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Full Length Research Paper

Evaluation of MODS culture in diagnosis of pulmonary tuberculosis

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Culture of *Mycobacterium* tuberculosis is the gold standard for diagnosis of tuberculosis (TB) that is a much more sensitive test than smear examination. There is a need to use the new methods in order for fast diagnostic way. The aim of this research was to compare microscopic observation drug susceptibility culture with Ziehl-Neelsen stain and Lowenstein-Jensen culture of sputum. Therefore, a diagnostic test was designed. If the patient's history revealed clinical criteria compatible with tuberculosis and the infectious specialist judgment was that of "TB" suspected case, the patient was considered as a pulmonary TB suspect. We did sputum Ziehl-Neelsen stain, culture in Lowenstein-Jensen and MODS media. 100 patients with mean of 52.9 ± 21.83 were evaluated. 40% of patients revealed positive Ziehl-Neelsen stain while 30 and 47% showed positive results in Lowenstein-Jensen and MODS media respectively. In comparison with sputum smear and Lowenstein-Jensen culture, MODS had sensitivity of 82.5 and 86%, specificity of 77 and 70%, positive predictive value of 70 and 55%, negative predictive value of 86 and 92%, respectively. MODS culture demonstrated faster recovery and higher negative predictive value. Therefore, it could be a simple and rapid method in diagnosis of pulmonary tuberculosis.

Key words: Tuberculosis, Ziehl-Neelsen stain, Lowenstein-Jensen culture, sensitivity.

INTRODUCTION

Every year, 1.7 million people die from tuberculosis (World Health organization, 2006). The patients with sputum smear positive tuberculosis continue to pass infection till it is to be treated (Espinal et al., 2000). MDR TB has increased mortality rates and failure of treatment (Espinal et al., 2000). WHO recommends to examine sputum smear with acid fast stain method in three times (sputum sample on day 1, overnight sample and a sample in the morning on day 2) to screen pulmonary tuberculosis (Enarson et al., 1996; API Consensus Expert Committee, 2006). Acid fast stain identifies carrier patients who transmit infection to others and also has a high specificity, although it has some limitations such as required instruments, qualified experts, time consuming procedure (15 min for each slide before negative report) make some difficulties for this method (Narain et al., 1971; Rouillon et al., 1976). Other diagnostic method for pulmonary tuberculosis is sputum culture which is gold standard diagnostic test and more sensitive than sputum smear because it can detect 10 to 100 viable mycobacterium per milliliter of sample (API Consensus Expert Committee, 2006) and in case of active disease they are found to have a sensitivity from 81% (API Consensus Expert Committee, 2006) to 84% (Moore et al., 2006) and a specificity from 98.5% (API Consensus Expert Committee, 2006) to 100% (Moore et al., 2006a).

Recognition of MDR TB by using the culture methods to evaluate sensitive and resistant strains is very difficult

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Table 1. Comparison of MODS culture in the sputum of suspected pulmonary tuberculosis patients with Ziehl-Neelsen stain.

Total	Patient with negative stain	Patient with positive stain	MODS culture
47	14	33	Positive
53	46	7	Negative
100	60	40	Total

Sensitivity: 82.5%, specificity: 70%, PPV: 70%, NPV: 86% and efficacy: 79%.

Table 2. Comparison of MODS culture results with Lowenstein –Jensen culture media.

Total	Patient with Lowenstein negative	Patient with Lowenstein positive	MODS culture
47	21	26	Positive
53	49	4	Negative
100	70	30	Total

Sensitivity: 86%, specificity: 70%, PPV: 55%, NPV: 92% and efficacy: 75%.

and laboratory errors make some troubles to identify sensitive and resistant strains (API Consensus Expert Committee, 2006; Burman et al., 1997). Therefore, new diagnostic instruments with high sensitivity and specificity are necessary for rapid diagnosis of tuberculosis and its sensitivity and resistant pattern. There are some studies that shows the MODS method and its sensitivity and specificity and also the possibility of rapid diagnosis of disease and recognition resistant pattern in the shortest period (Moore et al., 2006a,b, 2004; Park et al., 2002; Oberhelman et al., 2006) so thus, this study evaluated MODS culture in pulmonary tuberculosis and determined sensitivity, specificity, positive predictive value (PPV) and negative predictive value(NPV) in comparison to Ziehl - Neelsen stain and Lowenstein-Jensen culture of sputum.

MATERIALS AND METHODS

The research method was a clinical trial (diagnostic test) and the technique was observation-interview type. The procedure of sampling was continuous and it contains 100 suspected pulmonary tuberculosis (TB) patients in Masih Daneshvari hospital (who were ready to cooperate), Tehran, Iran. After taking a history, if the clinical criteria were compatible to tuberculosis, a chest x ray was done and also if the radiologist judgment revealed changes compatible with tuberculosis, the patient was entered to this study and a questionnaire form was completed. Then, sputum sample of patients were sent to the laboratory. In the first stage, sputum was decontaminated, then part of each sample was used for Ziehl-Neelsen stain for primary diagnosis and the other part of sample was sent for Lowenstein-Jensen culture and MODS (Microscopic Observation Drug Sensitivity) culture. Lowenstein stain culture, 250 cc of prepared sample was induced and incubated in 37°C and these samples were evaluated 2 times per week from 7th till 60th day in order to observe colony growth. In MODS method, we added liquid broth base media, 7H9, OADC (oxalic acid, albumin dextrose, catalase), PANTA (polymyxin, amphotaicin B, nalidixic acid, trimethoprim, azlocillin) into 24 squared plates, then, 720 µl of prepared sample was added to each block. 12 blocks were dedicated for each sample and positive and negative controls were considered.

