COMPARISON OF GENE XPERT VS SPUTUM SMEAR MICROSCOPY FOR TUBERCULOSIS DIAGNOSIS IN PEDIATRIC PATIENTS KEEPING CULTURE SENSITIVITY AS GOLD STANDARD

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ORIGINAL ARTICLE

ABSTRACT

Introduction: Tuberculosis (TB) is a granulomatous infection caused by Mycobacterium tuberculosis. Pakistan ranks 4th globally. Diagnosis is challenging due to children's inability to expectorate sputum and similar symptoms to other respiratory diseases. Rapid detection is crucial for poor prognostic outcomes. Gene Xpert is more accurate than sputum, but not routinely applied. **Objective:** Using culture as gold standard, compare the diagnostic precision of GeneXpert and Smear microscopy for the diagnosis of tuberculosis inpaediatric patients. **Methodology:** This cross-sectional validation study included 250 children and involved smear microscopy for 3 consecutive days. Preferred sample was expectorated sputum produced by deep cough. Alternate specimen was nasal secretions or gastric aspirate.Samples were stained for AFB and GeneXpert tests, and results were interpreted into invalid, negative, or positive categories. Positive results were categorized based on bacterial load(very low, low, medium, high) and rifampicin resistance. Results were recorded on the study proforma. **Result:** The mean age of patients was 8.51±2.29years wth 133 (53.2%) males and 117 (46.8%) females. The mean weight of patients was 28.07±7.05kg. On Smear, there were 8 (6.7%) patients with 1+ grade, 27 (22.7%) with grade 2+, 40 (33.6%) 3+ grade and 44 (36.9%) were grade 4+. The smear's diagnostic accuracy was 85.6% and specificity, PPV, NPV, and sensitivity were all above average. GeneXpert had a sensitivity of 95.5%, specificity of 95.7%, PPV of 94.6%, NPV of 96.4%, and diagnostic accuracy of 95.6%. **CONCLUSION:** GeneXpert is more accurate than smear for diagnosis of TB in children.

INTRODUCTION

Tuberculosis is granulomatous infection by Mycobacterium tuberculosis. It is a key health and economic problem in developing nations. Despite more than 50 years of effective TB medication and more than 90 years of vaccine use, TB is still a major public health concern.[1] Around 8.8 million people were affected by TB in 2010, and 1.4 million people died from it, including at least 50,000 children.[2] WHO estimated that TB caused 74,000 deaths in children globally in 2012.[3] Pakistan ranks 4th amongst high burden TB countries of the world.[4] Diagnosing TB in children is difficult due to non-specific symptoms. Diagnostic specimens are difficult to be collected in young children and more than 50% have negative TB cultures.[5] The diagnostic advancements of adult TB have not been validated in

children.[6] Several methods are used for testing Pulmonary TB, such as Chest Xray, tuberculin skin test (TST), bacteriological tests e.g Sputum smear microscopy, culture and molecular tests based on DNA i.e GeneXpert. The diagnosis is difficult due to inability of children to expectorate sputum and similarity of symptoms of TB with various other common childhood illnesses including pneumonia and other bacterial and viral infections including opportunistic infections related to HIV[7,8] and malnutrition.

Microbiological confirmation is important for diagnosing TB epidemic but often Smear microscopy is negative in children with culture-confirmed TB and since culture can take weeks, it can lead to delay in the initial treatment which can cause poor outcome. The main technique for TB detection and tracking treatment response is sputum microscopy.[8] Sputum microscopy, which has a positive predictive value of between 30 and 40%, is frequently the only diagnostic test available in endemic areas.[9] Pulmonary TB can be confirmed using the highly sensitive and specific Gene Xpert MTB test[10] which can also detect drug resistance rapidly . World Health Organization recommends GeneXpert to prevent the delay in initiation of appropriate therapy hence increasing cure rate and causing decreased mortality.[11] Mycobacterial culture is the gold standard, but it typically requires infrastructure and technical knowledge and takes 2 to 6 weeks to produce a final result.[12] Gene Xpert's overall sensitivity, specificity, PPV, and NPV were 86.8%, 93.1%, 78.5%, and 96%, respectively, while AFB smear microscopy's overall sensitivity and specificity were 22.2% and 78.5%, with a prevalence of 19%.[13] The rationale of this study is to compare the two diagnostic tests. It is difficult to collect the sample for smear microscopy from children for consecutive 3 days and is also time consuming while sample for gene xpert is easily collected, takes only a few hours for the result and is also precise and cost effect.

