A COMPARISION OF LABORATORY DIAGNOSTIC METHODS OF TUBERCULOSIS AND AETIOLOGY OF SUSPECTED CASES OF PULMONARY TUBERCULOSIS

Bhatt CP[1,2], Timalsina B,[2] Kutu B,[2] Pradhan R,[2] Maharjan B,[3] Shrestha B[3]

[1] Kathmandu Medical College Teaching Hospital, Sinamangal Kathmandu Nepal
[2] Nobel College, Sinamangal, Kathmandu Nepal
[3] German Nepal Tuberculosis Project, Kalimati, Kathmandu Nepal

ABSTRACT

Introduction: Tuberculosis remains a worldwide public health problem despite the highly effective drugs and vaccines are available making tuberculosis a preventable and curable disease. The objective of this study was to compare the different laboratory diagnostic methods of tuberculosis and determine its prevalence.

Methodology: Morning sputum samples were collected from suspected cases of pulmonary tuberculosis and proceeded for Ziehl Neelsen staining, fluorescent staining (auramin-O) and mycobacterium culture in Lowenstein Jensen medium.

Results: Total 78 suspected cases of pulmonary tuberculosis were included in this study among them 53 were male and 25 were female. Out of 78 cases, 46 cases were found to be culture positive. In culture positive cases (83%) were found to be *M. tuberculosis* and (17%) were found to be slow grower, fine colonies, AFB positive but niacin test negative (mycobacteria other than *M. tuberculosis*). In the direct microscopic examination by Ziehl Neelsen stained smear 26 samples were found to be acid fast bacilli and one sample was culture negative but acid fast bacilli positive. In fluorescent stained smear 34 samples were found to be positive for acid fast bacilli and 5 samples were culture negative but acid fast bacilli positive. Culture was accepted as gold standard, the sensitivity of direct microscopic examination was found 56.5% for Ziehl Neelsen staining and 73.9% for fluorescent staining respectively.

Conclusion: In culture positive cases *M. tuberculosis* and mycobacteria other than *M. tuberculosis* was found to be 83% and 17% respectively, it was found higher in male than female. Fluorescent microscopy is superior to Ziehl Neelsen microscopy but gives more false positive result than Z-N staining. Combining of Ziehl Neelsen and fluorescent staining is better than fluorescent staining alone.

Key words: Fluorescent Microscope, Mycobacterium Culture, *M. tuberculosis,* Ziehl Neelsen Stain.

INTRODUCTION

Tuberculosis is a disease of poverty affecting mostly young adults in their most productive years. The vast majority of TB deaths are in the developing world. The tuberculosis continues to be a great public health problem in Nepal. The number of new cases is increasing due to failure in early detection and drug resistance. These are the important problem in treatment and cure of the tuberculosis.[1] Cure of the disease is possible only with correct diagnosis and appropriate treatment. Early and accurate detection of active cases remains an important objective for improved implementation of chemotherapy and for reduction in the spread of the disease.[2] The diagnosis of tuberculosis is largely based on conventional approaches, which rely on clinical features and the results of X-ray, microscopy and culture examination. ZN staining or fluorescent staining allows highly accurate diagnoses which are widely available, simple and quick method.[3] The

Correspondence:
Dr. Chandra Prakash Bhatt
Associate Professor and Head of Department
Department of Microbiology, Kathmandu Medical College Teaching Hospital, Sinamangal
Kathmandu, Nepal
E-mail: drcpbhatt@yahoo.com, drcpbhatt@gmail.com

diagnosis traditionally depends upon identifying the infective organisms in secretions or tissues of diseased individuals. Sputum is the main sample for pulmonary tuberculosis.[4]

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 smears examination (Microscopy) is time honored and economical, but for this technique the yields requirement is between 5000 to 10,000 organisms per ml.[5] Conventional culture methods are sensitive and can detect 10-100 organisms per sample. However, culture methods are time consuming and take 6-8 weeks for the results. Furthermore, viable organisms are needed for culture.[6, 7]

Mycobacterium tuberculosis is the most important causative agent of tuberculosis (TB) while nontuberculous mycobacteria (NTM) may play a key role in etiology of TB-like syndromes.[8] Treatment of TB patients in most countries is based solely on the results of microscopic smear positivity. As such, all sputum smear positive diagnosed patients are indiscriminately placed on DOTS, the current international TB treatment strategy. The implication is that NTM is inappropriately managed with first-line anti-tuberculous drug worsening the patient's condition and raising the risk of drug resistance.[9, 10] Although it is known that most sputum smear positive patients are truly TB patients.[11] The continued increase in TB drug resistance raises the question on the impact of this indiscriminate use of TB drugs to treat all diagnosed sputum smear positive patients. In this study, the staining methods (Z-N and fluorescent staining) are compared and evaluated taking mycobacterium culture on Lowenstein- Jensen (LJ) medium as gold standard and determine the aetiology of suspected cases of pulmonary tuberculosis.

