Diagnostic value of Cobas Amplicor MTB and Rotorgene Real Time PCR for tuberculous meningitis: A six-year retrospective study

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ABSTRACT

Objective: Tuberculous meningitis (TBM) is the most severe and lethal form of tuberculosis (TB). Bacteriologic confirmation of TBM is difficult and slow. Therefore, most patients receive antituberculosis treatment based only on clinical and cerebrospinal fluid (CSF) characteristics. Rapid diagnosis of TBM is important to decrease morbidity and mortality. The aim of the study was to demonstrate that COBAS Amplicor MTB and Rotorgene Real Time (RT) PCR is a rapid method of diagnosing TBM before and after initiating anti-tuberculosis treatment.

Methods: A retrospective study was conducted between December 2002 and January 2009 in 468 patients with suspected TBM. Clinical specimens were collected from different hospitals in Ankara. All specimens were evaluated by smear microscopy and culture methods with Lowenstein-Jensen (LJ) and MGIT culture system.

Results: Using culture results as a gold standard, the sensitivity, specificity, positive predictive values (PPV), and negative predictive values (NPV) were 71.0%, 98.8%, 97.8% and 75.0%, respectively, for COBAS Amplicor MTB and 80%, 98.9%, 99.0% and 80.0%, respectively, for Rotorgene RT PCR. Statistical analysis showed no significant differences between the COBAS Amplicor MTB and Rotorgene RT PCR (p≥0.05). All isolates were susceptible to isoniazid, rifampin, streptomycin, and ethambutol with proportion method in LJ medium. All isolates were defined as LAM7-TUR by spoligotyping.

Conclusion: Retrospective analysis of COBAS Amplicor MTB and Rotorgene RT PCR found that both tests are effective in rapidly diagnosing MTB using CSF. It was concluded that Rotorgene RT PCR test is more sensitive (81.0%) than COBAS Amplicor MTB (71.0%).

Key words: Tuberculous meningitis, COBAS Amplicor MTB, Rotorgene Real Time PCR

Tüberkülöz menenjiti için Cobas Amplicor MTB ve Rotorgene Real Time PCR’in tanı değeri: 6 yıllık retrospektif çalışma

ÖZET

Amaç: Tüberkülöz menenjiti (TBM), tüberkülözun (TB) en şiddetli ve öldürücü formudur. TB’nin bakteriyolojik tanısı zor ve zaman alıcıdır. Bu nedenle, hastaların çoğu sadece klinik ve beyin omurilik sıvısı (BOS)’nın özelliklerine göre anitüberkülöz tedavisi almaktadır. TBM’nin hızlı tanı tanışı olum ve hastağın oranını azaltmasına önem taşmaktadır. Çalışmanın amacı tüberkülöz menenjitinin tanısının hızlı bir yöntem olarak COBAS Amplicor MTB ve Rotorgene Real Time PCR (RT PCR)’nın etkinliğini araştırılmıştır.


Bulgular: Kültür yöntemi altın standart olarak kabul edildiğinde duyarlılık, özgülük, pozitif belirleyicilik değerleri (PBD) ve negatif belirleyicilik değerleri (NBD) sırasıyla COBAS Amplicor MTB için %71,0, %98,8, %97,8 ve %75,0; Rotorgene RT PCR için %80,0, %98,9, %99,0 ve %80,0 olarak bulundu. İstatistiksel analizlerde COBAS Amplicor MTB ve Rotorgene RT PCR testi arasında anlamlı bir fark bulunmadı (p≥0.05). Löwenstein Jensen besiyerinde proporsiyon yönteminin gøre yapılan

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INTRODUCTION

Tuberculous meningitis (TBM) caused by M. tuberculosis is the most common form of Central Nervous System (CNS) tuberculosis (TB); the condition has very high rates of morbidity and mortality.\(^1\)\(^,\)\(^2\) TBM accounts for approximately 1% of total TB cases in the United States and causes death and severe neurological defects in more than half of those affected. The onset of TBM is insidious in children younger than 5 years of age, school-age children, and adolescents.\(^3\) Death from TBM occurs due to delays in diagnosis and treatment.\(^3\)

