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Research Article

THE DIAGNOSIS OF SUSPECTED TUBERCULAR LYMPHADENITIS CASES – ROLE OF SMEAR AND CULTURE OF ASPIRATED MATERIAL

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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, Ziehl Neelsen (ZN) staining and culture findings of clinically suspected tubercular lymphadenitis case. In the Present Study clinically suspected cases of lymphadenitis were undergoing Fine Needle Aspiration. The aspirate were examined cytologically followed by ZN staining and culture.

Results: 42 samples were collected from clinically suspected cases of tubercular lymphadenitis were processed for cytology which showed 57.1%, regular smear with ZN staining showed 23% and culture on Lowenstein Jensen (LJ) media showed 28% positive aetiological diagnostic results. Among the 12 culture positive for mycobacteria 9(75%) phenotypically identified as *M.tuberculosis*, rest three were *M.chelonae, M.abscessus* and *M. fortuitum.*

Conclusion: In spite of the diagnostic pitfalls, the results obtained on analysis 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.

INTRODUCTION

Tuberculosis has been and is a great problem throughout the world, especially in a developing country like India. Besides pulmonary tuberculosis, extra-pulmonary manifestations are relatively more common among the Asian populations, the most common being lymphadenitis especially in the cervical region. (Grange et al 1982) TB has been a major global public health problem from times immemorial. World Health Organization (WHO) estimates shows that globally there are 8.6 million incident cases of TB of which 80% are in 22 countries, with India ranked as the highest burden country. (Global tuberculosis report, WHO, 2013) Extra-pulmonary Tuberculosis (EPTB) constitutes about 15 to 20 % of all cases of TB.[2] The annual global incidence of EPTB has been increasing in the last decade due to the changing TB control practices, spread of HIV (human immunodeficiency virus), the population growth and the cure of infectious cases of TB might have resulted in a relative rise of annual EPTB case detection. HIV pandemic further complicates the situation, as EPTB constitutes more than 50 % of all cases of TB in HIV-positive patients. (Global tuberculosis control report, WHO, 2001) By and large; tissue diagnosis is the mainstay in the management of cases of EPTB. 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, anesthesia, surgical operation and hospitalization. It is very safe, trivial, cost-effective and at the same time conclusive.

Objective

The current study was conducted to determine efficacy of FNAC in detecting tuberculous lymphadenitis, to evaluate the role of Ziehl Neelsen's staining (ZN) and culture of aspirated materials tuberculous lymphadenitis. To correlate the gross appearance of aspirate and microscopic feature of lymph node aspirate with AFB positivity and culture.

MATERIALS AND METHOD

The present study consists of clinically suspected cases of tuberculous lymphadenitis attending the Outpatient department of N.R.S Medical College and Hospital from July to August 2015. The patients had been initially seen in the outpatient department of N.R.S Medical College and Hospital and was subsequently referred to FNA section for evaluation of their

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lymphadenitis. Each patient was subjected to complete clinical examination, routine hemogram with ESR of blood, ICTC testing and to FNA. The patients included in the study were HIV nonreactive. None of the patients were on anti-tubercular treatment. 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 2 smears at least, one for Hematoxyllin & Eosin (H & E) stain which was fixed immediately in cytofix containing equal volume of absolute alcohol and ether, and one for ZN stain. Remaining material was inoculated on Lowenstein – Jensen medium (LJ Medium) vial taking care to have at least 0.5 ml volume of test material in the vial. LJ Medium slopes were incubated at 37°C for 8 weeks. Any growth was subjected to smear examination by ZN staining method. Growths found to be positive for acid fast bacilli were identified on the basis of various culture and biochemical characteristics viz., (1) rate of growth, (2) growth at room temperature, and at 45°C, (3) pigments production. (4) catalase and peroxidase tests, (5) niacin test and (6) growth in paranitrobenzoic acid medium (PNB). If aspirate was found to be inadequate, FNA was repeated at the same time for better retrieval of aspirate.

