Positive Lateral Flow Urine Lipoarabinomannan Assay (LF-LAM) Result in Detection of Active Tuberculosis

Sara Puspita, Dewi Kartika Turbawaty, Nina Tristina, Leni Lismayanti
Department of Clinical Pathology Faculty of Medicine Universitas Padjadjaran
Dr. Hasan Sadikin General Hospital Bandung, Indonesia

Abstract

Lipoarabinomannan (LAM) is the main component of Mycobacterium tuberculosis (MTB) wall as result of MTB degradation by macrophages in the human body. In patients with active TB and HIV co-infection, a decrease in antibody responses may be apparent that some of LAM may not be bound with antibodies. In this condition, LAM can pass through the normal glomerular basement membrane and can be detected in the urine. One laboratory examination for detecting LAM is the Lateral Flow Urine Lipoarabinomannan (LF-LAM) assay that uses urine as the sample. The purpose of this cross-sectional observational descriptive comparative study was to compare the positivity rate of LF-LAM examination results in active TB patients with and without HIV infection. Random urine samples were collected from patients diagnosed with active TB with and without HIV infection who visited Dr. Hasan Sadikin General Hospital Bandung from August to October 2020. The proportion between the group with HIV and group without HIV was analyzed with the Chi-Square test. Subjects were 52 patients, consisting of 25 (48%) subjects with HIV infection and 27 (52%) subjects without HIV infection. The positive LF-LAM results were found in 11 (21%) subjects, consisting of 9 (36%) subjects with HIV infection and 2 (7%) subjects without HIV infection, with p=0.012. In conclusion, the positivity rate of LF-LAM results is higher in active TB patients with HIV infection compared to those without HIV infection.

Keywords: HIV, lateral flow urine lipoarabinomannan assay (LF-LAM), TB

Positivitas Hasil Pemeriksaan Lateral Flow Urine Lipoarabinomannan Assay (LF-LAM) Dalam Mendeteksi Tuberkulosis Aktif

Abstrak

Lipoarabinomannan (LAM) merupakan komponen utama dinding sel Mycobacterium tuberculosis (MTB) dan hasil produk degradasi MTB oleh makrofag di dalam tubuh manusia. Pada penderita TB aktif dengan infeksi HIV dapat terjadi penurunan respons antibodi, yang menyebabkan sebagian LAM dapat tidak terikat antibodi sehingga dapat melewati membran basal glomerulus normal dan terdeteksi di urine. Salah satu pemeriksaan laboratorium untuk mendeteksi LAM adalah LF-LAM, dengan bahan pemeriksaan urine. Tujuan penelitian ini adalah untuk mengetahui positivitas hasil pemeriksaan LF-LAM antara penderita tuberkulosis aktif dengan dan tanpa infeksi HIV. Penelitian ini merupakan penelitian observasional deskriptif komparatif dengan rancangan potong lintang. Bahan pemeriksaan penelitian berupa urine sewaktu dari penderita TB aktif dengan dan tanpa infeksi HIV di RSUP Dr. Hasan Sadikin Bandung pada bulan Agustus-Oktober 2020. Uji beda proporsi antara dua kelompok dianalisis dengan uji chi-square. Jumlah subjek penelitian adalah sebanyak 52 subjek terdiri dari 25 (48%) subjek dengan HIV dan 27 (52%) subjek tanpa HIV. Terdapat 11 (21%) subjek hasil pemeriksaan LF-LAM positif, terdiri atas 9 (36%) subjek TB aktif dengan HIV dan 2 (7%) subjek TB aktif tanpa HIV, dengan nilai p=0.012. Simpulan, positivitas hasil pemeriksaan LF-LAM lebih banyak pada penderita TB aktif dengan infeksi HIV dibanding dengan penderita TB aktif tanpa infeksi HIV.

