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d/a Laboratorium Patologi Klinik RSUD Dr. Soetomo Jl. Mayjend. Prof. Dr Moestopo 6–8 Surabaya, Indonesia
Telp/Fax. (031) 5042113, 085-733220600 E-mail: majalah.ijcp@yahoo.com, jurnal.ijcp@gmail.com
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Telp. (031) 5992246, 5992247, Fax. (031) 5992248. E-mail: aup.unair@gmail.com

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RESEARCH

DIAGNOSTIC VALUES OF MYCOBACTERIUM TUBERCULOSIS 38 KDA ANTIGEN IN URINE AND SERUM OF CHILDHOOD TUBERCULOSIS

(Nilai Diagnostik Antigen 38 kDa Mycobacterium tuberculosis Air Kemih dan Serum di Tuberkulosis Anak)

Agustin Iskandar[1], Leliawaty[2], Maimun Z. Arthamin[2], Ery Olivianto[3]

ABSTRAK

Diagnosis TB anak sangat sukar karena gambaran klinis tidak khas, foto paru juga sulit diinterpretasi. Di anak sulit mendapatkan dahak, jarang batuk dan jumlah kumannya sedikit. Deteksi antigen Mycobacterium tuberculosis merupakan sebuah pilihan yang tersedia untuk mendiagnosis TB. Teknik diagnosis TB secara serologis dan air kemih memberi banyak keuntungan karena mudah dikerjakan, biaya murah, cepat memberikan hasil dan mudah didapatkan, tidak menyakitkan serta tidak memerlukan spesimen dari jaringan yang sakit. Antigen 38 kDa merupakan antigen lipoprotein ekstraselular Mycobacterium sp memiliki potensi imunogen. Tujuan penelitian ini adalah membandingkan nilai diagnostik antigen 38 kDa Mycobacterium tuberculosis air kemih dan serum di tuberkulosis anak. Metode penelitian merupakan kajian potong lintang dengan pengambilan sampel secara berurutan (Juni 2013-Juni 2014). Subjek penelitian sebanyak 54 anak yang terduga TB. Dilakukan pemeriksaan kadar antigen 38 kDa Mtb air kemih dan serum dengan metode ELISA. Hasil telitian ini didapatkan rerata kadar antigen 38 kDa air kemih dan serum subjek dengan TB lebih tinggi dibandingkan kelompok bukan TB, rerata/Simpang Baku (SD) antigen 38 kDa Mtb air kemih [0,25(0,388)] vs [0,03(0,011)] p=0,002, AUC (84,3%), Cut-off point: 0,04 ng/mL (kepekaan 83% dan kekhasan 71,43%) dan antigen 38 kDa Mtb serum [14,21(13,335)] vs [4,189(0,386)] p=0,263, AUC (63,5%), Cut-off point: 4,25 ng/mL (kepekaan 53,2% dan kekahasan 57,1%). Antigen 38 kDa Mtb air kemih lebih baik daripada antigen 38 kDa Mtb serum untuk mendiagnostik tuberkulosis anak.

Kata kunci: *Antigen 38 kDa Mtb, air kemih, serum, TB anak*

ABSTRACT

Diagnosis TB in children is difficult because the clinical presentation is unspecific, the lung radiography is hard to interpret, and the sputum is hard to get. Detection of Mycobacterium tuberculosis antigen is one of the available choice for diagnosing TB. The serological methods is a better choice because it is more feasible and non-invasive. The 38 kDa antigen is an extracellular lipoprotein which has immunogen potention. The study aims to compare the diagnostic value of 38 kDa antigen of Mycobacterium tuberculosis between urine and serum of childhood tuberculosis. This study was a cross sectional study by using consecutively sampling methods. The study done on June 2013-June 2014. The subject of the study was 54 children with suspected TB. The level of 38 kDa antigen was measured by using ELISA method. The level of 38 kDa antigen in urine and serum of TB subject were higher than non TB. The mean (SD) of antigen 38 kDa Mtb in the urine was [0.25 (0.388)] vs [0.03 (0.011)] p=0.002, with AUC of 84.3%, By using cut off point 0.04 ng/mL the sensitivity of Mtb urine was 83% and specificity of 71.43%. While in the serum, the mean of 38 kDa Mtb antigen was [14.21 (13.335)] vs [4.189 (0.386)] p=0.263,with AUC (63.5%). By using cut off point 4.25 ng/mL, the sensitivity was 53.2% and specificity of 57.1%. 38 kDa Mtb antigen in the urine is better than those in the serum for diagnosing childhood TB.

