ORIGINAL RESEARCH PAPER

Volume-7 | Issue-7 | July-2018 | PRINT ISSN No 2277 - 8179

INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

URINARY LIPOARABINOMANNAN (LAM) IN THE DIAGNOSIS OF TUBERCULOSIS

Biochemistry

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ABSTRACT

Tuberculosis (TB) is an infectious disease caused by the Mycobacterium tuberculosis. It generally affects the lungs and other parts of the body. A diagnosis of TB is made by identifying M. TB in clinical samples (e.g., sputum, blood, pus, a tissue biopsy). However, the gold standard culture process for this slow growing organism can take 2-6 weeks for result. The detection of lipoarabinomannan (LAM), a 17.5-kDa glycolipid component of the mycobacterial cell wall, can be better approach to diagnose active TB. LAM is released from metabolically active & degrading mycobacterial cell wall and is detectable in urine. Detection of mycobacterial antigens in urine has several potential advantages compared with all currently used diagnostic method. The main aim of present study is to diagnose TB by detecting urinary LAM.

In this study we have taken microbiologically confirmed TB patients and healthy individual as control. 5 ml random urine sample was collected from Pulmonary TB patients, Extrapulmonary TB patient and normal healthy individual in sterile containers and quantitative estimation was done by ELISA. Urinary LAM of pulmonary TB (322.24 ± 195.73) pg/ml is high as compare to the Extrapulmonary TB (245.95 ± 217.92) pg/ml. LAM value of Pulmonary TB and Extarpulmonary TB as compared with healthy individual (control) (41.06 ± 9.77) shows statistically highly significant ($p<0.0001$). Urinary LAM can be used as a marker for the diagnosis of tuberculosis.

KEYWORDS

INTRODUCTION

Tuberculosis is one of the ninth leading cause of death among all the diseases. In 2016 it is estimated that 1.3 million TB death among HIV negative people (down from 1.7 million in 2000) and 3.74 million among HIV positive people. It is overall estimated that 10.4 million people were affected in 2016: among them 90% were adult, 65% were male, 10% people with HIV (74% in Africa) and 56% from five countries: India, Indonesia, China, Philippines and Pakistan [1].

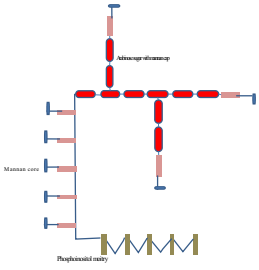
Tuberculosis is known to be a one of the serious global burden which can easily spread. Currently used diagnostic technique for TB are sputum microscopy and culture, TB skin testing (TST) and radiology are used since 1882. Sputum smear microscopy generally shows the high specificity in high TB prevalence setting and the technic has been the mainstay of TB control programme as its can identify the most chronic or active cases and is relatively low expensive and widely available[2]. However, to diagnose pulmonary TB the sensitivity shown by sputum smear methods ranges from 40% to 60%. The main problem is that the sputum smear microscopy cannot be used for the person who are unable to produce sputum like children and the person with extra pulmonary tuberculosis [3]. Cultures of sputum have low sensitivity and takes 6-8 weeks for these slow growing bacteria [4]. Other diagnostic tools like Polymerase Chain Reaction (PCR), Nucleic acid amplification, Interferon gamma and Lysozyme, are either time consuming, shows lack sensitivity or required technology is very expensive[5].

Due to delay in the diagnosis facility may lead to diseases transmission and chronic disability [6]. However, an accurate point-of-care test that could be used within peripheral clinical settings with limited laboratory facilities has not yet been successfully developed [7].

The detection of lipoarabinomannan (LAM) is an attractive approach to diagnosing active TB. LAM is a 17.5-kDa glycolipid component which is present in the mycobacterial cell wall [8]. Quantity of LAM in urine can reflect bacterial burden.

Lipoarabinomannan (LAM) is a structurally important heat-stable compound can account for up to 15% of the total bacterial weight and serves as an immunogenic virulence factor that is released from metabolically active or degrading bacterial cells during TB infection [9, 10]. Structure of LAM shows mannan polysaccharide backbone with branched oligoarabinosyl containing saccharide side chains; the former is covalently linked to a phosphatidyl inositol lipid moiety [11]. Variable capping of the arabinosyl side-chains with mannose residues results in a diversity of LAM molecules shows unique properties and functions. The presence of mannose capping (fig-1) allows mycobacteria to bind to mannose receptors on macrophages, which offer the preferred intracellular environment for the organism [12].

