Diagnosis of Tuberculous Lymphadenitis Using Fine Needle Aspiration Cytology: A Comparison Between Cytomorphology and GeneXpert Mycobacterium Tuberculosis Resistant to Rifampicin (MTB/RIF) Test

Tamanna E Nur, Shabnam Akther, Mohammed Kamal, Mohammad Sohel Shomik, Dinesh Mondal, Abu Khalid Muhammad Maruf Raza

Abstract

Background: Diagnosis of tuberculous lymphadenitis is challenging. Fine needle aspiration cytology (FNAC) can provide a diagnosis suggestive of tuberculosis (TB) when there are epithelioid cells with/without caseation necrosis. But epithelioid cells can occur in some other conditions as well. Xpert MTB/RIF test, a relatively newer technique, can detect bacteria specific DNA by polymerase chain reaction (PCR) and can also detect rifampicin resistance. This study was undertaken to see the role of Xpert test in the diagnosis of tuberculous lymphadenitis from fine needle aspiration (FNA) material, in comparison to cytomorphological diagnosis.

Methods: It was a cross-sectional study conducted in the Department of Pathology, BSMMU in collaboration with International Centre for Diarrhoeal Disease Research (icddr, b). Study population was clinically suspected cases of tuberculous lymphadenitis patient referred to Pathology Department, BSMMU. Total number of cases is 351. After FNA, cytomorphological diagnosis was done in Pathology Department, Xpert test in Microbiology Department and culture in icddr,b. Results of FNAC and Xpert test were compared. Kappa test was done to calculate percentage of agreement of FNAC and Xpert test with culture.

Results: Final analysis was done in 317 cases following exclusion criteria. Sensitivity, specificity, positive predictive value and negative predictive value of FNAC were 79.7%, 48.1%, 31.9% and 88.6%, respectively. Percentage of agreement of FNAC with culture was 0.183 (Kappa test) implying poor agreement. Sensitivity, specificity, positive predictive value and negative predictive value of Xpert were 95.9%, 60.5%, 42.6% and 98% respectively. Percentage of agreement of Xpert test with culture was 0.39 (Kappa test) implying a slight agreement.

Conclusions: In this study, sensitivity of Xpert was higher than FNAC. It was helpful in detecting TB in suppurative lymphadenitis where granuloma/caseation was not found. Cytomorphology, on the other hand, can diagnose granulomatous as well as reactive and other disease conditions. If facilities permit, both FNAC and Xpert test would better to be done.

Keywords: Mycobacterium tuberculosis; FNAC; Lymphadenitis; PCR

Introduction

Tuberculosis (TB) is a leading public health problem worldwide [1]. It remains to be one of the leading causes of morbidity and mortality in the developing countries [2]. Mortality rate in Bangladesh for TB is 51/100,000 people [3]. Commonly TB manifests clinically as pulmonary TB and less frequently as extrapulmonary TB (EPTB) [4]. Lymphadenitis is the most frequent form of EPTB usually occurring in the cervical region. The diagnosis of tuberculous lymphadenitis remains challenging in spite of the availability of various diagnostic tools. Fine needle aspiration cytology (FNAC) can provide a diagnosis suggestive of TB when there are epithelioid cells with/without caseation necrosis. But epithelioid cells can occur in some other conditions as well. Xpert MTB/RIF test, a relatively newer technique, can detect bacteria specific DNA by polymerase chain reaction (PCR) and can also detect rifampicin resistance. This study was undertaken to study the role of Xpert test in the diagnosis of tuberculous lymphadenitis from fine needle aspiration (FNA) material, in comparison to cytomorphological diagnosis. Objectives of the study were:

1) To calculate the sensitivity, specificity, positive predictive value and negative predictive value of FNAC were 79.7%, 48.1%, 31.9% and 88.6%, respectively. Percentage of agreement of FNAC with culture was 0.183 (Kappa test) implying poor agreement. Sensitivity, specificity, positive predictive value and negative predictive value of Xpert were 95.9%, 60.5%, 42.6% and 98% respectively. Percentage of agreement of Xpert test with culture was 0.39 (Kappa test) implying a slight agreement.