Key words: 38 kDa antigen, Mtb, urine, serum, childhood TB

[1] Department of Parasitology/Clinical Pathology, Medical Faculty of Universitas Brawijaya/Saiful Anwar Hospital, Malang, Indonesia. e-mail:agustin_almi@yahoo.co.id
[2] Department of Clinical Pathology, Medical Faculty of Universitas Brawijaya/Saiful Anwar Hospital, Malang, Indonesia.
[3] Department of Child Health, Medical Faculty of Universitas Brawijaya/Saiful Anwar Hospital, Malang, Indonesia.

INTRODUCTION

Tuberculosis (TB) is an infectious disease caused by *Mycobacterium tuberculosis* (M.tb). This disease has been considered as a public health problem throughout the world, and more than 90% of the cases arise in developing countries. World Health Organization (WHO) even has declared that TB becomes a global urgency and extraordinary incidence.[1] In Indonesia, TB is also considered as a major public health problem. WHO in 2012 reported that Indonesia was in the fourth rank of TB prevalence in the world after India, China and South Africa. The incidence of TB in Indonesia reached 0.4 to 0.5 million cases in 2011.[2]

Pediatric tuberculosis is an important health problem in developing countries because the number of children aged less than 15 years and suffering from TB was 40-50% of the total population. At least 500,000 children suffer from TB, and 20 children die every day from TB every year. Many children with TB, according to the DOTS program, do not get appropriate and correct treatment. Consequently, morbidity and mortality in children increase. Besides, the burden of pediatric TB cases in the world still has not been known because of the lack of "child-friendly" diagnostic tools and an inadequate system of recording and reporting of pediatric TB cases.[3]

Pulmonary tuberculosis is the most common type of TB disease. The diagnosis is confirmed by the presence of clinical symptoms, supportive pulmonary photo, microscopic bacteria detected through sputum staining and TB bacteria culture. Examination of sputum and culture is the main standard for the diagnosis of TB, but in some cases, the diagnosis can not be proven and confirmed in bacteriology, and the diagnosis is often based only on high suspicion of TB and a good response to anti-TB treatment.[4] Although the discovery of bacteria is the gold standard for a definite diagnosis of TB, the number of TB bacteria in pediatric TB is too few to be observed. As a result, the TB diagnosis in children is often based on complaints and symptoms, pulmonary photo, tuberculin skin test, and their contact with adult patients or more commonly known as pediatric TB Scoring System.[5]

In general, the pulmonary photo is used to detect pulmonary tuberculosis. And, hilar lymphadenopathy and changes in the parenchyma are usually obtained. Unfortunately, the sensitivity and specificity of pulmonary photo in children are still low (67% and 59%).[6] Purified Protein Derivative (PPD) used in the tuberculin test is antigen mixtures that are not only still rough and have limitations because they cross-react against Baccile Calmette-Guerin (BCG) and Non-Tuberculous Mycobacteria (NTM), but also have a low sensitivity, especially in patients with abnormal immune system suppression.[7] Tuberculin test, consequently, can not distinguish whether a child is "infected" TB or "sick" and in need of TB treatment.

Lately, new *in-vitro* and *in-vivo* techniques have been developed as alternatives to the tuberculin test, namely *T lymphocyte* examination by measuring interferon gamma (IFN-γ) production. Increased level of IFN-γ or higher production of IFN-γ can indicate the presence of TB bacteria. IGRA test is more accurate than tuberculin skin test, and shows the same sensitivity in detecting pediatric TB (70% to 90% sensitivity).[8] However, IGRA test requires cell culture technique and has limited resources. There are many differences between IGRA test and tuberculin skin test. Tuberculin test is mostly positive, while IGRA test is mostly negative; and like tuberculin test, IGRA test cannot distinguish between latent TB infection and active TB infection.[9]

TB diagnosis techniques conducted serologically, moreover, provide many advantages because they are easily worked, low cost, and available everywhere, they can give fast results, as well as they require no specimen of diseased tissue. Serologic test for tuberculosis was first discovered by Arloing in 1898, using hemagglutination technique. In general, antibody responses against *Mycobacterium tuberculosis* antigens in children are very varied with a sensitivity of 14% to 85% and a specificity of 86% to 100%. There are several factors affecting the accuracy of antibody detection tests, such as the age of children when they have a strong impact on their antibody response to antigens evaluated, isotype antibody evaluated, and the type of antigens tested.[10]

