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Review Article

Urine-lipoarabinomannan-might fill the gap in delayed diagnosis of life-threatening tuberculosis

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ABSTRACT

Mycobacterium tuberculosis bacilli is a causative organism for tuberculosis disease. The lungs are the most commonly affected organ with this infection but it can infect any vital organ of the body. The person with signs and symptoms of tuberculosis disease was identified with detection of acid-fast bacilli in the biological specimen and with rapid molecular testing. However, the limiting factor for the early tuberculosis (Tb) diagnosis is the running cost of the diagnostic test. It is the common reason preventing the advancement of diagnostic laboratories in low and middle-income countries. The person and community both suffered from this diagnostic delay. This delay causes significant morbidity and mortality in the patient. An alternate test and an alternate sample that can be easily obtained would be beneficial to prevent these diagnostic delay issues. Lipoarabinomannan (LAM) antigen detection in the urine sample is one such promising diagnostic test. Mycobacterium tuberculosis's inner layer is made up of glycolipids. LAM is derived from phosphatidylinositol. LAM is a heat-stable amphiphilic cell wall component. It is the precursor of phosphatidyl-inositol-mannosidase and lipomannan. An extra mannose cap is a characteristic feature of a virulent strain of Mycobacterium. LAM has immunogenic and immunomodulatory properties. It is a promising diagnostic test because it is simple to do, the assay can be performed at the patient's bedside and takes a while to perform. This assay sensitivity varies from 56% to 85% and it has greater than 88% specificity. In HIV seropositive patients, use of LAM assay can reduce 8-week mortality. LAM detection is also a very good assay for the detection of tuberculosis in renal failure and disseminated tuberculosis patients.

Lipoarabinomannan detection in the urine is a possible test that can prevent delay in diagnosis. This is a promising test because it's easy to perform, the test can be done with a card test besides the patient's bed. It can be used in HIV seropositive patients and various other forms of extra pulmonary tuberculosis. It can be a very useful test for diagnosing a critically ill patient who is not able to produce a target sample.

1. Introduction

Tuberculosis is an important global health issue. Mycobacterium tuberculosis bacilli is a causative organism for tuberculosis. According to WHO 2020 report, an estimated 10 million people suffering from tuberculosis, 5.6 million were male, 3.3 million were female and 1.1 million patients were children. It is the leading cause of death in India. Approximately 15 lakh people died from tuberculosis including 2.14 lakh people also were suffering from HIV in 2020.[1,2] The lung is a commonly affected organ with this infection but it can affect any organ of the body This disease infection is spread by coughing, sneezing and spitting by infected patients. A person gets infected when

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they inhale contaminated air. Tuberculosis infection in a host can cause either active or latent tuberculosis. About 1/4th of the world's population and 1/3rd of the Indian population are infected with Mycobacterium tuberculosis. Infected people have a 5-10% lifetime risk of falling sick with tuberculosis.[3] People suffering from malnutrition, diabetes, immunocompromised state and tobacco chewers have a high risk to develop active tuberculosis disease.[4] This disease development risk rises up to 10% yearly when patients also suffer from the human immunodeficiency virus.[5]

The person with signs and symptoms of tuberculosis disease was identified with detection of acid-fast bacilli in the biological specimen and with rapid molecular testing. The World Health Organization endorsed X-pert test has a sensitivity of 98.2% in smear-positive TB and 72.5% in smear-negative TB cases.[6,7] However, the limiting factor for the early TB diagnosis is the running cost of test, cost of reagents and cost of cartridges are the possible factors hindering the advancement of laboratories of many resource-limited settings. Many countries still use the AFB smear test to diagnose TB. The conventional test has low sensitivity as compared to mycobacterial culture. Even patients with advanced HIV infection have a much higher false-negative rate.[8] The elimination of goals TB will not be possible without new tests and technologies. Many time patient is not able to provide the targeted sample for prompt diagnosis of tuberculosis leads to a delay in the initiation of anti-tuberculosis treatment. The person and community both suffered from this diagnostic delay. This delay causes significant morbidity and mortality in the patient.[8,9] An alternate test and an alternate sample that can be easily obtained would be beneficial to prevent these diagnostic delay issues.

Urine-lipoarabinomannan (LAM) detection is one such promising diagnostic modality. It is a promising diagnostic test because it is simple to do, this assay can be performed at the bedside and takes a while to give results.[10,11]

2. The cell wall structure of Mycobacterium Tuberculosis

Cell wall is made up of four layers of variable composition lipid, carbohydrate and protein. The plasma membrane is the inner layer made up of phosphatidylinositol derived glycolipids such as lipomannan, lipoarabinomannan and phosphatidyl-Myo-inositol mannosidase. The second layer is periplasm. Periplasm is made up of proteins like EmbC and PonA1. The third layer is the cell wall which is made up of peptidoglycans and arabinogalactan and the last layer is porin and mycolic acids like trehalose monomycolates made outer membrane. The outermost covering is a capsule, which is made up of glucan matrix and secretory proteins. LAM is heat-stable and amphiphilic in nature. It is the precursor of phosphatidyl-inositol-mannosidase and lipomannan. LAM made approximately 15% weight of mycobacterium. LAM core is made up of branched arabinan polymer and chains. Mannose capped arabino mannan present in the surrounding capsule of mycobacterium tuberculosis.[8] Virulent, avirulent and non-tubercular mycobacterium strains have different LAM structures. The Virulency of LAM is due to mannose cap which is known as manLAM. So manLAM is a glycoprotein obtained from virulent strains of mycobacterium tuberculosis.