MATERIAL AND METHODS :

Setting: Study was conducted in NICU ,Benazir Bhutto Hospital, Rawalpindi

Study design: It was a cross sectional validation study

Duration of study: was 6 months from 20 January to 20 July 2019

Sample size: Sample size = 250, using sensitivity of smear microscopy= 22.2%[13] Sensitivity of smear microscopy= 78.5%.[13] Prevalence of TB in paediatric patients=19%. [14] Level of significance =5%. This sample size also covers the required sample size for gene xpert.

Sampling technique: Nonprobability consecutive sampling

Inclusion criteria: Children between ages 5-12 years, with positive signs and symptoms of TB.

Exclusion criteria: Patients undergoing anti-TB treatment and children with known chronic illness like cystic fibrosis.

Data collection procedure: After taking written informed consent from parents of the children, demographic information like name, age, sex and contact were noted in structured proforma attached as ANNEXURE 1. For each child, smear microscopy was done by early morning specimens for three consecutive days. Specimen size was 5-10 ml. Preferred sample was expectorated sputum produce by deep cough. Alternate specimens were nasal secretions or gastric aspirate. Ziehl-Neelsen stain was used to stain the sample for acid-fast microscopic examination. LJ medium was then used for culture, and for the same child the GeneXpert test was completed. The Gene Xpert module's single-use disposable cartridge was filled with 0.5 ml of the decontaminated and concentrated sample after treating with a sample solution of NaOH and isopropanol (1:3 ratio)at room temperaturefor 15 minutes duration. All results were categorised into three categories: invalid (if PCR inhibitors were found resulting in amplification failure), negative, or positive. Positive

results were scaled into 4 categories (very low, low, medium, and high)on basis of bacterial load, and according to the detection of gene mutations, susceptible or resistant to rifampicin. The findings of both tests were recorded in the study performa. **Data analysis plan:** Data was entered and analysed by SPSS version 20. Descriptive statistics were used for qualitative & quantitative variables. In the case of quantitative variables like age and weight of the patients, mean and standard deviation were calculated. Qualitative variables like gender, positive or negative smear microscopy ,geneXpert and culture and the grades of smear microscopy were given as frequency and percentages.

RESULTS

The mean age of patients was 8.51±2.29years. Table 1. There were 133 (53.2%) males and 117 (46.8%) females. The mean weight of patients was 28.07±7.05kg. Table 2 On Smear, there were 119 (47.6%) positive cases while 131 (52.4%) negative cases. On Smear, there were 8 (6.7%) patients with 1+ grade, 27 (22.7%) patients with grade 2+, 40 (33.6%) patients were 3+ grade and 44 (36.9%) patients were grade 4+. On GeneXpert, there were 112 (44.8%) positive cases while 138 (55.2%) negative cases. On culture, there were 111 (44.4%) positive patients while 139 (55.6%) negative patients. The smear's diagnostic accuracy was 85.6%, while its specificity, PPV, NPV, and sensitivity were all above average. Table 3 shows that GeneXpert had a sensitivity of 95.5%, a specificity of 95.7%, a PPV of 94.6%, an NPV of 96.4%, and a diagnostic accuracy of 95.6%. Table 4.

Table 1: Descriptive Statistics of age of patients

Age (Year)	n	250
	Mean	8.51
	Standard Deviation	2.29
	Minimum	5
	Maximum	12

Table 2: Descriptive Statistics of weight of patients

Wieght (KG)	n	250
	Mean	28.07
	Standard Deviation	7.05
	Minimum	18
	Maximum	42

Table 3: Accuracy of smear against culture

		Culture		Total
		Positive	Negative	
Smear	Positive	97	22	119
	Negative	14	117	131
Total		111	139	250

Sensitivity = 87.4%
Specificity = 84.2%
PPV = 81.5%
NPV = 89.3%
Diagnostic accuracy = 85.6%

Table 4: Accuracy of GeneXpert against culture

		Culture		Total
		Positive	Negative	
Gene Xpert	Positive	106	6	112
	Negative	5	133	138
Total		111	139	250

