Research article

The diagnostic value of urinary culture filtrate protein-*10* antigen in childhood tuberculosis

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ABSTRACT

Introduction and Aim: Childhood tuberculosis (TB) remains a major problem worldwide. However, diagnosis of tuberculosis in children is often complicated by the difficulty in obtaining a proper sputum specimens and low sensitivity of the gold standard diagnostic test to confirm the presence of *Mycobacterium tuberculosis (M.tb)* in this age group. Recently, *M.tb* antigen detection in urinary specimens has become a popular method. It is non-invasive and handling of specimen is simple. It was reported that urinary CFP-10, a specific protein of *M.tb*, has emerged as a potential biomarker in the future. However, its diagnostic value as a new biomarker in childhood TB remains poorly understood. The aim of the study is to determine the diagnostic value of urinary CFP-10 in childhood TB.

Methods: Seventy children with suspected pulmonary or extra pulmonary TB were enrolled. Tuberculosis was diagnosed by performing Tuberculin skin test, chest x-ray, microscopic examination, and microbiological culture obtained from sputum or gastric lavage specimen. The level of urinary CFP-10 antigen was analyzed by ELISA (Elabscience, China). Statistical analyses were performed using SPSS 21.0 and *p*-values of <0.05 were considered statistically significant.

Results: The levels of urinary CFP-10 in subjects diagnosed with TB was higher than that of the non-TB subjects, 4.13(0.62) vs 0.43(0.14) pg/mL, p=0.005. The cut-off value for urinary CFP-10 level reached 0.39 pg/mL (sensitivity 65% and specificity 67%). This value became 0.54 pg/mL (sensitivity 61% and specificity 62%) in microbiologically confirmed cases.

Conclusion: The urinary CFP-10 level has moderate diagnostic value for diagnosing childhood TB.

Keywords: childhood TB; biomarker; urinary CFP-10.

INTRODUCTION

Tuberculosis (TB) is a public health problem worldwide, with more than 90% of cases occurred in developing countries. According to World Health Organization (WHO) estimates, an estimated10 million people developed TB in 2017. Of this number, an estimated 1 million children became ill with TB(1-3).

Diagnosis of pulmonary TB in children is extremely challenging. Clinical symptoms, such as chronic cough or growth failure, are nonspecific (4-6). Chest X-ray interpretation may vary between clinicians, while tuberculin skin tests (TSTs) and IGRA cannot distinguish between latent TB infection and active disease. Furthermore, microbiological examination is hampered with difficulties in obtaining proper sputum specimens and paucibacillary nature of childhood TB infection. As a consequence, microbiological detection of *M.tb* in children lacks sensitivity compared to adults(7-10). The sensitivity is even worse when tests were carried out using non-respiratory specimens. Microbiological confirmation is the WHO-recommended gold standard for the diagnosis of TB disease. However, in many cases of childhood TB, diagnosis is often based on contact history with adult TB patients, clinical symptoms and signs, TST, as well as chest x-ray interpretations(11-13).

To improve the diagnosis of childhood TB, a number of serological tests for identification of *M.tb* antigens have been developed, including enzyme-linked immunosorbent assay (ELISA) and immuno-chromatography (ICT) methods. Culture filtrate protein10 (CFP-10) is a low molecular weight protein (10 kDa) and a specific antigen secreted in abundance by *M.tb*. It is freely filtered by the renal glomerulus and can be detected in urine. Because it is specific for *M.tb*, cross reactions with Non-tuberculous Mycobacteria (NTM) can be avoided and test results are unaffected by prior BCG vaccination(14-17). Despite considerable efforts to study the advantage of *M.tb* antigen detection for TB diagnosis, there is very limited research on urinary CFP-10 antigen detection as a non-invasive

alternative for TB diagnosis. Hence, this study aims to examine the diagnostic value of urinary CFP-10 antigen among children with TB disease.

MATERIALS AND METHODS

Research design

This study is an analytical observational study with cross-sectional study design.

Ethical issue

The study was approved by the Human Ethical Committee of Universitas Brawijaya, Malang, Indonesia. Written informed consent was obtained from all parents/legal guardians of participants.