Therefore, detecting *Mycobacterium tuberculosis* antigen is an option to diagnose TB and has advantages in reflecting mycobacterial material. Analysis of pulmonary tuberculosis antigens in urine is considered as a better choice than using sputum since urine samples are easily available and not invasive.[11]

There have been many researches on 38 kDa antigens, proteins with a high specificity (95%), using several methods, such as ELISA, agglutination test and immunochromatography. Senol *et al*[12] detected immunoglobulin G using recombinant antigens of 38 kDa and 16 kDa with ELISA method, and then the results showed a sensitivity of 52.5%, a specificity of 93.3%, a positive predictive value of 95.5% and a

negative predictive value of 39.7%.[12] For those reasons, this research aimed to determine and compare the diagnostic values of Mycobacterium tuberculosis 38 kDa antigens between in urine and in serum of pediatric tuberculosis patients.

METHODS

This research was a diagnostic study (observational analytic study with cross sectional design). This research was conducted in the Laboratory of Child Health, Faculty of Medicine, Brawijaya University (FKUB/RSSA Malang), Central Laboratory of RSSA and the Laboratory of Physiology, FKUB Malang. This research was performed from June 2013- June 2014.

The population of this research was all patients aged <14 years who were clinically suspected of having tuberculosis. The suspicion was based on a history of contact with adult people suffering from TB; a history of fever, chronic cough (≥2 weeks) and malnutrition; and/or a sign of extra-pulmonary TB, such as lump in the spine, enlarged lymph nodes in the neck, seizures, loss of consciousness, and diarrhea for ≥2 weeks. This research has received approval from the ethics committee of the Medical Faculty of Brawijaya University/Dr. Saiful Anwar Hospital, Malang. *Informed consent* was signed by a parent or a chaperone of the patients.

Moreover, there were several inclusion criteria used in this study. First, children were at the age of ≤14 years. Second, they had a history of contact with adult people suffering from TB. Third, they had pulmonary TB symptoms, such as cough for >2 weeks, fever, decreased weight, and decreased activity. Fourth, they had extra-pulmonary TB symptoms, such as a lump in the spine (gibbus), enlarged lymph nodes in the neck, seizures, loss of consciousness and diarrhea for ≥2 weeks. On the other hand, there were also some exclusion criteria. First, patients had diagnosed with tuberculosis before and had taken drug treatment of tuberculosis. Second, patients with a history of TB drug withdrawal.

Next, ±2 mL of blood samples and ±25 mL of *mid-stream* urine centrifuged at 3000 rpm for 20 minutes were taken from all of the research subjects. Meanwhile, the supernatant of the urine was taken and stored at a temperature of 2–8°C. Level of 38 kDa antigens in urine and serum were measured using ELISA method.

Afterward, data obtained were analyzed using SPSS version 20.0 to obtain the ROC. Finally, diagnostic values, such as sensitivity, specificity, positive predictive value, negative predictive value, Positive Likelihood Ratio (PLR) and Negative Likelihood Ratio (NLR) were determined.

RESULTS AND DISCUSSION

Data of 54 subjects aged 0–14 years with suspected TB were collected from June 2013 to June 2014. After the clinical and microbiological examinations, those subjects were grouped into TB patients and non-TB patients. Characteristics of the research subjects according to age, the results of tuberculin skin test (Mantoux), standard reference (microbiological and clinical confirmations) and nutritional status are shown in Table 1.

Table 1. Characteristics of the research subjects

Variables	Non-TB (n=7)	TB (n=47)
Age (months) Mean (SD)	14,6 (20.46)	87.0 (51.72)
Age group		
0–12 months	6/7	7 (14.9)
13–60 months	1/7	10 (21.3)
61–120 months	0	14 (29.8)
>120 months	0	16 (34.0)
Mantoux test results		
Negative	7	21(44.7)
Positive	0	26 (55.3)
Standard reference		
Microbiological	0	20 (42.6)
Clinical	0	27 (57.4)
Nutritional status		
Good	5	20 (42.6)
Poor	2	27 (57.4)

In this research, there were 34% of TB patients over the age of 10 years (120 months). This condition occurred not because of the epidemiology of TB patients most at that age, but because of the difficulties of sampling, especially urine in patients aged less than 5 years. Mtb infection in children generally occurs

due to transmission from contact with adult patients. Pediatric TB infection is usually considered as a primer infection.[13]

