DIAGNOSTIC ACCURACY OF GASTRIC ASPIRATE (GENEXPERT&ACID-FAST BACILLUS) IN PATIENTS WITH SUSPECTED PLEURAL TUBERCULOSIS.

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ABSTRACT

Introduction: Globally, pulmonary tuberculosis is a vital communicable disease. Amongst countries with the highest burden of Tuberculosis (TB), Pakistan is now on 5th position, together with the 4th greatest burden of drug-resistant TB worldwide. Detection of early-stage TB cases is important, for the successful treatment and reduction of transmission. For the rapid diagnosis of TB, the World health organization endorsed the GeneXpert MTB/RIF (Mycobacterium TB/Rifampin), in which an automated cartridge-based molecular technique detects MTB and rifampicin resistance within two hours. The study aimed to determine the diagnostic accuracy of gastric aspirate (GeneXpert and Acid-fast bacilli Smear) in patients with suspected pleural TB tanking pleural biopsy as the gold standard. **Materials and Methods:** This cross-sectional study was conducted at the department of pulmonology Jinnah Medical Postgraduate College, Karachi, Pakistan from July-December 2019 among 200 patients suspected with pleural effusion of 30-70 years of age with either gender. The gastric aspirate was sent for Acid-fast bacilli (AFB) smear andGeneXpert. Meanwhile,an ultrasound-guided pleural biopsy was also done. To check the diagnostic accuracy of AFBsmear and geneXpert, results were compared with pleural biopsy. **Results:** Mean of age and duration of symptoms was 35.38±6.32 years and 6.23±2.41 weeks respectively. The diagnostic accuracy of GeneXpert and AFB smear was 65.50% and 90.05% respectively forthe diagnosis of pleural effusion. The sensitivity, specificity, positive predictive value, and negative predictive value for GeneXpert was 29.03%, 97.20%, 90.00%, and 61.18%, respectively while for AFB sputum, the values were 83.87%, 93.48%, 85.25%, and 92.81% respectively. **Conclusion:** It is concluded that AFB smear has high diagnostic accuracy with high sensitivity and specificity as compared to GeneXpert. AFB is a cheap, readily available, and non-invasive modality that is reasonably good and relatively comparable with the biopsy in the diagnosis of pleural TB.

Keywords: Pleural TB, GeneXpert NPV, PPV, Sensitivity, Specificity, Diagnostic accuracy,

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INTRODUCTION

Globally, in 2009, Tuberculosis (TB) killed 1.7 million people, and according to an estimate by the World Health Organization, two billion people have latent TB at a certain point in time, out of which around 5-10% will become symptomatic in their lifetime. It is most prevalent in Africa, Eastern Europe, and the West Pacific. These areas are overwhelmed with elements believed to cause the spread of TB, multidrug-resistant (MDR) TB, and the Human immunodeficiency virus (HIV) infection[1,2]. Mycobacterium tuberculosis (MTB) is a pathogenic bacterial specie in the Mycobacteriaceae family and the contributory agent in many TB cases[3].

The pleural effusion related to TB comprises a comparatively fewer quantity of organisms, causing difficulty in diagnosis, and at times, the need to perform invasive procedures, such as pleural biopsy [4]. The pleural effusion can be confirmed via radiological studies like simple X-ray, computed tomography, or ultrasonography[5]], and with the help of improved culture media, it is possible to culture MTB from pleural tissue and fluid in as many as 70% of cases[6]. Gastric aspiration is done in children in those who cannot generate sputum even with aerosol inhalation. Nearly 50 ml of gastric contents are aspirated in the morning after 8 to 10 hours fasting and preferably when the patient is lying. Berger HW et al.found that 30% of patients with pleural TB grew MTB from gastric contents or sputum

culture [7]. Through signs and symptoms, it is very difficult to diagnose active TB, as is diagnosing the disease among immunosuppressed [8].It must be considered in those with constitutional symptoms lasting for at least two weeks along with the signs of lung disease. Multiple sputum cultures are performed for this acid-fast bacillus (AFB), along with chest X-ray (CXR), and are characteristically part of the primary assessment. Tuberculin skin tests and Interferon-γ release assays are not frequently used in developing countries[9]. Conventionally, TB is mostly being diagnosed by a mixture of the staining of sputum with special dyes followed by microscopy, CXR, the Mantoux test, and the growth of MTB in culture. The sputum smear microscopy (sputum AFB) test has some issues in HIV-positive patients, children, and also in patients with a low bacterial load. The GeneXpert MTB/RIF (Rifampin) test depicts high specificity and sensitivity forthe detection of pulmonary TB. An in vitro research showed a limit of detection of as few as 131 colony-forming units/mL of MTB, compared with nearly 10,000 colony-forming units/mL with conventional smear microscopy. Drug susceptibility can be diagnosed from the growth of MTB in culture as well but may take as long as six weeks, require high biosafety labs, and is not cost-effective. The identification of susceptibility of the drug is not irrelevant as MTB becomes progressively resistant to two of the key anti-tuberculosis drugs i.e. rifampicin and isoniazid. Such type of TB is called MDR-TB, which is rapidly increasing worldwide. MDR-TB cases require various antibiotics and are more complicated to treat due to higher costs and lengthier regimens[10,11].Being in a developing country, since the use of gastric aspirate can reduce both thecost anduse of invasive procedures, this study aimed to determine the diagnostic accuracy of gastric aspirate (GeneXpert and AFB Smear) for tuberculous pleural effusion.

