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Research Article

Cytology Of Tuberculous Lymphadenitis

Fine Needle Aspiration Cytology Study of Tuberculous Lymphadenitis with Ancillary Diagnostic Procedures- Analytical Study from A Resource Limited Domicile of East Coastal Region

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Abstract: Tuberculosis remains one of the major causes of morbidity and mortality in developing countries like India despite intense health campaigning and Government Programmes. Tuberculous lymphadenitis is the most common extrapulmonary manifestation of tuberculosis; the incidence is still on a higher margin, especially in resource-poor areas, which often goes unnoticed and remains to date as a diagnostic challenge to Pathologists. The novel aim was to advocate the best method for early and correct diagnosis of tuberculous lymphadenitis to initiate early appropriate treatment.: In the prospective study conducted in our institute, Puducherry, a total number of 145 cases clinically suspected of tuberculous lymphadenopathy were included, and patients with neoplastic lesions were excluded from this study. The lymph node aspirate collected was analyzed for tuberculous cytomorphological changes using routine cytological stains and correlated observations with the results of various ancillary diagnostic procedures. Among 145 cases studied, 25 aspirates showed classical tuberculous patterns on cytomorphology, correlating well with histopathology (100%), PCR (100%), and culture (92%), respectively. In the rest of the 120 cases carrying non-tuberculous patterns on cytology, further exploration by ancillary diagnostic procedures revealed tuberculosis in 35 cases on histopathology, which PCR confirms in 25 available cases and culture study.FNAC results in adjunction with histopathology, and PCR showed increased reliability and pick-up rate in diagnosing atypical presentations of tuberculous lymphadenitis. Therefore, the PCR procedure of even conventional method should be widely employed in endemic areas of resource-poor regions.

Keywords: Tuberculosis, Lymphadenopathy, Fine Needle Aspiration Cytology, Polymerase Chain Reaction, Histopathological Analysis

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1. INTRODUCTION

Tuberculosis remains a major global health problem and is prevalent in India; according to recent data from 2012, an estimated 8.6 million people developed tuberculosis, with an incidence of 1.3 million fatal cases [1,2]. The peripheral lymphoid organs, especially lymph nodes, are the commonest site for the extrapulmonary manifestation of tuberculosis and are identified as tuberculous lymphadenitis[TBLN][3]. Extrapulmonary tuberculosis accounts for two-thirds of tuberculosis in India [4], with cervical lymph nodes being the most commonly infected group by tubercular bacilli followed by axillary and inguinal nodes. The diagnosis of tuberculous lymphadenitis, apart from clinical evaluation, depends on several diagnostic techniques like conventional excision biopsy study of the node, Zeihl-Neelsenstain[ZN], Immunohistochemistry, biochemical parameters, Polymerase chain reaction[PCR], and each method having its advantages and limitations. The association of the Revised National Tuberculosis Control Programme (RNTCP) recommends Fine Needle Aspiration Cytology procedure [FNAC] along with ZN staining for demonstrating Acid Fast bacilli as the first line investigation for interpretation of tuberculous lymphadenitis[4]. FNAC is a simple, rapid, reliable, and minimally invasive diagnostic procedure. The diagnosis can be made on cytomorphological changes in lymph nodes and confirmed by the presence of epithelioid cell granulomas with multinucleated Langhans giant cells or caseous necrosis or acid-fast bacilli on ZN stain.[4] However, the clinical presentation of extrapulmonary tuberculosis, especially the interpretation of tuberculous lymphadenitis, remains a diagnostic challenge to pathologists with many differentials in microscopy. The efficiency of FNAC in diagnosing TBLN is highly variable, with many cytomorphological patterns mimicking other similar pathological entities. The interpretation of FNAC results is also highly influenced by several other factors, including the foci of aspiration, suction technique, treatment history, and the amount of aspirated material [5]. This present study is aimed to analyze the correlation between the cytomorphological patterns of TBLN with the other ancillary diagnostic methods, i.e., culture study, ZN stain, histopathology, and PCR study of the excision biopsy specimen, and to advocate the most reliable method in the diagnostic interpretation of tuberculous lymphadenitis. Fine Needle Aspiration Cytology (FNAC) is a widely used diagnostic procedure for evaluating tuberculous lymphadenitis, an infection of the lymph nodes caused by the bacterium Mycobacterium tuberculosis. In FNAC, a small sample of the lymph node tissue is obtained using a fine needle, which is then analyzed under a microscope to identify the presence of characteristic tuberculosis cells and other diagnostic markers. Ancillary diagnostic procedures such as Acid-Fast Bacilli (AFB) staining and polymerase chain reaction (PCR) can be used along with FNAC to improve the accuracy of the diagnosis. AFB staining can detect the presence of mycobacteria in the lymph node sample, while PCR can detect the genetic material of M. tuberculosis in the sample. Imaging studies such as X-rays or CT scans can also help identify the location, size, and number of the lymph nodes involved and monitor the response to treatment. It is important to note that a positive FNAC result does not necessarily confirm the diagnosis of tuberculosis, as other conditions such as lymphoma or fungal infections can also present similar cytological features. Hence, clinical correlation and other diagnostic tests are important in making the final diagnosis.

