ORIGINAL ARTICLE

Tuberculous Pleural Effusions: Efficacy Utilization and Comparison of Various Diagnostic Techniques

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ABSTRACT

Aim: To observe the efficiency and utilization of smear microscopy, AFB culture, GeneXpert, fluid cytology and adenosine deaminase levels in diagnosing TPE and comparing them with each other
Study design: Cross Sectional Study
Place and duration: Department of TB and Chest Medicine King Edward Medical University/Mayo Hospital Lahore from June 2016 to December 2017
Methods: Specimens of aseptically collected pleural fluids with a quantity of 20 ml or above were received in the lab and divided in at least 3 portions. One half of the each specimen was used for microbiology i.e. smear and culture, one fourth for cytology and biochemical examination and one fourth was used for GeneXpert MTB Rif Assay.
Results: Of the total 143 patients 70 (48.9%) were males and 73 (51.1%) were females with mean age of 34.63±15.93 years. Fourteen (9.8%) of the patients were categorized under relapse and 6 (4.2%) were defaulters while rest of the patients were new patients. History of contact was present in 87 (60.9%) cases. Response to ATT was taken as gold standard in present study therefore all patients were followed for 2 months, so 124 (86.7%) patients showed good response. Sensitivities of fluorescent microscopy, culture, GeneXpert, Lymphocyte counts and adenosine deaminase levels remained 9.7%, 21.8%, 17.8%, 79.1% and 93.8% respectively.
Conclusions: Although bacterial confirmation of TB bacilli is necessary for definite diagnosis but sensitivity is compromised and multivariate approach is necessary and still in use for timely management of TPE.
Keywords: Pleural Fluid, Tuberculosis, Extra-pulmonary tuberculosis, GeneXpert

INTRODUCTION

Tuberculosis (TB) caused by Mycobacterium tuberculosis complex (MTC) is responsible for considerable mortality and morbidity among both developed and developing countries. It is among the 10 upmost causes of death and foremost cause due to single most infectious agent[1]. This is a contagious disease and currently two billion people are suffering from TB around the world, moreover the organism is survived from more than 70,000 years[2]. A general concept about MTC is its origination from other organisms same genus and disease was named as consumption, phthisis, White Plague and Koch's disease in the history. WHO has place Pakistan amongst the top 20 high burden countries contained with 87% load of total global TB. Since the country consists 525000 new cases of active TB with mortality and incidence rates of 28 and 267 per 100000 respectively[1].

As MTC can infect each and every part of body however if it typically infects the bronchial parts of lungs such type of TB is called pulmonary while rest of all infected parts are categorized under extra-pulmonary TB. Burden of extra-pulmonary TB vary in various reports as 10-34% in Human immunodeficient virus (HIV) negative cases while 50-70% in HIV positive patients[3]. Recently WHO has reported a worldwide incidence of extra-pulmonary TB as 14% while 24% in Eastern Mediterranean Region that includes Pakistan[1]. Tuberculous pleural effusion (TPE) is the second most common type of extra-pulmonary TB which is characterized by an extreme chronic fluid accumulation in pleural space results in severe inflamation[4].

Many medical conditions other than TB can lead to pleural effusions which include congestive heart failure, cirrhosis, pulmonary embolism, leaking from other organs, cancer, infections and auto-immune disorders etc[5]. Shortness of breath, chest pain, fever and cough are the most common symptoms of pleural effusions. Chest X-ray, computed tomography (CT) scan and ultrasound can be used to monitor its presence but are not definite when cause is unknown or need to probe with suspicion to TPE[6]. Although TB has remained a main concern in medical diagnostics in current era of science and technology where many infectious diseases are diagnosed and monitored using molecular techniques but remained limited in case of TB[7].

Basic diagnostic test readily available in developing countries for screening of TB is smear for acid fast bacilli (AFB) by using Ziehl Neelsen (ZN) staining with compromised sensitivity of 55-70% reported in literature[8,9]. Due to paucibacillary nature of fluids large quantities are desired to centrifuge for the purpose of concentration of bacilli before smear microscopy even then the sensitivity remains low[10]. Auramine staining of smear through fluorescent microscopy although improved a bit but could not achieve desired results[8]. Culture on the other hand is still a gold standard but takes as long as 6-8 weeks for final

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diagnosis but GeneXpert MTB/Rif Assay an emerging molecular technique has excellent specificity however poor specificity in case of smear negative specimens especially TPE[11].

So formal guidelines lack for diagnosis and treatment of TPE therefore, a mix venture of x-ray, CT scan or ultrasound with smear and or culture are being used according to existing infrastructure in most of the healthcare facilities. GeneXpert is present at various sites is under estimated in this regard hence ignored. Present study was undertaken to observe the efficiency and utilization of smear microscopy, AFB culture, GeneXpert, fluid cytology and adenosine deaminase levels in diagnosing TPE and comparing them with each other.

