use of five tuberculin units produced stronger Mantoux reactivity and detected additional tuberculosis infections compared with the standard 2 TU test. The response was also better in children with severe acute malnutrition. Larger studies are warranted to determine whether higher tuberculin strength improves diagnostic screening for tuberculosis in children.

INTRODUCTION

India has one of the largest tuberculosis (TB) burdens worldwide, with approximately 2.2 million cases reported out of an estimated 9 million global cases.[1] Each year, approximately 1.2 million children worldwide develop tuberculosis, accounting for roughly 11–12% of all TB cases, and nearly 174,000 children under 15 years die of the disease annually.[2] Determining the true burden of pediatric TB remains difficult because confirmation of disease in children often lacks bacteriological evidence. Young children usually produce limited sputum samples, and the disease frequently presents with nonspecific clinical signs. Therefore, many diagnoses rely on combinations of clinical history, radiological findings, exposure history, and immunological tests. This diagnostic difficulty makes simple screening

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tools important in the evaluation of children suspected of having TB.

The tuberculin skin test (Mantoux test) is widely used in clinical settings to detect Mycobacterium tuberculosis infection. The test involves the intradermal injection of purified protein derivative (PPD) and the measurement of induration after 48–72 hours.[3] Health facilities in both urban and rural regions commonly use the Mantoux test because the procedure requires minimal equipment and basic training. Even with these advantages, negative reactions occur in 10–20% of patients with active tuberculosis, and the proportion of negative tests increases to nearly 70% in children with tuberculous meningitis.[3] Several biological and technical factors influence Mantoux reactivity. These include host immune response, severity of disease, nutritional status, and the dose of tuberculin antigen injected during the test.

Purified protein derivative RT23 (PPD RT23) remains the commonly used tuberculin preparation in most countries. Test interpretation generally uses an induration diameter of ≥ 10 mm as the threshold for a positive reaction when tuberculin strengths up to 5 tuberculin units (TU) are administered.[3] Earlier tuberculin surveys in many high-burden countries used 1 TU of PPD RT23 following earlier World Health Organisation recommendations. Subsequently, programmatic guidelines adopted 2 TU of PPD RT23 for tuberculin testing, and national pediatric tuberculosis guidelines in India also recommended this strength for clinical evaluation.[4] Studies that compared 1 TU and 2 TU preparations reported that several children who produced induration sizes < 9 mm with 1 TU showed induration sizes between 10 mm and 16 mm when tested with 2 TU.[5,6] These findings indicate that the strength of the tuberculin antigen can influence the size of the induration produced during the Mantoux reaction.

Undernutrition is common among children evaluated for tuberculosis in many parts of India. Protein-energy malnutrition alters cell-mediated immune responses and reduces delayed hypersensitivity reactions to mycobacterial antigens. Children with severe malnutrition, therefore, produce smaller Mantoux induration sizes and occasionally show negative reactions even in the presence of infection.[7] Experimental observations suggest that larger antigen doses can produce stronger delayed hypersensitivity responses in children with reduced immune reactivity.[8] National tuberculosis programme recommendations advise against using tuberculin doses above 5 TU, but clinical reports from India have used this strength in evaluating TB infection risk.[9]

Few pediatric studies have compared 2 TU and 5 TU Mantoux reactions using the same induration threshold in children undergoing evaluation for suspected tuberculosis, and information regarding the response in malnourished children remains limited. This diagnostic study compares the tuberculin reactivity produced by 2 TU and 5 TU PPD RT23 in children aged 3 months to 12 years with suspected tuberculosis. The study also examines the Mantoux response to these two tuberculin strengths in children with severe acute malnutrition, in which reduced immune responses may affect test performance.

MATERIALS AND METHODS

This diagnostic study included children with suspected tuberculosis attending a pediatric TB clinic and inpatient wards of a tertiary care children's hospital in South India. The study was conducted over a period of two years. Institutional Ethics Committee approval was obtained before the study commenced, and written informed consent was obtained from the parents for participation.