Fig 1: cartoon showing the structure of LAM



Tuberculosis is an infectious disease which is characterized by fever, weight loss, raised levels of acute phase reactants, and necrosis in lesions and skin-test sites. Activated macrophages are the likely source of TNF in tuberculosis patients. M. tuberculosis can trigger the release of TNF from human monocytes, particularly if they are first activated by exposure to interferon-y or 1,25-(OH)2-vitamin D3, lipoarabinomannan (LAM), a major component of mycobacterial cell walls has this property, both in vivo and in vitro, and is comparable to endotoxin in this respect [13].

Material and Method

The study is approved by Institutional Ethical Committee (ICE) MGM Medical College, Navi Mumbai. Patient in this study are included by giving their written and verbal consent.

Study Design:

The subjects for the present study are selected from Respiratory Department, General Medicine, MGM Hospital, Kamothe, Navi Mumbai diagnosed with Tuberculosis.

Group1: 30 patients with pulmonary tuberculosis.
Group2: 30 patients with extra pulmonary tuberculosis.
Group3: 30 normal healthy individual.

The aforesaid subjects of both genders (male and female) aged 22 to 55 years are enrolled from February 2017 to January 2018.

The patient pulmonary TB enrolled in our study are diagnosed and conformed by Microbiology Department, MGM Medical College by following test:

- Sputum smear microscopy
- Sputum culture
- by X-Ray

The patients with extrapulmonary TB are further confirmed by ultra-sound, and by X-RAY Radiology Dept. body fluid culture by microbiology Department and tissue biopsy histopathology, MGM Medical College, Navi Mumbai.

Sample collection:

- Urine samples are collected from pulmonary tuberculosis patients, extrapulmonary tuberculosis patient and normal healthy individual.
- 5 ml random urine samples are collected in sterile container from all subjects. Sterile plastic container with preservative.
- Samples were stored at -80°C upto 6 months.
- The sandwich ELISA kit is used for the accurate quantitative detection of Human Lipoarabinomannan (LAM) in urine sample.

Assay Principle

Quantitative estimation of LAM is done by the ELISA (Bioassay Technology Laboratory) according to the procedure. LAM is added to the wells pre-coated with LAM monoclonal antibody. After incubation a biotin-conjugated anti-Human LAM antibody is added and binds to Human LAM followed by the incubation, unbound biotin-conjugated anti-Human LAM antibody is washed away during a washing step. Streptavidin-HRP is added and binds to the biotin-conjugated anti-Human LAM antibody. After incubation unbound Streptavidin-HRP is washed away during a washing step. Substrate solution is then added and color develops in proportion to the amount of Human LAM. The reaction is terminated by addition of acidic stop solution and absorbance is measured at 450 nm. The data obtained was statistically correlated.

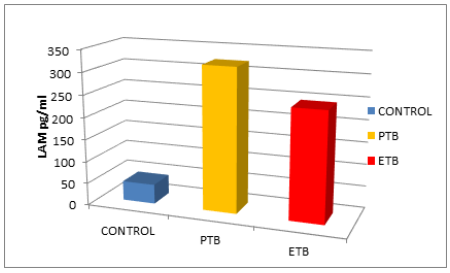
RESULT

Total 90 subjects are enrolled in present study out of this 60 participants were confirmed TB suspects. Mycobacterial isolates were recovered from the culture of respiratory or non-respiratory specimen and acid fast bacilli (AFB) smear, however caseating granuloma of TB could be detected through histopathologic examination of tissue biopsy. Extra pulmonary TB was diagnosed from pleural culture, isolated from lymph node, intervertebral lumber discs, ascetic fluid culture and CSF culture. The subjects for the present study are selected from Respiratory Department, General Medicine, MGM Hospital, NAVI-Mumbai diagnosed with Tuberculosis.

Table 1: Value distribution of Age, LAM, Tuberculin skin test, Smear positive, TB culture positive

Groups	Control (n=30)	Pulmonary TB (n=30)	Extrapulmonary TB (n=30)
Age in Years (Mean ± SD)	41.06 ± 9.77	39.6 ± 9.29	40.43 ± 9.47
Urinary LAM Pg/ml (Mean ± SD)	44.07 ± 20.75	322.24 ± 195.73	245.95 ± 217.92
Tuberculin skin test positive (%)	0 (0%)	24 (80%)	4 (14%)
Smear positive (%)	0 (0%)	11 (36%)	0 (0%)
TB culture positive (%)	0 (0%)	30 (100%)	30 (100%)
Smoking (%)	8 (26%)	11(36%)	5(16%)

Graph 1: Urinary LAM in Control, Pulmonary TB and Extrapulmonary TB



DISCUSSION

It is estimated that one-third of the world's population is infected with *M. tuberculosis* with approximately 8 million new cases of active TB and 3 million deaths per year. Such extensive infection of the human population requires a diagnostic test that is capable of discerning an active from as well as latent infection.