MATERIAL AND METHODS

This cross sectional study was undertaken in department of TB and Chest Medicine King Edward Medical University/Mayo Hospital Lahore from June 2016 to December 2017. Adult patients suspected to have TPE on the basis of clinical examination and history whom were referred to lab for fluid examination by physicians were included in this study. Patients having the history of congestive heart failure, liver cirrhosis, pulmonary embolism, cancer and auto-immune disorders were excluded from the study. Patients defaulted during intensive phase of treatment were also excluded from the study. A pre-designed questionnaire was used to collect all the information including demographic characteristics and history of patients.

After taking the informed consent from patients' specimens of aseptically collected pleural fluids with a quantity of 20 ml or above were received in the lab and divided in at least 3 portions. One half of the each specimen was used for microbiology i.e. smear and culture, one fourth for cytology and biochemical examination and one fourth was used for GeneXpert MTB Rif Assay. First half portion of each specimen was centrifuged at 3000 rounds per minute for 5 minutes in sterile test tubes and supernatant was separated while sediment was re-suspended by tabbing test tube on table top. A drop of re-suspended material was placed on each labeled glass slide to prepare smear and allowed to dry. Smears were heat fixed by using hot plate and stained by using Auramine staining technique and observed under fluorescent microscope[12].

Pertroff's method for decontamination and concentration was used before inoculation of specimens on labeled Lowestein Jensen (LJ) medium tubes and incubated the tubes for 4-8 weeks. Culture readings were done on weekly basis and culture tube containing a minimum of 3 colonies of AFB was considered as positive and reported accordingly[13].Lysing reagent provided with kits of GeneXpert was mixed with almost five ml of fluid, vortexed and allowed to stand for 15 minutes. Two ml of the mixture was then added to the cartridge provided with different chambers containing desired reagents and conditions for real time polymerase chain reaction[13]. Adenosine deaminase (ADA) levels were measure by the supernatant of already centrifuged fluids. Cytology was performed by doing cell counts and neutrophils & lymphocytes ratio using from third portion of fluid[14].

Data was entered and analyzed by using statistical package for social science (SPSS) version 20.0. Qualitative like gender, history and marital status etc. variables were presented in the form of frequency and percentages while quantitative variables like age and biochemical test values were presented in the form of mean ± standard deviation. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy of all the tests were also calculated by taking response to anti tuberculosis treatment (ATT) as gold standard.

RESULTS

A total of 152 patients suspected to have TPE brought their pleural fluid samples for analysis of which 4 have quantity less than 10 ml which was insufficient to perform all the tests and 5 patients were not found on follow up hence excluded. Of the remaining 143 patients 70 (48.9%) were males and 73 (51.1%) were females with male to female ratio of 1:1.04. Overall mean age of patients remained 34.63±15.93 while mean age of male patients was remained 35.10±15.90 and that of female was 34.45±15.85. Table I shows the distribution of patients according to their age groups among both genders.

Table I: Gender-wise distribution with age groups (n=143)

Age Group	Male	Female	Total
≤20 Years	11(15.7%)	16(21.9%)	27(18.8%)
21-30 Years	31(44.2%)	22(30.2%)	53(37.1%)
31-40 Years	12(17.2%)	13(17.8%)	25(17.5%)
41-50 Years	12(17.2%)	3(4.1%)	15(10.5%)
≥51 Years	4(5.7%)	19(26%)	23(16.1%)

Table II: Demographic characteristics of patients (n=143)

Affects	Frequency	%age
Marital status		
Married	91	63.6
Un-married	45	31.5
Others*	7	4.9
Education		
≤Primary	84	58.7
Middle	31	21.7
Matriculation	16	11.2
Higher Above	12	8.4
Socio-economic Status		
Poor	53	37.1
Middle	69	48.3
High	21	14.6
History of Smoking		
Smokers	52	36.4
Non Smokers	91	63.6
History of Contact		
Present	87	60.9
Not Established	56	39.1
Outcome of Previous anti-TB Treatment		
No History of Treatment	119	83.2
Defaulted	6	4.2
Relapse	14	9.8
Failure	0	-
Unknown	4	2.8
Family Size		
≤5	37	25.9
6-10	65	45.5
≥11	41	28.6

*Includes divorced, separated and widow

Fourteen (9.8%) of the patients were categorized under relapse as they had previous history of ATT while 6(4.2%) were defaulters while rest of the patients were new patients. History of contact was present in 87 (60.9%) cases. Other demographic characteristics of patients including marital status, education, socio-economic status etc. are presented in table II.

Fluorescent microscopy of pleural fluids just revealed 8.4% positivity in present study followed by GeneXpert 15.4% and culture on LJ medium showed 18.8% positivity. Lymphocytic counts of ≥70% were observed in 70.6% cases where as ADA levels ≥40 IU/L were observed in around 84% study subjects most of which (56.6%) had ADA levels ≥100 IU/L.

