Comparative Analysis of Various Diagnostic Techniques for Tubercular Lymphadenitis: A Pilot Study from a Resource Poor Country

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Abstract: Tuberculosis lymphadenitis (LNTB) is the most common presentation of extra pulmonary tuberculosis. The main causative agents reported were predominantly Mycobacterium tuberculosis (M.tb) and closely related Mycobacterium bovis (M.bovis) and non-tuberculous mycobacteria (NTM). Over the past decade, a set of tedious cytological and microbiological diagnostic tests i.e. fine needle aspiration cytology (FNAC), microscopic smear examination & culture were used. Although FNAC & smear examination were rapid, but none of these techniques were able to differentiate between M.tb and other members of Mycobacterium spp. which is highly essential for planning anti-microbial therapy programme. Methodology: In the present study, smear, FNAC, culture and hupB gene (Rv2986c) based PCR, were applied and each method was analyzed in terms of sensitivity, specificity, along with reliability and cost effectiveness. Results: Considering culture as a gold standard, all other diagnostic methods were compared. Direct PCR showed the sensitivity & specificity of 47% & 75% whereas when performed on culture isolates, the sensitivity rose to 76%. The sensitivity & specificity of FNAC were 60% & 49% respectively whereas direct smear examination was 50% and 70% respectively. Conclusion: We conclude that smear and FNAC are rapid, cost effective, easily available, but has lower specificity and may not be able to differentiate tubercular lymphadenitis from non tubercular lymphadenitis. PCR(hupB gene based) being a singular target for M.tb showed reliability and potential to rapidly detect & identify causative agent of LNTB, can help clinician to initiate correct and timely treatment.

Keywords: Tuberculosis lymphadenitis, culture, hupB gene, fine needle aspiration cytology (FNAC)

INTRODUCTION

Tuberculous lymphadenitis (LNTB) being one of the most frequent cause of lymphadenopathy, accounted for about half of 2,19,945 of total extra-pulmonary TB cases reported in the year 2008 in India. An Indian pediatric study showed prevalence of peripheral lymphadenopathy as 27.2/1000 children and that of LNTB as 4.43/1000 children. Although Mycobacterium tuberculosis complex (MTC) organisms i.e. Mycobacterium tuberculosis (M.tb), Mycobacterium bovis (M.bovis), Mycobacterium africanum and Mycobacterium microti were the main cause of mycobacterial lymphadenitis cases, nontuberculous mycobacterial (NTM) lymphadenitis (NTM-LN) with high frequency in human immunodeficiency virus type 1 (HIV-1)-infected individuals are reported to be an emerging causative agent. Cervicofacial lymphadenitis, the most frequent head and neck manifestation of NTM infection, often presents as chronic, unilateral lymphadenopathy with characteristic violaceous overlying skin changes. Lymphadenitis due to lymphadenitis often presents as chronic, unilateral lymphadenopathy with characteristic violaceous overlying skin changes. Lymphadenitis due to infection with the MTC is more chronic in nature, while NTM-LN often lacks the 12.7 kb fragment containing the mce3 operon whereas all M. tb isolates examined showed the presence of the 12.7 kb fragment, while all the M.bovis strains lacked this fragment. We exploited the differences in the organization of the mce3 operon in the two species.

METHODS

A collaborated pilot study was undertaken at Department of Histopathology and Microbiology, at Vardhman Mahavir Medical College & Safdarjung Hospital (VMMC & SJH), New Delhi, and Department of Biotechnology, at All India Institute of Medical Sciences (AIIMS), New Delhi, India during Jan, 2009 to August 2009. The study was divided in two parts. First phase involved patient registration, sample (aspirates) collection, smear preparation and staining and culture by both solid Lowenstein Jensen (L J) & Liquid broth based BacT/ALERT 3D automation and was performed at Safdarjung Hospital. The second phase involved DNA extraction & PCR from clinical aspirates and was performed in AIIMS.

