Correlation of Fine Needle Aspiration Cytology, Smear and Culture in Tuberculous Lymphadenitis: A Retrospective Study
Shazia Aslam, Sadia Hameed, Arif Hussain, Tariq Gulzar Bhatti, Mrs Nida Attique

Abstract
Tuberculous lymphadenitis is the commonest form of extrapulmonary tuberculosis. Fine needle aspiration cytology (FNAC) is a simple out-patient diagnostic procedure used for the diagnosis of tuberculous lymphadenitis. Over the last two decades, fine needle aspiration cytology has emerged as a simple outpatient diagnostic procedure for the evaluation of tuberculous lymphadenitis. This has replaced excision biopsy of lymph node. In this study, FNAC was complemented with smear examination and culture for AFB. It was observed that out of the 100 reported cases of tuberculosis on FNAC direct smear positivity on ZN staining was 3/100(3%). After the inoculation of residual aspirated material on LJ medium the culture yield was 27/100(27%). Thus FNAC had greater diagnostic efficacy, proved to be a rapid, less time consuming & non-invasive screening test for evaluation of tuberculous lymphadenitis. Settings and Design: A retrospective laboratory based study at, Meezan lab Faisalabad. Material and Methods: 100 patients of lymphadenitis which were diagnosed as cases of granulomatous inflammation on FNAC were included in this study. These cases were reported on cytology by using the Giemsa stain, H& E stain, Gram & Ziehl Neelsen stain. After the smear preparation the part of left over aspirated material was inoculated on LJ medium and were reported on the basis of morphological features by concerned microbiologist. Results: Out of these 100 selected reported cases of tuberculous lymphadenitis culture revealed growth of Mycobacterium on 27 of them. While direct microscopic examination of the ZN stained smears from these aspirates revealed the presence of AFB in only 3 out of 100 cases. Key Words: Mycobacterium tuberculosis, lymphadenitis, fine needle aspiration cytology & ZN staining (Ziehl Neelsen), Lowenstein Jensen medium.

INTRODUCTION
Tuberculosis is thought to be one of the oldest human diseases. The history of tuberculosis is almost as old as mankind. Evidence of its existence was seen in Egyptian mummies and statuaries in the form of Pott’s disease of spine. Since the identification of Mycobacterium tuberculosis as etiologic agent for tuberculosis by Robert Koch in 1882, there have been great advances in our understanding of many of the crucial aspects in its pathogenesis.
pulmonary tuberculosis is no doubt common, tuberculous lymphadenitis is the most common form of extrapulmonary tuberculosis. Tuberculosis waxed and waned in Europe during 18th and 19th centuries. During industrial revolution it claimed millions of lives in Europe and so was called ‘The White Plague’. According to World Health Organization tuberculosis still kills three million people every year in underdeveloped countries.  
Primarily considered to be a pulmonary disease, Tuberculosis can affect almost any organ or systems of human body. The term extra pulmonary tuberculosis has been used to describe isolated occurrence of tuberculosis at various body sites other than the lung. The most common sites of extra pulmonary tuberculosis consist of gastrointestinal tract (GIT), Lymph nodes, genitourinary system, bone, joint and central nervous system involvement followed by peritoneal and other organ involvement. 
Tuberculous lymphadenitis in the cervical region is termed as scrofula. The microbiological cause of scrofula was first appreciated by Bollinger, May and Demme in the mid to late 19th century when they noted that Mycobacterium bovis from cows was the cause of this ailment. The conventional methods of diagnosis for tuberculosis like examination of sputum for Acid fast bacilli and chest x-ray are fairly accurate in detecting the active pulmonary component of the disease. However they are not useful for detecting extra pulmonary components. The diagnosis of lymph node tuberculosis usually rests on a lymph node biopsy with or without bacteriological studies. 
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The unusual features of TB lymphadenitis are its gender and age distribution, as it is more common in females and in the younger age groups, in contrast to pulmonary tuberculosis which is more common in males and in the older age group. It has a peak age of onset at 20 to 40 years. Patients usually present with slowly enlarging lymph nodes which may otherwise be asymptomatic. Some patients may manifest systemic symptoms such as fever, weight loss, fatigue, and occasional night sweats. Fine needle aspiration cytology is now established as an alternative, easy and rapid method of tissue diagnosis. It also has a high degree of patient’s acceptance as FNAC avoids physical and psychological trauma occasionally encountered after biopsy due to anaesthesia, surgical procedure and hospitalization. It is very safe, cost effective and conclusive. Mycobacteria are slow growing and hence culture is not done routinely in all laboratories. Few studies have tried to correlate the cytological finding with microbiological results for the presence of acid-fast bacilli in smears and culture for mycobacteria. The present study was undertaken to correlate the cytomorphologic features of lymph node aspirates of tuberculous lymphadenitis to bacteriologic studies that included both smear and culture for acid fast bacilli (AFB).

