

EFFICACY OF FINE NEEDLE ASPIRATION CYTOLOGY, ZIEHL-NEELSEN STAIN AND CULTURE (BACTEC) IN DIAGNOSIS OF TUBERCULOSIS LYMPHADENITIS

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ABSTRACT

Background: Tuberculous Lymphadenitis is the commonest form of extra pulmonary tuberculosis and tissue diagnosis is the main stay in the diagnosis of extra-pulmonary tuberculosis. This study was conducted to compare cytology, ZN staining and culture findings of clinically suspected tuberculosis lymphadenitis cases.

Methods: In the Present Study clinically suspected cases of Lymphadenopathy were undergone Fine Needle Aspiration. The aspirate were examined cytologically followed by ZN staining and BACTEC culture.

Results: The cytology suggestive of tuberculous lymphadenitis was found in 46(76.6%) cases out of total 61 cases. Ziehl Neelsen stain demonstrated acid fast bacilli (AFB) in 14 (22.9%) cases and BACTEC isolated mycobacteria in 36 (59%) cases. Out of 61 cases as many as 15(24.5%) cases yielded pus and in 13 of those cases cytology of tuberculous lymphadenitis was found. Cases from blood mixed aspirate demonstrated AFB positivity in 2 (5.88%) and mycobacteria were isolated in 16 (48.48%) cases and cytology also suggest least numbers of tuberculous lymphadenitis i.e. 21 (63.63%) cases.

Seven out of Nine cases of necrosis with or without neutrophils show presence of AFB. Samples having epithelioid cell granuloma with or without necrosis show presence of AFB in 5 (16.66%) and 2 (25%) cases and mycobacteria was isolated in 22 (73.73%) and 5 (62.5%) cases respectively.

Conclusion: In spite of the diagnostic pitfalls, the results obtained on analytical examination of the study carried out reinforce the opinion that Fine Needle Aspiration Cytology serves as a potent and accurate diagnostic tool for patients presenting with Lymphadenopathy due to tuberculosis.

Key words: Fine Needle Aspiration Cytology, Ziehl-Neelsen Staining, BACTEC

INTRODUCTION

Tuberculosis is a very ancient disease and evidence of its existence was seen in Egyptian mummies and statues in the form of pott's disease of spine.¹ 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 as 'The White Plague'.² Robert Koch wrote that tuberculosis killed one third of Europeans of middle age. According to WHO tuberculosis still kills three million people every year in underdeveloped countries.² Tuberculosis still ravage in India even 100 years after the discovery of tubercle bacillus, with an annual incidence of 100/100,000 and a prevalence four times the incidence.³ AIDS is one of the important causes for change in etiological profile as well as increasing cases of extra pulmonary tuberculosis.⁴

Tuberculous Lymphadenopathy is the commonest form of extra pulmonary tuberculosis in region where mycobacterial infection is highly prevalent and presents commonly in lymphnodes draining the head and neck. The conventional methods of diagnosis for tuberculosis like sputum examination of 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. By and large, tissue diagnosis is the main stay in the management of cases of extra-pulmonary tuberculosis.

Fine needle aspiration cytology (FNAC) is now established as an alternative, easy and rapid method of tissue diagnosis. It also has a high degree of patient acceptance as FNAC avoids physical and psychological trauma occasionally encountered after biopsy, anaesthesia, surgical operation and hospitalization. It is

very safe, trivial, cost-effective and at the time 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 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
3. To correlate the gross appearance of aspirate and microscopic feature of lymph node aspirate with AFB positivity and culture.

MATERIAL AND METHODS

The present study consists of clinically suspected cases of tuberculous lymphadenitis attending the Outpatient department of SSG hospital, Baroda from August 2003 to October 2004. The patients had been initially seen in the outpatient department of SSG hospital Baroda and were subsequently referred to FNA clinic for evaluation of their Lymphadenopathy. Each patient was subjected to complete clinical examination, routine hemogram with ESR of blood and to FNA. Varying sites of Lymphadenopathy i.e. cervical, axillary, inguinal were aspirated using 22 gauge needle attached to 10ml disposable syringe under strict aseptic precaution. During each pass the needle was moved throughout the lesion several times while aspirating. Care was taken not to aspirate through dependent area of swelling to prevent sinus formation.

