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Role of Molecular Diagnostics in The Early Diagnosis of Tuberculous Meningitis

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ABSTRACT

Although tuberculosis (TB), caused by the bacteria *Mycobacterium tuberculosis*, affects the lungs in the majority of cases, it can also affect other parts of the body. It is most common in young children and HIV-infected patients, but it can also be found in adults with the condition. The most severe form of tuberculosis, tuberculous meningitis (TBM), is associated with the highest mortality and morbidity rates when compared to other forms of tuberculosis. Despite anti-tuberculosis treatment, tuberculosis-related mortality (TBM) remains a leading cause of death. TBM is difficult to diagnose early using immune-based and molecular-based methods because of the non-specific symptoms that occur in the disease and the low number of bacteria present in the target area, particularly the cerebrospinal fluid (CSF). TBM patients have a high sensitivity and specificity for tuberculosis, but there is currently no established diagnostic method that can detect the infection with high accuracy in a timely manner. In addition, the emergence of drug-resistant Mycobacterium tuberculosis strains complicates the diagnosis and treatment regimen for tuberculous bronchitis. It is the purpose of this review to summarise the difficulties associated with the currently employed molecular-based diagnostic methods as well as the potential future application of molecular diagnostic methods for TBM.

INTRODUCTION

Tuberculous meningitis is an extrapulmonary infection caused by the bacilli *Mycobacterium tuberculosis.* The infection is confined to the meninges. Once the bacteria infect the meninges, there is an increase in the pressure around the brain which will cause serious damage to the brain [1]. If TBM is not diagnosed rapidly, this leads to increased mortality and morbidity. MTB causes about 10.4 million new cases of tuberculosis and 1.5 million deaths per year, with an additional 0.4 million deaths in people who are already infected with human immunodeficiency. In comparison to non-infected people, patients who are HIV-positive have a 20-fold increased chance of contracting tuberculosis [1]. Due to the above severity, early diagnosis of TBM is necessary. In fact, a diagnostic technique with high sensitivity, specificity and a good positive predictive value is required. Statistical methods such as positive predictive value (PPV), negative predictive value (NPV) and Receiver operator characteristics curve can be used to understand the sensitivity and specificity of an ideal assay. TBM was almost fatal to all patients

universally before the introduction of chemotherapy [2,3] but TBM will be still fatal if the clinician does not provide a rapid and true positive diagnosis.

Even with modern culture techniques and more improved Ziehl-Neelsen technique and fluorescent detection methods, rapid diagnosis is still not possible because each technique mentioned above has its drawbacks. Firstly, 50% of the culture plates develop in 14 days and some plates completely develop in 28 days [11]. Secondly, Ziehl-Neelsen staining can miss 48% of the samples and has a chance of giving a false negative report [8] [9]. As the progression of TBM is fast, rapid diagnostics procedures are essential so the clinician can make quick decisions. Molecular diagnostic assays like PCR (Polymerase Chain Reaction), LCR (Ligand Chain Reaction), and next-generation nucleic acid analysis techniques (NAAT) like GeneXpert MTB/RIF. The Xpert MTB/RIF assay is a new test that is helping to revolutionize tuberculosis (TB) control by allowing for rapid diagnosis of TB disease and drug resistance [7]. In trials to understand how fast and specific these molecular diagnostic assays give [7], the test simultaneously detects Mycobacterium tuberculosis complex (MTBC) and resistance to rifampin (RIF) in less than 2 hours.

Other NAAT such as MDR/MTB ELITe MGB® Kit (ELITechGroup, Italy) which is a new multiplex, ultra-sensitive, real-time PCR assay (limit of detection 6 CFU/mL) used for the detection of MTBc DNA. This review article will be completely focused on how molecular diagnostic assays can be used for rapid diagnosis of the deadly tuberculous meningitis. The World Health Organization (WHO) declared Xpert as the preferred initial test to investigate TBM [4][5] The GeneXpert assay yields results very quickly, and minimum technical training is required. Moreover, this assay can identify multidrug-resistant TB. In recent years, Metagenomic Next-Generation Sequencing (mNGS), a sensitive technology capable of detecting pathological organisms from specimens such as cavity effusion, CSF, sputum, urine, and blood, has emerged as a sensitive technology.

METHODS

We searched Pubmed and Cochrane library from 01/01/2015 to 01/01/2021 using keywords Tuberculous meningitis, extrapulmonary TB, Xpert MTB/RIF and molecular diagnostics and then we sorted all available original articles on tubercul Data and key points addressing our topics were chosen and reviewed.

RESULTS AND DISCUSSION

Diagnosis of TBM

While TBM is a paucibacillary disease (containing fewer bacilli), CSF microscopy with the Ziehl Neelsen stain can easily validate the diagnosis by demonstrating acid-fast bacilli (AFB) has a low sensitivity (0-20 [1]. MTB culture results are extremely late to come by, with traditional solid media (Lowenstein-Jensen media) yielding results only after 10-35 days.[1] But WHO has recommended the culture method as the gold standard for the diagnosis [22].

