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Original Article

Evaluation of Diagnostic Accuracy of Gene Xpert MTB/RIF in Patients with Tuberculous Lymphadenitis using Fine Needle Aspirate in a Tertiary Care Hospital

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ABSTRACT

Tuberculous lymphadenitis form the largest proportion of extrapulmonay manifestation of tuberculosis and it's diagnosis always remains a challenge. Cytology and microscopy have suboptimal sensitivity and specificity, therefore accurate identification and timely cure of tuberculosis can lessen morbidity and mortality associated with TB lymphadenitis. The aim of this study was to evaluate the sensitivity and specificity of GeneXpert/RIF against smear and gold standard culture.

Objectives: The objective of this study was to demonstrate the diagnostic precision of GeneXpert MTB/RIF on fine needle aspiration cytology (FNAC) of cases clinically suspected to have tuberculous lymphadenitis and comparison with smear and culture as a yardstick.

Methodology: The present study is a cross sectional type piloted in Khyber Girls Medical College and the samples were collected from all the three tertiary care hospitals of Peshawar from June 2019- June 2020. Those patients who were suspected to have TB lymphadenopathy were included in this study and were subjected to FNAC. The sample was then processed for routine cytology and Ziehl Nelson staining, GeneXpert assay and Lowenstein Jensen culture. Rifampicin resistance was also determined by GeneXpert.

Results: A total of 100 cases with adequate aspirate on fine needle aspiration cytology were included in this study. Out of these 50(50%) showed cytological features consistent with mycobacterial infection. The sensitivity of Xpert equated to culture was 94 %(95% CI 75-97%) while specificity was 70% (95% CI 60-70%). The sensitivity of smear matched to culture was 37% (95 % CI 26-39 %) and specificity was 98% (95% CI 96-100 %). When the results of Xpert were equated with smear the sensitivity as well as specificity was 100% in those cases which were positive on both smear and culture. On the other hand cases which were negative on smear but positive on culture demonstrated sensitivity of 92% and specificity of 100 % when matched with Xpert. The outcome of RIF by Xpert was in total agreement with conventional drug sensitivity tests.

Conclusion: GeneXpert MTB/RIF is an excellent and time saving automated technique for confirmation of MTB in lymph node aspirates and at the same time determines rifampicin resistant cases.

Key Words: Fine needle aspiration cytology**,** GeneXpert MTB/RIF, Rifampicin, Mycobacterium Tuberculosis.

Introduction

Tuberculous lymphadenitis form the largest proportion of extrapulmonary manifestation of tuberculosis (TB) [1].The confirmation of tuberculous lymphadenitis (TBL) has been always arduous[2, 3]. The customarily utilized approaches (cytology and microscopy) have suboptimal sensitivity and specificity[4, 5, 6]. Accurate identification and timely cure of tuberculosis can lessen morbidity and mortality associated with TB lymphadenitis[7, 8, 9].

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Fine needle aspiration cytology (FNAC) provides a practical and harmless alternative for specimen collection[1].The utilization of cytology simultaneously with confirmation with Ziehl-Neelson (ZN) staining as well as mycobacterial recognition by culture provides outstanding yields but is restricted by lack of species verification, slow turnaround period, and be deficient in offering drug resistance information.[1]

ZN staining and cytological examination of aspirate has been used for initial diagnosis of TBL in resource limited settings[10, 11, 12]. Even though FNAC is simple, rapid and cost effective technique but it has low specificity due to the existence of analogous cytological findings in conditions supplementary to

TBL [2, 13, 14].WHO has recommended GeneXpert MTB/RIF to be used as preliminary diagnostic test in both pulmonary and extra pulmonary TB[2, 3,4,11].

The GeneXpert/RIF assay identifies DNA sequences precise for Mycobacterium tuberculosis and Rifampicin resistance by polymerase chain reaction[3, 15] .The results are obtained within 90 minutes with minimal biohazards[11, 16]. In this study I have assessed the accuracy of GeneXpert aimed at analysis of TBL by means of routinely done FNAC aspirate and compared the results with cytology and culture as a reference standard.

In Peshawar, there is scarce data available regarding Xpert MTB/RIF assay on fine needle aspiration and molecular characterization of rifampicin-resistant strains of Mycobacterium Tuberculosis.

Culture and sensitivity may help in decision making but it takes 2-4 weeks and rapid diagnosis and treatment is mandatory to reduce mortality and morbidity.