METHODOLOGY

This observational cross-sectional study was conducted at the department of pulmonology, Jinnah Postgraduate Medical Centre (JPMC), Karachi, Pakistan from July-December 2019, among 200 suspected patients of 30-70 years of age with either gender. The sample size of 196 was calculated by considering sensitivity as 47% (with a margin of error 7%), specificity as 93% (with a margin of error 6%), and a confidence level of 95% [12]. Suspected patients were defined as patients having pleural effusion on CXR (Unilateral or Bilateral). Patients receivingantituberculosis therapy for >2 weeks, lung cancer patients, and those with connective tissue diseases were excluded from the study. Patients who visited the department of pulmonology, JPMC with signs and symptoms of pleural TB for >2 weeks with unilateral pleural effusion on CXR, and fulfilled the rest of the inclusion criteria was included in the study. Informed consent was taken before enrollment in the study. The patient was Nil per Oral after midnight. A nasogastric tube (minimum 14 french) was placed in the patient.Stomach contents were aspirated. If less than about 10 ml of mucus was aspirated, sterile water of about 20-30cc was poured into the stomach through the tube quickly withdrawn. The tube was repositioned to maximize the yield of gastric contents. Gastric aspirates were placed in a special bicarbonate-containing gastric aspirate tube or regular specimen cup if the former was unavailable.The specimen was transported to the microbiology lab for AFB and GeneXpert. Meanwhile,an ultrasound-guided pleural biopsy was also done to check and compare the efficacy of gastric aspirate. All data were recorded by a principal investigator on a predesigned proforma. Confounding variables and biases were controlled by strictly observing exclusion and inclusion criteria.Data were compiled and analyzed using IBM Statistical Package for the Social Sciences (SPSS) Statistics for Windows, Version 22.0 (IBM Corp., Armonk, NY, USA). Frequencies and percentages were computed for categorical variables (like gender, Cough, Fever, Weight loss, and pleural effusion on histopathology). Quantitative variables like age and duration of symptoms were presented as mean ± standard deviation. Sensitivity, specificity, positive predictive (PPV), negative predictive value (NPV), and diagnostic accuracy were calculated for GeneXpert and AFB separately for diagnosis of pleural TB taking histopathology as the gold standard. Effect modifiers like age, gender, duration of signs and symptoms, cough, fever, weight loss were controlled through stratification. Post-stratification, sensitivity, specificity, PPV, NPV, and diagnostic accuracy were calculated.

RESULTS

In this study, 200 patients were included with a mean age of 35.38±6.32 years and a mean duration of symptoms 6.23±2.41 weeks. Out of the total, 104 (52%) were males and 96 (48%) were females. Weight loss was noted in 145(72.5%) patients. There were 172 (86%) patients complaining of cough while 168 (84%) complaining of fever. In histopathological examination, positive pleural effusion was found in 155(77.5%). As shown in Table 1, thediagnostic accuracy of GeneXpert was 65.50%, with a sensitivity of 29.03%, a specificity of 97.20%, PPV of 90.00%, and NPV of 61.28%. The diagnostic accuracy of AFB smear was 90.05%, with a sensitivity of 83.87%, a specificity of 93.48%, PPV of 85.25%, and NPV of 92.81%.

Table-1: Diagnostic accuracy of geneXpert and Acid-fast bacilli smear by using biopsy as a gold standard (n=200)

	Diagnostic accuracy of geneXpert		**Diagnostic accuracy of Acid-fast bacilli smear**	
		95% confidence interval		95% confidence interval
Sensitivity	29.03	0.1981-0.3826	83.87	0.7472-0.9303
Specificity	97.20	0.9407-1.0032	93.48	0.8936-0.9760
Prevalence	46.50	0.3959-0.5341	31	0.2459-0.3741
Positive predictive value	90	0.7926-1.0074	85.25	0.7635-0.9415
Negative predictive value	61.18	0.5385-0.685	92.81	0.8851-0.9710
Overall accuracy	65.50	0.5891-0.7209	90.05	0.8644-0.9456

Stratification for findings of GeneXpert and AFB smear was performed for age, gender, duration of disease, cough, weight loss, and fever as shown in table 2.

