RESEARCH ARTICLE

pISSN: 0126-074X | eISSN: 2338-6223
MKB. 2021;53(3): 169–73
https://doi.org/10.15395/mkb.v53n3.2265

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 M. 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 *M.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

Corresponding Author: Sara Puspita, Department of Clinical Pathology Faculty of Medicine Universitas Padjadjaran/Dr. Hasan Sadikin General Hospital Bandung, Jalan Pasteur No 38 Bandung, Indonesia, Email: sarawardhana3103@gmail.com

Introduction

The World Health Organization (WHO) has designated Indonesia as one of the countries with the most Tuberculosis (TB) cases 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 linked to smear negative pulmonary TB and extrapulmonary TB which are most likely caused by the delay in diagnosis and TB therapy.[2] A range of new diagnostic technologies have been endorsed by the WHO during the past decade for TB, including real-time polymerase chain reaction (PCR) assays, such as Xpert MTB/RIF® (Ultra) (cartridge-based); line probe assays (LPAs) such as GenoType® and Genoscholar™; loop-mediated isothermal amplification (LAMP), such as TB-LAMP; and antigen detection in a lateral flow format (biomarker-based detection) that includes, among others, Alere Determine™ TB LAM Ag (LF-LAM Urine Assay).[3] The problem found in performing these diagnostic technologies is the absence of appropriate facilities and infrastructure to meet the WHO recommendations on laboratory. Thus, there is an urgent 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 level.[4]

One of the alternatives for TB diagnostic examination is by detecting TB antigen, for example the examination of lipoarabinomannan (LAM) as the main component of MTB cell walls and a product of bacterial degradation by infected macrophage.[5,6] The LAM molecule with mannosylated caps (ManLAM) is one type of LAM for pathogenic mycobacteria species such as *Mycobacterium tuberculosis*, *Mycobacterium leprae*, and *Mycobacterium bovis*. They play an important role for the survival of MTB in cells.[4] Point of care test (POCT) for TB that is recommended by the WHO for detecting lipoarabinomannan is the lateral flow urine lipoarabinomannan assay or known as LF-LAM. This test uses urine as sample which is not invasive and easy and not complicated to do and without the need for various reagents. This makes the assay affordable and only requires a short time (±25 minutes) while showing increased sensitivity in active TB patient with HIV infection.[5] Based on Singhroy's study in 2020, some countries with high tuberculosis and HIV/AIDS burden have adopted and uptaken the lateral flow urine LAM test for detecting TB. These include Central of African Republic, Malawi, Myanmar, Uganda, and Zimbabwe.[7]

HIV infection 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). This damage causes LAM that is bound to anti-LAM antibodies to 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 higher in active TB and PLHIV compared to TB patients without HIV infection. The aim of this study was to determine the positivity of LF-LAM examination results in active TB patients, with and without HIV infection.

Methods

This was a descriptive comparative observational study with cross-sectional design. The inclusion criteria were adult patients (≥18 years), both outpatients and intpatients, who have been diagnosed with active TB with or without HIV, by a clinician in Dr. Hasan Sadikin General Hospital Bandung, Indonesia, based on the International Standard of Tuberculosis Care (ISTC) and by using various modalities diagnostic tools of TB, without considering whether the patients had received TB and/or ARV therapy or not. 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 using midstream random urine as sample, collected from the patient and stored in a sterile urine pot without preservatives. The samples were then aliquoted into a micro-plastic tube and stored at -80 °C at the Clinical Pathology Lab of Dr. Hasan Sadikin General Hospital Bandung, Indonesia, until the required sample size was met. Before processing (Alere Determine TB Ag), the frozen urines were thawed in room temperature (20–25°C) for one hour and then sentrifugated at 10,000 g for 15 minutes. Clear urine supernatants were obtained for about 60uL and dropped on the sample pad of the POCT. Result of the test must be read in 25–35 minutes. Data of the

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

Variable	Group		p-Value
	HIV (+) n(%) n=25	HIV (-) 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

two groups were analyzed using the Statistical Package for the Social Sciences (SPSS) software version 25.0 for Windows. The normality test was performed using the Saphiro wilk test and the proportion difference test was 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

There were 52 subjects in this study, consisting of 25 (48%) subjects with active TB with HIV and 27 (52%) subjects TB patients without HIV. Gender of the subjects was dominated by male subjects (n=29, 56%). The mean age of the two groups, with and without HIV were 34.38±6.579 and 37.85±12.895, respectively.

