A Comparison of Two Different Culture Methods for Use in the Diagnosis of Pulmonary Tuberculosis

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Abstract

Objective: Tuberculosis (TB) continues to be a significant health problem worldwide. Pulmonary TB is a contagious disease. To control the spread of TB, the disease must be diagnosed early and treated effectively.

Materials and Methods: In this study, we determined the rates and periods of TB bacterial reproduction using the Lowenstein-Jensen (LJ) and the Mycobacterium Growth Indicator Tube (MGIT) culture systems in respiratory specimens obtained from 105 suspected TB cases that applied to our service.

Results: Using either the LJ or MGIT method, the reproduction rates of TB cultures from 91 positively diagnosed cases were determined to be 69.2% and 92.3% (p=0.116), respectively. The reproduction period for these same cultures was determined to be 29.7±10.0 days and 12.1±6.1 days (p<0.0001), respectively. The culture positivity rate determined using both the LJ and MGIT methods together was found to be significantly higher than the rate determined using either LJ or MGIT separately (p<0.0001).

Conclusion: For the early diagnosis of pulmonary tuberculosis, which is essential for controlling the spread of TB, the routine use of the MGIT system, which is a rapid, automated and non-radiometric method, combined with the LJ method would effectively increase the diagnosis rate in order to control tuberculosis outbreaks.

Key Words: Pulmonary tuberculosis, mycobacterium growth indicator tube, Lowenstein-Jensen

Introduction

The causative agent, pathogenesis, and treatment of tuberculosis (TB) are well known, and currently TB can be effectively treated. However, TB is still a substantial public health problem worldwide because of the high mortality associated with this disease. A patient’s medical history, as well as the clinical and radiological findings, are significant indicators of TB infection. However, for the definitive diagnosis, the isolation and identification of the causative agent are required. For this purpose, suspected tuberculosis specimens submitted to the Clinical Microbiology Laboratory should be evaluated by direct microscopic examination, and the isolation, identification and antibiotic susceptibility tests should be performed as soon as possible. Timely laboratory results will contribute considerably to breaking the chain of infection and preventing the spread of disease [1].

For the control of TB, it is necessary that pulmonary TB, which is the primary form of infection, must be diagnosed early and treated effectively. Accordingly, recent studies show
that the Mycobacterium Growth Indicator Tube (MGIT) 960 culture system is a rapid, automated and non-radiometric method, which combines the advantages of antimicrobial resistance testing and bacterial identification with high sensitivity and specificity. Thus, routine laboratory use of the MGIT system has been suggested. Furthermore, the use of both the MGIT 960 culture system and the classical culture methods yields higher reproduction rates [2-8].

In this study, we compared the diagnostic rates and reproduction periods obtained by means of direct examination, Lowenstein-Jensen (LJ) or MGIT methods in respiratory specimens obtained from suspected cases of pulmonary tuberculosis that were admitted to our clinic.

Materials and Methods

One hundred and five patients, including 59 males and 46 females (56.2% and 43.8%, respectively, with a mean age of 40.99±19.60) who applied to the clinic of Chest Diseases, Faculty of Medicine, and Chest Diseases Hospitals between January 2008 and December 2009 with clinically and radiologically suspected pulmonary TB, were involved in the study. The pulmonary specimens collected (sputum, induced sputum and bronchial lavage) were processed at the department of Microbiology Tuberculosis laboratory. Throughout the study, only the most appropriate specimen from each case was evaluated. First, the specimens were stained by the Ehrlich Ziehl Neelsen (EZN) method, and the acid resistant bacilli (ARB) were identified by direct examination. Next, the presence of ARB was verified by the EZN staining method. After decontamination, homogenization and concentration, the sample was cultured in both the LJ medium and the MGIT 960 culture system.

The cost of both the LJ and MGIT 960 systems is equal in our hospital, approximately 22 TL (15 $ or 10 €) per specimen.

Statistical Analysis

All data recorded during the study were analyzed using the Statistical Package for the Social Sciences (SPSS) 11.0 Production Facility program. A chi-square test was used to analyze the categorical variables. The findings were considered to be statistically significant if a p value of <0.05 was obtained.