Kata kunci: HIV, lateral flow urine lipoarabinomannan assay (LF-LAM), TB
Introduction

The World Health Organization (WHO) stated that Indonesia is one of the countries with the most cases of Tuberculosis (TB) infection with and without the Human Immunodeficiency Virus (HIV).1 Tuberculosis is an opportunistic disease that is often found and causes death (±40–50%) in people living with HIV (PLHIV). This high mortality is especially in smear negative pulmonary TB and extrapulmonary TB which is most likely caused by the delay in diagnosis and TB therapy.2 A range of new diagnostic technologies have been endorsed by WHO during the past decade for TB are real-time polymerase chain reaction (PCR) assays – for example, Xpert MTB/RIF® (Ultra) (cartridge-based); line probe assays (LPAs) – for example, GenoType®, Genoscholar™; loop-mediated isothermal amplification (LAMP) – for example, TB-LAMP; and antigen detection in a lateral flow format (biomarker-based detection) – for example, Alere Determine™ TB LAM Ag (LF-LAM Urine Assay).3 The problem that occurs is the absence of facilities and infrastructure to meet WHO recommendations in the laboratory. There is a need for rapid point-of-care tests for TB with high diagnostic accuracy that can be readily used at all levels of the health system and in the community.4

One of the alternatives for TB diagnosis examination is detecting antigen of TB for example lipoarabinomannan (LAM) examination. Lipoarabinomannan is the main component of MTB cell walls and is a product of bacterial degradation by infected macrophage.5,6 Lipoarabinomannan molecule with mannosylated caps (ManLAM) is a type of LAM for pathogenic mycobacteria species such as Mycobacterium tuberculosis, Mycobacterium leprae and Mycobacterium bovis. These molecules have an important role for the survival of MTB in cells.4 Point of care test (POCT) TB that recommended by WHO for detecting lipoarabinomannan is known as the lateral flow urine lipoarabinomannan assay (LF-LAM). This test uses urine for the sample which is not invasive, the process is easy and not complicated, no need for various reagents, affordable, requires short time (±25 minutes) and shows increased sensitivity in active TB patient with HIV infection.5 Based on Singhroy’s study, in 2020, some of countries with high tuberculosis and HIV/AIDS burden has adopt and uptake of the lateral flow urine LAM test, such as Central of African Republic, Malawi, Myanmar, Uganda and Zimbabwe.7 Infection with HIV is a risk factor for a higher circulating burden of MTB and reflects a higher frequency of incidence of extra pulmonary (disseminated) TB.8,9 In renal TB, MTB infects the urinary tract and could damage the glomerular basement membrane (GBM). The damage of it causes LAM that is bound to anti-LAM antibodies pass through the GBM. In HIV infection there is also a decrease in the antibody response so that LAM may not bound to anti-LAM antibodies, and because LAM have low molecular weight so they can easily pass through the GBM.10,11 Thus, the results of LF-LAM will be more in active TB and PLHIV compared to without HIV infection. The aim of this study is to determine the positivity of LF-LAM examination results of active TB patients with and without HIV infection.

Methods

The study was a descriptive comparative observational study with cross sectional design. The inclusion criteria were adult patient (≥18 years) whom have been diagnosed active TB with or without HIV (inpatient and or outpatient), by clinician in Dr. Hasan Sadikin General Hospital Bandung based on International Standard of Tuberculosis Care (ISTC) and using various modalities diagnostic tools of TB, without considering the patient was getting or not getting therapy TB and or antiretroviral (ARV). The exclusion criteria were patients diagnosed with renal TB and urinary tract infection (UTI). The history of UTI syndromes based on anamnesis was also eliminated.