RESULTS

The total number of Samples (collected from ill-defined Lymph nodes of the suspected patients of Tubuerculous lymphadenitis in N.R.S Medical College and Hospital Department of pathology under FNAC section) was 42, of which total number of males (M) were 24, and that of females (F) were 18. These samples were used to perform Acid Fast Staining (Z-N staining) for detection of *Mycobacteria.* Also samples were spread on Lowenstein–Jensen medium for culturing. If organism grows, that confirms the presence of ***Mycobacteria*** on the sample. Age of the patients varies from 5yrs to 70 yrs. Most of the lymph nodes isolated were from cervical area (91.67%).

Table 1

Techniques	Percentage of Tuberculosis Diagnosis (n=42)
FNAC	57.1
AFB Culture	28.6
ZN staining	23.9

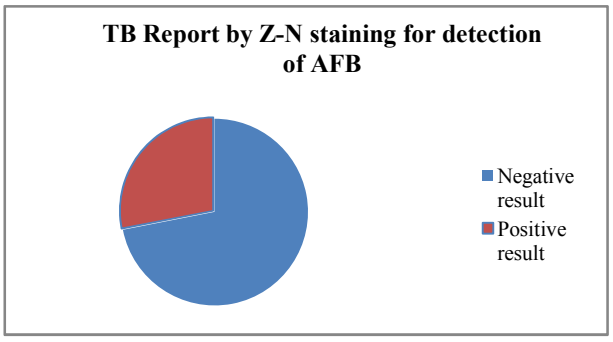

Fig.1 ZN Staining of smears results

Total 42 samples were used to perform Acid Fast Staining for detection of ***Mycobacteria,*** within 10 sample shows positive result.

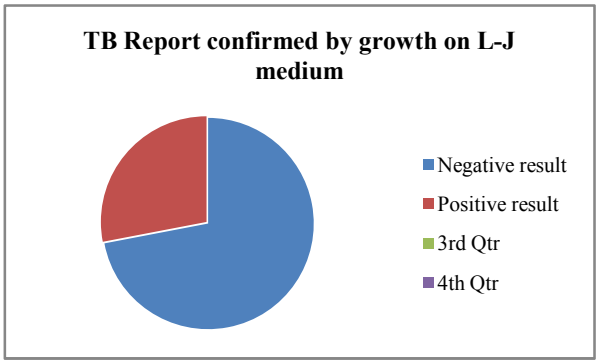

Fig. 2 LJ medium culture results

Total 42 samples were inoculated on L-J Medium for culturing, within 12 sample shows growth on the media.The total number of samples (collected from ill-defined Lymph nodes of suspected Tuberculous lymphadeopathy patients in N.R.S Medical College and Hospital department of pathology under FNAC section) was 42.Total 42 samples were used to perform Acid Fast Staining for detection of ***Mycobacteria ,*** within 10 sample shows positive result that is 23.9% of total. Total 42 samples were inoculated on **Lowenstein –Jensen** Medium for culturing, within 12 sample shows growth on the media that is 28.6 % of the total. Among the 12 culture positive for mycobacteria 9(75%) phenotypically identified as *M.tuberculosis*, rest three were *M.chelonae, M.abscessus* and *M. fortuitum.*

Four basic pattern of FNAC were observed: 1. Cellular Aspirate shows pyogenic lesion, Smears shows plenty of neutrophils, 2.Smears shows a typical epithelial cells clusters on a necrotic background (33.3%), 3. AFB Present in necrotic background (23.8%), 4. Smears shows cytological picture of adenocarcinoma in LN(9.6%). Among the cytological categories the category 2. showed 28.6%, and category 3. showed 80% culture positivity.