Definitive diagnosis of TBM requires detection of M. tuberculosis bacilli in CSF. Although culture technique is considered the "gold standard", it has limitation in rapid diagnosis due to late growth and to the length of time required to arrive at a clinical diagnosis.\(^4\) Microscopy is fast, but its sensitivity is variable (20%-80%).\(^5\) For this reason, microscopy can be used as rapid and accurate diagnostic methods for TB. Molecular techniques are thought to be promising approaches for the diagnosis of TBM due to their efficiency, sensitivity and specificity.\(^4\)

From nucleic acid amplification techniques, real time PCR used in the diagnosis of TBM has a short turn-around time.\(^6\)\(^,\)\(^7\) The COBAS Amplicor M. tuberculosis (MTB) PCR test (Roche Diagnostic Systems, Inc., Branchburg, NJ, USA) is an automated standardized commercial test used for the detection of MTB, based on the amplification of nucleic acids.\(^9\)

The COBAS Amplicor MTB PCR test is a rapid and highly specific diagnostic test for TBM.\(^10\) The Gen-Probe Amplified M. tuberculosis Direct Test (MTD) for detecting MTB complex in CSF is also thought to be efficient, sensitive, and specific.\(^11\)

The COBAS Amplicor MTB PCR and Rotorgene RT PCR tests are techniques commonly used to detect MTB using clinical samples. The COBAS Amplicor MTB PCR is validated for respiratory specimens, but many physicians also request PCR analyses for non-respiratory specimens. For this reason, we conducted a retrospective study to determine whether the COBAS Amplicor MTB PCR test and Rotorgene RT PCR can be used to rapid diagnose TBM before and after initiation of anti-tuberculosis treatment.

METHODS

A 6-year retrospective study was conducted between December 2002– January 2009 in 468 patients with suspected TBM in Refik Saydam Hygiene Center, National Tuberculosis Reference Laboratory, Ankara. The ages of the 468 patients ranged from 11 to 76 years, with a mean age of 39.3 years. One hundred and fifty three of 468 CSF samples were obtained from female, and 315 obtained from male. Clinical specimens were collected from different hospitals in Ankara.

All specimens were processed by conventional procedures for sterile specimens. For each specimen, smear microscopy was done and stained with the Ziehl Neelsen (ZN) method. The number of acid-fast bacilli seen in the whole slide was recorded. Five hundred microliters of each specimen was inoculated in BACTEC Mycobacteria Growth Indicator Tube (MGIT) 960 liquid medium (Becton Dickinson, USA) and 150 μl was inoculated onto Lowenstein-Jensen (LJ) slants. Slants were incubated at 37°C for about 8 weeks. LJ slants were evaluated for growth on a weekly basis, whereas the BACTEC MGIT 960 system provided continuous monitoring. Presumed mycobacterial growth was confirmed by ZN staining.\(^12\) If no growth was observed after 8 weeks of incubation, the specimen was reported as culture negative. Culture-positive specimens were evaluated by spoligotyping for molecular typing.

Drug susceptibility testing to rifampicin (R), isoniazid (I), streptomycin (S), and ethambutol (E) was performed by using standard proportion method on LJ medium.\(^12\)\(^,\)\(^13\)

COBAS Amplicor MTB (Roche Molecular Systems, Inc., Branchburg, NJ,) and Rotorgene Real Time PCR (Rotor-Gene® Q, Germany) and Artus® Real Time PCR Kits, were studied in accordance with the manufacturer’s instructions. (Rotor-Gene® Q and artus® PCR Kits-Pure Pathogen Detection-2010-Available from: www.qiagen.com).\(^14\)
Culture-positive specimens for molecular typing were evaluated by spoligotyping method.\textsuperscript{15} Spoligotyping was carried out with a commercial kit (Isogen Bioscience BV, Maarssen, the Netherlands) according to the manufacturer’s instructions.\textsuperscript{15} The 43 spacers between the direct repeats in the target region were amplified by using DRa biotinylated at the 59 end and DRb primers. The PCR product was hybridized to a membrane containing 43 oligonucleotides derived from the spacer sequences of M. tuberculosis H37Rv and M. bovis BCG P3 by reverse line blotting. M. tuberculosis H37Rv and M. bovis BCG P3 were used as controls for spoligotyping. Spoligotypes results were converted into octal code and in the SITVIT2 proprietary database at the Pasteur Institute of Guadeloupe, which is an updated version of the previously released SpolDB4 database (available at http://www.pasteurguadeloupe.fr:8081/SITVITDemo).