In each case the part of the aspirate was used for preparing 3 smears at least, one for Hematoxylin & Eosin (H & E) stain which was fixed immediately in cytofix containing equal volume of absolute alcohol and ether, one for giemsa stain and one for ZN stain. Remaining material was inoculated in BACTEC (Middlebrook 7H12B) vial taking care to have at least 0.5 ml volume of test material in the vial.

A record was made of all relevant findings including the gross appearance of the aspirate. The gross appearance of aspirate was described as caseous for cheese like or yellow-white aspirate, pusy for greenish-yellow or yellow aspirate and blood mixed when material was haemorrhagic.

Cytology smears and ZN stain smears were examined in cytology sections of Department of Pathology, Medical College, Baroda. BACTEC vial containing aspirated material was sent to Amin's laboratory without delay where first medium was supplemented with mixture of anti-microbials called PANTA, which contains polymyxin -B, amphotericin-B, nalidixic acid, trimethoprim and azlocillin to reduce the contamination. Then initial reading Growth Index (GI)

was taken and then incubated at 37°C. Readings of GI were taken on day 1, 3, 5, 7,9,12 for first 15 days and then weekly upto 45 days.

The diagnosis of tuberculous lymphadenitis was made when the following criteria's were met: the presence of epithelioid cell granuloma with or without necrosis and /or ZN smear positivity for Acid-Fast Bacilli (AFB) and/or positive culture for mycobacteria.

Data was recorded and statistically analyzed using chi square. Specificity, sensitivity, Positive Predictive Value (PPV), Negative Predictive Value (NPV) and likelihood ratio of smear and cytology were compared.

RESULT

The Present Study Consist Of 61 Cases Of Lymphadenopathy who attended the outpatient department of SSG hospital Baroda from august 2003 to October 2004. the FNA smears of all 61 patients were cytologically followed by ZN staining and BACTEC culture.

Culture reporting the minimum incubation time for isolation of mycobacteria through BACTEC culture was 14 days and maximum was 52 days (mean 24 days)

Table 1: Cytological findings in 61 cases of Lymphadenopathy

Total cases	61
Cytology suggestive of tuberculous lymphadenitis	46*
Cytology suggestive of nonspecific lymphadenitis	14 ⁺
Cytology of metastatic squamous cell carcinomas	01

*-in 1 case possibility of tuberculous lymphadenitis was given.

⁺-in 2 cases repeat after antibiotic therapy was advised to rule out tuberculosis

The cytology suggestive of tuberculous lymphadenitis was given in 46(76.6%) cases out of 61 cases, which also included one case where the possibility of tuberculous lymphadenitis was given. In 14 cases a cytology report suggestive of non specific or reactive of lymphadenitis was given which included two cases where repeat FNAC after antibiotic therapy to rule out tuberculosis was advised. One case showed metastatic squamous cell carcinoma on cytology.(Table 1)

Table 2: Correlation between cytology, ZN staining and culture findings

Total cases	61
Cytology suggestive of tuberculous lymphadenitis	46(75.4)
ZN staining demonstrating AFB	14(22.9)
Culture isolating mycobacteria	36(59)

Figure in bracket shows percentage

Ziehl Neelsen stain demonstrated acid fast bacilli mycobacteria in 36 (59%) cases (Table 2). (AFB) in 14 (22.9%) cases and BACTEC isolated

Table 3: Correlation between gross, cytology, ZN staining and culture features of aspirated material

Gross appearance of aspirate	Cases (%)	Cytology s/o TBLN (%)	AFB +ve on ZN stain (%)	Culture isolated mycobacteria (%)
Blood mixed	34(55.70)	21(61.70)	2(5.88)	16(47.05)
Cheesy	12(19.60)	12(100)	4(33.33)	8(66.60)
Purulent	15(24.50)	13(86.60)	8(53.33)	12(80.00)
Total	61	46(75.40)	14(22.95)	36(59.01)

Of 61 cases as many as 15(24.5%) cases yielded pus and in 13 of those cases cytodagnosis of tuberculous lymphadenitis was given. In two cases non specific lymphadenitis was given but in one of them ZN staining demonstrated AFB and culture isolated mycobacteria. Overall 8 (53.33%) cases from pus based aspirate demonstrated AFB on ZN staining and in 12 of these 15 cases mycobacteria were isolated on culture. This was followed by cheesy aspirate which demonstrated AFB in 4 (33.33%) cases and

mycobacteria isolated in 8 (66.66%) cases. Cases from blood mixed aspirate demonstrated least number of AFB positivity i.e. 2 (5.88%) and mycobacteria were isolated in 16 (48.48%) cases and cytology also suggest least numbers of tuberculous lymphadenitis i.e. 21 (63.63%) cases. (Table 3)

Of the 61 cases as many as 15(24.5%) cases yield pus and in 13 of those cases cytodagnosis of tuberculosis lymphadenitis was given.