There are several risk factors causing a child infected with TB or suffering from TB. The first risk factor is age. Children aged <5 years has a greater risk of progression of TB infection because their cellular immunity has not fully developed (immature). However, the risk of TB will be reduced gradually with age. In infants who are infected with TB, 43% will become ill with TB. In children aged five years old who are infected with TB, 24% will become sick of TB. In teens who are infected with TB, 15% will suffer from TB. Therefore, the highest risk of the progression of TB infection is for the first one year after infection, particularly during the first six months.[5]

Based on the nutritional status, the results showed that 27 TB patients (57.4%) had a good nutritional status, while 20 TB patients (42.6%) had poor nutritional status. It means that the relation between the nutritional status of children and TB is still unclear although malnutrition is an early predictor of disease associated with worse progressivity of TB. The results of a research conducted by Jaganath and Mupere[14] show that there are several factors triggering the progression of TB infection in children, such as the role of vitamin D receptor genotypes, the effects of malnutrition on the development of the immune system, as well as the presence of co-infection and respiratory infections. Further research on pediatric TB, thus, is needed, especially about how nutrition affects the risk and development of tuberculosis.[14]

In this research, moreover, the results of Mantoux test showed positive results in 26 TB patients (55.3%). According to Triasih and Graham[15], a history of positive contact with people with TB can trigger TB infection in children identified through a positive Mantoux test. But, accuracy of this test reduces in endemic areas. The use of TB Mantoux test to diagnose pediatric TB is still problematic in Indonesia because the test is not available in most health centers, especially in rural areas. Mantoux test and chest x-ray, therefore, are not required in a routine examination of suspected TB subjects with a history of positive contacts.[15]

Purified Protein Derivative (PPD) used in tuberculin test (Mantoux test), furthermore, has limitations because they cross-react against *Baccile Calmette-Guerin* (BCG) and *Non-Tuberculous Mycobacteria* (NTM), as well as provide low sensitivity to patients with abnormal suppression of the immune system.[7] False-positive test results, consequently, can be found after BCG vaccination and their cross-reaction with *non-mycobacterium tuberculosis*.[7]

In addition, pediatric TB diagnosis has many disadvantages although with the gold reference standard. Until now, Indonesia has used a TB scoring system, IDAI, for diagnosing TB even though there have been several researches claiming that this scoring system can cause inaccuracy problems in diagnosing pediatric TB. The results of a research conducted by Triasih and Graham[15] state that the scoring system has a sensitivity of 47% and a specificity of 68%. The results of these studies indicate that the use of a scoring system for diagnosing pediatric TB in Indonesia should be observed and reviewed.[15]

Clinical confirmation, furthermore, can be obtained if there are two of three clinical symptoms as follows: clinical results are consistent with TB; results of tuberculin test are positive (induration >10 mm or >5 on the state of immunosuppression, or; the patient is clinically improved with 2 month treatment using anti-TB drugs.[16]

In this research, 20 TB patients (42.6%) were diagnosed based on microbiological confirmation, while 27 TB patients (57.1%) were diagnosed based on clinical confirmation. The results indicate that pediatric TB is difficult to be diagnosed since the clinical picture of TB is not specific. As a result, the diagnosis is confirmed by the presence of clinical symptoms, supportive pulmonary photo, microscopic bacteria found through sputum staining and culture of TB bacteria. Sputum and culture examinations are the gold reference standards for the diagnosis of TB, but in some cases, the diagnosis can not be proven and confirmed bacteriologically; thus, the diagnosis is often based only on high suspicion of TB and a good response to anti-TB treatment.[4]

The difficulty of the diagnosis of pulmonary pediatric tuberculosis is due to the gold reference standard requiring the discovery of *Mycobacterium tuberculosis* directly from sputum smear examination and/or culture results. On the other hand, it is very difficult to get sputum in children. Even if sputum is obtained, the number of bacteria contained in sputum obtained is usually a bit. Therefore, it is not directly readable by staining technique. In other words, this is a challenge for researchers to be able to continue to develop the most easily diagnostic methods with high diagnostic value for the diagnosis of pediatric TB.