METHODOLOGY

A Prospective study was conducted at German Nepal Tuberculosis project; Kathmandu duration of the study was from July 2010 to December 2010. In this study 78 suspected cases of pulmonary tuberculosis were included.

Sample collection

Sputum specimen was collected in clean, sterile, leak-proof, wide-mouth containers. The processing of the samples was carried out in a bio-safety cabinet. The sample collected was evaluated in terms of its acceptability, proper labeling such as full name, age, sex, serial number of the patient, date of collection.

Microscopy

Morning sputum sample was collected, stained by Ziehl Neelsen staining and auramine-O fluorochrome method and observed under compound binocular microscope and fluorescent binocular microscope respectively.

Culture

For mycobacterium culture sputum sample were decontaminated and centrifuged by using 4% NaOH, according to modified Petroff method and inoculated into Lowenstein Jensen medium. Lowenstein-Jensen media were incubated at 37°C and left in the slanted position for 7 days to permit even distribution of the inoculum over the entire surface of the medium. The tubes are then placed upright and incubation at 37°C for 6-8 weeks.

Observation of colony morphology

The colonies of *M. tuberculosis* were rough, dry, 3-4mm in diameter, raised, and thick with wrinkled surface and an irregular thin margin. They were non-pigmented (off-white to faint buff), tenacious and not easily emulsified.

Identification of *M. tuberculosis*

For identification of *M. tuberculosis* rate of growth, colonies characters, AFB staining and niacin test were used as per manufacture instruction.

Niacin drop test

Niacin drop test was performed according to manufacturer instruction to identify *M. tuberculosis* and differentiated it from mycobacteria other than *M. tuberculosis.*

Ethical consideration

The research objective and methods were explained to the patient and informed consent was

taken from each participant before collection of sputum specimen.

Data analysis

Data was analyzed by EPI-Info version 3.3.2, document version 8.08 updated Sept 2005 and presented by chart and diagrams.

RESULTS

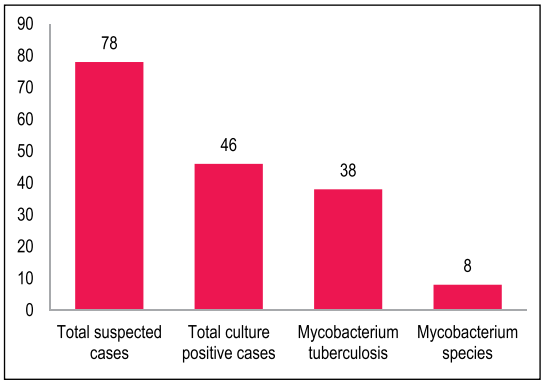

Figure 1. Total suspected and mycobacterium culture positive cases culture positive cases

Out of 46 cultures positive cases, 38 (83%) was found *M. tuberculosis* and 8 (17%) was found mycobacteria other than *M. tuberculosis.*

Forty six cases were found to be mycobacterium culture positive. In the direct microscopic examination by Ziehl Neelsen and fluorescent stained smear 26 and 34 samples were found to be acid fast bacilli and one and five samples were culture negative but acid fast bacilli positive respectively.

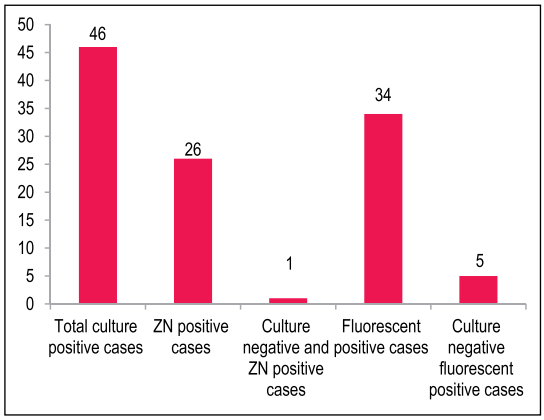

Figure 2. Comparison of mycobacterium culture, Ziehl Neelsen and fluorescent microscopy

Mycobacterium species was found higher in male than female and isolated maximum in age group 16-30 (43.8%) followed by the age group 46-60 (34.7%).

Niacin test was performed for 46 culture positive cases. Among them 38 was niacin positive (*M. tuberculosis*) and 8 was niacin negative (mycobacteria other than *M. tuberculosis*).