Cerebrospinal fluid characteristics such as cell count, protein and glucose level are usually used for diagnosis wherein the mean CSF cell count in the TBM population is 303.21 cells/mm^3, with mean CSF protein and glucose of 170.2 mg/dL and 38.3 mg/ With only CSF characteristics, a clinician can not decide.

Hence, more specific molecular diagnostics are necessary. The MPB64 gene and insertion sequence IS6100 of the *M. tuberculosis* is thought to be the most specific sequence for PCR assays in M. tuberculosis detection, and it has been used in several studies as a target sequence [12,15]. The sensitivity of multiplex PCR (PCR in which there is more than one primer) using both the insertion sequence IS6110 and the gene MPB64 is higher than that of IS6110 alone. In this research, the sensitivity for IS6110 primer was (75.4%) and MPB64 primer was (62.3 %) [15]. While there are studies conducted using ligase chain reaction assay but did not show convincing results [17,18].

In a study conducted by Janneke A. Cox and his colleagues, CSF from 91 patients was checked for definite histopathological TBM, LAM LFA had a sensitivity of 75%, ELISA had a sensitivity of 43%, and Xper Xpert ultra has shown a sensitivity of 72.05% and a specificity of 100% in a study consisting of 244 CSF samples [6]. Based on pooled data from 13 studies, including 709 CSF samples, Xpert MTB/RIF in CSF had a sensitivity of 80% and a specificity of 99%; a minority of these samples were from HIV-infected patients [13]. The above studies were conducted using uncentrifuged CSF samples [6],13]. But the identification of *Mycobacterium tuberculosis* was substantially improved by centrifugation of CSF before Xpert automated testing [19]. The sensitivity of Xpert on non-centrifuged CSF was 28% (5/18)[19]. The efficiency of Xpert was improved by centrifugation of the CSF to 72 % sensitivity and 95% NPV0020 [19].

Multi-targeted loop-mediated isothermal amplification (LAMP) LAMP has also been used in CSF samples. For confirmed (50 culture-positive) TBM cases, this test demonstrated a sensitivity and specificity of 96 and 100%, respectively. The sensitivity of probable TBM was 82%. The overall sensitivity was 88%, and the specificity was 100%. It has been used for the diagnosis of pulmonary TB but its diagnostic role in extrapulmonary TB is not clear.

Line probe tests (INNO-LiPA) (RIF & MYCOBATCERIA v2) are strip-based technologies used to detect MTB complex, RIF, and INH resistance. INNO-LiPA RIF TB commercial line probe test system (Innogenetics, Ghent, Belgium) The genotype MTBDR (Hain Life Sciences, Gmbh, Nehren, Germany) Other examinations are not recommended for commercial examinations [23]. TrueNAT had a positivity percentage of 48.5%, whilst Xpert had a positivity rate of 37.0%. In comparison to culture, the sensitivities of Truenat and Xpert were 88.3 and 79.7 per cent, respectively [24]. For countries with a high burden of TBM, biosensors with DNA hybridization reaction can provide a point of care testing that is rapid, economical, and handy.

Some studies have shown a prominent increase in sensitivity and specificity when biosensors are used [20]. The DNA nano biosensor shows good stability, high specificity, and provides new alternatives for clinical MTB diagnostics [21]. Even though some studies have reported that DNA biosensors can detect the presence of TB in low concentrations of DNA, very little evidence was provided differentiating latent TB, active TB, multidrug-resistance TB (MDR-TB) and extremely drug-resistance.

TB diagnosis has always been difficult for health care providers and clinicians. Although anti-TB therapy has been available for more than 60 years, it continues to be a leading cause of unacceptably high mortality rates.

Table 1. Summary of TBM detection methods.