Conclusions: In this study, sensitivity of Xpert was higher than FNAC. It was helpful in detecting TB in suppurative lymphadenitis where granuloma/caseation was not found. Cytomorphology, on the other hand, can diagnose granulomatous as well as reactive and other disease conditions. If facilities permit, both FNAC and Xpert test would better to be done.

Keywords: Mycobacterium tuberculosis; FNAC; Lymphadenitis; PCR
value, negative predictive value of FNAC in diagnosis of tuberculous lymphadenitis in comparison to Xpert MTB/RIF test taking culture as gold standard test; 2) To calculate the percentage of agreement of FNAC and Xpert MTB/RIF test with culture through Kappa test.

Materials and Methods

This study was a cross-sectional study which was conducted in the Department of Pathology, BSMMU from July 2013 to May 2015. This study was done in collaboration with International Centre for Diarrhoeal Disease Research (icddr, b). Clinically suspected cases of tuberculous lymphadenitis patient were the study population. Sample size of this study was 384. It was a purposive sampling method. Figure 1 shows detail study plan for this study.

Patient selection criteria

Those clinically suspected cases of tuberculous lymphadenitis were included in this study. Exclusion criteria included: 1) History of taking anti-TB drugs within 60 days of sample collection; 2) Lymphadenopathy due to suspected malignancies; 3) Inadequate sample (aspirated material); 4) Without consent.

Ethical consideration

The research proposal was approved by the IRB of icddr, b and IRB of BSMMU. Under a MOU icddr, b jointly worked with BSMMU team.

Results

A total of 351 clinically suspected tuberculous lymphadenitis patients were initially enrolled in the study. Out of those, 34 cases were excluded because of a diagnosis of malignancy like non-Hodgkin lymphoma, metastatic squamous cell carcinoma, metastatic adenocarcinoma etc. Finally 317 cases were included in this study for final evaluation. The patients’ age ranged from 5 to 75 years; mean age was 27.91 ± 13.157, majority between 20 and 40 years of age (56.2%), and male: female ratio = 1:1.58. In the present study, the sensitivity, specificity, positive predictive value and negative predictive value of Ziehl-Neelsen (ZN) stain for acid-fast bacilli (AFB) detection were respectively 17.6%, 98.4%, 76.5% and 79.7%. Cytomorphological diagnosis of TB was made when there was presence of epithelioid cells singly or in clusters (granuloma) with or without caseose necrosis. In this study it was found that sen-

Figure 1. Study plan for the study.

Figure 2. Results of FNAC, ZN stain, culture and GeneXpert in the study population.
Diagnosis of Tuberculous Lymphadenitis

Table 1. Sensitivity and Specificity of Cytomorphological Diagnosis (Culture Taken as Gold Standard) Test

<table>
<thead>
<tr>
<th>Culture</th>
<th>Cytomorphological diagnosis of TB</th>
<th>Total</th>
<th>Statistical test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>59 (79.7%)</td>
<td>185</td>
<td>P ≤ 0.001 (Pearson Chi-square test)</td>
</tr>
<tr>
<td>Negative</td>
<td>15 (20.3%)</td>
<td>132</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>74 (100%)</td>
<td>317</td>
<td></td>
</tr>
</tbody>
</table>

Sensitivity, specificity, positive predictive value and negative predictive value of cytomorphological diagnosis of TB are 79.7%, 48.1%, 31.9%, and 88.6% respectively. Percentage of agreement of cytomorphological diagnosis of TB with culture is 0.183 (Kappa test).

Table 2. Sensitivity and Specificity of GeneXpert (Culture as Gold Standard)

<table>
<thead>
<tr>
<th>Culture</th>
<th>GeneXpert test</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>71 (95.9%)</td>
<td>167</td>
</tr>
<tr>
<td>Negative</td>
<td>3 (4.1%)</td>
<td>150</td>
</tr>
</tbody>
</table>

Sensitivity, specificity, positive predictive value and negative predictive value of GeneXpert test are 95.9%, 60.5%, 42.6%, and 98% respectively. Percentage of agreement of GeneXpert test is 0.39 (Kappa test).

