



Comparison of Ziehl Neelsen Stain, Auramine Rhodamine Stain and Culture Sensitivity of AFB in Routine and Concentrated Pleural Fluid

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ABSTRACT

Background: Nearly one third of the global population is infected with mycobacterial tuberculosis. Pleural tuberculosis accounts for 20% of extrapulmonary tuberculosis. The diagnosis of tuberculous pleural effusion is difficult because of the low detection rate of different diagnostic tests like microscopy and culture. Current study aimed to compare the detection rate of different tests in non-concentrated and concentrated pleural fluid.

Methods: A hospital based prospective cross sectional study was carried out over one year duration in the Medicine Department of Bir Hospital. A total of 52 cases were enrolled. Detailed history taking and physical examination; radiological, hematological and serum biochemical investigations were performed. Thoracentesis was performed in all the patients; 20 ml pleural fluid was sent for microscopy with ZN and AR staining as well as for AFB culture. Up to 500ml of pleural fluid was heparinized and kept on cylindrical jar for two hours and 50 ml of sediment was also sent for microscopy and culture within one hour. The results obtained were documented and analysis was done.

Results: A total of 52 patients, 31 (59.6%) males and 21 (40.4%) females were included. Their mean age of study participants was 38.67 ± 17.71 (range 16-82 years). Common presentations were fever (94.2%), cough (92.3%), breathlessness (84.6%), chest pain (65.4%) and significant weight loss (59.6%). Haemoptysis was present in 13.5%, anemia in 48.1%, enlarged cervical lymph nodes in 5.8% of the patients. The detection rates of ZN stain, AR stain and AFB culture in non-concentrated pleural fluid were 3.8%, 9.6% and 11.5% respectively. The detection rates for the same tests using concentrated pleural fluid of the same patients were 7.7%, 25% and 17.3% respectively. Differences in detection rate with AR stain and AFB culture for non-concentrated and concentrated pleural fluid were statistically significant (p value <0.01).

Conclusion: The detection of AFB using microscopy with ZN and AR staining as well as culture in solid media is low. The detection rate was significantly increased by using concentrated pleural fluid sample for microscopy and culture.

Keywords: Diagnosis, Extra-Pulmonary tuberculosis, Pleural effusion



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INTRODUCTION

Tuberculosis (TB) is a major public health concern with nearly one third of the global population being infected with mycobacterial tuberculosis. Nearly 10 million people got infected with TB in 2019. It is the leading cause of death from single infectious agent.¹ In Nepal, about 45 percent of the total population is infected with TB. Every year, 40,000 people develop active TB, of whom 20,000 have infectious pulmonary disease who are able to spread the disease to others and 5,000-7,000 people still die per year from TB.²

Pleural effusion is a common complication of primary tuberculosis, alone or in conjunction with pulmonary infiltrate typical of post primary tuberculosis.³ Tuberculous

pleural effusion accounts for approximately 5 percent of all disease due to Mycobacterium tuberculosis and is the second most common form of extra-pulmonary tuberculosis. The effusions are thought to result from a delayed hypersensitivity reaction to mycobacterial antigens in the pleural space. The clinical presentation of TB can mimic several diseases and can be a diagnostic problem even in endemic areas. Virulence and load of the infecting mycobacterium, the immune status of the host, the organ system involved, all influence the clinical manifestations of tuberculosis. It usually presents as an acute febrile illness causing a nonproductive cough (94%)

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and pleuritic chest pain (78%) without an elevation in the peripheral white blood cell (WBC) count. Night sweats, chills, weakness, dyspnea, and weight loss can also occur.⁴

The physical signs of the presence of pleural effusions may identify patients who require further diagnostic procedures. In tuberculous effusion, the pleural fluid is an exudate with predominantly small lymphocytes. The diagnosis is established by demonstrating high levels of TB markers in the pleural fluid (adenosine deaminase (ADA), interferon, or positive polymerase chain reaction (PCR) for tuberculous DNA). However, cost and difficulty of these investigations make them less useful in developing countries. Alternatively, the diagnosis can be established by culture of the pleural fluid, needle biopsy of the pleura, or thoracoscopy.⁵ A definitive diagnosis of tuberculous pleural effusion requires the demonstration of AFB in sputum, pleural fluid or pleural biopsy tissue or demonstration of granulomas in pleural tissue.⁶