In addition, Mtb 38-kD antigen is an extracellular lipoprotein antigen of *Mycobacterium sp,* which has potential immunogens, *phosphate-binding protein*

similar to the *periplasmic phosphate-binding protein* of *Escherichia coli* acting as receptors for active transport, and can be found in other Gram-negative bacteria.[17] A research conducted by Tandya[18] shows that hemagglutinin protein of 38-kDa has the highest hemagglutination titer and is considered as a protein adhesion.[18]

38-kDa antigen is also considered as a primary protein that appears on culture filtrates of *Mycobacterium tuberculosis*. Specific antigens originating from other culture filtrates are *early secretory antigenic target* 6 (ESAT-6) and *culture filtrate protein* 10 (CFP-10), both of which are encoded by *RD-1* genes (*region of difference 1*) and *TB10* antigens.[4] All of those three are classified into family *ESAT-6* and those three are immunodominant antigens mostly found in TB patients. Powerful ESAT-6 antigens are identified by lymphocyte cells producing *interferon gamma* (IFN-γ). ESAT- 6 protein as an antigen has a variety of epitopes recognized by T cells in various populations with different genetics.[19]

Based on the results of the normality test, Kolmogorov-Smirnov test, level of Mtb 38 kDa in serum and urine had a significance value of 0.000 ($p<0.05$). It means that the distribution of data of Mtb 38 kDa antigen level in serum and urine was not normal. Thus, the comparative test, t test, could not be conducted to obtain the mean of Mtb 38kDa antigen level in serum and urine of patients with TB and without TB because the assumption of normality of data distribution was not met. Therefore, Mann-Whitney U test was used to obtain the mean of 38 kDa antigen level in urine and serum of patients with TB and without TB as seen in Table 2.

Table 2. Mean of 38 kDa antigen level in urine and serum

Mean (SD)	Subjet group		P
	TB (n=47)	Non-TB (n=7)	
Urine (ng/mL)	0.25(0.388)	0.03(0.011)	0.002
Serum (ng/mL)	13.21(0.385)	4.19(0.34)	0.263

Table 2 shows that the Mtb 38 kDa antigen level in urine of TB patients was 0.25 (0.388) ng/mL, while in non-TB patients was 0.03 (0.0114) ng/mL. It indicates that there was a significant difference in the level of Mtb 38 kDa antigens in urine ($p=0.002$) between in the TB group and in the non-TB group. On the other hand, the mean of Mtb 38 kDa antigen level in serum of the TB group was 13.21 (13.34) ng/mL, while in the non-TB group was 4.19 (0.038) ng/mL. It means that there was no significant difference in the level of Mtb 38 kDa antigens in serum ($p=0.002$) between in the TB group and in the non-TB group ($p=0.263$). In other words, the results of this research showed that Mtb 38 kDa level in urine and serum of the TB patients were higher than in the non-TB patients.

There are actually some weaknesses of TB serodiagnosis. First, there are false positive results against multiple antigens. Second, there are still several antigens not only specific to TB bacteria alone. These antigens can be found in NTM (*non-tuberculous mycobacteria*) and NPM (*non-pathogenic mycobacteria*). These antigens include *38-kDa, KP_{90}, $LAM^{55, 60}$* and *A60*.[19] Therefore, Mtb antigen level in serum of non-TB patients are as high as in the case of this research.

Factors determining filtered molecules, moreover, are molecular size, molecular charge, bonding with protein, configuration and rigidity. Small molecules with a Molecular Weight (MW) of <7000 Dalton (such as water with a MW of 18; and all ions including sodium, potassium, chloride, phosphate, magnesium and calcium) are filtered without restriction. Large molecules, such as myoglobin with a MW of 17,000 are less filtered. Meanwhile, very large molecules, such as plasma proteins with a MW of 70,000 Dalton cannot pass through normal glomerular membrane.[20]

The mean of Mtb 38 kDa antigen level in serum in this research was higher than in urine. This result is supported by a theory stating that the presence of Mtb in systemic area will bind to plasma protein (immune complexes formed), thereby inhibiting filtration in the kidneys to pass the glomerular membrane. Mtb antigens released into the circulation are not bound to antibodies so that they can pass through glomerular filtration and be detected in urine.[21]

In addition, reference standard in this research used microbiological confirmation (BTA staining and/ culture) and/or clinical confirmation by a pediatrician. Since the sensitivity and specificity of microbiological examination are very low and sputum sampling is difficult to be obtained in children, this research used microbiological confirmation and/clinical confirmation as a reference standard. Clinical confirmation of TB is conducted by a pediatrician when obtained two or more of the following criteria, namely symptoms and signs supporting the diagnosis of TB; Radiological thorax photograph accordance with TB; close contact with TB people, positive results of Tuberculin Skin Test (TST); and positive response/clinical improvement after administration of anti-TB drugs for two months.