Discussion

FNAC is an easy, reliable outpatient procedure for the diagnosis of tuberculous lymphadenitis, especially with palpable superficial lymph nodes. It is ideally suited for use in resource limited settings, especially in developing countries where tuberculosis is a major cause of morbidity and mortality [5]. Cytomorphological diagnosis of TB was made when there was presence of epithelioid cells singly or in clusters (granuloma) with or without caseous necrosis (Figs. 3-5). Several conditions, including mycosis, bacterial, and viral adenitis, can present similar cytologic features as tuberculous lymphadenitis. In this study, additional laboratory tests may be essential to identify the cause of such adenopathy correctly for a definitive treatment. Demonstration of tubercle bacilli in cytology smears made from fine needle aspirates can be done with ZN stain (Fig. 6). Also culture of the aspirated material can be done to confirm the presence of mycobacterium species along with drug sensitivity results. And now, aspirated material is also being tested with GeneXpert to detect the presence of *Mycobacterium tuberculosis* along with information about rifampin resistance. All these methods essentially help to establish a correct diagnosis of tuberculosis leading to a proper treatment, thereby reducing morbidity and mortality of the disease.

This present study was done to confirm the usefulness, reliability and diagnostic accuracy of FNAC in comparison to Xpert MTB/RIF test for investigating patient with suspected tuberculous lymphadenitis. The study evaluated 317 patients with suspected tuberculous lymphadenopathy. The patients were referred for FNAC of their enlarged lymph nodes to the

Figure 3. Photomicrograph of granuloma (PAP stain, × 400).

Figure 4. Photomicrograph of caseous necrosis (PAP stain, × 400).
Department of Pathology, BSMMU. The aspirated material was used for cytological examination (with Pap stain), detection of AFB (with ZN stain) and culture (with Lowenstein-Jensen (LJ) media), and GeneXpert test.

The age range of the patients was from 5 to 75 years. The most commonly affected age group was the third decade of life (34.4%) followed by the fourth decade (21.8%). Patients below 30 years was altogether (34.4% + 21.5% + 4.7%) = 60.6%. Majeed and Bukhari (2011) found 47% of the patients’ age to be 20 years or below and 62% below age 30 years [6]. Bezabih et al (2002) also found 69% below age 30 [7]. Overall results suggest that tuberculosis is more commonly seen in young population [7]. Male: female ratio of the study population is 1:1.58. Female patients were more in number, a fact in concordance with other studies [6, 8, 9]. However, slight male predominance was also reported in a study [7]. In the study cervical lymph node involvement was found most commonly (95.9%). Other similar studies also found cervical lymphadenopathy in the majority followed by axillary lymphadenopathy [7, 8, 10, 11].

Cytomorphological diagnosis of TB was made when there was presence of epithelioid cells singly or in clusters (granuloma) with or without caseous necrosis. FNA has become the first-line diagnostic technique in endemic areas, where the mere presence of epithelioid cell/granuloma indicates tuberculosis until proven otherwise. The reported sensitivity of FNAC from endemic areas ranges from 97% to 100% and the specificity ranges from 88% to 100% [12-14]. But in non-endemic areas sensitivity and specificity of FNAC was found to be low. Ellison et al (1999) reported FNAC sensitivity was 25% and a positive predictive value of 65% in USA [15]. Knox et al (2012) found sensitivity of cytology 38% in a non-endemic area [8]. In this study it was found that sensitivity, specificity, positive predictive value and negative predictive value of FNAC was 79.7%, 48.1%, 31.9% and 88.6% respectively. In the present study, unlike other studies from endemic areas, FNA gave a relatively low sensitivity of 73%. A study in Egypt reported the overall diagnostic sensitivity, specificity, positive predictive value, and negative predictive value of FNAC of cervical lymph nodes to be 90.9%, 67.2%, 82.6%, and 81.3% respectively [16]. Another study in Ethiopia found sensitivity, specificity, positive predictive value, and negative predictive value of FNAC to be 81%, 50%, 54.2%, and 78.6% respectively [17]. Percentage of agreement of FNAC with culture is calculated to be 0.183 (Kappa test) implying poor agreement.

