

Comparison of Two Conventional Techniques used for the Diagnosis of Tuberculosis Cases

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ABSTRACT

The present study was conducted to investigate and evaluate the Tuberculosis (TB). A total of 1351 samples including pulmonary and extra-pulmonary were collected at the Center. Two commonly used microscopic and macroscopic tests for the identification *Mycobacterium tuberculosis* (MTB), AFB smear examination (Zeihl Neelsen staining) and culturing on Lewenstein-Jensen (L.J) medium by concentrated method, were used and their results were compared to evaluate the percentage of specificity and sensitivity. Out of 1351 samples 3.55% were AFB positive and 25.84% were culture (L.J media) positive. In AFB smear examination (Microscopy) 43 were true positive (positive on culturing) and 5 false positive (negative on culturing). By this study the sensitivity of AFB smear (Microscopy) was detected 12.32% as compare to culturing.

Key Words: Tuberculosis; Diagnosis; AFB smear staining microscopy; L.J media culturing

INTRODUCTION

Tuberculosis (TB) is a chronic, communicable, bacterial disease caused by tubercle bacilli (Cohen, 1995). WHO in 1993 clearly demonstrated the enhancing trend of tuberculosis worldwide and called upon all the member states to immediately intervene to control the spread of TB disease. TB is not new born disease but it is disease of ancient and had caused more deaths than any other infectious disease and is known the disease of poor areas where the facilities are not so good, about 95% of these deaths are in the developing countries (Kumarison, 1994). TB is a disease of great antiquity, the age of TB can be estimated from the presence of TB lesions in the vertebrae of Neolithic man in Europe and of Egyptian mummies perhaps as early as 3700 BC (Morse *et al.*, 1964). TB is present with an extreme high prevalence in Asian countries, where 60 to 80% children below the age of 14 years are infected (WHO, 1993). The annual incidence of the disease is expected to be increased 41% between 1998 to 2020 (from 7.4 million to 10.6 million cases per year) and achievement of WHO targets by 2010 would prevent 23% (15-30 million) cases by 2020 (Dye *et al.*, 1999).

In mid nineteenth century the vaccination of TB (BCG) and improved medical services had impressively accelerated the decline of TB in economically developed countries although in developing countries the situation remain grim (Crofton & Dovgles, 1975). Even still the disease is fully alive and killing more people worldwide than Malaria and HIV (Brown, 1993). With the co-infection of TB and HIV the incidence rates and deaths are being pushed sharply upward (Javaid, 1997). According to (WHO, 1993) 3.1 million people are co-infected with TB and HIV. Without expanding efforts to control TB nearly 30 million people will die by this decade including 2.9 million deaths

expected in North Africa and Middle East. The often delayed and inadequate TB diagnosis problems are the main facts of TB increasing trend. In this way drug resistant strains of MTB are also increasing at an alarming rate which is a devastating threat to TB control (Karamat *et al.*, 1995).

Isolation of organisms is the only definitive currently available mean for the diagnosis of TB. It has specificity that approaches 100% and also permits susceptibility testing of the isolates. But due to the poor availability of the culturing facilities, the diagnosis is often delayed and majority of the cases are mismanaged by unwise use of anti-TB drugs and other antibiotics, which may also result in developing the resistance. Although AFB smear examination (Microscopy) is time honored and economical, but for this technique the yield requirement is between 5000 to 10,000 organisms per ml (Levy *et al.*, 1989). With merits and demerits Zeihl-Neelsen acid fast staining is mostly used and is known a reliable test for TB diagnosis. According to one survey 57.69% doctors prefer AFB staining as an important test for the diagnosis of TB and only 3.84% doctors were familiar with culturing facility (Iqbal *et al.*, 2001). So, this study is designed to aware the doctors with the importance of culturing and stress them to prefer this test for the diagnosis of TB cases and not rely only on smear examination. Although the diagnosis of TB is also done on molecular basis (PCR), but it is expensive and not so common in under developed countries. This study will also be helpful to compare two well-known and commonly used techniques (AFB smear & culturing).

MATERIALS AND METHODS

This study was conducted at TB Research Center of Pakistan Medical Research Council (PMRC) which is affiliated with the Institute of Chest Medical King Edward

College, Mayo Hospital, Lahore Pakistan. The fresh specimens were collected at the Center either directly from the patients or indirectly from TB ward, OPD and other wards of Mayo Hospital at the morning. The patients were instructed to send the specimen in sterile container. The patients from where specimens were collected were from different areas of the country. All the specimens were recorded with full history of the patient including patient name, father/husband name, sex, age, address ward/bed No. and treatment history. All the specimens were proceeded for AFB smear and L.J media culturing on the same day as follows: The specimens received were assigned a code number and 5 mL specimen was added in 50 mL centrifuge tube containing 25 mL 4% NaOH and placed for 15 min in water bath at 37°C to mix and digest. The tubes were filled with phosphate buffer (pH 6.8) and centrifuged at 3000 rpm for 20 min. Supernatant was discarded remaining 2-3 mL of sediment for making suspension (Mohon & Manuselis, 1995). Smear was prepared placing two drops of that suspension on clean glass slide. Then the smear was dried overnight and after fixing in alcohol (methanol) transferred to special staining racks and poured one percent solution of fuchsin on the slides heated gently and left in contact with smear for 10 minutes until the formation of steam, then it was alternatively washed gently by water and acid alcohol until no more stain come out. The slides were then counter stained with 0.1% methylene blue for 1 minute and then air-dried after gentle washing. At least 200-300 fields under oil immersion lens were screened for AFB and the results were reported as 0-1, 1-10, 10-1000 or more AFB per high power field. Two tubes of Lowenstein Jensen medium were also inoculated from this suspension and were incubated at 37°C. The culture results were read till 6 weeks with weekly interval. The results were reported negative with actual colony count if less than 50 colonies, + for 50- 99 colonies, ++ for 100-200 colonies, +++ for 300 colonies and ++++ for confluent growth (more than 500) (Idrees *et al.*, 1998).