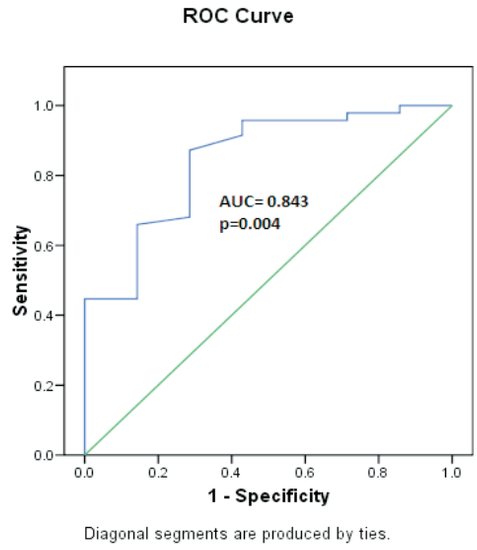

Figure 1. ROC curve of Mtb 38 kDa antigen level in urine.

Note:
blue line = Mtb 38 kDa antigen level;
green line = reference line

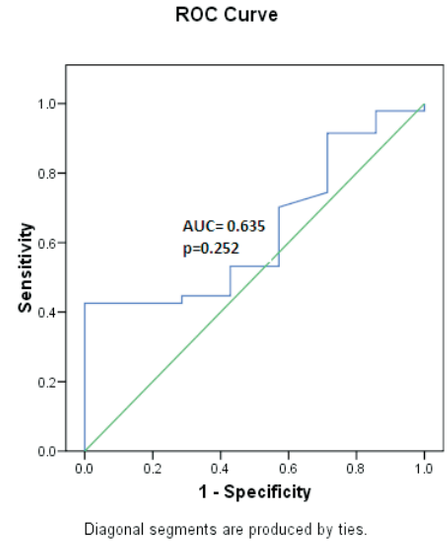

Figure 2. ROC curve of Mtb 38 kDa antigen level in serum.

Note:
blue line = Mtb 38 kDa antigen level;
green line = reference line

Analysis of the ROC curve depicting Mtb 38kDa antigen level in urine and serum using the reference standard with microbiological confirmation and/or clinical confirmation showed that the Area Under Curve (AUC) of Mtb 38 kDa antigens in urine was 84.3% (p=0.004), while in serum was 63.5% (p=0.252).

The *cut-off* value of Mtb 38 kDa antigen level used in this research was 0.04 ng/mL for urine and 4.25 ng/mL for serum. The results of the diagnostic test on 38 kDa antigens in urine showed a sensitivity of 85.11% and a specificity of 71.43%, while in serum showed a sensitivity of 53.19% and a specificity of 57.14% (see Table 3).

Table 3. Results of diagnostic test on Mtb 38 kDa level in urine and serum using microbiological confirmation and/or clinical confirmation as the reference standard

Variables	Sensitivity %	Specificity %	PPV %	NPV %	PLR	NLR
Mtb 38 kDa level in serum	53.2	57.1	89.3	15.4	1.23	0.82
Mtb 38 kDa level in urine	85.1	71.4	95.0	42.0	2.93	0.21

PPV : *Positive Predictive Value*
NPV : *Negative Predictive Value*
PLR : *Positive Likelihood Ratio*
NLR : *Negative Likelihood Ratio*

Table 4. Results of diagnostic test on Mtb 38 kDa level in urine and serum using microbiological confirmation only as the reference standard

Biomarker	Sensitivity %	Specificity %	PPV %	NPV %	PLR	NLR	Accuracy
Mtb 38 kDa level in serum	48.0	44.8	42.8	39.3	0.87	1.16	46.3
Mtb 38 kDa level in urine	80.0	20.6	46.5	54.5	1.00	0.97	48.1

PPV : *Positive Predictive Value*
NPV : *Negative Predictive Value*
PLR : *Positive Likelihood Ratio*
NLR : *Negative Likelihood Ratio*

Table 3 shows the results of the diagnostic test on Mtb 38 kDa antigens in urine and serum using microbiological confirmation and/or clinical confirmation as the reference standard.