The diagnosis of tuberculous pleural effusion can be difficult to make because of nonspecific clinical presentation and paucibacillary nature of the disease even with numerous advancements in diagnostic modalities.⁶ The detection rate of various diagnostic tests like Ziehl Neelsen stain, Auramine Rhodamine fluorescent stain and culture sensitivity of mycobacterial tuberculosis is very low.⁷ With this study we aimed to find out whether concentrating the pleural fluid at bed side increases the yield of AFB. The main objective of this study was to compare the detection rate of Ziehl Neelsen, Auramine Rhodamine and AFB Culture in routine and concentrated pleural fluid.

METHODOLOGY

This was a hospital based cross sectional study conducted over one year duration at Bir Hospital, National Academy of Health Sciences (NAMS). The study was conducted after ethical approval from Institutional Review Committee (IRC). Patients aged 18 years or more admitted to medical ward with provisional diagnosis of tuberculous pleural effusion based on clinical and investigational support were included in the study. Those patients who were already on ATT or have multiple pathology of pleural effusion; those with renal insufficiency and/or liver insufficiency and the patients refusing consent were excluded from the study.

Detail history and examination were performed for all the patients. For all the patients routine investigations required for management including WBC, ESR and chest x-ray, sputum for AFB, were sent. Thoracocentesis was performed for all the patients. Twenty milliliters of pleural fluid was sent for routine ZN and AR staining as well as AFB culture. The pleural fluid was also sent for pleural fluid cytology, protein and ADA estimation. Then, up to 500 ml of pleural was collected in a cylindrical jar and regular heparin was added to the collected sample at a ratio of 1ml of heparin (1:1000) for every 50 ml of pleural fluid.⁸

The heparinised sample thus collected was allowed to stand aside undisturbed for two hours to allow sedimentation to occur. The lowermost 50 ml of the sediment was obtained from the reservoir and sample was sent for microbiological analysis (ZN, AR staining and culture) within one hour. The sample was further centrifuged and then decontamination was done by standard method in lab. The ZN, AR staining and cultures of AFB were done by standard methodology. The reports of these three tests were noted for each patient.

The collected data were entered into the master chart and then analyzed using Statistical Package for Social Science (SPSS) Version 11.5. The frequency distribution was calculated for categorical variables and the continuous variables were expressed as mean \pm SD. The correlation between diagnostic tests producing nominal data was done by Chi-square test and Fischer Exact test wherever applicable. The p value <0.05 was considered statistically significant.

RESULTS

Total of 55 patients with provisional diagnosis of pleural effusion were included in the study. Three patients had alternative diagnosis on follow up visit so were excluded from the analysis. Therefore, remaining 52 patients were taken for analysis. The mean age of the patients was 38.67 ± 17.71 years with 40.3% being less than 30 years of age. The male: female ratio was 1.46.

The most common presenting symptoms were fever and cough noted in 94% and 93% of patients respectively. The presenting symptoms and signs are depicted in Table 1.

Table 1: Presenting symptoms and signs of patients with tuberculous pleural effusion.

Symptoms and signs	N (%)
Fever	49 (94.2)
Cough	48 (92.3)
Shortness of breath	44 (84.6)
Chest pain	34 (65.4)
Weight loss	31 (59.6)
Pallor	25 (48.1)
Hemoptysis	7 (13.5)
Enlarged lymph node	3 (5.8)
History of smoking	28 (53.8)
Positive family/ contact history	12 (23.1)

The pleural fluid sample was positive for ZN staining in 3.8% in non-concentrated sample as compared to 7.7% of concentrated

sample. Similarly, AR staining was positive in 9.6% of routine sample compared to 25.0% of concentrated sample and AFB culture was positive in 11.5% of routine as compared to 17.3% of concentrated sample as presented in Table 2.

Table 2: Comparison of positive rate for different microbiological tests in routine and concentrated sample

Microbiological tests	Positive rate in non-concentrated sample N (%)	Positive rate in concentrated sample N (%)	P-value
ZN stain	2 (3.8)	4 (7.7)	0.15
AR stain	5 (9.6)	13 (25.0)	<0.01
AFB Culture	6 (11.5)	9 (17.3)	<0.01

The association between the results obtained from analysis of non-concentrated pleural fluid and concentrated pleural fluid is presented in Table 3.