Total 90 subjects are included in present study, they are comprised of 30 normal healthy control, 30 pulmonary TB, and 30 Extrapulmonary TB suspects. The aim of present study is to assess the diagnostic accuracy of urinary LAM among tuberculosis infected patients either pulmonary or extrapulmonary.

In view of role of urinary LAM in TB diagnosis this study is planned to investigate the diagnostic value of urinary LAM in Indian community. In this study we try to find out the sensitivity of urinary LAM in three groups Healthy individual, Pulmonary TB, Extrapulmonary TB subjects.

Our study reveals that patient with pulmonary TB has higher urinary LAM level (322.24 ± 195.73) pg/ml than Extrapulmonary TB (245.95 ± 217.92) pg/ml (table-1), however this change is not statistically significant ($P \geq 0.05$). The cut off range of this study is 490 pg/ml.

Our study shows the overall sensitivity in microbiologically confirmed pulmonary TB patient (n=30); LAM sensitivity observed is 36%, and specificity 70%. In microbiologically conformed extra pulmonary TB (n=30) patient observed sensitivity of LAM is 26% and specificity 73% .We observe that urinary LAM positivity is more in pulmonary TB patient than Extrapulmonary TB patient.

We correlated the levels of LAM with various authors with our result; Mohammed A. Agha, Rana H. EL-Helbawy et all (2013) studied the quantitative analysis of urine lipoarabinomannan in the diagnosis of tuberculosis, shows that the urinary lam level in pulmonary TB (0.58 ± 0.53 ng/ml) is higher than extrapulmonary TB (0.17 ± 0.11 ng/ml) [14].

Agustin Iskander, Erlin Nursiloningrum, et all studied the diagnostic value of urine Lipoarabinomannan (LAM) Antigen in childhood Tuberculosis, shows that value of LAM in TB patient (1.80 ± 1.02 mg/l) seems to be more than non TB group (0.46 ± 0.3 mg/l) with sensitivity of 33% and specificity of 60%[15].

Boehme and colleagues used a prior version of the existing urinary LAM essay to evaluate 231 TB suspects (69% HIV-positive) and 103 healthy controls, sensitivity was 80.3 % 2005. Most of the studies on the urine LAM test have focused on patient co-infected with TB and HIV[16].

Gompol, kamon, et al. 2017 shows the sensitivity of urinary LAM is 40% in HIV positive patient and 20% in HIV negative patient which are somehow nearby with our study with HIV negative patient with TB and other studies reported previously (Nakiyingi et al, 2014; Fielding et al, 2015; Lawn et al, 2013) conclude that urinary LAM shows high sensitivity in TB patient with HIV [17].

Our study shows low sensitivity i.e. 36% (pulmonary) and 26% (extrapulmonary). We compare our findings with other studies, we conclude that the value of LAM shows low sensitivity with TB patient without HIV but on comparing with TB patient with HIV; the sensitivity is increased by 83% like as Boehme and colleagues (2010).

There are different reasons which explain the higher sensitivity of LAM assay in immunocompromised patients. One theory cites the correlation of higher sensitivity with greater bacillary burden and assumes relatively greater multiplication of M. tuberculosis bacilli in

patient with impaired immune function. Alternatively, due to the general lack of cavity formation in immunosuppressed patients, the bacteria are forced to replicate in tissue that would facilitate the diffusion of shed LAM in the circulation. Second explanation is that a larger degree of antigen antibody complex formation in TB patient without immune suppression interferes with LAM excretion in the urine[18].

LAM is degradable product of Mycobacterium which is excreted through urine. The amount of urinary LAM generally reflects the bacterial burden. Various study have shown that person with TB with HIV shows the high quantity of urinary LAM then the HIV negative with TB, this means that the person with immunosuppressed generally have high quantity of LAM.

The benefits of using a noninvasive, easily collected specimen such as urine would be greatly appreciated in the diagnosis of pediatric TB. Considering the inadequate diagnostic yield of sputum based diagnostic in young children, further evaluation of urinary LAM assay in pediatric population is warranted.

CONCLUSION

We conclude that the diagnostic value of urinary LAM in Pulmonary and Extrapulmonary TB shows the low sensitivity 36% and 26 %. But it was statistically high significant on comparing with control group. It can be considered as point of care diagnostic test for TB. Quantitative LAM increases progressively with bacillary burden. Usage of quantitative urine LAM test result may thus offer additional clinical insight into the degree severity of TB diseases that cannot be observe from qualitative results alone.

The combination of urinary LAM and sputum smear microscopy may have an additional benefit in diagnosis of TB, while the urine LAM test is unlikely to stand alone for definitive TB diagnostic testing. Qualitative and quantitative estimations of urine LAM may have future utility as biomarkers, reflecting response to TB treatment.

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