After incubation in 37°C, every sample was daily evaluated by invert light microscope; positive samples were identified through cord formation which was hallmark of BK growth. We also added different antibiotic with different concentration to the plate in order to evaluate resistant pattern of them.

RESULTS

100 patients (48 male, 52 female) with an average age (52/9±21/83) years, (minimum 15 and maximum 87) were evaluated. 48% of patients were 20 to 60 years old, 43% were older than 60 and 9% were younger than 20 years. 96% of patients had cough, 87% had sputum, 85% had weight loss, 75% had fever, 74% had sweating and 25% had hemoptysis; chest x-ray revealed: apex involvement (88%), middle and lower lobes involvement (79%), bilateral parenchyma involvement (64%), cavitory lesions (55%), miliary type (7%) and hilar lymphadenopathy (4%). Ziehl –Neelsen stain of sputum was positive in 40% of patients, culture of TB in Lowenstein-Jensen media was positive in 30% while 47% of patients had positive culture in MODS media. Isoniazid, ethambotul, rifampin and pirazinamide resistance were reported at 62, 61, 60 and 55% respectively. Sensitivity and specificity, PPV, NPV and efficacy of MODS culture compared to Ziehl – Neelsen stain has been shown in Table 1 and also sensitivity and specificity, PPV, NPV and efficacy of MODS culture in comparison with sputum culture has been shown in Table 2. Positive culture obtained in 8 to 10 days by MODS method and in 3 to 4 weeks by Lowenstein-Jensen media.

DISCUSSION

In this research, positive Ziehl-Neelson stain was reported

with the rate of 40% while the positive cultures of *Mycobacterium tuberculosis* in Lowenstein-Jensen and MODS media were observed in 30 and 47% of cases respectively. Moore et al. (2004) evaluated 5771 samples and reported a positive result of 94 and 87% in MODS media and Lowenstein-Jensen media. Arias et al. (2007) reported positive Ziehl -Neelsen stain with the rate of 35.9% whilst the results in Lowenstein-Jensen media and MODS method in their study were positive in 44.4 and 41.5% of patients. Their research was performed in countries with high prevalence of tuberculosis in a multi-centric study. Moore et al. (2004) and Arias et al. (2007) reported a similar rate of positive results in Lowenstein-Jensen and MODS cultures. Oberhelman et al. (2006) research that performed in the children less than 12 years old with pulmonary TB symptoms and they evaluated gastric aspirate, nasopharyngeal aspirate and stool specimens. MODS and Lowenstein-Jensen cultures were positive in 86.8 and 55% of them and revealed that the rate of positive MODS was much more than Lowenstein -Jensen culture. In our study, positive *M. tuberculosis* by MODS method obtained on 8 to 10 days that is similar to Moore research (Moore et al., 2004) who obtained on 8 days but shorter than Arias study (Arias et al., 2007) that showed obtaining after 2 weeks. Mengatto et al., (2006) obtained them in less than 10 days.

In our research, MODS culture sensitivity in comparison to Ziehl-Neelson was 82.5% and its specificity was 77% while Arias et al. (2007) reported 94% sensitivity and 90% specificity for MODS culture in comparison to Ziehl-Neelson stain and Moore et al. (2006b) reported 99.9% specificity that was more than our study. Also, our study revealed the PPV of 70% for MODS culture in comparison to Ziehl –Neelsen stain which means if MODS culture in pulmonary tuberculosis suspected patients is positive, Ziehl-Neelsen stain will be positive with a possibility of 70%. PPV of MODS culture in comparison with Ziehl-Neelsen stain in Arias et al. (2007) study and Moore et al. (2006b) research were 94 and 99% respectively. In this research, NPV of MODS in comparison to Ziehl-Neelsen stain was 86% which means if MODS culture is negative in a pulmonary TB suspected patient, Ziehl-Neelsen stain will be negative with a probability of 86%. It is in agreement with Arias et al. (2007) results which reported a NPV of 90%. Finally, efficacy of MODS culture in comparison to Ziehl-Neelsen stain in our study was 79% that was less than Arias et al. (2007) result with the rate of 85%. The sample size of their study might explain this difference. In our research, sensitivity and specificity of MODS culture in comparison to Lowenstein culture were 86 and 70% that were less than Arias et al. (2007) findings with the sensitivity and specificity of 96.5 and 92.6%. This research showed a PPV (55%) and NPV (92%) of MODS culture in comparison to Lowenstein-Jensen media which means if MODS culture in pulmonary TB suspected patients becomes positive, Lowenstein-Jensen culture will be positive with 55% probability and if MODS culture becomes negative, Lowenstein-Jensen culture will be negative with a probability of 92%. In Arias research (Arias et al., 2007), PPV and NPV of MODS were 90 and 95% that are more than our findings. In our study, efficacy of MODS culture in comparison to Lowenstein-Jensen media was 75% that was less than Arias result (94%) (Arias et al., 2007).

Evaluation of resistance of *M. tuberculosis* to INH, RIF by use of MODS culture in Moore (Moore et al., 2004), Mengatto (Mengatto et al., 2006) and Park(Park et al., 2002) studies has been suggested this method as an exact test and more rapid than reference method with good sensitivity (more than 90% till 100%). In our study, resistance to isoniazid, ethambotul, rifampin and pirazinamide on MODS culture positive samples was detected in a short time that can be important and useful in identification of MDR TB.

Conclusion

In terms of obtaining the result of MODS culture in a short time and its high NPV (92%) versus Lowenstein-Jensen method, this seems that the evaluation of pulmonary TB suspicious patient's sputum by MODS culture, not only is a simple and rapid test but also can identify resistant and sensitive Mycobacterium to isoniazid, ethambotul, rifampin and pirazinamide in a short time.

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