This study may help in analysing the importance of XpertMTB/RIF assay for rapid diagnosis of tuberculous lymphadenitis as well as determine strains resistant to rifampicin.

Objectives

1) To identify XpertMTB gene in fine needle aspirate of patients with TBL

2) To evaluate the sensitivity and specificity of GeneXpert/RIF against smear and gold standard culture.

Methodology

This is a cross sectional study conducted in Hayatabad Medical Complex which is a tertiary care hospital and Khyber Girls Medical College Peshawar from June 2019 to June 2020. Sample Size was 100, calculated by WHO sample size calculator using confidence interval 95%, error 0.05 and prevalence 79%. A non-probability consecutive sampling technique was used.

Ethical clearance and letter of permission is obtained from concerned departments. Informed consent is taken from all the patients who were enrolled in this study.

A total of 100 patients clinically suspected of TBL from two tertiary care hospitals of Peshawar were enrolled. The demographic and medical data was compiled via pre-verified inquiry form.

The FNA specimen was gathered by a cytopathologist in the diagnostic pathology laboratory by using 23-25gauge needle attached to 10 ml syringe in two passes. Few drops were used for cytology and AFB smears. Smears were checked in lieu of adequacy for cytological diagnosis and cases were omitted from the study if smears have inadequate material for diagnosis. ZN staining was performed for direct finding of mycobacterial on every specimen labeled as adequate.

The remaining aspirate is used for Lowenstein Jensen (L J) culture and Gene Xpert assay. For culture sample was processed by standard N-acetyle-L-cysteine and sodium hydroxide technique with a final NaOH concentration of 1%. After centrifugation step, Lowenstein-Jensen (LJ) slant was injected with 0.1 ml suspension and incubated at 37◦C.

For Xpert gene detection a cartridge computerized device was utilized. Specimens were processed directly by gene Xpert device. Sample mixture was blended with the unprocessed sample at a proportion of 2:1 and later on agitated by hand and set aside for 10min at room temperature. It was then mixed once more and set aside for 5 min. Afterward 2ml of inactivated material was shifted to the test cartridge and this was then inserted into the test platform. The data analysis from these tests was software dependant and not user based.

All patients clinically suspected to have tuberculous lymphadenitis were included in this study while patients who had taken anti-tuberculous treatment or clinically suspected to have lymphadenopathy due to other causes were excluded from this study.

Data regarding age, gender, indication for FNAC, past history and family history of TB, clinical features and hematological parameters (hemoglobin level, total leukocyte count and ESR) was analyzed for the study. Clinical data such as tuberculin test, chest x ray and ultrasound of enlarged lymph nodes were taken from patient's records. The results of Xpert MTB/RIF were also analyzed

Data was analyzed using SPSS software version 21. Performance calculations such as sensitivity, specificity were done to match the analytical performance of Xpert MTB/RIF to the positive cytology and culture for mycobacterium tuberculosis. Chi square test was applied for association between different variables.

Results

A total of 100 patients clinically suspected to have tuberculous lymphadenitis and had adequate smears were included in this study. There were 40(40%) males and 60(60%). female patients Out of these 60(60%)

cases had cytomorphological features suggestive of TB. 20(20%) patients showed non specific reactive lymphadenopathy on cytology. 10(10%) cases were diagnosed as acute bacterial lymphadenitis while 10(10%) showed epithelial inclusion cyst on cytology. Distribution of cases on the basis of age, sex and cytological diagnosis is shown in table 1.

Table 1: Distribution of Patients According to Age, Gender and Cytological diagnosis.

Characteristics	No of cases n=100	% of cases
Age		
< 5 years	5	5%
5-20 years	10	10%
>20 years	85	85%
Gender		
Male	40	40%
Female	60	60%
Cytological features		
Cases with cytology consistent with TB	60	60%
Reactive lymphadenopathy	20	20%
Acute bacterial lymphadenopathy	10	10%
Epithelial inclusion cyst	10	10%

In our study family history of tuberculosis was seen in 64(64%). All the patients gave history of fever and night sweats (100%). Other clinical features like lethargy were noted in 97% cases while cough was present in 5% cases. Past history of pulmonary TB was seen in 56% of the cases as shown in table 2.

Table: 2 History &Clinical Features of study cases n=100

Features	Present
Family Hx of TB	64 (64%)
Contact history	52(52%)
Fever and night sweats	100 (100%)
Lethargy	97 (97%)
Weight loss	65(65%)
Loss of appetite	56(56%)

In current study 50 out of 100 cases were positive on both smear and culture. There were 20 cases which were positive on culture but negative on smear. While 25 cases were negative on both smear and culture. A total of 5 cases were culture adulterated. The results of Xpert assay were positive in 68/70 culture positive cases and in12 out of 25 culture negative cases. It was positive in 2/5 culture adulterated cases.

