

DETECTION OF MYCOBACTERIUM TUBERCULOSIS IN CLINICAL SAMPLES BY SMEAR AND CULTURE

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Objective: A retrospective study was carried out in order to compare the smear stained by ZN and Lowenstein–Jensen (LJ) medium for the detection of Mycobacterium in clinical samples from different categories. **Study Design:** Laboratory based, Retrospective. Place and Duration: Sir Ganga Ram Hospital/ Fatima Jinnah Medical College, Lahore over a 5 year period between Jan 2001 and June 2006. **Material and Methods:** A total of 798 clinical samples were collected from patients of both sexes and all ages with a provisional diagnosis of tuberculosis. A Ziehl-Neelsen stain (ZN) and culture on LJ medium was performed for the detection of Mycobacterium. The specimen categories were sputum, pus, lymph node aspirate, urine and endometrial curetting. **Results:** Out of 5 types of 798 specimens received over a period of five years, only 46.3% (n=369) were respiratory whereas the remaining 53.7% (n=429) were non respiratory tract category samples including sputum, pus, lymph node aspirate, urine and endometrial curetting. All were examined for the presence of acid-fast bacilli (AFB) in ZN smear. Among these 3.578% gave a positive ZN stain while 11.65% were positive on culture. Out of a total of 369 respiratory tract category samples, 38 (10.3%) sputum samples were positive for AFB on both ZN and culture. Among the non respiratory tract category, 47 (28.2%) pus, 26 (31%) LN aspirate, 5 (15.6%) urine, 5 (3.42%) endometrial curetting were reported positive. Only 15.16% of clinical samples belonging to 5 different categories of specimens received from patients of both sexes with a provisional diagnosis of tuberculosis, tested positive for Mycobacterium by both ZN stain smear and culture on LJ medium. Among these, 3.57% were positive for AFB on ZN smear and 11.65% were positive on culture on LJ medium. **Conclusion:** These conventional techniques have proved to be reliable testing tools for detection of Mycobacterium tuberculosis in our settings but there is an urgent need to promote the use of Bactec and PCR for early detection and drug susceptibility keeping in view the rise in the number of multi-drug resistant cases of tuberculosis in the last few years. **Keywords:** Mycobacterium tuberculosis, Ziehl-Neelsen staining (ZN), Löwenstein-Jensen medium (LJ), Tuberculosis (TB)

INTRODUCTION

Tuberculosis (TB) is a major air-borne infectious bacterial disease. It remains a major world wide health problem with global mortality ranging from 1.6 to 2.2 million lives per year. The situation is further exacerbated with the increasing incidence of drug resistant TB. After HIV, it is the leading cause of death in the world. Tuberculosis flourishes where ever there is poverty, crowding and chronic debilitating disease.¹ The diagnosis of TB depends on the history, physical and radiographic evidence or the presence of AFB in acid fast smears and cultures.² Most standard laboratory text books and guidelines for laboratories suggest that at least three specimens, preferably collected on three consecutive days, should be submitted to the laboratory for acid-fast bacilli (AFB) smear and culture. The detection of AFB in direct smears prepared with concentrated sputa, urine and specimens of other body fluids has considerable clinical and epidemiological value and remains the most widely used rapid diagnostic test for TB in most developing countries.³

While new radiometric and molecular diagnostic techniques have been developed and are widely used in the developed world, it is estimated that

between 60% and 70% of all TB cases are diagnosed by means of sputum smear examination.^{4,5}

Pulmonary tuberculosis is the most important form of the disease, although infection may also occur by way of the intestinal tract, tonsils and skin. Oral lesions consist of persistent ulcers or granuloma.⁶ Cutaneous infections with Mycobacterium tuberculosis with an atypical clinical appearance have become more common because of the increasing number of immunocompromised patients.^{7,8}

