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CASE REPORT

An Unusual Case of Tuberculous Lymphadenitis and Comparison of Various Methods for Diagnosis of Tuberculous Lymphadenitis

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Abstract

Although tuberculous lymphadenitis (TBLN) is extremely common in India, sometimes it may pose a diagnostic dilemma even after multimodality testing. This case report is about a patient who had undergone multiple fine needle aspirations (FNAC), Ziehl-Neelsen staining of aspirate, GeneXpert Nucleic acid amplification test (TBNAAT), biopsy and immunohistochemistry before arriving at a provisional diagnosis by hematoxylin-eosin staining of additional deeper sections. Additional information obtained by TBNAAT is whether the isolated strain is sensitive to Rifampicin. Emergence of multidrug resistance tuberculosis (resistance to rifampicin and INH) and extensively drug resistant strains implying resistance to fluoroquinolones and second-line injectable drugs can be a huge problem in the management of the disease. She is now under treatment and follow up. This case report highlights that caseation necrosis on FNAC even in the absence of acid-fast bacilli and a negative TBNAAT is strongly suggestive of tuberculous aetiology and may be used for initiating definitive treatment. A comparison of results of ZN stain of aspirate with TBNAAT for 22 cases of clinically suspected TBLN is also brought out.

Key Words: *Tuberculous Lymphadenitis; Fine needle aspiration; Ziehl-Neelsen stain; Nucleic acid testing*

Introduction

Extra Pulmonary Tuberculosis accounts for approximately 15% of all tuberculosis infections [1]. Fine needle aspiration cytology (FNAC) is usually the first investigation in a suspected case of tuberculous lymphadenitis. Sensitivity and specificity of FNAC for diagnosis of TB lymphadenitis has been reported as 79.7% & 48.1% when compared to acid fast bacilli (AFB) culture [2]. With histopathological examination as reference standard the corresponding values were 90.9% and 67.2% respectively [3]. Although easy to perform and specific, Ziehl-Neelsen (ZN) stain lacks sensitivity, requiring 10^3-10^4 bacilli/mL of sputum to be visualized [4]. Our protocol for suspected tuberculous lymphadenitis includes FNAC followed by both

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Leishman-Giemsa and ZN stain of the aspirate. Submission of the sample for Gene Expert Nucleic acid amplification test (TBNAAT) has been recently added to existing protocol because it further confirms the diagnosis and also provides additional information regarding sensitivity to Rifampicin. The test is available free of cost in local government hospital. Histopathological examination is done only if the results are inconclusive. Treatment of TB requires five drugs for a minimum of six months with potentially serious numerous side effects and necessitates high degree of patient compliance. It is commenced after obtaining a definitive diagnosis. At our centre multidrug resistant and extensive drug resistant cases are very rare. In the local government hospital out of a total of 480 patients with tuberculosis eight (1.6%) exhibited rifampicin resistance. Data for MDR and XDR TB is not available as only very recently testing for the former has commenced.

Case Report

A 21 yrs old female patient presented in medical OPD with history of multiple left sided cervical lymph nodes for five months along with history of unquantified weight loss and mild anorexia. In spite of undergoing fine needle aspirations thrice at two different places the reports were inconclusive. Fine needle aspiration cytology done in this hospital showed a cellular aspirate with small and large lymphocytes, macrophages, occasional plasma cells and neutrophils with focal necrosis resembling caseous necrosis. A provisional diagnosis of necrotizing lymphadenitis-possibly tuberculous was given. Both Ziehl-Neelsen (ZN stain) and TBNAAT were negative. Surgical excision of the lymph node was performed for histopathological diagnosis prior to initiating anti -tubercular treatment. On gross examination the tissue was light brown, soft measuring 1 X 0.5 X 0.4cm. Microscopy showed presence of predominantly reactive lymphoid cells, plasma cells, occasional Reed Sternberg like cells, small monomorphic cells with hyperchromatic nuclei and irregular nuclear membrane and paracortical extension were observed without any necrosis (Figure 1). A provisional diagnosis of lymphoproliferative disorder was made, and immunohistochemistry (IHC) with lymphoma panel was advised. A IHC panel comprising of CD 3,CD 20,CD 21 and CD 30 was carried out on tissue-blocks which stained reactive T cells, B cells, follicular dendritic cells respectively but the Reed Sternberg cell marker (CD30) score was zero (Figure 2-4). CD 21 microphotograph has not been provided. Additional deeper sections of the tissue were cut as a definitive diagnosis was not made on IHC. Occasional necrotizing epithelioid granuloma were observed on microscopy which enabled a revised diagnosis of necrotizing granulomatous lymphadenitis. The patient was initiated on anti-tubercular treatment (ATT), has improved with treatment and is under follow up in Medical OPD.