DISCUSSION

The present study consists of 42 clinically suspected cases of tuberculous lymphadenitis with a M: F ratio of 1.33:1 and in the age group of 5 to 70 years, who attended the outpatient department of N.R.S Medical College and Hospital, Kolkata. In a study by Ahmad *et al*, the youngest patient was two-year-old and the oldest being 95 years. [4]Majority of the patients (75%) were in the second to fourth decades of life. Similar age distribution was seen in a study by Ergete andBekele[5], Purohit *et al* [6] and Dandapat *et al* [7] female predominance was noted by Pamra *et al* [8], Ergete and Bekele [5] and Purohit *et al* [6] while male predominance was noted by Rajsekaran *et al* [9], and Ahmad *et al* [4]. Clinically, in our study, cervical region was the most commonly affected region, involved in 91.7% of cases. This was in concordance with Bezabih *et al* [10] who observed cervical involvement in 74.2% of cases. While matted lymph nodes seen in majority of cases (60%) by Ahmad *et al* [4,] Single lymph node enlargement was seen in 48.6% tubercular lymphadenopathy by Aggarwal *et al* [11] .We noted a much

higher incidence (50%). The patients were examined clinically and fine needle aspiration of Lymphadenitis was carried out, material obtained was used for cytological examination, ZN smears and culture, also the gross appearance of aspirate was noticed as either purulent or cheesy or blood mixed.

The smear positivity rate was relatively low (23.9%) probably due to only one staining technique used. Fluorescent staining along with Ziehl Neelsen method could have improved the smear positivity rate. The smear positivity rate among the cytologically diagnosed cases was 41.67% (10 out of 24).Culture isolation of mycobacteria was better in our study (28.6%). Positive cultures are usually obtained only 30-50% of all such cases[12].

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. The overall ZN staining positivity for AFB was 23.9% and in 28.6% cases mycobacteria were isolated by culture. In all the culture positivity was significantly higher than ZN smear positivity (p value =0.00005%). Among the 12 culture positive for mycobacteria 9(75%) phenotypically identified as *M.tuberculosis*, rest three were *M.chelonae, M.abscessus* and *M. fortuitum.*

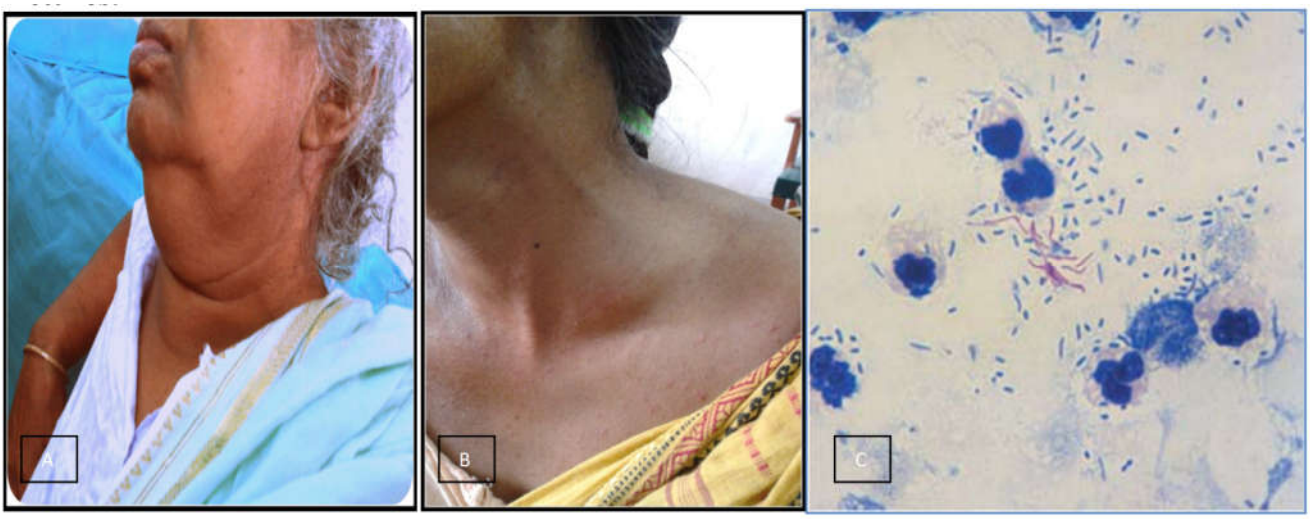

Fig 3A&B Clinical presentation of suspected tubercular lymphadenitis patients

Fig 3C Ziehl Neelsen smear from Lymph node aspirated materials show Acid Fast Bacilli