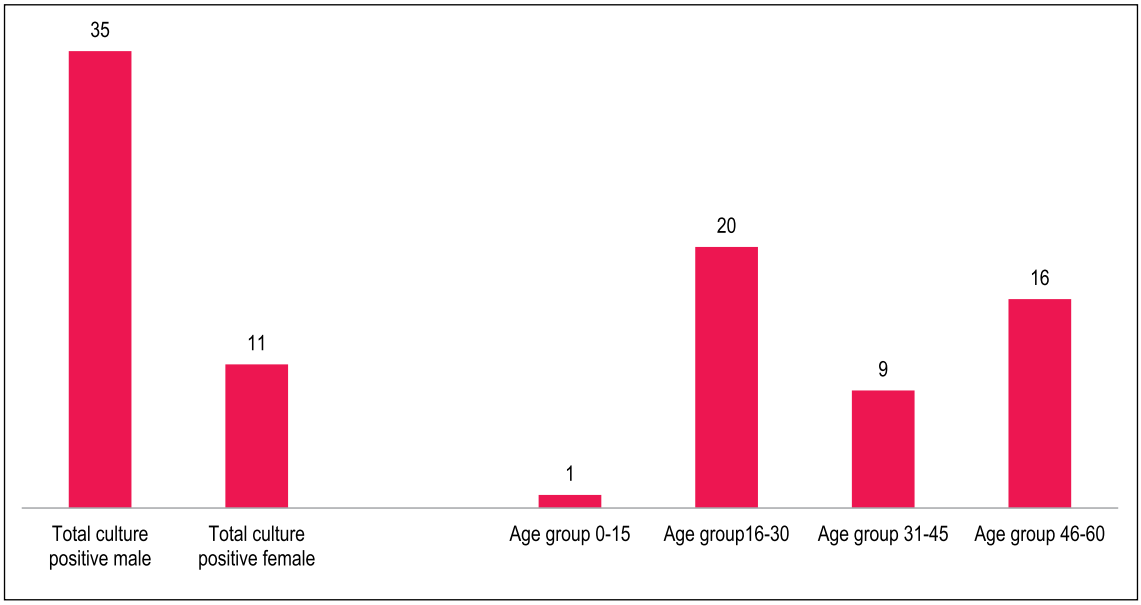

Figure 3. Gender and age wise distribution of positive cases

DISCUSSION

According to the WHO guidelines for TB control, patient with more than three weeks history of cough should be screened for pulmonary tuberculosis with direct sputum smear examination for *M. tuberculosis*. Because the clinical signs and symptoms of pulmonary tuberculosis are not specific, the fundamental principal for the diagnosis of tuberculosis is the accurate demonstration of *M. tuberculosis* in a suitable specimen from the suspected cases of pulmonary tuberculosis for the adequate treatment. Either presumptive diagnosis is based on the demonstration of tubercle bacilli in the sputum or *M. tuberculosis* may be demonstrated by culture.[12] 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. Though smear positivity correlates well with infectivity, much of the transmission occurs before the level of bacilli reach 10^5/ml in the sputum.[13] ZN stain can detect bacilli when they are in the order of 10^5/ml of the sputum whereas a more sensitive fluorescent stain can detect in the order of 10^4/ml of sputum.[14]

The findings of present study showed that out of 78 cases 46 (58.97%) cases was culture positive. In culture positive cases 83% was found to be *M. tuberculosis*. In culture positive cases 34 (73.9%) were positive for fluorescent staining (auramin-O) and 26 (56.5%) were positive for Ziehl Neelsen staining. This finding agrees with the study done in the other parts of the world fluorescent stain is superior to Ziehl Neelsen stain. A study conducted by Laifangbam *et al* showed that out of 102 patients suspected of pulmonary tuberculosis, 44.1%, 71.6% and 79% were found positive by ZN, auramine O staining and culture respectively.[13] Similar study conducted by Jain *et al* found sensitivity of ZN was 32.7% and auramine O staining was 41.6%[14], Githui *et al* found sensitivity of ZN was 65%, fluorescent staining was 80%[15], Ulukanligil *et al* found sensitivity of ZN was 67.6% and fluorescent staining was 85.7% [16] and Prasanthi and Kumar found sensitivity of ZN was 50% and fluorescent was 69%.[17] Fluorchrome stain is more efficient over ZN stain in detecting tubercle bacilli in sputum, especially the paucibacillary cases. Since screening is done under lower power of magnification (400X), fluorescent microscopy has been found to be less time consuming as compared to ZN method (1000X) in the diagnosis of tuberculosis. The fluorescing bacilli are easily identifiable and cause less eye strain. The advantages of sputum smear microscopy is that it has very close relation with infectiousness, patient who are sputum smear positive and culture positive are more likely to be infectious than culture positive but smear_negative. Thus the detection of AFB in sputum smear examination by microscopy play an important part in tuberculosis control programs as transmission of the disease is due mainly to patients whose sputum contains so many organisms that they are detectable by direct microscopy of sputum smear and is helpful to control highly transmission of tuberculosis patients. Ziehl Neelsen staining is rapid and inexpensive but requires a high amount of organisms in the specimens. Fluorescent microscopy is more significant than ZN microscopy but even has chance of observing false negative. Culture along with fluorescent microscopy should be the method of choice for the detection of TB cases in-spite 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. Finding of this study suggested that at least fluorescent microscopy and culture must be recommended and should not be rely on clinical symptoms only for the treatment of tuberculosis cases.