Results

One hundred and five cases, consisting of 59 males and 46 females (mean age 40.99±19.60), were involved in the study. The presence of ARB and the culture positivity were determined in 91 of these cases including 50 (54.9%) males and 41 (45.1%) females (mean age 41±20) (Table 1, 2).

There was contact history in 13.2% of the cases. New cases comprised 86.8% of the study population, and relapsed cases comprised 12.1%. Only one case was identified as a chronic case. Of the total number of patients, 58.2% were fied protein derivative (PPD) positive.

Of the total number of patients, 76.9% were determined to be ARB positive. The percentage of positive cultures detected when using either the LJ or the MGIT systems was 95.6%, when using only one culture system was 29.7% and when using both systems was 65.9%. Although four cases were positive for ARB, bacterial growth was not identified in the cultures. The reproduction numbers and rates in the culture systems are shown Table 3. Despite the fact that the MGIT method detected higher numbers of positive cultures compared to the LJ method, this difference was not statisti-
Table 4. Evaluation of the cases according to their previous treatment history

<table>
<thead>
<tr>
<th>Case Type</th>
<th>Turkey (%)</th>
<th>Erzurum (%)</th>
<th>Present study (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NEW CASE</td>
<td>87.6%</td>
<td>87.3%</td>
<td>86.8%</td>
</tr>
<tr>
<td>Previously TREATED CASE</td>
<td>12.3%</td>
<td>12.7%</td>
<td>13.2%</td>
</tr>
<tr>
<td>Relapse</td>
<td>9.4%</td>
<td>7.7%</td>
<td>12.1%</td>
</tr>
<tr>
<td>Treatment after default</td>
<td>2.1%</td>
<td>4.9%</td>
<td>0.0%</td>
</tr>
<tr>
<td>Failure</td>
<td>0.5%</td>
<td>0.0%</td>
<td>0.0%</td>
</tr>
<tr>
<td>Chronic</td>
<td>0.1%</td>
<td>0.0%</td>
<td>1.1%</td>
</tr>
</tbody>
</table>

Table 5. Comparative reproduction periods for the LJ and MGIT methods

<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th>LJ reproduction period (day)</th>
<th>MGIT reproduction period (day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chien and et al. [13]</td>
<td>2000</td>
<td>30.7</td>
<td>10.7</td>
</tr>
<tr>
<td>Ozturk and et al. [12]</td>
<td>2001</td>
<td>20.8</td>
<td>11.1</td>
</tr>
<tr>
<td>Our study</td>
<td>2008-2009</td>
<td>29.7</td>
<td>12.1</td>
</tr>
</tbody>
</table>

LJ: Lowenstein-Jensen; MGIT: mycobacterium growth indicator tube


cally significant (p=0.116). However, the number of positive cultures detected using both the LJ and MGIT together was significantly higher than either the LJ or the MGIT method alone (p<0.0001). In addition, the reproduction period as determined by MGIT (12.11±6.10 days) was significantly shorter than that determined by the LJ method (29.71±10.03 days) (p<0.0001).

Discussion

Despite being a known and treatable disease for many years, tuberculosis is still a substantial health problem threatening humanity.

In our study, a positive smear or culture was detected in 91 of 105 cases (86.6%). In similar studies, all specimens submitted to a particular microbiology laboratory were combined; thus, the percentage of positive smears and cultures detected was lower (8-50%) [2-4, 8]. The inclusion of clinical specimens from suspected cases of pulmonary TB, together with clinically and radiologically identified pulmonary tuberculosis, increased the number of positive cases in our study compared to others.

The previous treatment history of the pulmonary tuberculosis cases that appeared in Erzurum and Turkey in 2007 as well as the cases presented in this study is available in Table 4 [9].

The sensitivity of direct smears for the detection of tuberculosis has been declared to be 22-78%. The direct smear positivity rate is higher for respiratory tract specimens compared to others. The factors affecting sensitivity are: the quality of the specimen, loss during centrifugation, the staining method, the culture method and the case group evaluated. For the detection of pulmonary tuberculosis, the sensitivity of a direct smear compared to a culture is approximately 50%. The use of multiple specimens increases the sensitivity. The sensitivity for single-sputum specimen is 30-40%, but increases to 65-75% when multiple specimens are used [10, 11].