All the cases after clinical and diagnostic assessments were put on ATT by physician, as response to ATT was taken as gold standard in present study therefore all patients were followed for 2 months. Out of 143 patients 124 (86.7%) patients showed good response to the treatment hence were considered true TPE cases. Sensitivity, specificity, PPV, NPV and accuracy of all the 5 parameters were calculated and shown in Table IV. Cut off value for lymphocyte count was taken as ≥70% while for ADA levels it was ≥40 IU/L. Highest sensitivity and accuracy was shown by ADA levels while the lowest was shown by fluorescent microscopy.

Table III: Outcome of Diagnostic Techniques applied on pleural fluids

Results	Frequency	%age
Fluorescent Microscopy for AFB		
Positive	12	8.4
Negative	131	91.6
Culture on LJ Medium		
Positive	27	18.8
Negative	116	81.2
GeneXpert MTB Rif Assay		
MTB Detected	22	15.4
MTB Not Detected	121	84.6
Lymphocyte Count		
≥70%	101	70.6
<70%	42	29.4
ADA Levels		
≤40 IU/L	23	16.1
41-100 IU/L	39	27.3
>100 IU/L	81	56.6

Table IV: Sensitivity, Specificity, PPV, NPV and Accuracy of Diagnostic Techniques

Diagnostic Technique	Sensitivity%	Specificity%	PPV%	NPV%	Accuracy%
Fluorescent Microscopy	9.7	100	100	14.5	21.7
Culture on LJ Medium	21.8	100	100	16.4	32.2
GeneXpert MTB Rif Assay	17.8	100	100	15.7	28.7
Lymphocyte Count	79.1	84.2	97.0	38.1	79.7
ADA Levels	93.8	73.7	95.8	60.9	90.2

DISCUSSION

Definite diagnosis of TPE alike other TB cases is also based on the presence of bacilli on smear and/or LJ culture but present study showed a much lower sensitivity of both tests as 9.7% and 21.8% respectively. Although the results are much better than a previous study from same settings which showed positivity rates of 1.02% and 2.04% for smear and culture respectively[15]. Culture on LJ medium is still used as gold standard for diagnosis of TB but promptness is compromised due to long incubation time of 4-8 weeks which can delay the treatment considerably. On the other hand smear microscopy is cheaper, easy and readily available throughout the healthcare facilities lacks the sensitivity which is further declined in fluids as an older study reported a wide range of 0-75% sensitivity of smears among fluids[16].Other studies have reported batter sensitivity rates of 35.7% and 33.7% respectively[7,17], but these studies include pleural fluids and other extra-pulmonary TB cases.

GeneXpert MTB Rif Assay showed a far improved sensitivity of 17.8% as compared to smear in present study and is an emerging technique in developing countries. Although the test is not readily available but network has been expanded to at least secondary healthcare level throughout the country[18].As GeneXpert technique is PCR based and its Sensitivity has been reported to pick as low as 2fg of TB bacilli which is equal to only two MTB[19] nonetheless the presence of viable or non-viable organism is necessary which is a great drawback in case of pleural fluids as it is not a disease itself but an indication of presence of underlying etiology[15].

Cell counts and ADA levels are not definite diagnostic tests for TB but their importance has been valued in case of TPE in many studies[6,14,20]. Lymphocytosis in suspects of TPE has been considered as an indicator of underlying disease and a cut of value for ≥70% lymphocytes in pleural fluids was considered in present study and revealedsensitivity, specificity and accuracy of 79.1%,84.2% and 79.7% respectively. Similarly ADA levels of >40 IU/L are considered in present study showed sensitivity, specificity and accuracy of 93.8%, 73.7% and 90.2% in present study. Reactivation of previous TB infection, positive history of contact and high prevalence areas of TB are commonly useful in assessment of patients with pleural effusions[15]. A study has suggested algorithm for high prevalence TB countries having ≥125 patients/100000 population and recommended pleural biopsy for the cases who are bacteriological negative and have lymphocytic fluid with ADA levels of >40IU/L[6].

Poor socio-economic status was found to be in 37.1% of study participants, followed by smokers 36.4% and patients having previous history of ATT as 14% are also associated factors; comparable to other similar studies[13,21,22]. Overall mean age of 34.63±15.93 among study subjects denotes the occurrence of disease among younger people more over most of the study subjects (37.1%) were in the age range of 21-30 years are also in agreement with other studies[21,22]. No gender bias was observed in present study as there were 48.9% male and

51.1% female subjects enrolled are contrary to other studies[21,22].

Although bacterial confirmation of TB bacilli is necessary for definite diagnosis but sensitivity is compromised and multivariate approach is necessary and still in use for timely management of TPE. Contribution of latest GeneXpert technique in developing countries also remained unable to meet the halfway. The ground is still empty to get a great breakthrough in diagnosis and management of TPE.

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