Subjects

This study involved subjects aged 0-14 years old with presumptive pulmonary or extra pulmonary TB disease. The presumptive criteria for pulmonary disease included history of TB contact, cough and unexplained fever that lasts for more than two weeks, lymph node enlargement, growth failure, and undernutrition. For extrapulmonary TB disease, the criteria included symptoms such as joint swelling, decreased consciousness, cervical lymph node enlargement, and so forth. This study excluded subjects who had been treated with anti-tuberculosis for at least two weeks as well as subjects with HIV coinfection or suffered from renal diseases.

Procedures

A thorough physical examination was conducted, including measurement of nutritional status and growth using the WHO child growth standards for children under five years of age and CDC 2000 growth charts for children aged five years and older. Tuberculin PPD RT23 (Staten Serum Institute, Copenhagen) was used for TST. The test dose (0.1 mL) of Tuberculin PPD was injected intradermally at the volar area of the lower arm. A positive test was defined as $\geq$10 mm diameter of induration when read 48-72 hours after injection. Chest x-ray might be suggestive of TB when perihilar or paratracheal lymph node enlargement, airway narrowing, or military type lesion were present.

Diagnosis of pulmonary TB should be performed by a pediatrician (EO) and reported as confirmed TB when microbiological examination such as acid-fast bacilli (AFB) smear or culture was positive. In the case of negative AFB smear or culture despite the presence of clinical symptoms of pulmonary TB, positive TST, or chest x-ray changes suggestive of TB, it was reported as a clinically-diagnosed TB.

A 5 mL random urine specimen was collected and centrifuged in 2,000-3,000 rpm for 20 minutes. The supernatant was recentrifuged if there was residual sediment. Urinary CFP-10 was measured by ELISA using a double-antibody sandwich technology against *M.tb* CFP-10 antigen (Urine CFP-10 Antigen Test, Elabscience, China).

Data analysis

Statistical analyses were performed using SPSS for Windows version 21.0. *P*-values of less than 0.05 were regarded as statistically significant.

RESULTS

Characteristics of subjects

During June 2018 to June 2019, 78 subjects with presumptive TB were enrolled in the study. Eighty one percent of which were finally diagnosed as having pulmonary or extrapulmonary TB. The most prevalent type of disease was pulmonary TB (71%), followed by tuberculous meningitis (6%), tuberculous lymphadenitis (6%), tuberculous spondylitis (5%), tuberculous peritonitis and tuberculous appendicitis (4%). Other extra pulmonary TB such as kidney TB, tuberculous pericarditis, and tuberculous coxitis, accounted for 2% each. Table 1 shows the characteristics of the study subjects in detail.

Levels of urine CFP-10

The levels of urinary CFP-10 in subjects diagnosed with TB was significantly higher than that of the non-TB group, 4.13(0.62) vs 0.43(0.14) pg/mL, respectively (Mann-Whitney test; p=0.005).

Diagnostic value of urinaryCFP-10

The AUC of urinary CFP-10 levels reached 73.3% (95%CI 61.2-85.4) with 65% sensitivity and 67% specificity (cut-off point of 0.39 pg/mL) when reference standard included both clinical diagnosis and microbiological examination (Fig. 1). The AUC was 61.0% (95%CI 48.3-73.5) with 61.1% sensitivity and 61.9% specificity (cut-off point of 0.54 pg/mL) when microbiological examination, i.e., the gold standard was the only reference standard used (Fig. 2).