2. MATERIALS AND METHODOLOGY

2.1 Study primer

The prospective study was conducted in the department of Pathology over a year and aimed to find a novel technique for diagnosing TBLN. The declaration of Helsinki did the study.

2.2 Ethical concerns

After institutional ethical committee approval, the study was initiated. (IHEC:426/2011/36.) As the study involved an invasive procedure like FNAC, complete informed consent was taken from all the patients. Informed consent: Patients must be fully informed about the risks and benefits of the procedure and any available alternative options before giving their consent for the procedure to be performed. Risk of harm: While FNAC is a relatively safe procedure, there is a small risk of complications such as bleeding, infection, and pain. Patients was informed of these risks and provided with appropriate pain management and infection control measures. False positive results: FNAC has a small but significant risk of false positive results, leading to unnecessary further diagnostic tests and treatments, causing anxiety and harm to the patient. False-negative results: False-negative results may delay the correct diagnosis and treatment of the condition, putting the patient at risk of a delay in treatment or progression of the disease. Privacy and confidentiality: Patients must be informed that their personal and medical information will be collected and used for the study and that their privacy and confidentiality will be respected and protected. All the above concerns were addressed.

2.3 Inclusion and exclusion criteria

A total number of 145 cases suspected of tubercular lymphadenitis clinically with significant lymphadenopathy were included in the study. All the neoplastic lesions of lymph nodes were excluded. Patients were informed, and willing patients were included.

2.4 Data collection

A detailed history, including clinical data, physical examination, and other details of each patient, was obtained as a proforma sheet with ethical clearance. Lymph nodes were palpated, and an aspiration procedure was performed with a 22/23 gauge needle per the standard guidelines proposed by Orell et al. Total of six parallel smears were made from the aspirates and subjected to various cytological staining. Two dry Smears were stained by Grunwald Giemsa stain and the other by Papanicolaou stain. Two smears were used for ZN staining to demonstrate acid-fast bacilli. All the staining procedures were performed according to the standard protocol concerning standard textbooks.[6]

2.5 Culture studies

Under strict sterile conditions, a portion of the aspirated material was transferred to the Department of microbiology for culture study. The specimen was inoculated on Lowenstein Jensen[LJ]

medium and incubated at 37 C. This LJ slant was checked for growth every day in the first week and then every week till the sixth week. The mycobacterial growth was identified based on colony morphology, pigmentation, and growth rate. The colony was further screened for Acid Fast Bacilli on ZN stain.[5,6] In cases with clinical suspicion for TBLN with negative cytomorphology for tuberculosis and poor clinical progression with the given treatment of antibiotics, a simple excision biopsy of the lymph node was advised. The biopsy specimens were fixed in 10% formalin and processed by a tissue processing machine adopting the 24-hour scheduling. Microscopical sections of 3 to 4 microns were cut for conventional histopathological evaluation with hematoxylin and eosin stains. The histopathological diagnosis of tuberculous lymphadenitis was based on the morphological presence of typical caseating necrosis with epithelioid granuloma. A ZN stain was performed to look for the presence of bacilli.[7]