Methods	Objectives	Results
Microbiological method, Zn staining	To evaluate the microbiological diagnosis of tuberculous (TB) meningitis in a referral hospital in Indonesia.	ZN-microscopy lacks sensitivity for diagnosis of TB meningitis. 12.2% (86/703) for microscopy, 42% (73/174) for Xpert MTB/RIF, 46.0% (163/354) for solid culture, 48.8% (332/680) for liquid culture, and 64.0% (212/331) for in-
The Polymerase Chain Reaction	PCR technique, using primers directed against the insertion sequence IS6110 and MPB64 gene for the detection of Mycobacterium tuberculosis in Cerebrospinal fluid (CSF), for rapid diagnosis of TBM patients.	The Multiplex PCR system using primers targeting IS6110 and MPB64, for the detection of M. tuberculosis. DNA in CSF samples has higher sensitivity than any one of them alone and could be used for the early detection of TBM in CSF
Ligase Chain Reaction	Rapid detection of TBM in suspected patients using ligase chain reaction by using extrapulmonary samples (CSF)	The LCx assay is more sensitive than both direct smear and culture. Traditional diagnosis techniques such as acid-fast staining and culture should be retained. The sensitivity, specificity, positive predictive value and negative predictive value of the LCx assay were 55.5, 100, 100 and 92.9%, respectively.
Multitargeted LAMP	Evaluating LAMP assay using insertion sequence (IS) 6110 and MPB64 targets for the Mycobacterium tuberculosis complex (MTC) for the rapid diagnosis of TBM	Multitargeted LAMP had a sensitivity and specific The sensitivity of IS*6110* polymerase chain reaction (PCR), IS*6110* LAMP and MPB64 LAMP for probable cases was respectively 70 (70%), 78 (78%) and 82 (82%)
Xpert RIF/MTB	The Xpert MTB/RIF assay is a rapid and simple test, which has been endorsed by the World Health Organization as an initial diagnostic test for the diagnosis of TBM. However, evidence still lacks for its performance in cerebrospinal fluid (CSF) for the diagnosis of TBM, especially from India.	The sensitivity and specificity of the Xpert MTB/RIF assay in comparison to MGIT-960 was 55.1% (95%, CI: 40.2–69.3) and 94.8% (95%, CI: 90.9–97.4) respectively. Overall, the Xpert MTB/RIF assay detected 38 (14.2%) as positive for MTB of which 4 (10.5%), 31 (81.6%) and 3 (7.9%) were found to be rifampicin-resistant, sensitive and indeterminate respectively.
Point of care testing, Biosensors	Use of a non-PCR technique for rapid diagnosis of TBM	Nano biosensors with DNA probe hybridization have shown a prominent increase in diagnosis and are rapid and consume less time

Extrapulmonary tuberculosis often has life-threatening effects as a result of delayed diagnosis and ineffective treatment. To address this problem, prompt diagnosis and appropriate care are critical. GeneXpert, which has been endorsed by the WHO, has emerged as a game-changing tool for diagnosing EPTB, but there is not much research on its diagnostic validity in effusions. With the available literature of the last 5 years, we have tried to assess the efficiency of the present molecular techniques and addressing the drawbacks of other diagnostic tools, we have understood that the Xpert MTB/RIF assay is a very efficient assay for rapid diagnosis of TBM. A summary is given in **Table 1**.

Even though Xpert has very good specificity, each of its sample runs is costly. So, the Xpert assay should be innovated so that it becomes affordable for large scale sample testing. It is still difficult to diagnose tuberculous meningitis (TBM) in people who have been infected with the human immunodeficiency virus (HIV). Moreover, TrueNAT has more positivity and also has more sensitivity than Xpert [24]. Other molecular diagnostic tools are needed, despite the current scale-up of the Xpert® MTB/RIF assay, particularly in low-and middle-income countries where Xpert testing is not available [16]. LAMP has been used for the diagnosis of pulmonary TB but its diagnostic role in extrapulmonary TB is not clear. More study has to be done on LAMP [22].

Although metagenomic Next-Generation Sequencing (mNGS) has been used to detect pathogens in infectious disorders, its utility in the quick diagnosis of tuberculous meningitis (TBM) has not been established based on large samples [25]. In that case, with some disadvantages of NAAT, which is considered a good diagnostic method, biosensors come into the picture which has shown good sensitivity, but they have some drawbacks to be answered, such as differentiating between TB and multidrug-resistant TB.

The MDR/MTB ELITe MGB® Kit, in conjunction with the ELITe InGenius®, is used to diagnose MTB complex as well as Rifampicin and Isoniazid resistance in both pulmonary and extra-pulmonary samples. This automated technology streamlines laboratory workflow has a quick turnaround time (less than 3 hours), and aids in the selection of suitable therapeutic therapy and patient care. This has a great advantage over others [26].

In conclusion, the most severe form of tuberculosis, tuberculous meningitis (TBM), is associated with the highest mortality and morbidity rates when compared to other forms of tuberculosis. Despite anti-tuberculosis treatment, tuberculosis-related mortality (TBM) remains a leading cause of death. TBM is difficult to diagnose early using immune-based and molecular-based methods because of the non-specific symptoms that occur in the disease and the low number of bacteria present in the target area, particularly the cerebrospinal fluid (CSF). TBM patients have a high sensitivity and specificity for tuberculosis, but there is currently no established diagnostic method that can detect the infection with high accuracy promptly. In addition, the emergence of drug-resistant Mycobacterium tuberculosis strains complicates the diagnosis and treatment regimen for tuberculous bronchitis.

It is the purpose of this review to summarise the difficulties associated with the currently employed diagnostic methods such as microbiological method, Zn staining, The Polymerase Chain Reaction Ligase Chain Reaction, Multitargeted LAMP, Xpert RIF/MTB and sensing-based methods, as well as the potential future application of molecular diagnostic methods for TBM.

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