International Scholars Journals

Advanced Journal of Microbiology Research ISSN 2241-9837 Vol. 12 (5), pp. 001-004, May, 2018. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Study of sputum and bronchoscopic lavage for acid fast bacilli in patients with pulmonary infections

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Accepted 23 January, 2018

The diagnosis of tuberculosis is based on the detection of *Mycobacterium tuberculosis* on clinical specimens with different methods. Although there are many diagnostic techniques, such as culture and molecular methods, however, sputum smear microscopy for demonstration of acid fast bacilli remains the most important diagnostic method for tuberculosis in high prevalence countries due to its speed, easy performance, and low cost. The aim of this study was determination of prevalence of acid fast bacilli (AFB) in specimens of suspected patients of pulmonary infections. In total, 2872 specimens of sputum and bronchoscopic lavage were collected. For smear preparation, the specimens were decontaminated and processed. Prepared smears were stained by Ziehl-Neelsen staining method as per standard guideline and examined under the light microscope for the presence of acid fast bacilli. From total specimens examined, 1726 (60%) were isolated from male patients and 1146 (40%) were from females. One hundred and eighty three (6.4%) were positive for acid fast bacilli. These were identified in 81.4% of sputum specimens and 18.4% of bronchoscopic lavages. The majority of smears were graded as 3+ according to criteria for AFB smear reporting. The results of the present study indicated that Ziehl Neelsen stain is preferable method for all suspected tuberculosis cases in absence of culture.

Key words: Sputum-bronchoscopic lavage, Ziehl Neelsen, acid fast bacilli, tuberculosis.

INTRODUCTION

Tuberculosis (TB) is a major health problem worldwide, with an estimated eight million new cases and two million deaths occurring every year (WHO, 2006). In most industrialized countries, the control of tuberculosis has improved dramatically during the last century due to availability of effective chemotherapy, and the subsequent development of case management strategies aimed at efficiently reducing transmission of *Mycobacterium tuberculosis*. However, the disease still have high burden in developing countries (Dye et al., 2005).

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The diagnosis of tuberculosis is based on the detection of *M. tuberculosis* in clinical specimens. The most commonly used and reliable specimen for bacteriological examination is sputum for the diagnosis of pulmonary TB (WHO, 2003). When the patient cannot expectorate sputum, several methods, such as laryngeal swab, sputum induction, gastric aspiration, and bronchoscopic lavage, can be used to obtain specimens for smear or culture examination for acid-fast bacilli (AFB). Acid-fast stain and culture of gastric aspiration specimens is mainly used in pediatric patients (Saka et al., 2006).

Although there are many sophisticated techniques for diagnosis of the disease such as molecular methods and direct examination of clinical specimens on light microscopy, cultures are superior among them (Dunlap et al., 2000). Culture is considered a gold standard in

clinical bacteriology. Despite the method is sensitive and the specificity of the method is close to 100%, however, it is time consuming and take 6 to 8 weeks for the results, and identification by biochemical tests makes the process longer. The culture method is also not cost efficient (Shah and Rauf, 2001; Chakraborty, 2003; Schoch et al., 2007).

Sputum smear microscopy for demonstration of AFB remains the most important diagnostic method for TB in high prevalence countries. Because of its speed, its high specificity even under adverse conditions, and its efficiency in detecting the main sources of transmission, it constitutes one of the pillars of the DOTS strategy (Selvakumar et al., 2005). In developing countries, sputum AFB smear microscopy is the primary tool for detecting pulmonary TB. The Ziehl-Neelsen (ZN) method is commonly used for staining sputum smears because of its simplicity and low cost (Karin, 1998). In spite of several modifications have been attempted to improve its sensitivity since Robert Koch first described it in 1882, still the disadvantage of the method is lower sensitivity compared to culture (Selvakumar et al., 2002). However, for developing countries with a large number of cases and financial constraints, evaluation of rapid and inexpensive diagnostic methods like demonstration of AFB in smears has great importance (Laifangbam et al., 2009). Besides, due to the risk of the spread of disease and the potential for the emergence of drug resistant strains, the rapid diagnosis of TB is very important (Khagi et al., 2009).

The aim of present study was investigation of prevalence of AFB in sputum and bronchoscopic lavage samples of patients with pulmonary infections and determination of the reliability of the method.

MATERIALS AND METHODS

During 14 months between November 2008 to March 2009, 2872 sputum and bronchoscopic lavage specimens belonging to 2191 hospitalized patients in pulmonary and internal medicine wards of Imam Khomeini teaching hospital, Ahvaz, Iran, and outpatients with symptoms of pulmonary infections referred to a private clinic were collected.