2.6 PCR procedure

A Polymerase Chain Reaction (PCR) procedure was applied in selected lymph node biopsy specimens with the available resources in the study area covering the rural regions. The DNA extraction was done for a part of a fresh biopsy sample by kit for rapid extractions for formalin-fixed specimens using the standard protocol described by *Eisenach et al.* [8]. In addition, the mycobacterium tuberculosis[MTB] complex IC kit with the specificity of 100% in vitro nucleic acid amplification using Insertion sequence6110[IS6110] oligonucleotide was applied in the detection of the bacilli. The specificity of the PCR results was reassured by using positive and negative control provided along with the kit.

3. STATISTICAL ANALYSES

The study was an analytical observational prospective study with a simple convenience sample of 145 patients. The data collected were entered into an excel sheet and transferred to SPSS software 20.0 IBM – USA for descriptive statistics and analyses. The mean and standard deviation were expressed, and any significance was analyzed using ANOVA. The correlation was analyzed using the Kruskell-Wallis test, and a p-value of less than 0.05 was considered significant.

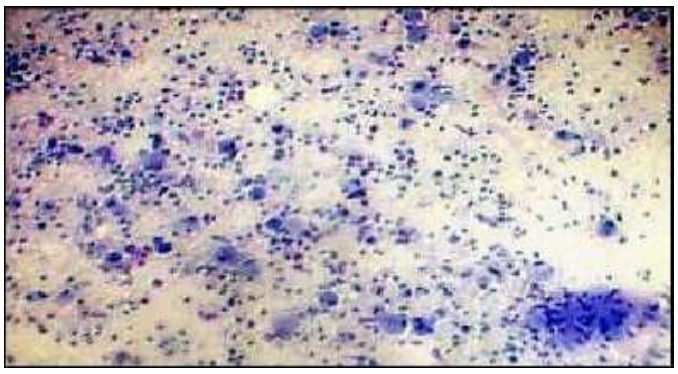
4. RESULTS AND OBSERVATIONS

In this prospective study, a total number of 145 cases were subjected. The present study showed the female preponderance. The cervical group of lymph nodes was predominantly (80 cases) involved among the cases studied. Based on the cytomorphological study of all the cases, 25 (17.24%) cases were reported with classical tubercular pattern with strong evidence of TB [Fig.1A] and 120(82.75%) cases with a non-classical presentation with the possibility of tuberculosis. Among 25 classic TB cases observed on the cytology pattern, subsequent histopathology and PCR demonstrated 100% positivity. However, corresponding the culture study from aspirates showed only positivity of 92%. ZN stain showed positivity only in 60% of cases previously found to have strong evidence of TB on cytology patterns. [Table.1]

Table 1. Competence of Various Methods In Diagnosing Classical Cases Of TB LN

Methods	Total no. of cases studied	No of positive cases %
Cytomorphology	25	25(100%)
Histopathology	05	05(100%)
PCR	05	05(100%)
ZN stain	25	15(60%)
Culture	25	23(92%)

The 120 cases with non-tuberculous patterns demonstrated by FNAC were categorized as Reactive lymphoid hyperplasia (50 cases), Acute suppurative (20 cases), Non-necrotizing granulomatous (20 cases), Necrotizing granulomatous (15cases) based on cytomorphological pattern [Fig.1B] and Scanty hemorrhagic aspirate (15 cases). All these cases were further explored and investigated for the possibility of tubercular lymphadenitis using Histopathology, Culture, PCR, and ZN stain. The maximum positivity of 100% (35/35) was confirmed by histopathology [Fig.1C] and by PCR. The IS 6110 oligonucleotide used in the detection of MTB with standard positive control and the results obtained are shown in [Fig.1D].



