Original Article

Sensitivity and Specificity of Auramin-Rhodamin Stain for Diagnosis of Pleural Tuberculosis

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Abstract

Background*: Tuberculosis remains a major public health problem in Bangladesh. Among the extra-pulmonary tuberculosis (EPTB), pleural is the second most common site. Tubercular pleural effusion may cause a diagnostic dilemma as mycobacterium could not be detected in most cases due to paucibacillary nature of effusion. Sensitivity of detection of AFB in pleural fluid by microscopy is very low.* ***Objective****: To evaluate the sensitivity and specificity of Auramine-Rhodamine staining.* ***Materials and Methods****: This cross-sectional analytical study was based on 183 specimens of pleural fluid which was collected from the patient of suspected pleural tuberculosis at Institute of Disease of the Chest and Hospital (NIDCH). After collection, the specimens were processed and stained with Auramine-Rhodamine staining method and cultured on MGIT960 medium.* ***Results****: Among 183 specimens, 09 (4.9%) were found positive by Auramine-Rhodamine stain and 16 (8.7%) samples were positive for growth on MGIT960 medium. Sensitivity and specificity of Auramine-Rhodamine staining was 56.3% and 100% respectively.* ***Conclusion****: This study demonstrates that Auramine-Rhodamine staining method may be a useful method where culture facilities are not available.*

Key words*: Tuberculosis; Auramine-Rhodamine staining; Tubercular pleural effusion*

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Introduction

Tuberculosis (TB) continues to remain a major public health problem causing significant morbidity and mortality worldwide.[1] It causes nearly nine million new active diseases and two million deaths worldwide every year. Geographically the burden of TB is highest in Asia (40% of total TB cases are in south East Asia) and Africa (26% of all cases). Bangladesh ranks the sixth position among 22 highest TB burden countries in the world and also one of the 27 high multidrug resistant tuberculosis (MDR-TB) burden countries where about 70,000 people die every year due to TB.[2] The WHO global TB report showed that the incidence of extra pulmonary-TB (EPTB) has increased to approximately 17% of all new and relapse cases of

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TB. Among the different types of EPTB, tuberculous pleural effusion (TBPE) is one of the most common forms.[3]

Various methods such as chest radiograph, different types of microscopy, cultural techniques, immunological techniques, nucleic acid based techniques and histopathological examination of pleural tissue for granulomatous inflammation can be used for diagnosis of TB as well as pleural TB.[4] Diagnosis of EPTB by detection of *Mycobacterium tuberculosis (*MTB) in the pleural fluid is considered difficult because of the paucibacillary characteristics of the disease.[3] A faster, simpler, more accurate and cost effective method of diagnosis is necessary for the successful treatment of the TB cases as wells as to prevent its spread in the community.[5] Despite the significant advances in molecular diagnosis of TB during last two decades, mycobacterial culture of the body fluid or biopsy specimens remains the gold standard for the diagnosis of EPTB, enabling complete post-culture antimicrobial susceptibility testing and genotyping.[6] Though cultural technique is considered as gold standard method, it also has low sensitivity (24–58%) and is limited by the lengthy delay in obtaining results.[4,7] Serological techniques have the disadvantage of lack of sensitivity and specificity. Molecular techniques such as polymerase chain reaction, although rapid, are costly to be routinely used in developing countries.[8] Smear microscopy is the simplest and quickest currently available procedure to detect Acid Fast Bacilli (AFB) in clinical specimens. Conventional Ziehl-Neelsen (ZN) stain for AFB plays a key role in the diagnosis and also for the monitoring of treatment in tuberculosis. Its major disadvantages are low (ranging from 20% to 43%) sensitivity. Fluorescent microscopy using Auramine-Rhodamine (AR) staining plays an important role for detection of Mycobacteria and has been considered to be superior to ZN staining. Though it has some limitations, it can provide rapid, safe and inexpensive technique for early provisional diagnosis of mycobacterial infection in cytological specimens.[9]