The study was conducted from August 2020 to October 2020. The used sample is midstream random urine, collected from the patient and stored in a sterile urine pot without preservatives. The samples then aliquoted into a micro-plastic tube and stored at -80 ℃ until the number of research samples is met at clinical pathology laboratory of Dr. Hasan Sadikin General Hospital Bandung. Before processing the test (Alere Determine TB Ag), the frozen urines were thawed in room temperature (20–25°C) for one hour and then sentrifugated at -80 °C until the number of research samples is met at clinical pathology laboratory of Dr. Hasan Sadikin General Hospital Bandung. Before processing the test (Alere Determine TB Ag), the frozen urines were thawed in room temperature (20–25°C) for one hour and then sentrifugated at 10000 g for 15 minutes. Clear urine supernatants were taken for about 60uL and dropped on the sample pad of the POCT. Result of the test must be read in 25–35 minutes. The data of two groups were analyzed using Statistical Package for the Social Sciences (SPSS) software version 25.0 for Windows. The normality test performed using
Saphiro wilk test and the proportion difference test performed with Chi-Square test. The study was approved by the Health Research Ethics Committee of Dr. Hasan Sadikin General Hospital Bandung through the issuance of the ethical approval no. LB.02.01/X.6.5/215/2020.

Result

Subjects of the study were 52 subjects with a composition of 25 (48%) subjects active TB patients with HIV and 27 (52%) subjects TB patients without HIV. Gender of the subjects was dominated by men, 29 (56%) subjects. The mean age of the two groups, with and without HIV infection respectively were 34.38±6.579 and 37.85±12.895.

The positive result of LF-LAM test was 11 (21%) from all groups. In the active TB group with HIV infection there were more positive LF-LAM results than the active TB group without HIV infection.

Table 1 Differences Results of LF-LAM between Patients Active Tuberculosis With and Without HIV Infection

| Variable | HIV (+) | HIV (-) | p-Value |
|----------|---------|---------|----------|
|          | n(%) n=25 | n(%) n=27 |          |
| LF-LAM   |         |         | 0.012    |
| Positive | 9 (36)  | 2 (7)   |          |
| Negative | 16 (64) | 25 (93) |          |

LF-LAM=lateral flow urine lipoarabinomannan assay; p<0.05 with chi-square test

Table 2 Characteristics of Subject with Positive LF-LAM Results

| Characteristic | Group | HIV(+) | HIV(-) |
|----------------|-------|--------|--------|
|                |       | N(%) N=9 | N(%) N=2 |
| Gender         |       |        |        |
| Men            |       | 8 (89)  | -      |
| Female         |       | 1 (11)  | 2 (100) |
| TB Classification |   |        |        |
| Pulmonary TB   |       | 4 (45)  | 2 (100) |
| Extra Pulmonary TB | |        |        |
| Lymph          |       | 1 (11)  | -      |
| Meningitis     |       | 1 (11)  | -      |
| Abdomen        |       | 1 (11)  | -      |
| TB Pulmonary + Extra Pulmonary TB | |        |        |
| Pulmonary + Lymph | | 2 (22)  | -      |
| Therapy (+)    |       |        |        |
| TB drugs +ARV  |       | 4 (44)  | -      |
| TB drugs       |       | 1 (11)  | 2 (100) |
| Therapy (-)    |       |        |        |
| TB drugs       |       | -      | -      |
| ARV            |       | 1 (11)  | -      |
| TB drugs +ARV  |       | 4 (45)  | -      |

TB= tuberculosis; ARV=antiretroviral; Therapy (+)=had therapy, Therapy (-)=had not therapy
infection, that was 9 subjects (36%), depicted in Table 1.

The proportion difference test between the two groups was statistically significant (p=0.012). Characteristics subjects of positive LF-LAM results can be seen in Table 2.

In Table 2, the results showed that some subjects who had received TB drugs and ARV therapy when they were tested for LF-LAM were still able to show positive results.

Discussion

Overall, the gender characteristics of the subjects of this study were dominated by men, as many as 29 (56%) subjects. This is in accordance with the WHO annual report in 2019 which reports that the composition of TB sufferers in Indonesia is dominated by men compared to women.1

The result of positive LF-LAM examination in this study was 11 (21%), this is in accordance with the WHO statement which has conducted an analysis of 15 previous studies and concluded in theory that out of 1000 subjects with active TB with HIV, only 189 subjects (18.9%) showed positive LF-LAM results.5

The difference in proportion between the two groups showed statistically significant results (p=0.012). This is in accordance with the study by Suwanpilmolkul et al.12 which examined LF-LAM examination in the active TB group with and without HIV infection in Thailand with the proportions of each group being 37.2% and 7.4%, respectively.