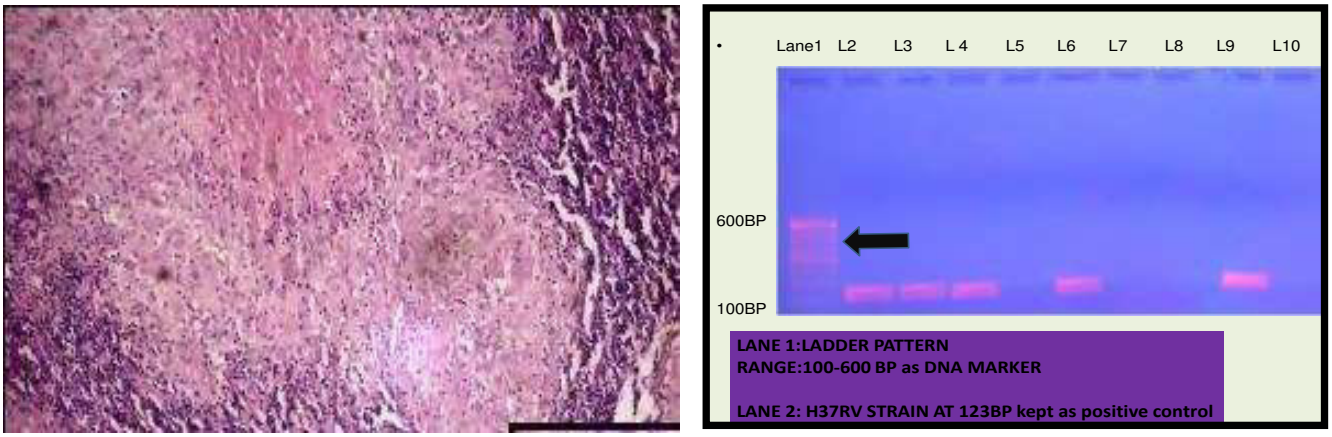


Fig 1: A- Cytomorphology showing sole-shaped epithelioid granuloma, Pap, 40X; B- Acute Suppurative Necrotizing Granuloma, Giemsa stain, 40X; C- Caseating necrotizing granuloma aggregates, H&E,40X; D- PCR with IS610 probe with ladder pattern and H37RV as

Among 25 cases subjected to control, 24 demonstrated positivity which previously showed a non-tuberculous pattern on cytomorphology. The culture and ZN stain was positive in 67.5% (27/40) and 29.8% (11/37), respectively. [Table.2; Fig2A&2B]. On the other hand, when applying PCR as a gold standard for comparing the other variables, in terms of sensitivity and specificity, Histopathological diagnosis was to be 100%, and ZN stain was 63%, respectively.

Table 2: showing PCR as a standard for comparing other variables

PCR	Cytomorphology	No of cases	Histopathology	Culture	ZN
Acute suppurative	20	10/10	7/10	3/10	6/7
Necrotizing granulomatous	15	05/5	3/5	3/10	5/5
Non necrotizing granulomatous	20	10/10	7/10	4/10	6/6
Reactive lymphoid hyperplasia	50	7/7	6/8	1/7	2/2
Scanty hemorrhagic aspirate	15	3/3	4/7	ND	ND
Total	120	35/35	27/40	11/37	19/20

ND- Non Diagnostic aspirate; ZN- Zeihl Neelsen stain;

Fig 2: A-Culture positive for TB bacilli, L-J media; B- Acid Fast Bacilli, ZN stain, Oil Immersion power