In the study by Ozturk et al. [12], ARB positivity was detected in 66% of sputum samples obtained from 118 cases with pulmonary tuberculosis. In our study, ARB positivity was detected in 66 patients (75.8%) of 87 culture-positive cases. According to the Turkey Tuberculosis Control Report, smear positivity for all pulmonary tuberculosis cases in 2007 was 64.3%, whereas the rate in our study was 76.9% [9]. The higher positivity rate can be attributed to the use of multiple samples; at least 3 sputum samples from each case were submitted, and all samples were respiratory material.

According to the Turkey Tuberculosis Control Report, the cases with positive smears but negative cultures were 3.5% of the total. In our study, ARB positivity was detected in 4 (4.4%) cases and was not reproduced using both culture methods. Shortening the culture incubation period by prolonged and intensive sample decontamination leads to samples that are positive when directly prepared but negative when cultured. Specimens from properly treated pulmonary tuberculosis patients are positive by direct preparation and negative by culture for 2-10 weeks [11]. As patients receiving treatment were not involved in our study, the smear positive-culture negative cases seem to be associated with the prolonged and intensive decontamination process.

As diagnosed patients live in crowded environments and have symptoms for a prolonged period, it is necessary to diagnose TB patients as soon as possible to break the chain of infection and prevent spread of the disease. For this purpose, rapid culture methods were developed and studies that compared different culture methods, including LJ and MGIT, were conducted.

Kocaybek BS [4], Mycobacterium tuberculosis was isolated in 61 of 648 samples. ARB positivity was detected in 32 samples, and MGIT detected a significantly rapid reproduction period.

Rishi et al. [3] detected reproduction using both the MGIT and the LJ method in 50.6% of 500 specimens in 2007. The reproduction rate was determined to be 34.1% and 1.93% for the MGIT and the LJ methods, respectively. The MGIT method seems to identify a more rapid reproduction period (9.66 days
for MGIT and 28.81 days for LJ) and had greater sensitivity, and thus it was suggested that the two culture systems be applied together for optimal results.

A study conducted by Sorlozano et al. [8] in 2009, which involved 1770 clinic patterns, revealed that 156 cases showed reproduction using one of the two culture methods. The MGIT was considered to be more statistically significant in both the sensitivity (86.5%) and reproduction period (15.3 days). This group concluded that applying the LJ and the MGIT methods together increased both the sensitivity (95.5%) and the specificity (99.6%).

In our study, the total reproduction rates when using either the LJ or the MGIT systems was 95.6%, when using only one culture system was 29.7% and when using both of them was 65.9%. MGIT culture positivity was determined to be higher than LJ, 92.3% and 69.2%, respectively. Culture positivity assessed by using LJ and MGIT together was greater when compared to either MGIT (p<0.0001) or LJ alone. Although these results were not statistically significant, (p=0.116), they suggest that MGIT is a rapid and efficient method that detects a high number of positive tuberculosis cases. In addition to these methods, classical culture plating methods can increase in the rate of reproduction considerably.

A comparison of the mean reproduction periods showed that the growth period for MGIT (12.1±±6.10 days) was significantly shorter than for LJ (29.71±10.03 days) (p=0.003). In similar studies (Table 5), reproduction periods determined by LJ method were 2-3 times longer than those determined by MGIT. For TB, in which early diagnosis is vital to public health, we suggest that rapid culture methods must be used.

Consequently, tuberculosis is a public health problem requiring rapid and correct diagnosis and treatment. According to data from the Turkey Tuberculosis Control Report (2007), 69.5% of all tuberculosis cases were pulmonary tuberculosis and that drug sensitivity testing was applied to only 35.9% of these cases [9]. Particularly when pulmonary TB is suspected clinically and radiologically, respiratory materials must be examined by direct smear and then plated for MGIT analysis, which is a rapid, automated and non-radiometric method. There is no difference in cost between the LJ and MGIT methods accompanied by LJ analysis increases the reproduction rate significantly.

**Ethics Committee Approval:** Ethics committee approval was received for this study from the ethics committee of Ataturk University Faculty of Medicine (14.11.2008/6).

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