In this study, 10 subjects (20%) had positive LF-LAM examination results and 1 person (2%) equivocal or indefinite LF-LAM. The equivocal or indefinite result was from active TB subjects with HIV infection and the researcher included it into groups with positive LF-LAM results, so that the total positive LF-LAM examination results were 11 subjects (21%). Based on the research of Siddiqi et al.,13 3 (19%) of 16 active TB study subjects were accompanied by HIV infection, with equivocal or indefinite LF-LAM results then continued to MTB culture from cerebrospinal sample at the same time showed results positive of TB.

World Health Organization states that LF-LAM is better used in patients with active TB with HIV infection who showing clinical symptoms of TB (pulmonary and/or extra pulmonary) with a CD4 count <100 cells/μL, respectively 9 cells/μL, 12 cells /μL and 63 cells/μL.

Based on the theory, various mechanisms that can cause LAM to be detected in urine are the result of MTB infecting the kidneys (renal TB), LAM molecules are not bound to immune complexes (free LAM molecules, small molecular weight of LAM) so that they can pass through the normal glomerular basement membrane, and also because immune complexes of LAM or MTB can cross the damaged glomerular basement membrane. Lipoarabinomannan (LAM) molecules which are not bound to antibodies can be caused by an impaired immune response to form antibodies or there is an imbalance in the amount of LAM with immunoglobulins in the body.10,11 In HIV infection there is a decrease in the number of CD4+ T cells in HIV infection will cause an increase in IL-7 which will disrupt the regulation of B cell maturity. The number of immature B cells will increase and will decrease the response of B cells to antigens. The HIV infects T helper and causes a decrease in IL-21 production which will have an impact on B cell differentiation, decreased B memory cell formation and antibody response.14

One of the subjects who showed LF-LAM positively, had diagnosed with systemic lupus erythematosus (SLE). People with SLE have a 6x higher risk of being infected with TB. Systemic lupus erythematosus can become an immunocompromised condition due to immune system abnormalities, immunoglobulin and complement deficiency, defects in chemotaxis, phagocytosis and abnormalities in cellular immunity, and is supported by receiving high steroid therapy which causes immunosuppression.15 The research of Suwanpimolkul et al.,12 the LF-LAM examination can be used for an active TB population accompanied by severe immunocompromised conditions and further research is still needed.

In this study, 2 subjects (12.5%) of 16 active TB subjects were immunocompromised without having HIV infection.

Fortyone subjects were found to have negative LF-LAM examinations. Based on the theory states by Cox et al.19 states that LAM is an immunogenic molecule and has a low molecular weight. When LAM binds to anti-LAM antibodies, the total molecular weight will be large so that it cannot pass through the normal glomerular basement membrane.

The study had some limitations including the absence of routine urine examinations and urine
culture to rule out UTI diagnosis more accurately, also there were no complete CD4 count data and the study did not examine CD4 cell count of the subjects, total of the CD4 data in this study were only 12 (48%). Over all, the positivity of the LF-LAM examination results in this study was low (21%). However, the difference proportion test showed significantly result between active TB with and without HIV infection (p=0.012), it showed that active TB with PLHIV will give more higher positive results of LF-LAM.

To conclude, because of the low of positivity LF-LAM urine assay, the test should be used more in HIV patients with clinical symptoms of TB (pulmonary and/or extra pulmonary) with a CD4 count <100 cells/µL or seriously ill with or without known CD4 cell count, as an add on or alternative test for diagnosing TB and it should be used in combination with other TB modality tests in establishing the TB diagnosis.