3. Role of LAM in Active and Passive Immunity

LAM is an important virulent factor that has immunogenic and immunomodulatory properties. LAM is identified by antigen-presenting cells receptors of both innate and adaptive immune cells. Mannose cap attenuates host immune response by inhibiting the production of proinflammatory cytokine production, phagolysosome biogenesis inhibition, apoptosis inhibition of host cell and autophagy.[12–14] LAM have a profound effect on both innate and adaptive immunity. Mannose capping also mediates the binding of Mycobacterium tuberculosis bacteria with the host cell and subsequent entry into macrophages. LAM is an independent major histocompatibility complex T-cell epitome.[15] LAM and its variants are recognized by CD1b restricted T-cell. It also activates these cells for the generation of immunity. CD1b cell induces B-cell and production of antibodies against LAM antigen.[12,16,17] LAM is released in circulation by live bacilli and excreted by the kidney. Hence, urine might be used to detect TB.

3.1. Anti-LAM antibodies

Anti-LAM antibodies were found in 18.5% of tuberculosis patients. The positivity of Anti-LAM antibodies is different in new and relapse Mycobacterium tuberculosis patients. Anti-LAM antibodies also can induce by BCG vaccination and Mycobacterium tuberculosis and non-mycobacterium. So immune response against LAM shows tuberculosis protection. Mannose capped arabinose-mannan moiety of LAM is a future candidate against mycobacterium tuberculosis as the vaccine.[14,16]

3.2. Development in LAM assay test

First-time ELISA method used for detection of urine LAM. In the past, no point of care test for diagnosis was available worldwide and the reason behind this is cross-reactivity with non-tuberculous mycobacteria and the use of tests based on antibody detection tests based on an animal model. First Sandwich ELISA was 'Clearview TB ELISA' also known as alereLAM. The field evaluations of alereLAM are promising. The smear-positive pulmonary tuberculosis sensitivity of this test is 7-80% and specificity of this test is 86-99% for. The sensitivity of the Clearview improve and

Table 1:

S.No.	Test name (antigen/antibody)	Author/year	Patient Profile HIV serology	Sensitivity of test	Specificity of test	Result
	FujiLAM		Negative	53.2%	98.9%	Detect 5 time more patient AlereLAM, high positive predictive value
1	AlereLam	Broger et al, 2020	Negative	10.8%	92.3%	
	EclLAM		Negative	66.7%	98.1%	
2	EclLAM	Broger T et al,2019	Negative	Urine-93% Serum-55%	>97%	LAM assay can be detected in both Urine and Serum
3	Clearview TB-ELISA	Hanifa Y et al, 2015	Positive and negative	7.1 vs 0%	98.5% vs 99.8%	-
4	Clearview TB-ELISA	Shah M et al,2009	Positive/negative	42% overall 56% in sputum negative	-	-
5	Clearview TB-ELISA(concentrated)	Savolainen L et al, 2013	Negative	57%	89%	100 fold Concentration of urine increase sensitivity but reduce specificity
6	Clearview TB-ELISA	Dheeda K et al,2010	Positive /negative	21% in positive 6% in negative		
7	Clearview TB-ELISA	Talbot E et al, 2012	Positive	65%	86%	Urine LAM has higher sensitivity than sputum smear
8	TB-ELISA	Boehme C et al, 2005	-	80.3%	99%	-
9	IPCR-ELISA	Mehta p et al, 2017	negative	74%	91.5%	-
10	Co-agglutination technique	E Sada et al, 1992	-	88% in sputum positive 67% in sputum negative		Its accurate test for diagnosis of tuberculosis
11	AlereLAM	Peter JG,2012	Positive	66%(grade 1 cutoff)	96%(grade 1 cutoff)	
12	ELISA-LAM	Gounder CR, 2011	Positive	32%	98%	Inadequate to replace culture
13	LAM ELISA	Reither K et al, 2009	Mixed	50.7 overall 60.2%positive	87.8%	-
14	LAM-ELISA	Tessema T et al, 2001	negative	74%	86.9%	Urine LAM assay in TB patients may improve case finding
15	ELISA-LAM	Mutetwa R et al, 2009	Mixed	44% Overall 52% positive	89%	Urine LAM has greater sensitivity for detection for HIV-related TB
16	ELISA-LAM concentrated	Lawn SD, 2009	positive	67%, 53% and 21%, CD4 cell counts of less than 50, 50-100 and more than 150 cells/ml, respectively.	-	LAM assay has substantially superior sensitivity to sputum microscopy

specificity reduces by using 100 times concentrated urine. (19) Point-of-care AlereLAM assay's advantage is its cost, which is approximately $1/4^{th}$ of the available nucleic acid amplification tests (NAAT). [18–25]