Out of 46 culture positive cases 35 were male and 16 were female. In this study 76% of patients accounted male is higher incidence than the study conducted by Bhatt *et al* (64%)[18] and Bam (65%).[19] This finding agrees with the study conducted in National Tuberculosis Center Thimi, Bhaktapur, Nepal by Bhatt *et al*[20] it was found that the incidence of pulmonary tuberculosis was higher in male 75% than female 25%. In almost all areas where the TB is the public health problem, the incidence of TB among women is less than man. Gender is not merely the biological difference but the differences between men and women in their roles, behaviors, expectations and opportunities within a social cultural and economic context. Nepalese society encounters gender disparities profoundly in many aspects in their lives such as, education, job opportunities, food & nutrition, morbidity & mortality pattern of diseases and health care. The low status

accorded to women in male dominated country like Nepal, their limited decision making power, restricted mobility and poor access to health care resources make them particularly vulnerable to ill health and reduce opportunities in accessing basic and available health care.

The majority (67.3%) of patients belongs to economically active young age group 16-45 years. The proportion was nearly same as reported by Bhatt *et al*[18] (62.7%) of the respondents belong to 21-50 years age group and Bam[19,20] reported (95%) was 15-54 years age group. More than 90% of global TB cases and death occur in the developing world, where 75% of cases are within the economically most productive age group (15-54 years). An adult with TB (in the developing world) loses on average 3-4 months of work time and the economic losses to the family and community are staggering. The estimates suggest a loss of 20-30% of annual household income and, if the person dies of the disease, an average of 15 years of lost income.[21] The finding of this study showed that *M. tuberculosis* was found to be 83% and mycobacteria other than *M. tuberculosis* was 17%. Similar study conducted by Shanker *et al* found 7.9% were atypical mycobacterium.[22] Kumar and Khurana found incidence of *M. tuberculosis* and atypical mycobacteria 91.6% and 8.4% respectively.[23] Other study conducted by Bhatt *et al* found all the isolates were *M. tuberculosis.*[18] Mycobacteria have been isolated from many sources including soil, animal and human faces, marshland, water (including lakes, rivers, estuaries, swimming pools, aquaria, and domestic water supplies), vegetation and human skin.[24] Definitive diagnoses of pulmonary nontuberculous mycobacteria infection are difficult. Because the organisms are often saprophytes, they may colonize airways rather than infect them. Cultures can be falsely positive in patients with chronic lung disease and falsely negative in infected patients without cavities.[25] Thus, identification of acid-fast bacilli at microscopy or isolation of nontuberculous mycobacteria in culture by itself is not enough evidence for establishing the diagnosis.[26] Further there is cross-reactivity between MAC and *M. tuberculosis* on the purified protein derivative standard test.[27]

Sample size of this study is small and for identification of *M. tuberculosis* rate of growth, colonies characters, AFB staining and biochemical test only niacin test was performed. So that it was not possible to identify niacin negative mycobacterium species (mycobacteria other than *M. tuberculosis)*. It is therefore recommended that a large sample size that would cover rural and urban area of Nepal to compare different diagnostic methods, determine prevalence of *M. tuberculosis* and identification of mycobacterium species different biochemical tests should be considered in further investigation.

CONCLUSIONS

The findings of this study showed that the efficacy of fluorescence microscopy proved to be much higher than conventional light microscopy. Combining of Ziehl Neelsen and fluorescent staining was better than fluorescent staining (auramine-O) alone both the staining significantly improve sensitivity and percentage of false negative result. In culture positive cases *M. tuberculosis* and mycobacteria other than *M. tuberculosis* was found to be 83% and 17% respectively, it was found higher in male than female. Diagnosis of pulmonary atypical mycobacterium infection may be difficult. The clinical and radiographic manifestations of infection are variable and frequently overlap. For the starting appropriate treatment of atypical mycobacterium diagnosis is the first step that assures full chances of cure because atypical mycobacterium are resistant to standard regime for tuberculosis.

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