Table 4: Correlation of morphology on smears, AFB positivity and culture positivity

Morphology on cytology	Cases (%)	AFB positive (%)	Culture positive (%)
Epithelioid cell granuloma without necrosis	8 (17.02)	2 (25.00)	5(62.50)
Epithelioid cell granuloma with necrosis	30(63.82)	5(16.66)	22(73.33)
Necrosis without granuloma	9(19.14)	7(77.77)	9(100.00)
Total	47	14(29.78)	36(76.59)

Based on present study criteria 47 cases could be classified as tuberculous lymphadenitis. These cases are sub divided into three groups on the basis of presence of necrosis and epithelioid cell granuloma as shown in table- 5. 7 out of 9 cases showing necrosis with or without neutrophils show presence of AFB and in same all 9 cases mycobacteria could be isolated. The other two groups i.e. epithelioid cell granuloma with or without necrosis show presence of AFB in 5 (16.66%) and 2 (25%) cases and mycobacteria could be isolated in 22 (73.73%) and 5 (62.5%) cases respectively. In all cases culture positivity was significantly higher than smear positivity (with p value 0.00005%).

DISCUSSION

The present study consists of 61 clinically suspected cases of tuberculous lymphadenitis with a M:F ratio of 1.25:1 and in the age group of 5 to 55 years ,who attended the outpatient department of SSG hospital, Baroda.

The patients were examined clinically and fine needle aspiration of Lymphadenopathy was carried out. The material obtained was used for cytological examination, ZN smears and BACTEC culture, also the gross appearance of aspirate was noticed as either purulent or cheesy or blood mixed. Diagnosis of tuberculous

lymphadenitis was made when following criteria were met: epithelioid cell granuloma with or without necrosis and /or smear positivity of acid fast bacilli and /or culture isolating mycobacteria

The results obtained were in the range that was observed by other authors, who carried out same procedure. When gross appearance of aspirate was correlates with AFB and culture positivity, maximum positivity was observed in cases with purulent aspirate for both. On cytomorphological correlation maximum positivity for AFB (77.77%) and culture (100%) was found in smears showing necrosis without epithelioid granuloma. The overall ZN staining positivity for AFB was 22.95% and in 59.01% cases mycobacteria were isolated by culture. In all the culture positivity was significantly higher than ZN smear positivity (p value = 0.00005%)

When culture was taken as the Gold Standard the diagnostic parameters for cytology were as follows: Sensitivity 97.2% Specificity 56% Accuracy 80.32%. Sensitivity of FNAC was higher and diagnostic accuracy was comparable with other studies.

The diagnostic difficulties encountered were parallel to those experienced by different authors^{5,6} working on similar projects, a case in point being false negative cytology diagnosis in case with purulent aspirate which

calls for ZN staining in every case suspected of tuberculous in origin.

CONCLUSION

In spite of the diagnostic pitfalls, the results obtained on analytical examination of the study carried out reinforce the opinion that Fine Needle Aspiration Cytology serves as a potent and accurate diagnostic tool for patients presenting with Lymphadenopathy due to tuberculosis.

This approach carries numerous advantages, the principal ones being enhanced patient compliance obtainable samples, easily aspirated tissue and an inexpensive yet accurate outcome. Another essential and practical feature includes the staining of aspirated smears by Zeihl-Neelsen technique especially in purulent material for an improved diagnostic accuracy. These procedures facilitate a reduction in operative morbidity as well as the time span for a definitive diagnosis.

Finally, culture methods for the specific mycobacteria proved to be the mainstay in the diagnosis of tuberculosis.

In conclusion, FNAC serves as one of the most accurate frontline method for the diagnosis of

tuberculous lymphadenitis. It is more than adequately supplemented by the mandatory lymphadenitis. It is more than adequately supplemented by the mandatory Ziehl-Neelsen technique carried out on smears as well as culture methods which can be relied upon in conjunction with the above procedures. However, newer diagnostic techniques would be more than welcome in improving the diagnostic yield of tuberculous lymphadenitis.

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