In this study, 89 clinical suspected patients (52 male and 37 female) of tuberculous lymphadenitis were included, after taking ethical clearance and informed written consent from patients or from parents (in case of children). There were 23 (25.8%) cases of children <15 yrs. The duration of lymph adenopathy varied from 10 days to 20 months. Fine needle aspirations (approx. volume 0.5-2 ml) from all enrolled patients were performed in the Department of Histopathology under sterile aseptic conditions on the suspected lymph nodes. Two smears were prepared and...
stained by Giemsa stain and acid fast staining by Ziehl Neelsen (ZN) method as per the approved guidelines and the findings were recorded. 0.1-0.2 ml aspirate was inoculated on Lowenstein-Jensen (LJ) slant & 0.2-0.5 ml (depending upon total volume of sample aspirated) was put into BacT/ALERT enriched bottles for cultivation of bacilli. The growth on LJ slant was checked everyday in first week to look for rapid growers bacteria and thereafter on weekly basis till sixth week. The growth was finally confirmed by ZN stain for the presence of AFB. In the same way, when BacT/ALERT system flashes positive signal, the bottle was taken out and confirmed by ZN stain for the presence of AFB. In the same way, when BacT/ALERT system flashes positive signal, the bottle was taken out and confirmed by ZN stain for the presence of AFB.

**RESULTS**

The aspirated lymph nodes included cervical (67%), supraclavicular (11 %), submandibular (6 %), auricular (11 %), submental (5 %) of the total cases. The final diagnosis was made if FNAC showed epithelial cell granuloma with necrosis and/or smear positivity for AFB and/or growth on LJ or bactec, were seen1. Based on this, 54 cases were termed as positive. Considering culture as a gold standard, this assay when applied directly on clinical aspirates, showed the sensitivity & specificity of 47% & 75% whereas when performed on culture isolates, the sensitivity rises to 76%. The sensitivity & specificity of FNAC were 60% & 49% respectively. The overall acid-fast bacilli positivity in fine needle aspiration smears was 38.4% of the total cases and in 50% of all culture-positive aspirates whereas of direct smear examination was 50% and 70% respectively (Table 3). LN-PCR was positive in 47% of the aspirates from patients, while PCR on culture isolates, showed sensitivity Table 3: Comparison of sensitivity, specificity of PCR & other tests with culture. 

<table>
<thead>
<tr>
<th>Test</th>
<th>Sensitivity</th>
<th>Specificity</th>
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<tr>
<td>PCR(Clinical samples)</td>
<td>47%</td>
<td>75%</td>
</tr>
<tr>
<td>PCR(Culture isolates)</td>
<td>76%</td>
<td>N.A*</td>
</tr>
<tr>
<td>FNAC</td>
<td>60%</td>
<td>49%</td>
</tr>
<tr>
<td>Smear</td>
<td>50%</td>
<td>70%</td>
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* Can't calculate as it is performed only on positive culture isolates to 76%.

**DISCUSSION**

In endemic areas like India, the detection of LNTB with traditional diagnostic tools is always a major challenge. In the past decade, various studies describing lymph node PCRs from fine needle aspirates or biopsy specimens have consistently shown improved sensitivity (61-78%) when compared with conventional microbiologic methods. The PCR assay described in the study is based on the hupB gene of M. bovis and M. tb. The specificity of the hupB-based PCR assay to detect and identify M. bovis and M. tb has been established and the sensitivity were reported to detect as low as 10-20 picogram DNA of the tubercle bacilli.

In the present study, the LN-PCR was positive in 47% of the aspirates from patients. This lower sensitivity may be attributed either to the small volume of aspirate remaining after distributing the sample for the microbiological and cytological assays or due to presence of PCR inhibitors. To confirm the cause, we again performed PCR on culture isolates, the PCR sensitivity then jumps to 76%. This was expected as inadequate sampling predominantly influences the assay. We performed PCR from the leftover aspirate after performing conventional parameters and identified M. tb in the vast majority of positive cases whereas M. bovis was not found. There were three pediatric cases in which only culture & PCR came positive whereas both FNAC & smear microscopic were negative. These results indicate that culture-enhanced PCR is a highly sensitive and specific method for the detection of M. tb in extrapulmonary specimens especially in children and would diminish the chance of open biopsy.