Table 1: Characteristics of the study subjects

Variables	Non-TB (n=15)	Diagnosed as TB (n=63)
Age (year), mean (SD)	2.04 (2.91)	7.36 (4.0)
Gender		
Male	10 (66.67%)	37 (58.73%)
Female	5 (33.37%)	26 (41.27%)
TST results		

Negative	15 (100%)	26 (41.3%)
Positive	0 (0%)	37 (58.7%)
Reference Standards		
Microbiology	0 (0%)	37 (58.7%)
Clinically TB	0 (0%)	26 (41.3%)
Microbiology results		
Culture (+)	0 (0%)	4 (6,3%)
AFB (+)	0 (0%)	33 (52.4%)
Culture/AFB (-)	15 (100%)	26 (41.3%)
Nutritional Status		
Good	7 (46.7%)	21 (33.3%)
Bad	8 (53.3%)	42 (66.7%)
Mean of creatinine	11.22 mg/dL	27.79 mg/dL (pulmonary) 48.98 mg/dL (extra-pulmonary)

*Result in year; SD= Standard Deviation

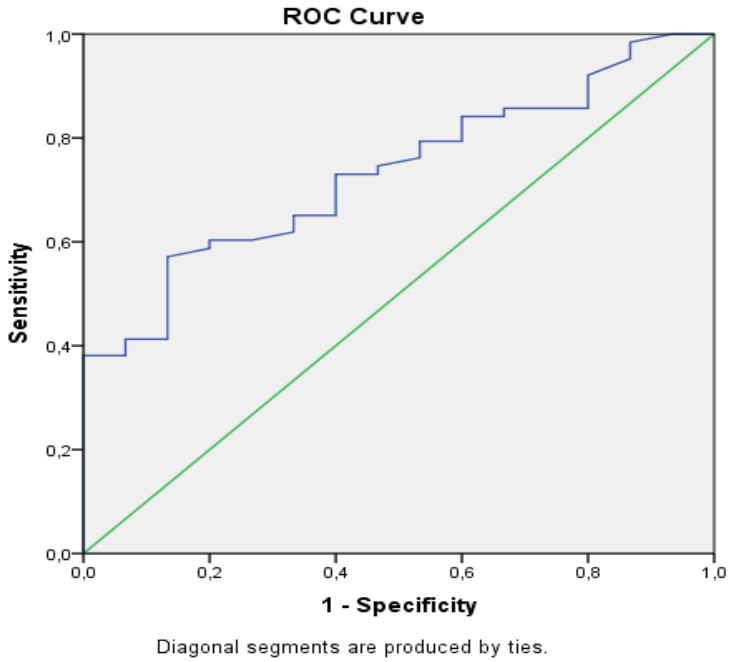

Fig. 1: ROC curve for CFP-10 levels using clinical diagnosis and microbiological examination as a reference standard

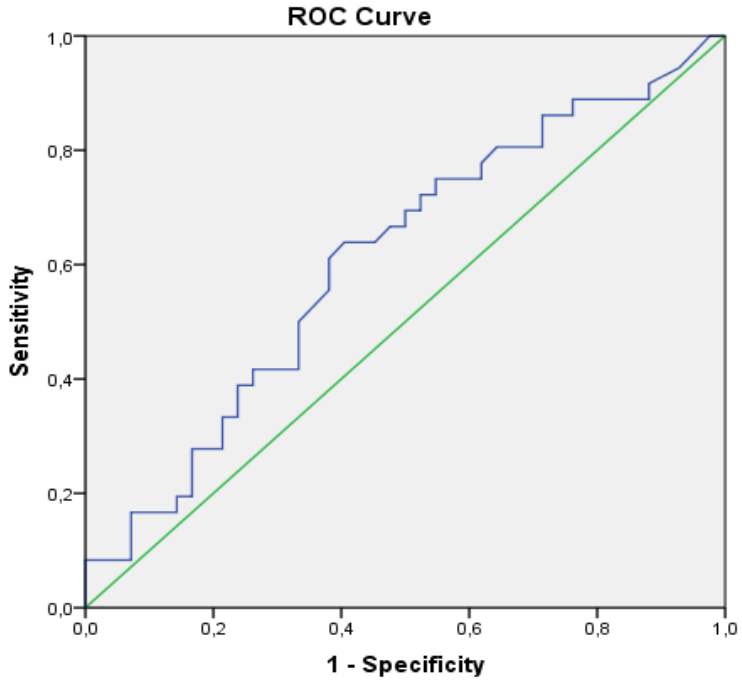

Fig. 2: ROC curve forCFP-10 levels using only microbiological examination as a reference standard