Sample size calculation

Previous reports in children have shown Mantoux positivity rates of 10% with 5 TU and 1.42% with 2 TU at ≥10 mm induration. With 95% confidence and 80% power, the calculated sample size was 130 children. Considering the 22.5% prevalence of undernutrition in NFHS-4, the sample size increased to 450 suspected TB children.

Inclusion and exclusion criteria

Children aged 3 months to 12 years with suspected pulmonary or extra-pulmonary tuberculosis were included, in whom pulmonary TB was suspected in children with fever or cough lasting more than two weeks, weight loss or failure to gain weight with or without contact with an adult TB case, and extra-pulmonary TB was suspected in those with organ-specific symptoms, such as lymph node swelling, joint pain or swelling, neck stiffness, or altered mental status.

Children who had undergone Mantoux testing in the previous 12 weeks were excluded because the earlier test could enhance the skin response to a subsequent test due to a boosting effect.

Methods: Each child underwent two Mantoux tests to compare the responses produced by the two tuberculin strengths. Purified protein derivative RT23 (PPD RT23) in strengths of 2 TU and 5 TU was obtained from a commercial manufacturer in India (Span Diagnostics Ltd.). To maintain blinding, the labels on the vials were concealed, and a pharmacist who was not part of the research team relabeled them as vial A (5 TU) and vial B (2 TU). Tuberculin from vial A was injected intradermally into one forearm, and tuberculin from vial B was injected into the opposite forearm at a site approximately 5 cm below the elbow.

The injection sites were encircled and marked with A or B on the child's skin to allow identification during reading. Parents were instructed not to remove the markings until the test was completed. Both the technician administering the injection and the investigator measuring the reaction were unaware of the tuberculin strength used in each forearm. Induration was measured 48–72 hours after injection using a ball-point pen method, and the diameter was

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recorded in millimetres. All tests were administered by the same technician and read by the same physician to reduce observer variation.

An induration of ≥ 10 mm was considered a positive reaction according to the National Tuberculosis Elimination Programme pediatric guidelines (2019). Local reactions, such as blistering, necrosis, or lymphangitis, were also recorded. The primary outcome measured was the sensitivity of the Mantoux reaction with 5 TU compared with the standard 2 TU. The study also assessed the response to the two strengths among children with severe acute malnutrition. Children with a positive Mantoux reaction but normal clinical and radiological findings were classified as having TB infection, whereas TB disease was diagnosed when clinical features, radiological findings, exposure history, or microbiological tests, such as culture or CBNAAT, supported active tuberculosis.

Statistical analysis: Analysis was done with SPSS v21. Statistical significance was set at $P < 0.05$. The diagnostic accuracy of 5 TU versus 2 TU in detecting TB infection was analysed using receiver operating characteristic analysis.

RESULTS

A total of 450 children with suspected tuberculosis were enrolled, of whom 431 (95.8%) completed the evaluation after 19 were lost to follow-up (4.2%). The mean age was 60 months (range, 3–144 months), with 237 males (55%) and 194 females (45%). Respiratory symptoms were the most common presentation (154 children, 35.7%), followed by asymptomatic contacts evaluated for LTBI (111 children, 25.8%) [Table 1].

Table 1: Baseline characteristics and presenting complaints of children with suspected tuberculosis (n = 431)

Variable	Category	N (%)
Study participants	Children enrolled	450
	Lost to follow-up	19
	Completed evaluation	431
Age	Age range	3-144 months
	Mean age	60 months
Sex	Male	237 (55%)
	Female	194 (45%)
Presenting complaints	Respiratory symptoms	154 (35.7%)
	Referred for ruling out LTBI (asymptomatic contacts)	111 (25.8%)
	Fever	64 (14.8%)
	Lymphadenitis	61 (14.2%)
	Neurological symptoms	17 (3.9%)
	Failure to thrive	11 (2.6%)
	Abdominal symptoms	8 (1.9%)
	Bone and joint symptoms	3 (0.7%)
	Hypersensitivity reactions	2 (0.5%)

Of the 431 children, 175 (40.6%) had a history of contact with an adult tuberculosis case. Most children were normally nourished (281, 65.2%), while 136 (31.6%) were undernourished. A BCG scar was present in 332 children (77%), and 18 (4.2%) had chest X-ray findings suggestive of tuberculosis. On evaluation, 210 children (48.7%) had no evidence of infection, 127 (30%) had tuberculosis infection, and 58 (13.4%) were diagnosed with tuberculosis disease [Table 2].