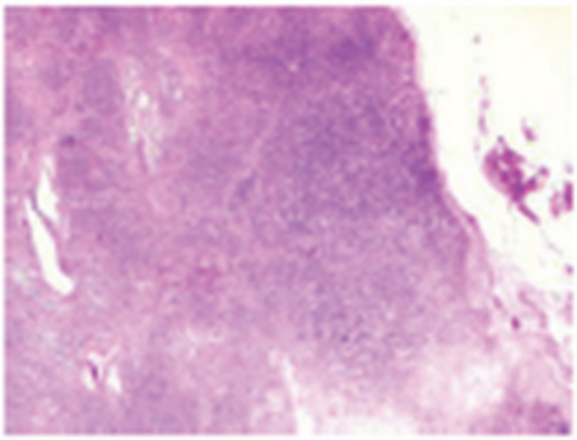

Figure 1) *Showing Hematoxylin and Eosin-stained microphotograph at 20X.*

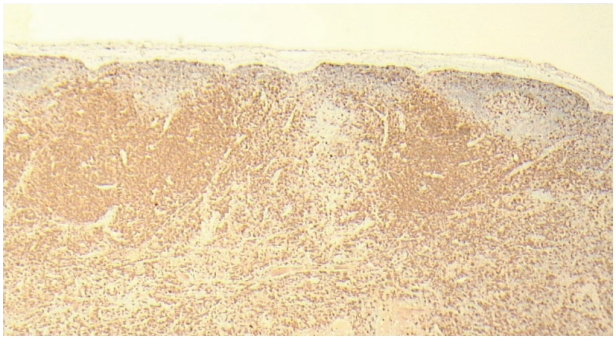

Figure 2) *CD 3 staining of T cells light brown colour, unstained cells are blue.*

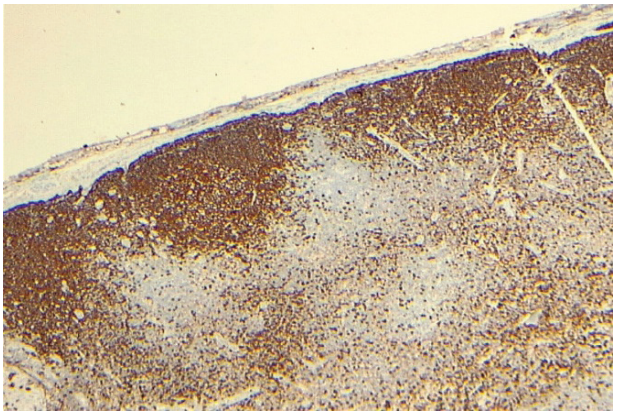

Figure 3) *CD 20 staining of follicular B cells indicated by arrow.*

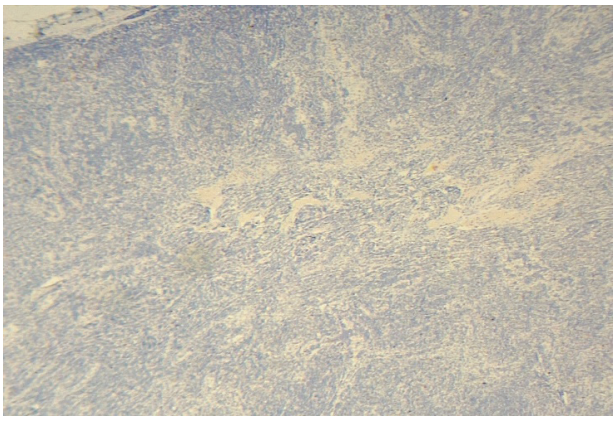

Figure 4) *CD 30-no brown staining noted which confirms absence of Reed Sternberg cells.*