Table III: Diagnostic accuracy of geneXpert and acid-fast bacilli smear by using biopsy as a gold standard (n=200)

		Diagnostic accuracy of geneXpert						**Diagnostic accuracy of acid-fast bacilli smear**					
		Sen	Spe	P	PPV	NPV	A	Sen	Spe	P	PPV	NPV	A
Age (years)	**30-45**	29.09	96.88	46.22	88.89	61.39	65.55	83.78	93.33	62.18	95.38	77.8	87.39
	> 45	30.56	95.56	44.44	84.62	63.24	66.67	86.79	92.86	65.43	95.83	78.79	88.89
Gender	**Male**	29.82	95.74	54.81	84.97	52.94	59.62	83.87	92.86	59.62	94.55	79.59	87.5
	Female	28.00	92.31	49.02	77.78	57.14	60.78	84.00	91.30	52.08	91.30	84.00	87.50
Symptom duration (weeks)	**3---6**	29.35	97.44	54.12	93.10	53.90	60.59	83.95	92.13	47.65	90.06	86.32	88.24
	>6	29.41	92.31	56.67	83.33	50.00	56.67	83.33	88.89	40.00	83.33	88.89	86.67
Cough	**Yes**	28.09	96.34	52.05	89.29	55.24	60.82	83.33	93.18	48.84	92.11	85.42	88.37
	No	27.27	88.24	39.29	60.00	65.22	64.29	83.33	93.75	42.86	90.91	88.24	89.29
Weight loss	**Yes**	29.58	95.95	48.97	87.50	58.68	63.45	83.33	92.41	45.52	90.16	86.90	88.28
	No	28.57	92.59	50.91	80.00	55.56	60.00	83.33	92.00	54.55	92.59	82.14	87.27
Fever	**Yes**	29.35	93.42	54.76	84.38	52.21	58.33	83.78	93.62	44.05	91.18	89.29	89.29
	No	29.17	87.5	75.00	87.50	29.17	43.75	80.00	90.91	31.25	80.00	90.91	87.50

Sen, Sensitivity;Spe, Specificity; P, Prevalence; PPV, Positive Predictive Value; NPV, Negative Predictive Value; A, Accuracy.

DISCUSSION

Despite an increasing number of studies exhibiting encouraging results of Xpert to rapid detection of MTB respiratory specimens, there are comparatively few data on the use of Xpert to assess specimens, particularly those obtained from gastric aspirate, and of these data, there are varied findings of the diagnostic performance of Xpert[13–19]. In the present study, we used the Xpert assay for direct MTB detection in gastric aspirate in a high TB-endemic country. To date, this study is the only of its kind, evaluating the performance of Xpert using gastric aspirate for the diagnosis of pleural TB. It demonstrates that AFB smear performs well in biopsy specimens for rapid and accurate diagnosis of pleural TB. In our study,for the diagnosis of pleural TB, using biopsy as gold standard, diagnostic accuracy of GeneXpert and AFB smear of gastric aspirate was 65.50% and 90.05%; sensitivity 29.03%, 83.87%; specificity, 97.20%, 93.48%; PPV, 90.00%, 85.25%; and NPV, 61.18%, 92.81% respectively.Our results are comparable with most national and international studies. The sensitivity of the Xpert assay for detecting pleural TB using a gastric aspirate sample was slightly higher than previously reported[13–15,19], whereas the specificity was similar. Pleural TB is a paucibacillary form of the disease, as indicated in a previous study[18,20]. Techniques based on nucleic acid amplification are currently being studied for the diagnosis of pleural TB, to improve the specificity and sensitivity [21]. In patients' samples, precise quantification of the MTB load might permit the assessment of the evaluation of the disease severity, patient's infectiousness, and treatment monitoring. One limitation of this technique is that, in detecting MTB DNA, they cannot distinguish between viable and nonviable microorganisms. However, higher Xpert assay loads were found to be associated with reduced Mycobacterial growth indicator tube culture time to detection of positive growth, in line with earlier data indicating that the Xpert assay's semiquantitative findings might be used to estimate the MTB load[20]. One limitation of this study is that the cost-effectiveness and turnaround time of the Xpert assay were not evaluated. Overall, Xpert may be a potentially useful additional tool using biopsy specimens for rapid diagnosis of pleural TB in patients with AFB smear-negative sputum results and allowing these vulnerable individuals to get onto TB treatment earlier. Further studies should be done to determine the clinical impact of the Xpert assay, involving outcomes assessment and influence on clinical practice decisions, the development of new diagnostic algorithms, and the feasibility of implementing the assay. Since our inclusion and exclusion criteria were stringent therefore, the strength of this study was the use of consecutive sampling best fitted for this research design and sample selection. Because of non-probability sampling,a small number of patients, and a short duration of the study, it is hard to generalizethese findings. This study was hospital-based; therefore the figure may not exhibit the actual severity of the disease.

CONCLUSION

It is concluded that AFB smear has high diagnostic accuracy with high sensitivity and specificity as compared to GeneXpert. AFB is a cheap, readily available, and non-invasive modality that is reasonably good and relatively comparable with the biopsy in the diagnosis of pleural TB.

ETHICS APPROVAL: The ERC gave ethical review approval

CONSENT TO PARTICIPATE: written and verbal consent was taken from subjects and next of kin

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