Spot and/or early morning sputum specimens from each hospitalized and outpatients were collected in clean, sterile, leak-proof, wide-mouth containers. From some of the hospitalized patients, 2 or 3 sputum specimens were obtained. Bronchoscopic lavage specimens belonged to hospitalized patients whom could not expectorate sputum and were provided by relevant wards of the hospital for bacteriological examination and decontamination. The processing of the samples was carried out by the Petroff's method for decontamination in a biosafety cabinet (Kent and Kubica, 1985). The specimen was spread evenly over a 1 to 2 cm area of a new slide, left to dry, and fixed over the flame of a spirit lamp. Prepared smears were stained by Ziehl- Neelsen staining method as per standard guideline (WHO, 1998; Enarson et al., 2000). Briefly, the slides were allowed to stand in 1% hot fuchsin stain for 5 min and were then washed in running tap water. The slides were differentiated by using 1% acid alcohol for 1 to 3 min and washed again in running tap water. The smear was stained with 1% methylene blue as counterstain for 30 s. The stained smears were drained and air dried thoroughly and examined under the light microscope with 100 objective and oil immersion. In AFB stain, bright pink to red, beaded or barred forms are seen in *M. tuberculosis* while the tissue cells and other organisms are stained blue. The grading for the number of observed bacilli was recorded according to the recommendations of the International Union against Tuberculosis and lung Diseases (IUATLD) as represented in Table 1 (Rieder et al., 1998).

Table 1. Criteria for acid fast bacilli smear reporting (Rieder et al., 1998).

Report of slide	Number of AFB in microscope fields
Negative	0 AFB were seen
Doubtful	1-2 AFB in 300 fields
1+	1-9 AFB in 100 fields
2+	1-9 AFB in 10 fields
3+	1-9 AFB in field
4+	>9 AFB in fields

RESULTS

In present study, 2758 (96%) sputum and 114 (4%) bronchoscopic lavage specimens were screened for AFB. The specimens were obtained from 1314 (60%) male patients and 877 (40%) females. One hundred and eighty three (6.4%) of total specimens, each belonged to individual patients were positive for AFB using Ziehl Neelsen staining. These were identified in 81.4% of sputum specimens and 18.4% of bronchoscopic lavages. Positive specimens belonged to 60.7% of male and 39.3% of female patients. Figure 1 represents the percentage and frequency of number of positive AFB specimens according to patient's sex.

The frequency and distribution of AFB seen in Ziehl-Neelsen (ZN) stain smears from sputum and lavage samples are shown in Figure 2. Majority of smears were graded as 3+ according to criteria for AFB smear reporting, which belonged to specimens from male patients, while the smear grading in female patients was mainly 2+ and 1+.

DISCUSSION

Detection of smear positive cases is the highest priority in any TB control program, as these cases are infectious and contribute to transmission of disease (Prasanthi and Kumari, 2005).

In our study, 6.4% of the total specimens examined were positive for AFB. We did not provide culture for the specimens since in Iran, TB laboratory services are organized down to the district hospital level providing only smear microscopy, but culture examination was available

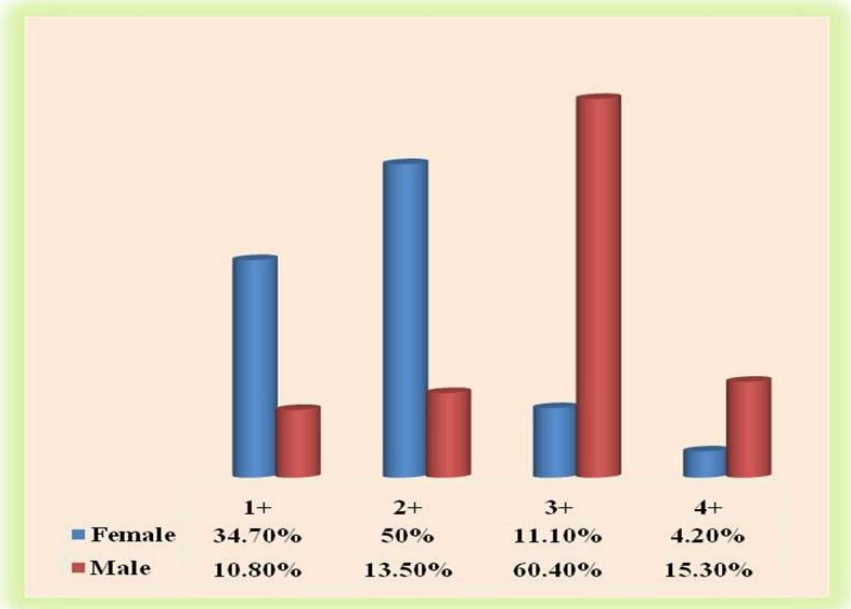

Figure 1. Distribution of positive specimens according to patients' sex.