FujiLAM test has better sensitivity as compared to AlereLAM. Studies suggest that FujiLAM is a cheap, strong potential and promising point of care test that can make bedside definitive diagnosis of tuberculosis possible. At present, FujiLAM has been recommended to be used in malnourished or HIV seropositive children. Recent studies proved that the application of X-pert and LAM test together for hospitalized HIV people is an economical strategy as compared with serial testing and CD4-count dependant testing. Besides, the use of Xpert plus LAM test is attributed to a better chance of survival and it is possible because a combination test strategy obtains a rapid and reliable TB diagnosis. [2]

Another comparative study of FujiLAM, AlereLAM and eclLAM shows that the sensitivity of these tests is 53.2%, 10.8% and 66.7% respectively and specificity in HIV negative patients is also very high. (Table 1) So it shows potential to be used for the diagnosis of tuberculosis. [26,27]

Many other technologies emerged for the detection of LAM antigen as a point-of-care test. Photonic biosensors are one of them. Ultra-low concentrations of LAM can be detected by a plasmonic fibre optic absorbance biosensor. [2]

Using Nanocage technology detection of MoAb1 antibody testing is another technique that might be used in the paediatric population for the detection of pulmonary and extra pulmonary tuberculosis. This technique identifies different LAM epitopes in the urine sample. [2]

Advance technology is necessary to increase the antibodies detection. Compared to conventional ELISA, the test sensitivity can improve by 50 to 100-folds using anti-LAM-magnetic nanoparticle-conjugates immunoassay that concentrates the urine antigen. In the same manner, the use of nanotechnology with copper complex dye within a hydrogel nanocage in pre-treatment urine specimen raises the sensitivity of detection of LAM 100–1,000-times. [2]

The gas chromatography/mass spectrometer method and electrochemiluminescence platform are other alternative techniques for LAM assay. This method has comparable sensitivity and specificity as compared to the classic ELISA. [2] The immuno-polymerase chain reaction (I-PCR) is another advanced technique for the detection of ultralow urinary LAM. It has the simplicity and versatility of enzyme-linked immunosorbent assay and the exponential amplification capacity and sensitivity of PCR. Hence, this test has many-fold higher sensitivity than ELISA. This technique can detect very low LAM levels. This test has a sensitivity of 74% and a specificity of 91.5%. [28]

The sensitivity and specificity of the LAM test depend upon the concentration of LAM antigen in Urine. The available LAM antigen kit has maximum sensitivity from 0.05-10 μg and the specificity of the test varies at different LAM concentrations. A large amount of LAM reduces the sensitivity of the test. It can justify by the "shielding effect". This is because of biasing created by epitopes, antibodies against LAM and the presence of various other biomolecules.

3.3. LAM assay and HIV

Especially in advanced HIV infection patients with disseminated TB and for those patients who have CD4 cell count of fewer than 50 cells/mm^3. The sensitivity varies from 56-85%. In HIV Seropositive patients, application of LAM assay reduces 8-week mortality. [9]

3.4. LAM assay and renal failure

Hemodialysis patients have a risk to get tuberculosis. Identification and prompt initiation. Renal failure patients release a high amount of LAM antigen in urine. Hence, the LAM assay might improve the identification of TB in renal failure. [29]

3.5. LAM assay and tubercular pleural effusion

Tubercular pleural effusion is another form of extrapulmonary tuberculosis. Diagnosis is tubercular pleural effusion becomes easy with the advent of adenosine deaminase assay. Urine LAM assay in tubercular pleural effusion did not show a very good result.

3.6. LAM assay and tubercular meningitis

CNS tuberculosis is also a very serious form of extrapulmonary tuberculosis. CSF-LAM also show good sensitivity.

LAM is not only specific for mycobacterium tuberculosis, it can come positive in mycobacterium leprae and non-mycobacterium tuberculosis bacilli also.

Point of Care LAM assay is still waiting for implementation because of several difficulties related to cost, the sensitivity of the assay, various methodology for detection and unclear research results. A review of all these researches shows that urine LAM antigen detection has possibilities for advancement and opportunities to improve the sensitivity and specificity of the assay still remain.

4. Conclusions

The Lipoarabinomannan levels in the urine can prevent a delay in diagnosis. The assay is a promising method because it's easy to perform, the test can be done with a card test beside the patient's bed. It can be used in HIV seropositive patients and various other forms of extra pulmonary tuberculosis. It can be a very useful test for diagnosing a critically ill patient who is not able to produce a target sample for mycobacterial or molecular testing.

LAM is not only specific for mycobacterium tuberculosis, it can come positive in mycobacterium leprae and non-mycobacterium tuberculosis bacilli also. Hence, Urine-Lipoarabinomannan might fill the gap in the delayed diagnosis of Life-threatening Tuberculosis.

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6. Conflict of Interest

None.

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