The mycobacterial growth indicator tube (MGIT) system, is part of the new-generation of rapid tests for detection of mycobacteria. This technique is based on fluorescence detection of mycobacterial growth in a tube containing a modified Middlebrooks 7H9 medium together with fluorescence quenching-based oxygen sensor.[10] MGIT-960 systems is a fully automated, non-radiometric, and used to incubate and monitor 960 samples at a time with automated result-reporting system. The methods designed to shorten turnaround time with good recovery rate than conventional solid TB culture system.[11] Studies on automated MGIT 960 systems have shown maximum recovery of mycobacteria. It has the advantage of high throughput, rapidity (4–13 days) and easier interpretation of results. All these aspects would be suitable for the TB detection in high-burden and resource-poor settings.[10]

Conventional fluorescence microscopy is more sensitive (10%) than the Ziehl-Neelsen and takes less time, but it is limited by the high cost of mercury vapor light sources, the need for regular maintenance, and the dark room requirement. Light-emitting diodes (LED) have been developed to offer fluorescence microscopy without the associated costs. It was more sensitive than conventional Ziehl-Neelsen microscopy and it had qualitative, operational and cost advantages over both conventional fluorescence and Ziehl-Neelsen microscopy. Based on these findings, the WHO recommends that conventional fluorescence microscopy be replaced by LED microscopy and that LED microscopy be phased in as an alternative for conventional Ziehl-Neelsen light microscopy.[12]

Considering cultural technique as the gold standard method for diagnosis of tuberculosis, present study was attempted to evaluate the sensitivity and specificity of Auramine-Rhodamine as a routine diagnostic tool of Pleural tuberculosis.

Materials and Methods

This cross-sectional analytical study was based on 183 specimens of pleural fluid which were collected

from the patients who attended indoor and outdoor of National Institute of Disease of the Chest and Hospital (NIDCH), Mohakhali, Dhaka during July 2017 to June 2018. This study was carried out in the National Tuberculosis Reference Laboratory (NTRL), Mohakhali, Dhaka. Clinically and radiologically diagnosed pleural effusion and suspected cases of tuberculous pleural effusion, irrespective of age and sex, who undewent thoracocentesis were included in this study. Known case of haemothorax, empyema thoracic and diagnosed case of heart failure, cirrhosis of liver, renal failure were excluded from this study. Before collecting specimens, each patient was interviewed; informed written consent was taken from patients or their legal guardians. Statistical analyses were carried out by using the Statistical Package for Social Sciences for Windows Version 21.0 (SPSS Inc., Chicago, Illinois, USA).

Specimen collection and processing

Pleural fluids were aspirated aseptically with a sterile syringe and about 50 mL aspirated fluid was taken in a sterile Falcon's tube for microscopy and culture. After sample collection, all the steps including sample processing, smear preparation and culture were performed in a class II safety cabinet in Bio-safety level-3 (BSL) laboratory. Rest of the pleural fluid was then centrifuged in a sterile Falcon's tube at 3000 rpm for 15 minutes and deposit was used for Auramine-Rhodamine staining and culture in MGIT-960 media.

Results

Table I shows the age distribution of this study population. Mean age of study population was 38.73 (±19.45) years, minimum age was 7 and maximum age was 90 years. Maximum study subjects (75, 41%) were found in the age group between 21−40 years, followed by 43(23.5%) in the age group 41−60 years, 37(20.2%) subjects in the age group ≤ 20 years and 28(15.3%) subjects belonged to age >60 years. Among the study population 115 (62.8%) were male 68 (37.2%) were female with male female ratio 1.7:1.

Table II shows the result of microscopic examination of Auramine-Rhodamine stain. Out of 183 samples 09 (4.9%) were found positive.