Table 2: Clinical characteristics and diagnostic outcomes among children with suspected tuberculosis (n = 431)

Variable	Category	N (%)
History of contact with an adult TB case		175 (40.6%)
Nutritional status	Normal nutrition	281 (65.2%)
	Undernourished (SAM and MAM)	136 (31.6%)
	Overweight/obese	14 (3.2%)
	BCG scar present	332 (77%)
	Chest X-ray suggestive of TB	18 (4.2%)
Diagnostic outcome	No infection or disease	210 (48.7%)
	TB disease	58 (13.4%)
	Microbiological confirmation (CBNAAT / TB culture)	10 (17.2% of TB disease cases)
	Tissue biopsy suggestive of TB	21 (4.9%)
	TB infection (positive Mantoux with either 2TU or 5TU)	127 (30%)

Table 3: Mantoux test results with 2 TU and 5 TU in relation to tuberculosis disease status (n = 431)

Variable	Mantoux result	TB disease +	TB disease −
2 TU Mantoux	Positive	47	131
	Negative	11	242
5 TU Mantoux	Positive	49	162
	Negative	9	211

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Of the 58 children with tuberculosis disease, 47 (81.0%) showed a positive reaction with 2 TU Mantoux, while 49 (84.5%) were positive with 5 TU Mantoux (Table 3). Of the 373 children without TB disease, 131 (35.1%) were positive with 2 TU and 162 (43.4%) were positive with 5 TU Mantoux [Table 3].

The sensitivity and specificity of 2 TU Mantoux and 5 TU Mantoux in identifying TB disease were 81.03% and 64.88%, and 84.48% and 56.57%, respectively. ROC curves were constructed for both Mantoux tests. The ROC curve for the 5 TU Mantoux test had an AUC of 0.745, while the ROC curve for the 2 TU Mantoux test had an AUC of 0.715. [Figure 1 and 2].

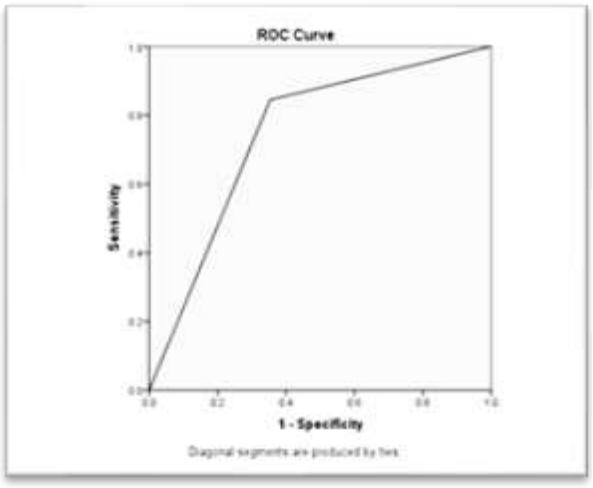


Figure 1: ROC for 5TU Mantoux

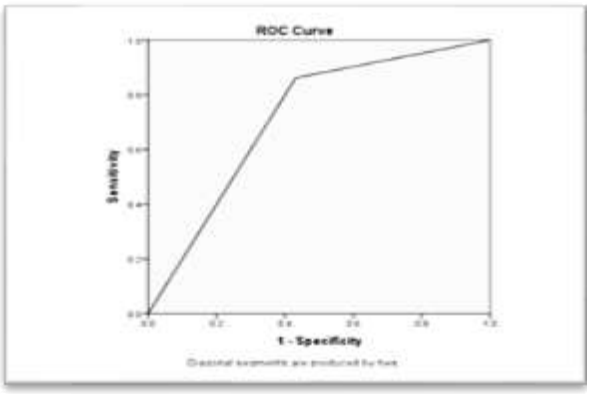
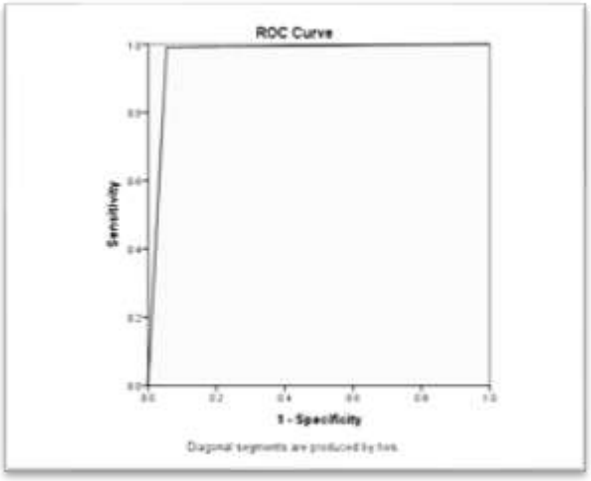