References

1. WHO. Global Tuberculosis Report 2019. France: WHO; 2019.
2. Keputusan Menteri Kesehatan Republik Indonesia Nomor Hk.01.07/ Menkes/90/2019 Tentang Pedoman Nasional Pelayanan Kedokteran Tata Laksana HIV. Kemenkes RI: Jakarta; 2019.
3. WHO Guidelines Approved by the Guidelines Review Committee. WHO consolidated guidelines on tuberculosis: Module 3: diagnosis – rapid diagnostics for tuberculosis detection. Geneva: World Health Organization; 2020.
4. Lawn SD. Point-of-care detection of lipoarabinomannan (LAM) in urine for diagnosis of HIV-associated tuberculosis: a state of the art review. BMC Infec Dis. 2012;12(1):103.
5. WHO. Lateral Flow Urine Lipoarabinomannan Assay (LF-LAM) for the diagnosis of active tuberculosis in people living with HIV Policy update (2019). 2019.
6. Iskandar A, Nursilingingrum E, Arthamin MZ, Olivianto E, Chandrakusuma MS. The diagnostic value of urine lipoarabinomannan (lam) antigen in childhood tuberculosis. J Clin Diagn Res. 2017;11(3):EC32–EC35.
7. Singhroy D, MacLean E, Kohli M, Lessem E, Branigan D, England K, et al. Adoption and uptake of the lateral flow urine LAM test in countries with high tuberculosis and HIV/AIDS burden: current landscape and barriers. Gates Open Res. 2020;4:24.
8. Broger T, Sossen B, du Toit E, Kerkhoff AD, Schutz C, Ivanova Reiold E, et al. Novel lipoarabinomannan point-of-care tuberculosis test for people with HIV: a diagnostic accuracy study. Lancet Infect Dis. 2019;19(8):852–61.
9. Talbot E, Munseri P, Teixeira P, Matee M, Bakari M, Lahey T, et al. Test characteristics of urinary lipoarabinomannan and predictors of mortality among hospitalized hiv-infected tuberculosis suspects in Tanzania. PLOS ONE. 2012;7(3):e32876.
10. Cox J, Lukande R, Kalungi S, Van Marck E, Van de Vijver K, Kambugu A, et al. Is urinary lipoarabinomannan the result of renal tuberculosis? assessment of the renal histology in an autopsy cohort of Ugandan HIV-Infected adults. PLOS ONE. 2015;10:e0123323.
11. Wood R, Racow K, Bekker L-G, Middelkoop K, Vogt M, Kreiswirth BN, et al. Lipoarabinomannan in urine during tuberculosis treatment: association with host and pathogen factors and mycobacteriuria. BMC Infect Dis. 2012;12:47.
12. Suwanpimolkul G, Kawkitinarong K, Manosuthi W, Sophonphan J, Gatechompol S, Ohata PJ, et al. Utility of urine lipoarabinomannan (LAM) in diagnosing tuberculosis and predicting mortality with and without HIV: prospective TB cohort from the Thailand Big City TB Research Network. Int J Infect Dis. 2017;59:96–102.
13. Siddiqi OK, Birbeck GL, Ghebremichael M, Mubanga E, Love S, Buback C, et al. Prospective cohort study on performance of cerebrospinal fluid (CSF) Xpert MTB/RIF, CSF Lipoarabinomannan (LAM) Lateral Flow Assay (LFA), and urine LAM LFA for diagnosis of tuberculous meningitis in Zambia. J Clin Microbiol. 2019;57(8):e00652–19.
14. Ruffin N, Pham T, Rethi B, Nilsson A, Chiodi F. The impact of inflammation and immune activation on B cell differentiation during HIV-1 infection. Front Immunol. 2012;2:90.
15. Maduemem KE, Adedokun CO, Vatca A. Combined diagnosis of systemic lupus erythematosus and tuberculosis in an Irish adolescent female. Case Rep Pediatr. 2018:2031219.