The only limitation found was unlike culture, the PCR technique does not distinguish between live and dead mycobacteria, a feature that is of the utmost importance when screening for viable mycobacteria in samples such as dairy products following pasteurization or pre-exposure of broad spectrum antibiotics such as amoxicillin-clavulanic acid & fluoroquinolones known to inhibit M. tb.

Revised National Tuberculosis Control Programme (RNTCP) mainly recommends cytology & ZN smear microscopy for the diagnosis of LNTB. In FNAC, diagnosis is based on the presence of granulomas, central necrosis and if possible, demonstration of acid-fast bacilli by staining. Cytology has a sensitivity of approximately 32–59%. In our study, it was 60%. But when compared with culture, FNAC showed low specificity (49%). However, absence of specific cytologic findings of granulomatous lymphadenitis or negative acid-fast bacilli (AFB) smears requires additional open biopsy or repeated FNAC, thus this method has limitations in clinical situations. Another shortcoming of FNAC lies in the difficulty of differentiating tuberculosis from other granulomatous diseases or nontuberculous mycobacterial. Lymphadenitis caused by nontuberculous mycobacterial species usually resistant to anti-tubercular drugs and they would be misdiagnosed as multi-drug resistant tuberculosis (MDR-TB). However, this technique...
provides an easy way for collecting materials for bacteriological examination. The concentration of organisms in the clinical specimen has a direct relationship with the sensitivity of the ZN stain and a concentration of ≥10^5 organisms/ml would normally guarantee a positive smear. The overall acid-fast bacilli positivity in fine needle aspiration smears can vary from 37.4% to 59.4%. In the present study, it was 38.4% of the total cases and in 30% of all culture-positive aspirates. The low sensitivity was probably due to the low concentration of mycobacteria in the aspirate.

Culture reports from different studies detect fine needle aspirates between 39% to 80% positive in the clinically suspected TB-L cases. Our observation also falls in between the reported range (43.1%). However, low sensitivity and extensive time requirements of culture studies limit its usual application. Traditionally, culture followed by a panel of biochemical tests has been used for speciation of mycobacteria but has inherent shortcomings. In the present study, the time consumed for primary isolation on LJ media ranges from 4 to 6 weeks and 2-3 weeks by liquid broth based automated BacT/ALERT system. Although BacT Alert 3D system recovers mycobacteria rapidly even this is too long as it is necessary to commence treatment as soon as possible. These results confirm that PCR from the remainder of fine-needle aspirate could be a good initial diagnostic tool. Given the availability of a thermal cycler, the rest of the procedure has a cost similar to other routine assays for onset of both human and bovine tuberculosis and it can reduce the need for more invasive diagnostic approaches.

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Maternal Obesity and Pregnancy Outcome: A Prospective Analysis

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Objective: To analyze whether the obese women have an increased risk of pregnancy complications and adverse fetal outcome. Methods: The longitudinal prospective study was carried out in the Obst and Gynaec. department, IPGME and R, Kolkata. The study enrolled 422 pre-pregnant obese women with pregnancy as study population and equal number of non obese pregnant mothers as controls. Body mass index (BMI) was e ≥ 30.0kg/m2 and 20-22 kg/m2 in obese and control group respectively. Results: In comparison to average weight pregnant women, obese pregnant women were at increased risk of gestational diabetes mellitus (19.43 vs 3.79%; p<0.001), pregnancy induced hypertension (12.32 vs 2.36%; p<0.001), pre-eclampsia (8.76 vs 3.31%; p<0.001), preterm labor in less than 34 week gestation (7.58 vs 3.55%; p<0.001), cesarean section (36.72 vs 17.53%; p<0.001), instrumental deliveries (12.32 vs 5.21%;p<0.001) and postpartum infection morbidity (9.95 vs 3.79%; p<0.001). These women were more prone to develop overt diabetes (2.36% vs 0) and chronic hypertension (5.21 vs .47%) in future as well. Neonates of obese women were mostly large for gestational age, macrosomic and they had high incidences of birth injuries, shoulder dystocia, premature deliveries, late fetal deaths and congenital malformations particularly spina bifida, cleft lip, cleft palate and heart defect. Conclusion: As obesity is considered to be a modifiable risk factor, preconception counseling and creating awareness regarding health risks associated with over weight and obesity should be encouraged.

LITERATURE REVIEW