**Statistical Analysis**

The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the nine NAATs were calculated by using culture results as the reference standards. Statistical comparisons of the nine methods were performed by using the chi-square test; a P value of <0.05 was considered significant. The analysis was performed by using of SPSS, version 14.0 (SPSS).

**RESULTS**

The positivity rates of microscopy, culture and PCR tests (COBAS Amplicor MTB and Rotorgene RT PCR test) are shown in Figure 1.

![Figure 1. Positivity rates of microscopy, culture and PCR tests.](image)

Using MGIT 960 culture results as a gold standard, the sensitivity, specificity, positive (PPV) and negative predictive values (NPV) of the COBAS Amplicor MTB and Rotorgene RT PCR were 71%, 98.8%, 75% and 98.6%, respectively, for COBAS Amplicor MTB and 81%, 98.8%, 77% and 99 %, respectively, for Rotorgene RT PCR (Figure 2, Figure 3).

![Figure 2. Results of PCR in Cerebrospinal Fluid.](image)

![Figure 3. Sensitivity, specificity, positive predictive value and negative predictive value of 468 samples.](image)

All isolates were found to be susceptible to isoniazid, rifampin, streptomycin, and ethambutol. In this study, all isolates were defined LAM7-TUR as a common MTB type (Table 1).

**Table 1. MTB types of isolates defined by spoligotyping method**

<table>
<thead>
<tr>
<th>Results of Spoligotyping</th>
<th>Octal Number</th>
<th>SIT</th>
<th>Family</th>
<th>Pattern Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>777737404760771</td>
<td>41</td>
<td>LAM7-TUR</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>777737404760771</td>
<td>41</td>
<td>LAM7-TUR</td>
<td>2</td>
<td></td>
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</tbody>
</table>
DISCUSSION

Rapid diagnosis of TBM is highly important to decrease morbidity and mortality. TBM is still a complex issue because of inconsistent clinical presentations and a lack of a rapid, sensitive, and specific test. Various nucleic acid-based amplification tests are available for the detection of MTB, but few data are available on their use to diagnose TBM. The COBAS Amplicor MTB and Rotorgene RT PCR are useful for the rapid diagnosis of MTB using CSF.

Erdem et al. analysed 506 TBM cases with microbiologically confirmed in the Haydarpasa-I study. They reported that the sensitivities of the tests were 90.2 % for interferon-gama release assay (Quantiferon TB gold in tube® ), 81.8 % for automated culture systems (AMC), 72.7% for Lowenstein-Jensen medium (L-J), 29.9% for adenosine deaminase (ADA), and 27.3 for Ehrlich-Ziehl-Neelsen staining (EZNs), respectively. In this study it is reported that cerebrospinal fluid (CSF) -ACS was superior to CSF L-J culture and CSF-PCR with statistically significant. As a results, CSF-L-J culture was superior to CSF-PCR in this study. In addiyion to this result, they reported that fair and inverse agreement was detected for CSF-ADA and CSF-PCR. They concluded that diagnostic accuracy of TB was increases when both ACS and L-J solid cultures were used together and non-culture tests such as CSF-PCR, CSF-ADA and EZNs tests contributed TBM diagnosis with different ratios. Consequently, they stated that the diagnostic approach to TB can be individualized according to the technical capacity of each institions.

In our study, we used both COBAS Amplicor MTB and Rotorgene RT PCR tests to detect MTB from CSF samples. According to the our results, 20 of 468 CSF samples were found positive by COBAS Amplicor MTB, while 22 were positive by Rotorgene RT PCR method. 448 samples were negative by COBAS Amplicor MTB, while 446 were negative by Rotorgene RT PCR. In comparison with culture as the reference method, 5 false-positive results and 6 false-negative results were detected by COBAS Amplicor MTB assay, while 5 false-positive results and 4 false-negative results were found by Rotorgene RT PCR. The sensitivity, specificity, PPV and NPV for MTD were 93.8% and 99.3%, respectively. PPV and NPV for MTD were 38%, 99%, 96%, and 66%, respectively. MTD was more sensitive than ZN staining. However, it appeared that the sensitivities of ZN staining, MTD, and the two tests combined were improved to 64%, 59%, and 83%, respectively, when repeated sampling. They concluded that ZN staining of the CSF is at least as good as MTD for the rapid diagnosis of TB, but the combination of these methods on serial samples detected more cases.