Table I: Age distribution of the study population

Age in years	Number of patients	Percentage
≤ 20 years	37	20.2
21−40 years	75	41.0
41−60 years	43	23.5
> 60 years	28	15.3
Total	183	100.0
Mean±SD	38.73 (±19.45)	

Table II: Result of Auramine-Rhodamine stain (n=183)

Auramine-Rhodamine stain	Number	Percentage
Positive	09	4.9
Negative	174	95.1
Total	183	100

Table III shows the rate of detection of *M tuberculosis* in liquid media (MGIT 960). Out of 183 samples 16 (8.7%) samples were growth positive and 167 (91.3%) showed no growth.

Table IV shows the sensitivity, specificity of Auramine-Rhodamine stain. Among the 16-growth positive by liquid culture (MGIT 960) 9 were also positive by Auramine-Rhodamine stain. All growth negative in liquid (MGIT 960) medium were also negative by Auramine-Rhodamine stain. We found Auramine-Rhodamine stain was 56.3% sensitive and 100% specific.

Table III: Isolation of *M. tuberculosis* in Liquid (MGIT 960) medium (n=183)

MGIT 960 media	Number	Percentage
Positive	16	8.7
Negative	167	91.3
Total	183	100

Table IV: Comparison between Auramine-Rhodamine staining with MGIT 960 medium

Auramine-Rhodamine stain	MGIT 960 medium		Total	Sensitivity (%)	Specificity (%)
	Positive	Negative			
Positive	09	00	09 (4.92)	56.3	100
Negative	7	167	174 (95.0)		
Total	16 (8.70)	167 (91.40)	183 (100)		

Discussion

In this study mean age was 38.73 (±19.45) years, minimum age was 7 years and maximum age was 90 years. Maximum (75, 41%) subjects were found between 21−40 years, followed by 43 (23.5%) in the age group 41−60 years, 37 (20.2%) belonged to age ≤ 20 years and 28 (15.3%) belonged to age >60 years. This observation was in agreement with the study of Lakshmi et al[13] which showed majority of the patients (48%) were between the age group of 21 and 40 years, followed by 11−20 years (17%) and 41−50 years (15%). Lusiba et al[14] also reported similar to the present study in which mean (±SD) of age was 34±13 years. Approximately similar result was reported by Zeka et al.[15]

This study showed male were predominant (115, 62.8%) and female were 68 (37.2%) with male:female ratio 1.7:1. Lakshmi et al[13] reported that male was found 64.0% and female was 36.0%. Male: female ratio of 1.8:1. Study of Lusiba et al[14] showed males were found 57.0% and females were 43.0%. The study of Laskar et al[16] reported, among 107 clinically suspected pulmonary tuberculosis cases 64 (59.81%) were male and 43 (40.19%) were female and male female ratio was 2:1.34. Torrea et al[17] reported that out of 247 pulmonary tuberculosis patients, 160 (64.78%) were male and 87 (35.22%) were female with male female ratio 1.83:1. Findings in all these studies are similar to the present study about gender distribution.

The reason of higher male tuberculosis cases than female cases might be explained by the fact that males are actively populated in the community and may come in contact with TB infected persons more frequently. But female members in Bangladesh still reside at home and therefore the chance of exposure is comparatively less. Smoking is also common in male.

In present study, out of 183 samples 09 (4.9%) were found Auramine-Rhodamine positive and 174 (95.1%) samples showed negative result. In study of Mistry et al[18] reported among 115 samples 6(5.21%) were positive by Auramine-Rhodamine staining method. This result is in agreement with the present study.

In present study showed *M. tuberculosis* detection from pleural fluid samples by liquid culture (MGIT 960) was 16 (8.7%) more than Auramine-Rhodamine staining 9 (4.9%). Among 9 fluorescence stained were positive cases all yielded growth in liquid culture (MGIT 960) media. All negative in liquid (MGIT 960) media were also negative in Auramine-Rhodamine staining. This correlates with the study of Siddiqui et al.[19]

In conclusion, Auramine-Rhodamine staining method offers several advantages: highly specific, rapid and does not require high expertise. It is much more convenient for screening a large number of samples in the routine clinical laboratories have to test every day. Thus, it is recommended that where fluorescent microscope is available, Auramine-Rhodamine method should be used for detection of mycobacteria.

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