Cytological diagnosis of tuberculosis and culture negative results in this study could be due to suppurative inflammation where granuloma and or caseous necrosis identification was difficult. It might be due to the inhibitory effect of prior-broad spectrum antibiotics [12, 18]. Cytomorphology is an expression of immune response which may exist even in other conditions such as sarcoidosis, fungal infection, leprosy, foreign body granuloma etc. Or it might be that patients with cytological diagnosis of tuberculosis but negative culture were in various stages of disease where viable mycobacteria might not exist in the lymph node.

On ZN staining, Mycobacterium tuberculosis appeared as red/pink beaded rod-shaped bacteria against a blue background. The concentration of organisms in the sputum in pulmonary tuberculosis has a direct relationship to the sensitivity of the ZN stain and a concentration of ≥ 10⁴ organisms/mL would guarantee a positive smear [19]. This is applicable to the aspirates from tuberculous lymphadenitis as well. In the present study, the sensitivity, specificity, positive predictive value and negative predictive value of ZN stain for AFB detection were respectively 17.6%, 98.4%, 76.5% and 79.7%. Derese et al (2012) reported sensitivity and specificity of ZN stain for AFB to be 22.9% and 92.4% respectively [17]. Such low degree of sensitivity is consistent with the findings of other studies with sensitivity ranging from 20% to 43% [20, 21]. The quality of the smear as well as the scanty bacilli found in the FNA could be the main factor for decreased sensitivity, but as expected, the specificity was the highest. However, in this study four of the 17 AFB positive FNA smears were culture negative. This result may be due to the presence of dead bacilli that failed to grow on culture.

The Xpert MTB/RIF (Xpert) test is a novel automated molecular diagnostic test recently endorsed by the WHO for rapid diagnosis of TB. Performance related data of Xpert test from high TB prevalence regions to investigate clinically suspected TB lymphadenitis are limited. Culture served as a standard for growth of MTBC and phenotypic and MTBDR plus drug susceptibility testing for detecting RIF resistance. The Xpert test identified the rpoB mutations associated with RIF resistance. Furthermore, the test is simple and suitable to use in remote and rural areas for the diagnosis of TB lymphadenitis directly from FNA sample.
In this study the sensitivity, specificity, positive predictive value and negative predictive value of Xpert test were 95.9%, 60.5%, 42.6% and 98% respectively. Percentage of agreement of GeneXpert with culture is calculated to be 0.39 (Kappa test) implying a slight agreement. In this study, Xpert test also detected eight positive cases with rifampicin resistance. The sensitivity of this study is in concordance with those reported by other studies [4, 22-24]. The presence of an unrepresentative FNA specimen, the scant number of organisms in the lymph node lesion, nonviable organisms due to a decontamination process, and the presence of amplified false positive signals [1, 25] might all account for the reduced specificity.

Conclusions

In this study, FNAC has been found to be an effective diagnostic method of tuberculous lymphadenitis. FNAC is a diagnostic test which can diagnose granulomatous inflammation as well as reactive and other disease conditions including malignancies, thereby providing more information about the patient’s disease status. Besides, it is a safe outpatient procedure and cost effective. GeneXpert, on the other hand, proves to be very efficient in detecting Mycobacterium tuberculosis with rifampicin resistance. GeneXpert was very helpful in the cases with suppurative lymphadenitis where granuloma or caseation was not found. The limitation of GeneXpert is that it cannot detect other mycobacterium species. It also cannot identify any other disease processes involving lymph nodes. In the perspective of Bangladesh where incidence of tuberculosis is very high, GeneXpert and FNAC both would be very useful in the diagnosis of tuberculous lymphadenitis.

Conflict of Interest

None of the author has any conflict of interest.

References

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