AIMS & OBJECTIVES 
The current study was conducted with following objectives. 
1. To determine efficacy of FNAC in detecting tuberculous lymphadenitis. 
2. To evaluate the role of ZN staining and culture of aspirated material in detecting tuberculous lymphadenitis.

MATERIALS & METHODS 
The cases of this study came from those patients with lymphadenopathy referred to Meezan lab for routine aspiration diagnosis from November 2011 to April 2013. All of the 100 aspirates diagnosed as tuberculous lymphadenitis were subjected to mycobacterial culture & ZN (Ziehl Neelsen) stain for AFB on directly prepared smears. Fine needle aspiration cytology was performed aseptically by using 24 G needle and 5ml or 10ml BD syringe. The smears were stained by Giemsa stain, H& E stain and by the Ziehl Neelsen stain.
technique for AFB. The contents of the needle were also inoculated on Lowenstein Jensen medium. Morphology of the growth appearing after 2-3 weeks of incubation was checked by AFB smear. The negative cultures were incubated for 6-8 weeks before discarding them. The criteria used for making the diagnosis of tuberculosis on FNA aspirates were presence of granulomas comprising of epithelioid cells with or without giant cells and presence of necrotic material with or without epithelioid cells and /or ZN smear positivity for Acid Fast Bacilli (AFB) and/or positive culture for Mycobacteria.

RESULTS
The study comprises of 100 cytologically diagnosed cases of tuberculous lymphadenitis at Meezan lab Faisalabad. The age of the patients ranged from 3.5 to 57 years, the majority being in the age group of 10-30 (70%). The male to female ratio was 38:62. The majority of aspirations were from cervical lymph nodes (52%) followed by supraclavicular (14%) and submandibular lymph nodes (14%) (Table no 1).

The nature of the material aspirated was variable. Thin liquefied pus like material, frankly purulent material, typical caseous material or solid particles were noted on gross examination. Based on the cytomorphology smears were categorized into three types:

1. Necrotic or caseous material only
2. Necrotic material with typical epithelioid granulomas; or
3. Epithelioid granulomas or cells in background of superadded pyogenic infection.

Culture isolates were 27/100(27%) (Figure-1). The isolates on culture was Mycobacterium tuberculosis in all the positive cases proved by ZN staining which was also 27%.

As the direct smears of the aspirates were also subjected to acid fast staining by Ziehl Neelsen technique(Table no 2) which gave a lower positivity of 3/100(3%). So the total ZN stained smear positivity was 30/100 (30%) (Figure-2). Among these majority cases 17/100(14%) were belonging to chronic granulomatous inflammation with superadded pyogenic infection (category 3) (Figure 3, 4), 10/100 cases were of the chronic granulomatous lymphadenitis group (category2) (Figure 5-7) and 3/100 cases were belonging to class of necrotic or caseous material (category 1).

Table-1
Frequency of granulomatous inflammation / Tuberculosis at various sites

<table>
<thead>
<tr>
<th>Site</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cervical lymph nodes</td>
<td>52%</td>
</tr>
<tr>
<td>Supraclavicular lymph nodes</td>
<td>14%</td>
</tr>
<tr>
<td>Submandibular lymph nodes</td>
<td>14%</td>
</tr>
<tr>
<td>Axillary lymph nodes</td>
<td>09%</td>
</tr>
<tr>
<td>Preauricular lymph nodes</td>
<td>03%</td>
</tr>
<tr>
<td>Submental lymph nodes</td>
<td>01%</td>
</tr>
<tr>
<td>Inguinal lymph nodes</td>
<td>01%</td>
</tr>
<tr>
<td>Gluteal region swelling</td>
<td>02%</td>
</tr>
<tr>
<td>Left shoulder swelling</td>
<td>01%</td>
</tr>
<tr>
<td>Swelling in front of neck</td>
<td>01%</td>
</tr>
<tr>
<td>Discharging sinus cervical region</td>
<td>01%</td>
</tr>
<tr>
<td>Breast swelling</td>
<td>01%</td>
</tr>
</tbody>
</table>