The sensitivity of Xpert equated to culture was 94 %(95% CI 75-97%) while specificity was 70% (95% CI 60-70%). The sensitivity of smear matched to culture was 37% (95 % CI 26-39 %) and specificity was 98% (95% CI 96-100 %). When the results of Xpert were equated with smear the sensitivity as well as specificity was 100% in those cases which were positive on both smear and culture. On the other hand cases which were negative on smear but positive on culture demonstrated sensitivity of 92% and specificity of 100 % when matched with Xpert. As shown in table 3 and 4.

Table 3: Results of Xpert, Culture and Smear. N=100

	Culture+ Smear+	Culture– Smear-	Culture Adulterated
	70 (70%)	25 (25%)	5 (5%)
Xpert Positive	68 (68%)	12 (12%)	2 (2%)
Xpert Negative	2 (2%)	13 (13%)	3 (3%)

Table 4: Sensitivity and Specificity outcomes

	Sensitivity 95% CI	Specificity 95% CI
Xpert equated to Culture	94% (74-97%)	70% (60-70%)
Smear equated to culture	37% (24-39%)	98% (96-100%)
Xpert equated to smear+ culture+	100% (80-100%)	100% (95-100%)
Xpert equated to smear –culture +	92% (75-95%)	100% (96-100%)

Among the 68 cases positive on Xpert 8(11.76%) cases showed RIF resistance. This showed total agreement with conventional drug sensitivity tests. Among culture negative cases, 3 demonstrated RIF resistance on Xpert.

Discussion

Tuberculous lymphadenitis is a grave public health issue globally and is a diagnostic dilemma. The most important obstacle towards the diagnosis is the unusual appearance often mimicking malignant or inflammatory diseases. In addition to this the lymph node aspirate often yields small number of bacilli and therefore results in low sensitivity of smear and culture[9]. Traditional laboratory approaches such as direct microscopy is much less sensitive and mycobacterial culture is long running and arduous for the diagnosis of tuberculosis. Therefore it is desirable to evolve innovative techniques for prompt recognition of the Mycobacterium tuberculosis in

lymph node aspirates. The WHO has endorsed the utilization of GeneXpert for rapid recognition of MTB in lymph node aspirates.[3]

Xpert MTB/RIF manifested improved sensitivity than smear cytology when equated with gold standard culture technique and can be thought of as a trustworthy technique for early diagnosis of extrapulmonay TB including TB lymphadenitis.[17,18] The XpertGene is a valuable supplement to the investigative apparatus for fast detection of pulmonary and extrapulmonary TB altogether as it has significantly reduced the period of diagnosis to almost two hours which is far less than other techniques. This precedence is reflected into medical management for patients with smear negative TB.[18]

Our study showed high sensitivity and specificity (sensitivity 94% & specificity 70%) for XpertMTB/RIF technique on fine needle aspiration cytology (FNAC) of patients with suspected tuberculous lymphadenopathy when compared to culture. While the sensitivity and specificity approached 100% when equated to smear. A current research by Hillemann et al. established the efficacy of this procedure for extrapulmonary samples with collective sensitivity and specificity of 77.3% and 98.2%. [19]Another study conducted by Tortoli et al. on extrapulmonay samples in low incidence country like Italy demonstrated overall sensitivity and specificity of Xpert to be 81.3% and 99.8% compared to culture.[20] In addition to MTB gene detection GeneXpert also determines RIF resistance which is useful in management of patients with MDR TB in TB endemic areas.[21] In current study the Xpert results for RIF showed complete agreement with usual methods used for drug sensitivity. RIF resistant strain was detected even in culture negative cases. This is in accordance with studies done by Boehme et al and Steingart et al.[22, 23]

In conclusion FNAC is a painless and easy practice that can be conducted in an outpatient locale by trained cytologists or clinical workers or even nurses.[1]It is idyllic for use in resource constrained setup including pastoral areas and is safe, cheap with nominal biohazards. If we use transfer vials practically no sample preparation is requisite and there is negligible menace of contagion. Moreover the transference hazard to the worker may likewise be abridged. Combining FNAC with GeneXpert would significantly perk up approach to apt diagnosis and management for patients with tuberculous lympahadenitis.[1,9,19].

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