TBNAAT was carried out on 22 clinically suspected TBLN cases of which four (18.2%) were subjected to histopathological examination. (Table 1) shows a comparison of the ZN and TB NAAT results of the ten cases in which both TB NAAT and ZN stain were negative, tuberculous etiology was suspected for four cases on FNAC, while remaining six had a confirmed nontuberculous cytodiagnosis. Positive concordant results were obtained in nine patients. Negative ZN stain with positive TBNAAT for one patient can be explained by higher sensitivity of molecular method. Of two aspirates that tested negative for TBNAAT and were positive on ZN stain, one had high bacillary load (+3). In the second patient with discordant results, after extensive microscopic examination by two technologists, only single bacterium was on the aspirate. Both these patients have shown clinical response to ATT. FNAC and biopsy diagnosis agreed for all four patients.

TABLE 1

Ziehl-Neelsen and TBNAAT results for 22 patients.

Ziehl Neelsen Status	TB NAAT Positive	TB NAAT Negative
Ziehl Neelsen Positive	9	2
Ziehl Neelsen Negative	1	10*

*Six had nontuberculous pathology on FNAC evaluation with a clinically suspected TB.

TB NAAT- Nucleic acid testing by GeneXpert.

Discussion

Most cases of TB lymphadenitis can be diagnosed by FNAC followed by ZN stain of the aspirate whereas histopathological examination may be useful if a repeat aspirate is inconclusive [5]. In one study caseation was identified in 83% of lymph node biopsies of which acid-fast bacilli were detected in 61.7% of cases [6]. Diagnosis by TBNAAT is usually available within one hour and the treating physician also gets information regarding Rifampicin resistance. Weiler *et al.* reported superiority of FNAC to histopathology for diagnosis of tuberculous lymphadenitis (85.7 *vs* 66.7%) in a small study of 21 cases [7]. Acid fast bacilli culture is the gold standard and may be done on solid or liquid media using the BACTEC Mycobacteria Growth Indicator Tube (MGIT) system, with the former taking 20-25 days and the latter considerably shorter time of 10 days [8]. The analytical sensitivity for MGIT is 81.5% compared to Lowenstein Jensen solid media of 67% [9]. The sensitivity and specificity of GeneXpert was found to be 78.5% and 64.9% when compared to culture MGIT [10]. Spiked-sputum studies have shown that GeneXpert assay has analytical limit of detection equivalent to 131 CFU/ml which is superior to the culture of concentrated specimens can detect very low concentrations of organisms-as low as 10 to 100 CFU/ml [11].

Concordance between ZN stain and TBNAAT of aspirate was observed in 19/22 (86.3%) cases. This however is based on very limited

data which includes six patients with non-tuberculous lymphadenitis, and larger studies need to be carried out. Raja *et al* (2020) reported sensitivity and specificity of 82.6% and 85% respectively for TBNAAT when compared with histopathological diagnosis [12]. This is likely to be true for early diagnosis and not for follow up. Biopsy could have been avoided by either a repeat FNAC for confirmation of diagnosis or empirical ATT for three patients (including above patient). In the remaining case with a cytological diagnosis of reactive lymph node, biopsy should have been deferred until evaluation of clinical response of the patient-which was not done for want of patient compliance. The possible reason for TBNAAT negative with a positive ZN stain is difficult to explain by amplification failure because the test was not repeated. A possible cause could be that it was a different strain for which there were no primers and therefore the requirement for multi-modality testing. The incidence of rifampicin resistance in local population was 1.6% which is slightly lower than that reported by World Health Organization for India-2.5% for newly diagnosed cases [13]. Collection of rifampicin resistance data in this hospital has been started recently and a clearer picture shall emerge after some time.

In this case report microscopy of the FNAC slides was ambiguous in that it had no granuloma, showed a picture of reactive lymph node except for focal caseation necrosis. A definitive diagnosis was not made as ZN stain failed to show acid fast bacilli and TB NAAT was negative. Initial biopsy was suggestive of lymphoproliferative disorder. Additional cost and worries for the patient could have been avoided by repeat aspiration and or examination of deeper sections before initiating IHC.

Conclusion

Fine needle aspiration cytology is a useful method for diagnosis of tuberculous lymphadenitis and the value of the test is further augmented by subjecting the aspirate to Ziehl-Neelsen staining and TBNAAT.

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Conflict of Interest: The authors declare no conflict of interest.

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