The positive result of LF-LAM test was found in 11 (21%) subjects from all groups. In the

Table 2 Characteristics of Subject with Positive LF-LAM Results

Characteristic	Group	
	HIV(+) n(%) n=9	HIV(-) n(%) n=2
Gender		
Male	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)	-
Pulmonary TB + 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 (+)=under therapy, Therapy (-)=not under therapy

active TB group with HIV infection there were more positive LF-LAM results than the active TB group without HIV infection (n=9, 36%) as depicted in Table 1.

The proportion difference test between the two groups resulted in a statistically significant difference with p=0.012). The characteristics of subjects with a positive LF-LAM result are listed 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, male subjects dominated the subjects of this study (n=29, 56%). This is in line with the WHO annual report in 2019 which reported 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%), which supports the WHO statement, based on an analysis of 15 previous studies and concluded in a theory, that out of 1,000 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 was statistically significant (p=0.012), which is in accordance with the study by Suwanpilmolkul et al.[12] that examined LF-LAM examination results in the active TB group with and without HIV infection in Thailand, with the proportions of 37.2% and 7.4%, respectively.

In this study, 10 subjects (20%) had a positive LF-LAM examination result and one (2%) had an equivocal or indefinite LF-LAM result. The equivocal or indefinite result was from an active TB subject with HIV infection and the researcher included him into the group with positive LF-LAM results, bringing to a total positive LF-LAM examination results of 11 subjects (21%). Based on a study of Siddiqi et al.,[13] 3 (19%) on 16 active TB study subjects with HIV infection, the equivocal or indefinite LF-LAM results were followed by the MTB culture from cerebrospinal sample and at the same time showed a positive results for TB.

The World Health Organization stated that LF-LAM is better used in patients with active TB and HIV infection who show clinical symptoms of TB (pulmonary and/or extra pulmonary) with a CD4 count of <100 cells/uL or seriously ill with or without a known CD4 cell count.[5] In this study, three of nine subjects in the active TB group with positive LF-LAM test results were known to have a CD4 count of <100 cells/uL, i.e., 9 cells/uL, 12 cells /uL and 63 cells/uL, respectively.

Based on the theory, various mechanisms that can cause LAM to be detected in urine are originating from the MTB infecting the kidneys (renal TB). The 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 that can cross the damaged glomerular basement membrane. It is possible that an impaired immune response to form antibodies prevents the LAM molecules from binding to antibodies, but it is also possible that the failure in binding is caused by an imbalance in the amount of LAM and immunoglobulins in the body.[10,11] In HIV infection the number of the CD4+ T cells decrease and may lead to an increase in IL-7, which will disrupt the regulation of B cell maturity. The number of immature B cells will increase and the response of B cells to antigens will decrease. The HIV infects T helper and causes a decrease in IL-21 production, which will have an impact on B cell differentiation, decreasing B memory cell formation and antibody response.[14]

One of the subjects who showed a positive LF-LAM result had been diagnosed with systemic lupus erythematosus (SLE). People with SLE have a six-time higher risk of being infected with TB. Systemic lupus erythematosus is an immunocompromised condition caused by the abnormalities in the immune system; deficiency in immunoglobulin and complement; chemotaxis defects; phagocytosis; and abnormalities in the cellular immunity, which is further worsened by the high steroid therapy provided to the SLE patients, causing immunosuppression.[15]S study by Suwanpimolkul et al.[12] demonstrated that the LF-LAM examination can be used for an active TB population that is accompanied by severe immunocompromised conditions; yet, further research is still needed. In this study, twos (12.5%) of the 16 active TB subjects were immunocompromised without being infected by HIV.

Forty-one subjects were found to have a negative LF-LAM result. Based on the theory from Cox et al.[10], 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 it cannot pass through the normal glomerular basal membrane.

This 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 as data on CD4 were only available for 12 (48%) subejcts. Overall, the positivity rate of the LF-LAM examination results in this study was low (21%). However, the difference proportion test showed significantly different result between patients with active TB with and without HIV infection (p=0.012), with PLHIV who is experiencing active TB will give a higher positive result in LF-LAM.

To conclude, because of the low of positivity rate of LF-LAM urine assay, the test should be used more in HIV patients with clinical symptoms of TB (pulmonary and/or extra pulmonary), a CD4 count <100 cells/uL, or seriously ill with or without known CD4 cell count as an add-on or alternative test for diagnosing TB. This assay should also 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, Nursiloningrum 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 Reipold 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.