Table-2
Correlation of cytomorphologic features with smear AFB positivity in cytologically proved lymphadenitis cases

<table>
<thead>
<tr>
<th>Cytologic category</th>
<th>Number</th>
<th>Smear positivity (%)</th>
<th>Culture positivity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Necrosis only</td>
<td>07</td>
<td>03</td>
<td>03</td>
</tr>
<tr>
<td>Granuloma with necrosis</td>
<td>40</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Granuloma with superadded pyogenic infection</td>
<td>53</td>
<td>17</td>
<td>14</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>30(30%)</td>
<td>27(27%)</td>
</tr>
</tbody>
</table>
Figure-1
Photograph revealing growth on LJ medium inoculated from part of aspirate

Figure-2
Cytological smear revealing AFB positivity on ZN stain

Figure-3
Cytological examination revealing granuloma formation in a background of mixed inflammatory cell infiltrate

Figure-4
Granulomatous inflammation with Superadded pyogenic infection

Figure-5
Cytological smear revealing granuloma formation within a lymph node

DISCUSSION

Figure-6
Cytological examination revealing granulomas undergoing the process of caseation necrosis
DISCUSSION
The cytological diagnosis of tuberculous lymphadenitis is usually based upon the demonstration of conventional epithelioid cells, with or without multinucleated giant cells and with or without caseous necrosis. Even in the absence of epithelioid cells, necrotic material has proved to be useful as it provides the highest yield of Acid fast bacilli (AFB) positive cases. In our study the majority (17%) of the positive cases were of the category 3 in which the granulomatous inflammation accompanied by necrosis was superadded by pyogenic infection. In many studies diagnosis of Tuberculosis has been made on aspiration cytology. Cervical lymph node was the most common site of involvement in most of the studies followed by axillary lymph nodes. Our study was also consistent with above studies in terms of cervical lymph node involvement (87%) as the most common anatomic site of granulomatous inflammation. Female gender was slightly more affected (62%) in current study and was in concordance with other studies. However, there was slight male predominance in a study of Bezabih et al which Out of our 100 cases, 60% patients were of 10-30 years, 25% were above 30 years of age among them the maximum age was 58 years while 10% patients were below 10 years of age & out of these 10% cases the minimum age was 11 months. These findings were close to the results by Bezabih et al in which 69% were below 30. Based on the facts, it can be inferred that tuberculosis was more commonly seen in young population. Using the Ziehl-Neelsen technique on direct smears the AFB positivity was 3% in our study compared with the 23.5% & 40-50% reported in earlier studies. Prior treatment by antituberculous drugs could be one of the reason for this different result. However in smears with characteristic cytomorphology failure to demonstrate AFB does not exclude a diagnosis of tuberculosis as it is known that for microscopic demonstration the number of AFB should be 10,000-100,000/ml of material. Culture for Mycobacteria is the gold standard to confirm the etiology. In our study the rate of isolation of Mycobacteria from aspirates was 27%. This is slightly lower than the culture positive isolates in a study reported by Radhika et al which was 35%. The overall ZN staining positivity for AFB was 30% which is slightly higher than a study by Ninama GL et al. in which it was 22.95%. Our experience showed that FNAC is a reliable diagnostic tool in cases of tuberculous lymphadenopathy and it should be used as a first line of investigation.

CONCLUSION
FNAC is an excellent diagnostic tool for diagnosis of tuberculosis in patients with lymphadenopathy. It is simple, safe, quick, reliable, accurate, minimally invasive, cost effective and most suitable in our country where tuberculosis is highly prevalent. Moreover with FNA one can obtain suitable material for ZN stain and culture in patients of tuberculous lymphadenitis. With FNA one can avoid scar mark of biopsy and its complications.
REFERENCES


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