Figure 2: ROC for 2 TU Mantoux

The diagnostic accuracy of 5 TU Mantoux was compared with that of 2 TU Mantoux using the receiver operating characteristic (ROC) curve, and it was found that the area under the curve (AUC) for 5 TU was better (97 %) than that of 2 TU (90%), implying better overall performance of 5 TU [Figure 3 and 4].



Figures 3: The diagnostic accuracy of 5 TU was analysed using the ROC

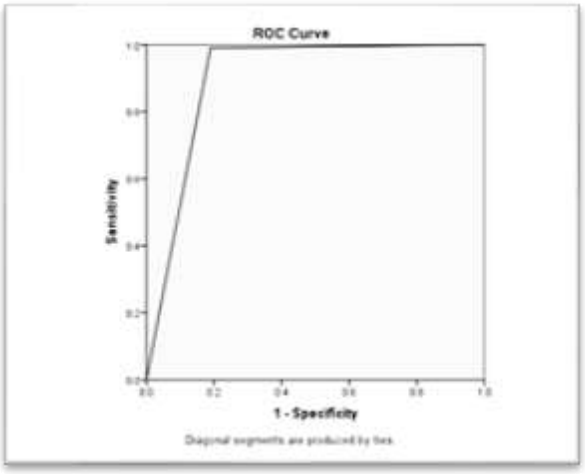


Figure 4: The diagnostic accuracy of 2 TU was analysed using the ROC

The mean induration size was higher with 5 TU (8.60 ± 7.724 mm) than with 2 TU (6.75 ± 6.826 mm), with a mean difference of 1.89 mm, which was significant (p = 0.000). In 37 children with severe acute malnutrition, the mean induration was also higher with 5 TU (7.27 ± 8.164 mm) than with 2 TU (5.46 ± 6.772 mm), with a mean difference of 1.81 mm (p = 0.000).

5 TU Mantoux performed better than 2 TU Mantoux in children with severe malnutrition (Pearson Chi-square test, 19.956), which was significant (p = 0.000).

Table 4. Comparison of the size of the Mantoux induration of 5TU and 2TU Mantoux in children with severe acute malnutrition

Variable	Category	2 TU Mantoux	5 TU Mantoux	p value
All children (n = 431)	Range of induration (mm)	0-30	0-35	-
	Mean ± SD (mm)	6.75 ± 6.826	8.60 ± 7.724	0
	Secondary reactions	16 (3.7%)	19 (4.4%)	-
	Mean difference in induration	-	1.89 mm (SD 3.212)	-

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Children with severe acute malnutrition (n = 37)	Mean ± SD (mm)	5.46 ± 6.772	7.27 ± 8.164	0
	Mean difference in induration	-	1.81 mm	-

DISCUSSION

Mantoux testing is a simple and widely used method for detecting tuberculosis infection. It is commonly used when evaluating children suspected of having tuberculosis, identifying children who need preventive therapy, and during epidemiological surveys. However, the result of the tuberculin skin test depends on several factors that can affect its interpretation and response.[11]

The present study aimed to compare the reactivity of 2 TU and 5 TU PPD RT23 in children with suspected tuberculosis and to determine whether 5TU could detect more cases of tuberculosis infection and disease. The study also examined the performance of these two tuberculin strengths in children with severe acute malnutrition. Protein malnutrition is known to reduce the immune response to tuberculin antigens because of antigen-specific anergy. Pelly et al. reported that children with reduced body protein reserves show weaker delayed hypersensitivity response to tuberculin.[12] Similarly, Taushanova et al. showed that nutritional status influences Mantoux induration in children.[13] Earlier work by Lloyd suggested that a higher antigen dose can produce a stronger reaction in malnourished children.[14] However, studies directly comparing 2 TU and 5 TU in children are limited.