Sensitivity = 95.5%
Specificity = 95.7%
PPV = 94.6%
NPV = 96.4%
Diagnostic accuracy = 95.6%

DISCUSSION

For clinical and epidemiological reasons, TB diagnosis at an early stage is essential. To successfully control TB and prevent disease transmission, it is essential to identify patients early and provide them with effective care. Culture of specimen is the gold standard test for TB diagnosis. Nevertheless, the majority of laboratories use direct ZN microscopy as their primary detection method for Mycobacterium

tuberculosis due to the lack of accessibility to culture facilities and the lengthy turnaround times associated with it. In our study, the mean age of patients was 8.51±2.29years. There were 133 (53.2%) males and 117 (46.8%) females. The mean weight of patients was 28.07±7.05kg. On Smear, there were 119 (47.6%) positive cases while 131 (52.4%) negative cases. On GeneXpert, there were 112 (44.8%) positive cases while 138 (55.2%) negative cases. On culture, there were 111 (44.4%) positive patientss while 139 (55.6%) negative patients. On Smear, there were 8 (6.7%) patients with 1+ grade, 27 (22.7%) patients with grade 2+, 40 (33.6%) patients were 3+ grade and 44 (36.9%) patients were grade 4+. In our study, NPV was 89.3%, PPV was 81.5%, sensitivity of smear was 87.4%, specificity was 84.2%, and diagnostic accuracy was 85.6%. GeneXpert had a sensitivity of 95.5%, a specificity of 95.7%, a PPV of 94.6%, an NPV of 96.4%, and a diagnostic accuracy of 95.6%. Gene Xpert's overall sensitivity, specificity, PPV, and NPV were 86.8%, 93.1%, 78.5%, and 96%, respectively, while the acid-fast bacilli smear microscopy's overall sensitivity and specificity were 22.2% and 78.5%,[12] with a prevalence of 19%.[13] On the GeneXpert platform, a real-time PCR assay for Mycobacterium tuberculosis was created that also detects rifampicin resistance. In the study, a single direct MTB/RIF test revealed 92 percent of cases of pulmonary tuberculosis, including 75 percent of cases with smear-negative disease, which is comparable to the solid culture results. The Gene Xpert's diagnostic accuracy was confirmed by Andrea Rachow.[15] In patients with a positive culture, the Xpert MTB/RIF Assay had an 88.4% (95%CI = 78.4% to 94.9%) sensitivity rate and a 99% (95%CI = 94.7% to 100.0%) specificity rate. Based on these findings, the author came to the conclusion that the Gene Xpert is a highly sensitive, precise, and quick diagnostic tool for TB that has the potential to complement the current gold standard for TB diagnostics and

enhance its overall sensitivity.[15] A study from China assessed Gene Xpert's effectiveness. According to study results, Gene Xpert had a sensitivity and specificity of 94.4% and 90.2% for MTB detection in suspected non-multidrug resistant cases. Smear-negative cases had a sensitivity of 88.8%. Previous MTB/RIF studies that evaluated concentrated, decon-taminated sputum pellets or sputum samples consistently reported test sensitivity of 72–75% in smear-negative TB cases and 98–100% in smear-positive TB cases.[16] Zeka et al. discovered that the Gene Xpert had a sensitivity of 70%, a specificity of 100%, an NPV of 90.6%, and a PPV of 100%. The MTB/RIF test had a sensitivity and specificity for pulmonary cases of 82.3% and 100%, respectively. For smear-positive and smear-negative specimens, the sensitivities were 100% and 68.6%, respectively.[17]

CONCLUSION

It has been concluded that GeneXpert is more accurate than smear for diagnosis of TB in pediatric patients. Smear microscopy is less diagnostic than geneXpert as it is difficult to collect the sample from children for consecutive 3 days and it also take much time for the result which delay the management of the patients while sample for geneXpert is easily collected and take only few hours for the result and it is also precise and cost effective as well. Now we have got local evidence in favor of GeneXpert. In future, we will apply GeneXpert instead of sputum smear for diagnosis of Pulmonary Tuberculosis among pediatric patients.

AUTHORS CONTRIBUTION

SR: Conception of study, experimental work, **SA**: Experimentation, **IM:** Manuscript write up, **TR:** Analysis and critical review, **NUAU:** Manuscript write up, **AI:** Manuscript write up

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