Bacteriological investigation of non-pregnant endometrial curetting was carried out for detection of tuberculous endometritis in group of women with the complaints of infertility, abnormal uterine bleeding and miscellaneous conditions (pelvic pain and pyometra). It was observed that bacteriological study was of greater value in doubtful cases where the tuberculous granuloma or epithelioid cells were absent but there was presence of non-specific inflammatory cells along with variable degree of necrosis of glandular epithelia in the histopathology slides. Thus simultaneous use of culture with histopathology yielded better results.⁹

Mycobacterium Tuberculosis was found to be excreted intermittently in the urine of infected patients, and single specimens were more likely to be false

negative than a 24-h sample. The best method appeared to be the concentration of a large volume of urine.¹⁰

Among patients with laparoscopic findings suggestive of tuberculosis, 60% of those with a probable diagnosis and 33% of those with incidental findings were positive by PCR.¹¹ Culture remains the gold standard for the definitive diagnosis of tuberculosis because it allows testing of drug susceptibility.¹² The conventional method of ZN staining of smears for detection of acid fast bacilli is found to be the method of choice for the diagnosis of tubercular bacterial infection in developing countries.¹³

MATERIAL AND METHODS

Laboratory based, retrospective study conducted from Jan 2001 to June 2006. A total of 798 clinical samples were collected from patients of both sexes and all ages with a provisional diagnosis of tuberculosis. A ZN stain smear and LJ media based cultures was performed for the detection of Mycobacterium. The specimen categories were sputum, pus, lymph node aspirate, urine and endometrial curetting. Cases where data of smear and culture media were available for inclusion in the evaluation study.

All sputum samples were decontaminated and concentrated using the N Acetyl-L-Cysteine-Sodium Hydroxide procedure recommended by the Centre of Disease Control and Prevention.³ The sputum, urine and other specimens were prepared using the conventional centrifugation method and the smears were stained with carbol fuchsin (ZN). The sputum, urine and other specimen were inoculated on to Lowenstein-Jensen media and incubated at 37 °C in 5% CO₂ for up to 8 weeks. The culture was used as the reference method and compared with the ZN technique.

RESULTS

The results of current study showed that a higher number of female patients were suspected of tuberculosis infection than men (Table-1). Out of 5 types of 798 specimens received over a period of 5 years, only 46.3% (n=369) were respiratory, whereas the remaining 53.7% (n=429) were non respiratory tract category samples including sputum, pus, lymph node aspirate, urine and endometrial curetting.

All specimens were examined for the presence of acid-fast-bacilli (AFB) in ZN smear. Among these 25 (3.1%) gave a positive ZN stain while 96 (12.3%) were positive on culture. Only 15.16% of clinical samples belonging to 5 different categories of specimens received from patients of both sexes with a provisional diagnosis of tuberculosis, tested positive for Mycobacterium both by ZN smear and culture on LJ medium. Out of a total of 369 respiratory tract categories 38 (10.3%) sputum samples were positive for AFB on both ZN and culture. Among the non

respiratory tract category, 47 (28.2%) pus, 26 (30.95%) lymph node aspirates, 5 (15.6%) urine, 5 (3.42%) endometrial curetting were reported positive (Table-2).

In males, 8.6% were positive on ZN while only 1.96% females gave a positive ZN smear (Table-3). The rate of detection was highest in lymph node aspirates, followed by pus and sputum (Table-3). In contrast 64 females and 29 males gave a positive culture results (Table-4).

Table-1: Sex distribution of 798 cases

	Males	Females	Total
Sputum	91	278	369
Pus	4	118	167
Lymph node	26	58	84
Endometrial Curetting	0	146	146
Urine	20	12	32
Total	186	612	798

Table-2: Comparison of ZN positive case and Culture positive case

	ZN	Culture	Total	Percentage
Sputum	9	29	38	10.30
Pus	9	38	47	28.2
Lymph node	3	23	26	30.95
Endometrial Curetting	1	4	5	3.42
Urine	3	2	5	15.63
Total	25	96	121	15.16

Table-3: Distribution of ZN Positive Cases (n=798)