In this study, 5 TU Mantoux detected additional cases of latent tuberculosis infection that were not detected with 2 TU alone, including 7.8% of children with LTBI. This finding is important because the National Tuberculosis Elimination Programme recommends preventive therapy for children aged 5–18 years with latent tuberculosis infection. Detecting more infected children can reduce the number of individuals with latent infections in the community. Better reactivity with higher tuberculin doses has been reported in previous studies. Likewise, Jo et al. also observed stronger Mantoux reactions with 5 TU compared with 2 TU in comparative testing.[15] Adams et al. reported that a Mantoux induration cut-off of 10 mm showed good sensitivity for identifying latent tuberculosis infection in endemic settings.[16]

In this study, the Mantoux test was administered by the same technician, and induration was measured by the same investigator. Both were unaware of the tuberculin strength used in each forearm. This helped maintain a uniform technique and reduce variations in reading. A small number of children reacted to 2 TU but not to 5 TU, which does not have a clear biological explanation. Possible reasons include minor technical variations during administration or interpretation of the test. As both reagents were obtained from the same lot, technical variation is the most likely reason for this observation.

In our study, the induration size was larger with 5 TU Mantoux than with 2 TU. Similar findings have been previously reported. Jo et al. who observed greater reactivity with higher tuberculin doses.[15] Goel et al. also reported larger induration sizes when higher tuberculin strengths were used in children.[17] A larger induration size may have diagnostic importance. Kendig et al. showed that tuberculin reactions are sometimes underestimated by health care workers during routine reading.[18] Reichman et al. commented on this problem and described the under-reading of Mantoux reactions as “scandalous incompetence.”[19] A larger induration produced by 5 TU may therefore help identify more infected children while remaining below the strength at which cross-reaction with nontuberculous mycobacteria becomes common.

Malnutrition and tuberculosis remain important health problems in India. Our study showed that severe acute malnutrition can cause immune suppression and may lead to false-negative Mantoux tests. Likewise, Pelly et al. showed that antigen-specific anergy occurs in children with reduced body protein levels.[12] Lloyd et al. also observed that higher tuberculin strengths produce better reactions in malnourished children.[14] In the present study, the reactivity of 2 TU and 5 TU Mantoux tests was compared in children with severe acute malnutrition, especially those under five years of age, where nutritional status has a greater influence on immune response. The results showed better reactivity and larger induration with 5 TU Mantoux in this group.

group. Low serum protein levels in malnourished children is associated with reduced size of Mantoux induration and nutritional support reversed this anergy.[18,19] Griffin et al. reported that delayed hypersensitivity responses improved after nutritional support.[20] Kardjito et al. also found that Mantoux induration size correlated with serum protein levels.[21] To our knowledge, there are very few studies comparing 2 TU and 5 TU Mantoux tests in children with severe acute malnutrition. Since Mantoux reactivity is generally reduced in malnourished children, using a higher tuberculin strength may help detect more cases of infection and disease in this group, as shown in this study.

Limitations:

Microbiological confirmation was limited in several cases. Interpretation of the Mantoux test may still vary despite efforts to standardise test administration and reading.

CONCLUSION

The study showed that 5 TU Mantoux produced greater reactivity and detected more cases of tuberculosis infection than the standard 2 TU test in children with suspected tuberculosis. Larger induration size with 5 TU improved the identification of positive reactions. The test also performed better in children with severe acute malnutrition. These findings suggest that a higher tuberculin strength may

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help detect infection in vulnerable groups. Further studies in larger and more diverse populations are required to confirm these observations and guide future diagnostic practice.

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