Table 4 shows the results of the diagnostic tests on Mtb 38 kDa antigens in urine and serum using microbiological confirmation only as the reference standard. In this research, the *cut-off* value of Mtb 38 kDa antigen level used was >4:25 mg/L for serum, and >0:04 mg/L for urine.

M.tb antigen detection is an option available to diagnose TB and has some advantages, such directly reflecting Mycobacterium material, conducted either by ELISA or ICT and preventing more invasive examination in patients suspected with extrapulmonary TB. Thus, antigen detection using urine samples is a better choice for children, who may find it difficult to obtain sputum.

Enzyme-linked immunosorbent assay (ELISA), moreover, is a serological test commonly used in a variety of laboratory immunology, and has several advantages, such as relatively simple, economical and highly sensitive construction techniques. ELISA was introduced in 1971 by Peter Perlmann and Eva Engvall to analyze the interaction of antigens with antibodies in a sample by using enzymes as label.[22]

Based on the data of M.tb 38 kDa antigens in urine using ROC and AUC, the *cut-of* value of Mtb 38 kDa antigens in urine was ≥0.04 ng/mL. This value indicates a sensitivity of 85.11% and a specificity of 71.43%. On the other hand, based on the data of M.tb 38 kDa antigens in serum using ROC and AUC, the *cut-of* value of Mtb 38 kDa antigens in serum was ≥4.25 ng/mL. This value indicates a sensitivity of 53.19% and a specificity of 57.14. As a result, it can be concluded that the diagnostic value of 38 kDa antigens in urine is clinically better than in serum, so it is quite satisfactory for the diagnosis of TB in children.

Positive predictive value of M.tb 38 kDa antigens in urine, furthermore, was 95%, while in serum was 89.29%. This value indicates the probability of a person suffering from TB. On the other hand, negative predictive value of M.tb 38 kDa antigens in urine was 15.38%, while in serum was 71.42%. This value indicates the probability of a person not suffering from TB. These results support the conclusion that the diagnostic value of M.tb 38 kDa antigens in urine is better than in serum.

Although there are still not many theories explaining how 38 kDa antigens are detected in the urine, the high diagnostic value of 38 kDa antigens in urine in this research can also be related to the low molecular weight of the antigens so that they can easily pass through glomerular filtration and then are detected in the urine.

In addition, many serological researches on Mtb 38 kDa antigen reported that various results (16–94%), mostly dependent on sputum examination results and disease manifestations. Specificity value in patients with TB was 88% -100%, but sensitivity value was 33–89% for positive sputum smear examination results, and 16–54% for negative sputum smear examination results.[12]

Krishna *et al*[24] conducted a research for tuberculosis diagnostics by using a combination of antibodies in serum and urine. The research aims to determine the presence of antibodies against *Mycobacterium tuberculosis* contained in TB patients and to evaluate the feasibility of antibody-based diagnostic tests in urine. The results of this research showed that M. tuberculosis culture filtrate proteins, such as MPT 32, 81 kDa protein and protein GlcB detected in the urine of TB patients, have lower sensitivity than antibodies in serum (68–77%).[24]

The research also showed that the diagnostic values of 38 kDa antigens in serum and urine are lower when microbiological confirmation only is used as the reference standard. This is presumably due to microbiological confirmation of TB in children that is not routinely performed, difficulties to get the samples and poor staining performance of microscopic examination. Diagnosis of TB is also based mainly on clinical examination of samples by staining acid-resistant bacteria (AFB) in adults, but in children with pulmonary TB usually cannot cough up sputum because children usually do not produce a lot of sputum and the procedure is uncomfortable. There are chances on pausibasiler conditions (few bacteria). Finally, it can be said that in order to determine the diagnosis of TB in children, microbiological confirmation cannot be used alone, but must be combined with clinical confirmation.[13]

CONCLUSION AND SUGESSTION

Based on the results of this research, it can be concluded that M.tb 38 kDa antigen level in urine and serum of patients with TB are higher than in urine and serum of patients without TB. Diagnostic value of M.tb 38 kDa antigens in urine is also considered to be better than in serum (urine: a sensitivity of

85.11% and a specificity of 71.43% with a *cut-off* value of 0:04 ng/mL versus serum: a sensitivity of 53.19% and a specificity of 57.14% with a *cut-off* value of 4.25 ng/mL). However, further research had better use more samples, POCT method, that is faster and easier, and other antigens, which level are higher than urine to enhance these results.

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