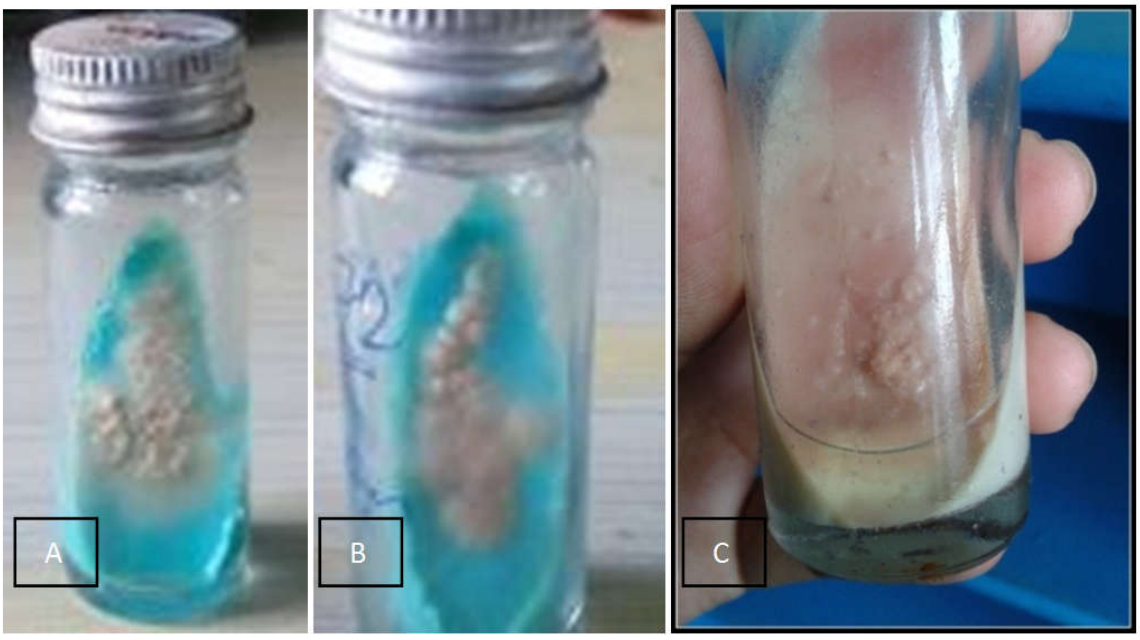

Fig 4 Culture in Lowenstein Jensen Media showing growths of A. M.tuberculosis, B. M. abscessus and C. M.fortuitum respectively.

This may be due to the low number of organisms in lymph node lesions. In addition, natural healing process, previous anti-tuberculosis treatment and unrepresentative specimens of the lymph node material used for culture can all account for more negative cultures. The results obtained were in the range

The identification of species of mycobacteria would help to study various biological properties of mycobacteria including drug sensitivity and therapeutic approach. The diagnostic difficulties encountered were parallel to those experienced by different authors 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. The tubercular lymphadenitis patients were treated with CAT –I antitubercular regimen under DOTS. Other patients were treated with Clartithromycin and Ethambutol based regimen, both group of patients responded well.

CONCLUSION

Tubercular lymphadenitis is the commonest among the suspected patient group and also among other the Non Tuberculous Mycobacteria are also coming up. So identification up to the species level is important in the management of the patients. In spite of the diagnostic pitfalls, the results obtained on analysis 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

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