Group	ZN positive	ZN negative	Total	Percentage
Male	16	170	186	8.60%
Female	12	600	612	1.96%
Total	28	770	798	3.51%

Table-4: Distribution of Culture Positive Cases (n=798)

Group	Positive	Negative	Total	Positive %
Male	29	157	186	15.59%
Female	64	548	612	10.46%
Total	93	705	798	11.65%

DISCUSSION

Among communicable diseases, tuberculosis (TB) is the second leading cause of death worldwide, killing nearly 2 million people each year. It is estimated that about one-third of the world population are infected with TB (2 billion people) and about 10% of this figure will progress to disease state. Most cases are in the under developed countries of the world.¹²

Seven hundred and ninety-eight (798) biological specimens were examined for the presence of acid-fast-bacilli (AFB), out of which 121 specimens were positive (15.16%). Among these, 25 (3.1%) were AFB smear positive with ZN stain and 96 (12.03%) were AFB positive on culture with LJ medium. In contrast, a group of workers examined 81 samples of lymph node biopsies from clinically suspected cases of lymphadenitis for AFB. They reported that rate of positive cases on culture and AFB smear was 13.6%

and 28.4% respectively.¹⁴ However according to a study bacteriological and/or histological confirmation of tuberculosis was obtained in 88% of the cases.¹⁵

The results of current study showed that a higher number of female patients were suspected of tuberculosis infection than men (Table-1). In males, 8.6% were positive on ZN while only 1.96% females gave a positive ZN smear (Table-3). In contrast 64 females and 29 males gave a positive culture results (Table-4). A major number of the sputum specimens, (278, 3.8%), were of females with a provisional diagnosis of pulmonary tuberculosis who remain at increased risk of developing active tuberculosis being disadvantaged and marginalised population due to social problems in Pakistan like a delay in seeking medical care. Our study also included 146 endometrial specimens. Similarly, the results of a study showed that the rate of infection is more in men than in women.¹⁶ On the other hand, a recent study also showed that men and women were equally affected.¹⁷ Another study found that the incidence among women peaks at 25–34 years of age. In this age group, rates among women are usually higher than those among men.¹⁸

Our data indicate that in the diagnosis of Tuberculosis, culture has greater sensitivity than ZN. In particular, in case of a single specimen, the diagnostic value of culture is quite significant. It is, therefore, possible to conclude that both ZN and culture can be used for the diagnosis of TB, however, if only one or two specimens are available, culture is preferable. The ZN method has commonly been used around the world, particularly in developing countries, because of its simplicity and low cost.⁵ One disadvantage of the technique is that it may sometimes yield false positive results. However, most of these can be prevented by re-staining the smear.³

Our study showed that the rate of detection of Mycobacterium was highest in lymph node, and pus than sputum. In contrast some other studies found that the sputum is the major infectious sample, followed by biological liquids.^{7,16}

The results of a study show that ZN smear examination has a sensitivity of 33.79% and a specificity of 100%. For LJ media, sensitivity was 48.9% and specificity was 100%.¹⁹ A group of workers also found that the ZN stain is the primary procedure for detection of Mycobacterium.²⁰ However, other studies found that besides bacteriology, histopathology is a complimentary diagnostic tool for detection of TB granuloma in tissues.^{15,21}

CONCLUSION

The data suggests that in TB epidemic areas, most of the cases of TB can be diagnosed correctly by simple and cheap methods which are generally available at district hospital level. Although acid fast bacilli (AFB)

microscopy, and conventional Lowenstein Jensen (LJ) culture remain the cornerstone of the diagnosis of TB, these traditional bacteriological methods are either slow or their sensitivity is quite low, especially with clinical samples that contain small number of organisms. This can affect treatment by either delaying it or causing inappropriate empiric therapy for TB to subjects without Mycobacterial infections or with Atypical Mycobacteria.¹⁹ There is an urgent need to promote the use of Bactec for early detection and drug susceptibility and real time PCR for even more rapid diagnosis in our country.

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