Table 3: Association between pleural fluid analysis by different microbiological tests in routine and concentrated pleural fluids

	Concentrate		Total	P-value
	Positive	Negative		
Routine	ZN staining (n=52)			
	Positive	1	1	2
	Negative	3	47	50
	AR staining (n=52)			
	Positive	5	0	5
	Negative	8	39	47
	AFB Culture (n= 52)			
	Positive	5	1	6
	Negative	4	42	46

*Fischer Exact test used.

DISCUSSION

Tuberculosis is a major public health problem globally as well as in Nepal. About 45 percent of the total population in Nepal is infected with TB, of which 60 percent are adults² Tuberculous pleural effusion is one of the most common forms of extrapulmonary tuberculosis (TB).⁹ In this study, we have attempted to compare the microbiological tests in routine and concentrated pleural fluid in provisional diagnosis of tuberculous pleural effusion.

Among 52 patients, 60% were male with male: female ratio 1.46:1. The mean age of the patients was 38.67 ± 17.71 years. Many other studies have also demonstrated male preponderance and mean age of the participants are middle aged.¹⁰⁻¹² The most common presenting symptoms were fever, cough shortness of breath and chest pain in decreasing order. Similar findings were reported in other studies too. However, our study has higher percentage of patients having shortness of breath.^{11,13,14}

The microscopic examination pleural fluid smear can identify AFB in less than 10% of the cases.^(11,15) Similar results were obtained in our study with Ziehl-Neelson stain and Auramine Rhodamine staining. However, the detection rate was almost double for ZN staining and almost three times more for AR staining if concentrated pleural fluid was used. While analyzing the detection rate according to age group, AR staining was positive in 4 out of 10 patients in patients aged more than 50 years if concentrated pleural fluid was used. Culture of pleural fluid can be performed using either solid media or liquid media. In our study we used the solid media. With solid culture media the sensitivity is low (12-30%) in contrast to 70% sensitivity with liquid media.⁷ In our study the detection rate of AFB culture was 11.5%. The detection rate was 17.3%, significantly higher while using concentrated pleural fluid. However, different studies have reported pleural fluid culture positive rate up to 36% without concentrating the pleural fluid and pleural tissue culture positive rate of 56.4%.^{16,17}

In our study using concentrated pleural fluid detected six percent of patient who were considered negative by ZN staining in routinely sent pleural fluid sample. Similarly with the use of AR staining 17% patient considered negative were found to be positive while using concentrated pleural fluid. With concentrated pleural fluid, culture for AFB was also positive in around 9% patients who had negative culture result with non-concentrated pleural fluid. Even though our study demonstrated significant increase in yield rate while using pleural fluid concentrates from larger volume (11.5 vs 17.3; p<0.01), other studies have not demonstrated significantly improved detection rate with larger volume of pleural volume (53.5% vs. 50% respectively; p=0.75).¹⁸ The reason behind this may be because we use 50 ml concentrated sample from 500 ml of pleural fluid while the cited study used 100 ml of non-concentrated sample. Some other studies have demonstrated

higher yield of culture with bedside inoculation as compared to laboratory inoculation.¹⁹

The AFB detection rate of pleural fluid culture was higher than AR staining which was further higher than ZN staining in non-concentrated pleural fluid. This may be because fluorescence microscopy can scan about 15 times as many fields as by conventional microscopy in the same period. Therefore, there is a higher probability of finding AFB, particularly if a smear contains only a few bacilli.²⁰ In concentrated pleural fluid detection rate of AR stain is more positive than that of ZN stain and AFB culture. This was probably due to delay in transport of pleural fluid and long duration of decontamination of fluid.

The study demonstrated that the detection of AFB by different microbiological tests is improved if concentrated pleural fluid is used. Nevertheless, the study is limited by small sample size. Also with marginal improvement in detection rate, generalized use of this method in clinical practice is still to be established.

CONCLUSION:

The detection of AFB using microscopy with ZN and AR staining as well as culture in solid media is low. The detection rate was significantly increased by using concentrated pleural fluid sample for microscopy and culture. Nevertheless, studies with larger sample size are required to derive definite conclusion.

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