Table 2: Diagnostic performance of urinary CFP-10 levels

	Reference Standards	
	Clinical diagnosis and microbiology examination	Microbiology examination
Sensitivity, %	65	61.1
Specificity, %	67	61.9
Positive predictive value, %	89	58
Negative predictive value, %	32	65
Positive likelihood ratio	1.97	1.71
Negative likelihood ratio	0.52	0.63
Accuracy, %	65	62

DISCUSSION

Due to the paucibacillary nature of childhood TB, the detection of *M.tb* is complicated. Moreover, children tend to have reduced tussive force compared to adults, making it difficult to obtain a proper respiratory specimen. Attempts had been made to develop biomarkers to detect the presence of microorganisms from an alternative, easy-to-obtain non-respiratory specimens as well as sensitive antigen-specific diagnostic tools(18).

This study is the first to determine the performance of urinary CFP-10 levels to diagnose active TB in pediatric subjects. Hong *et al.,* had demonstrated an exponential relationship between *M.tb* numbers and urinary CFP-10 levels in TB patients using surface plasm on resonance (SPR) spectroscopy. Thus, indicating the potential for CFP-10 antigen to be diagnosed in urinary specimens of children with active TB disease(19).

In our study, the levels of urinary CFP-10 in the TB group were higher than that of the non-TB group. These results suggested that the urinary secretion of CFP-10 produced by *M.tb* was also detectable in children with active TB disease. Similar results were obtained in s study using an immunochromatography method to detect *M.tb* specific antigens in urinary specimens of children with active TB disease(20).

The AUC value of 73.3%, which was obtained when both clinical diagnosis and microbiological examination used as a reference standard, indicated that the levels of urinary CNP-10provided good diagnostic value. The AUC value was reduced to 61.0% when microbiological examination (the gold standard) used as the only reference standard. Nevertheless, these results revealed that urinary CFP-10 levels can be used to diagnose active TB disease in children, where most of cases were diagnosed clinically or radiological in the absence of positive microbiological results.

A higher sensitivity of 71% was found in a study involving adult population, using TB antigens cocktail, ESAT-6, CFP-10, and MPT-64 rapid immunochromatography test(21). However, it remained unclear whether it was due to the multiple specific antigens used in the cocktail (compared to a single antigen in our study) or higher concentration of CFP-10 in urinary specimens of adult TB patients, which typically showing more positive results on *M.tb* culture.

In this study, the sensitivity of urinary CNP-10 levels was greater compared to that of microbiological examination. However, due to lower specificity, it was not meant to replace the conventional microscopic examination and *M.tb* culture. To increase the sensitivity and positive predictive value of urinary CFP-10 level, it should be used in conjunction with microbiological examination(21). A positive result on urinary CFP-10 levels would provide better diagnostic predictive value in both clinically and microbiologically confirmed TB cases than those only microbiologically confirmed.

This study has its own challenges. The difficulty in obtaining proper sputum specimens causing microbiological examination results to be less reliable as a reference standard. In addition, collection of urinary specimens in some children was inconvenient because they were wearing diapers.

In the future, the collection of urinary specimens should be carried out at approximately the same period, i.e., in the morning, in order to obtain a relatively similar urine concentration (homogeneous) among the study subjects. For smaller children and babies, specimens can be obtained using urine collection bag designed for this age group.

CONCLUSION

The diagnostic value of urinary CFP-10 antigen in childhood TB with reference to standard microbiologic examination with or without clinical confirmation reached 65% sensitivity, 67% specificity, 89% positive predictive value, and 32% negative predictive value. The positive likelihood ratiowas1.95, whereas the negative likelihood ratio was 0.52. The accuracy was 65% with a cut off value of 0.39 pg/mL. The diagnostic value of urinary CFP-10 in children with regards to microbiological confirmation reached61% sensitivity, 62% specificity, 58% positive predictive value, and 65% negative predictive value. The positive likelihood ratio was 1.6, while the negative likelihood ratio was 0.63. The accuracy was62% with a cut-off value of 0.54 pg/mL.

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CONFLICT OF INTEREST

Authors declare there is no conflict of interest.

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