RESULTS

A total of 1351 clinical samples collected at PMRC were induced in this study. Out of 1351 samples 349 (25.84%) were detected positive and 1002 (74.17%) negative on L.J medium culturing. Only 48 (3.55%) specimens were AFB positive on smear microscopy and 1303 (96.45%) were negative (Table I). Out of 349 culture positive specimens 43 (12.32%) were positive and 306 (87.68%) were negative with microscopy. There were also interesting results that out of 1002 culture negative samples 5 (0.50%) were positive and 997 (99.50%) were negative

Table I. Results of 1351 specimens

	Positive	Negative
Culture	349 (25.84%)	1002 (74.17%)
Microscopy	48 (3.55%)	1303 (94.45%)

with microscopy (Table II). When evaluation of Z-N staining (microscopy) was done for diagnosis of AFB, there were 43 true positive (also positive on L.J medium) 5 false positive (Negative on L.J medium). 997 were true negative (Negative on L.J medium). 306 were false negative (Positive on L.J medium). Sensitivity was detected 12.32% (Microscopy and culture positive) (Table III). In this way the specificity of microscopy was detected 99.50% (out of 1002 culture negative 997 were also negative at microscopy).

Table II. Microscopy and culture results

Culture Results	Microscopy		Total
	Positive	Negative	
Positive	43	306	349
Negative	5	997	1002
Total	48	1303	1351

Table III. Evaluation of microscopy for the diagnosis of AFB

True positive	43
False positive	05
True negative	997
False negative	306
Sensitivity	12.32%
Specificity	99.50%

DISCUSSION

In this study the concentrated method was used because higher positive rate was declared in many studies done by concentrated method (Salmon *et al.*, 1977). The need of a sensitive and specific test for adequate treatment of TB should be acknowledged, but commonly the laboratory diagnosis of TB is based on AFB smear microscopy examination (Zeihl Neelsen AFB staining) especially in the poor areas of the country (Pakistan). By this study it is clear that AFB staining is only 12.32% sensitive as compare to culturing which shows that only AFB staining for diagnosis is not so reliable. Out of 1351 samples only 48 (3.55%) were detected on microscopy. This low amount of Positive results on microscopy may be due to low amount of organisms, because to detect AFB there should be present 5000-10,000 organisms per ml or greater (Yeager *et al.*, 1967). There was also an interesting thing that out of 1002 culture negative 5 (0.50%) were positive on microscopy which is near to negligible, but this positive detection on microscopy may be due to many reasons, these results may be false positive as has also been reported in many other studies (Boy & Marr, 1975). In other reason, the samples may be collected from the patients which were on anti-TB drugs and the organisms (bacteria) were not able to grow on the media or the culture medium and environment were not ideal for the growth of tubercle bacilli. In this way 306 (87.68%) were negative by microscopy, out of 349

culture positive. High culture positive rates as compare to smear examination has also been reported in the literature (Joseph *et al.*, 1969). These negative smears may be due to low AFB amount in the specimens that were missed on the microscopy detection. The smear examination of fresh sputum specimen shows that there should be at least 5×10^4 tubercle bacilli per ml to detect a single AFB (Takahashi, 1975). Thus those specimens, which contain less than 5×10^4 AFB/ml are missed on direct smear and may be detected by culture. This highlights the importance of culturing in tuberculosis, especially in fresh cases. All the strains of culture positive samples will also be analyses on molecular level using PCR technique. Then study will also be extended for the detection of mutation in Isoniazid (INH) anti-tuberculosis drug related genes. In this way we may be able to develop a technique not only for the detection of TB cases but also the resistance against anti-TB drugs. In this way we may be able to short the detection time of TB cases from 6 weeks to one day and in resistant cases from 12 weeks to two days.

CONCLUSION

This study predicts that Zeilhl-Neelsen staining is rapid and inexpensive but lacks sensitivity and specificity because it cannot be used to distinguish between the various members of the mycobacterium and also requires a high amount of organisms in the specimen. Due to its low sensitivity there are high chances of false negative. So, to treat the tuberculosis patients on the basis of AFB staining results is not the proper way. Culturing should be a method of choice for the detection of TB cases inspite of its time consuming demerit. The detection of TB cases on molecular level is also in practice in our country but due to the lack of molecular expertise, highly expensive equipment need and high test charges these techniques are not so common. So, it is highly suggested that at least culturing must be recommended and should not be rely only on AFB for the treatment of tuberculosis cases.

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