Wei and coworkers used the nested PCR assays developed previously to detect and identify MTB from CSF samples of patients with suspected TBM and non-tuberculous patients in their study. From 11 CSF samples analyzed, 5 samples were from patients with suspected TBM and 6 samples were from the clinically diagnosed non-tuberculous patients. Surprisingly, two of the non-tuberculous patients were shown as MTB positive. This finding supported that nested PCR was an important test for the diagnosis of TBM.

In the study performed by Reischl and coworkers, COBAS Amplicor PCR was used to detect MTB in respiratory and nonrespiratory clinical specimens sent to their laboratory for routine MTB testing. Of the 77 CSF samples collected, 3 samples were PCR positive, 74 samples were PCR negative, one sample was false positive, and one sample was false negative, in comparison with culture as the reference method. The authors stated that the number of false negatives could be further reduced by raising the portion of the original sediment introduced to PCR testing.

A study has been conducted in 2000 patients to assess the role of COBAS Amplicor PCR test in the early diagnosis of TBM. Eighty-three CSF samples were collected prospectively from 69 patients with suspected TBM. In this study, it was concluded that the COBAS Amplicor TB PCR test was a rapid and highly specific diagnostic test for TB.

Thwaites et al. and coworkers compared their performance by ZN staining, Gen-Probe amplified MTD test, and culture from 341 CSF specimens. They found that sensitivity, specificity, and PPV and NPV for MTD were 38%, 99%, 96%, and 66%, respectively. MTD was more sensitive than ZN staining. However, it appeared that the sensitivities of ZN staining, MTD, and the two tests combined were improved to 64%, 59%, and 83%, respectively, when repeated sampling. They concluded that ZN staining of the CSF is at least as good as MTD for the rapid diagnosis of TB, but the combination of these methods on serial samples detected more cases.

In Chedore and Jamieson’s 5-year retrospective study, the utility of MTD in detecting MTB complex was evaluated. From 311 CSF samples, 17 were positive. When compared with culture as the gold standard, the sensitivity and specificity of the MTD test were 93.8% and 99.3%, respectively. PPV
and NPV for TBM were 88.2%, and 99.7%. The study found that MTD test was rapid, sensitive, and specific for TBM. These results were corroborated with our results.

Pai and coworkers\(^2\) did a systematic review and meta-analysis to establish the diagnostic accuracy of nucleic acid amplification (NAA) tests for TBM from 49 studies. The summary estimates in 14 studies with commercial NAA tests were sensitivity 0.56 (95% CI 0.46, 0.66), specificity 0.98 (0.97, 0.99), positive likelihood ratio 35.1 (19.0, 64.6), negative likelihood ratio 0.44 (0.33, 0.60), and diagnostic odds ratio 96.4 (42.8, 217.3). It was thought that the summary accuracy could not be established with confidence because of wide variability in test accuracy in the 35 studies with in-house (“home-brew”) tests. It was stated that commercial NAA tests showed a potential role in confirming TBM diagnosis, although their overall low sensitivity precluded the use of these tests to rule out TBM with certainty. This meta analysis provided valuable information that helped us arrive at the conclusion of our study.

The limitations of our study were the lack of some epidemiological, clinic, and laboratory dataes of all TBM. Therefore, a detailed analysis could not be performed based on clinical data. In our study, all culture-positive samples isolates were detected as LAM 7 TUR by spoligotyping for molecular epidemiologic evaluation. Our data were consistent with the findings from studies conducted in the different geographical regions of our country.\(^{15,22-24}\)

We concluded that COBAS Amplicor MTB and Rotorgene RT PCR tests are effective for the rapid diagnosis of TBM using CSF. We thought that Rotorgene RT PCR test was better than COBAS Amplicor MTB, and also DNA extraction was the most important step for test performance.

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