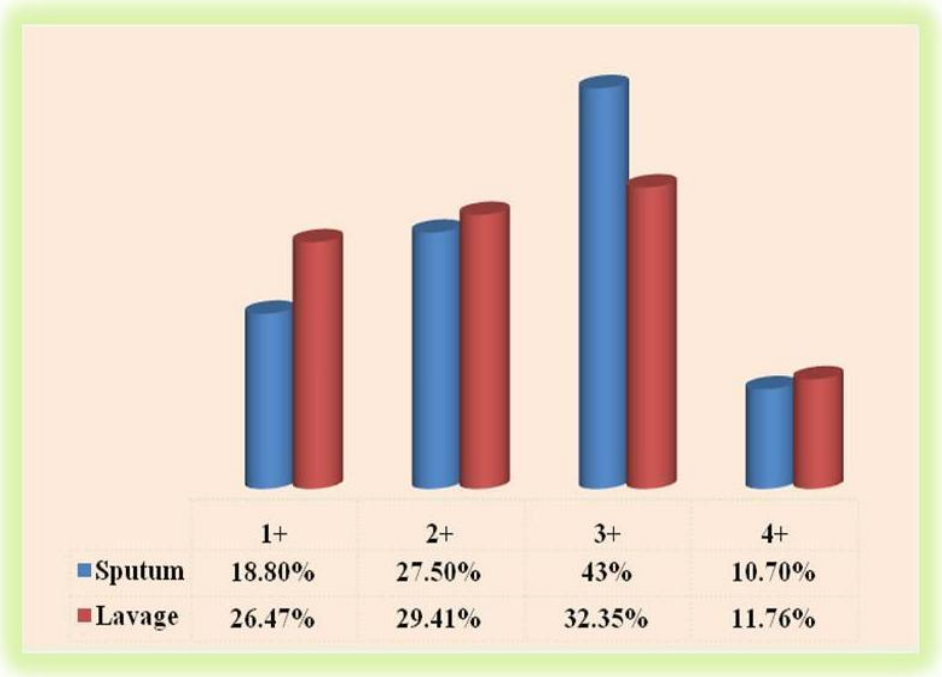

Figure 2. Distribution of positive specimens based on reporting criteria.

at the provincial reference TB centers and sensitivity tests are performed at the central level. So in this survey, we reported the prevalence of smear positivity. Although based on our experience from Tb reference laboratory in this province, the results of smear microscopy and culture is closely related. As reported in study of Kumar et al. (1998), 85% of the culture positive cases could be diagnosed by microscopy alone. However, the sensitivity of smear microscopy, which is extensively used in many developing countries with satisfactory results as previously reported, varies from 30 to 80%, depending on various factors (Peterson et al., 1999). For instance one

of the factors is physical and chemical injury to the integrity of the cell wall, which is known to cause tubercle bacilli to lose their acid-fastness (Kim, 2002), and we believe that there is no need for the sputum specimens' decontamination process used for ZN staining without culture. We did not attempt to modify the integrity of standard ZN staining method, since referring to literature, there were reports of lesser positive rate by modifying the concentration of fuschin from 1 to 0.5% or 1 to 3% (Selvakumar et al., 2002; Selvakumar et al., 2005). The majority of our examined smears were 3+ in grading. The disadvantage of acid fast stain is that it has low sensitivity for smears with scanty number of bacilli, but it works well for smears with higher bacilli contents and in this study we could detect all smears with 1 to 3+ in grading, which was in agreement with clinical symptoms of the patients. In the present study, we did not screened the positive samples for non tuberculous mycobacteria. This was normally performed by culture and identification tests, which was not applicable and was not included in our study goals. However, we were not bothered about that since the prevalence of non tuberculous mycobacteria was very low in our region based on previous culture-based surveys with the latest fingerprinting study with 9% (Khosravi et al., 2009)

The results of present study indicated that ZN stain is the preferable method for all suspected tuberculosis cases especially in settings that the number of specimens is high and the culture is not available.

ACKNOWLEDGEMENTS

This work was supported by a grant (No. S.53-88) from Research Affairs, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran. We thank Miss Afrooz Morvaridi from Central Laboratory, Imam Khomeini Teaching Hospital, Ahvaz, Iran, for providing smear slides and technical assistance.

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