5. DISCUSSION

As proposed by WHO, the incidence of extrapulmonary manifestation of tuberculosis is highly on an increasing margin, with high morbidity and mortality with the emergence of Multi-drug resistance[MDR]. FNAC procedure is a useful rapid diagnostic method for detecting the etiology of lymphadenitis. It is considered a simple, safe, and economical method[9]. Inflammation of the lymph node is one of the common clinical presentations and an indicator of various pathological diseases. This study aimed to analyze the efficacy of various diagnostic methods in diagnosing classic and non-classical cases with underlying evidence of tuberculous lymphadenitis. In the past decades, various diagnostic methods have been evaluated for their efficiency in diagnosing tubercular lymphadenitis[10]. In our study, female predominance was observed, which concurs with the other research studies [11-13]. In the present study, histopathology and PCR methods showed accurate results in all the classic tubercular cases, which were previously positive by FNAC. This result was in close concordance with the study by Chakravorty et al. [14]. The microbiological methods, i.e., culture (92%) and ZN stain (60%), were comparatively noncompeting in diagnosing tubercular lymphadenitis. The Zeihl Neelsen stain has lower cytohistological sections sensitivity than molecular procedures. The reason attributed is the bacterial load, foci of aspiration, and treatment history. It has been explored that a minimum of 10000bacilli/uL is required for demonstration for ZN stain, which is highly uncommon in most cases, as observed in the study[8]. Cases with the non-classical mode of TBLN presentation showing non-specific cytological patterns suggesting different underlying etiology were categorized into groups for further correlation. With the available resources, selected cases were further screened for tubercular lymphadenitis, and it was found that histopathology and PCR showed maximum positivity of 100% (35/35) and 95% (19/20), respectively, which is in concordance with other previous studies. [15, 16] A study by Eseinach et al. showed high specificity and low sensitivity of PCR in detecting TBLN in comparison with ZN stain, which is in concordance with the present study. Juan Rodriguez et al. proposed an observation that showed high sensitivity and specificity of histopathology and PCR in diagnosing TBLN, correlating with similar observations of the present study[8]. The culture and ZN stain was positive only in 67.5% and 29.7%, respectively. The present study came out with minimal sensitivity of microbiological assay when correlated with the previous studies in TBLN[17,18]. Although microbiological assays are considered the gold standard and direct evidence of tubercular infections, they are commonly used to diagnose pulmonary TB. In different pulmonary conditions like tubercular lymphadenitis, the productivity of these methods is low. It may be because of lower bacilli load, blood-stained, inadequate specimen, immune status, treatment history, resistant strains, dead bacilli, relapse, and remission conditions.[17] In developing countries like India, with the limited establishment of diagnostic tools, especially in rural areas, laboratory diagnosis of TB is solely based on histomorphological evidence, culture study, and ZN stain to confirm the presence of bacilli, with each method having its own advantages and pitfalls. Another area for improvement in carrying out a culture study is the time period taken for about eight weeks for growth with a high risk of contamination, giving false positive results. However, the most important edge of culture study is identifying atypical mycobacterial species, which other ancillary methods could not demonstrate.

5.1 Values of FNAC

FNAC has a high diagnostic yield in patients with tuberculosis and allows for the rapid diagnosis of the condition. In addition, it can help to differentiate tuberculous lymphadenitis from other conditions that can cause lymph node enlargements, such as lymphoma or metastatic cancer. FNAC can also be used to determine the activity of the disease by analyzing the degree of caseation, granulomatous reaction, necrosis, and neutrophils in the lymph node. This information is useful for guiding therapy and assessing treatment response. FNAC is also relatively inexpensive, non-invasive, easy to perform, and well tolerated by patients, making it an accessible diagnostic option in a resource-limited setting.

5.2 Limitations of FNAC

It's important to note that a positive FNAC result does not necessarily confirm the diagnosis of tuberculosis, as other conditions, such as lymphoma or fungal infections, can also present similar cytological features. Hence, clinical correlation and other diagnostic tests such as AFB stain, PCR, and culture should be used in parallel to make the final diagnosis.

6. CONCLUSION

The observations of the present study strongly advocate the judicious use of PCR and histopathology in diagnosing atypical and complicated cases of tubercular lymphadenitis, which showed non-specific patterns in cytology. The PCR procedure being a robust, quicker method, could be used as a routine diagnostic tool for highly endemic regions with resource-poor parts. It is advisable to reassure with the PCR method before documenting TBLN negative. The present study also recommends that in cases of atypical presentation of all superficial lymphadenitis, the FNAC procedure should be further thoroughly explored for underlying tuberculosis infection.

7. AUTHOR CONTRIBUTION STATEMENT

Anandraj vaithy.K, Keerthika Sri. E, S. Shanmugasamy.K, and Sowmya. S conceptualized and gathered the data with regard to this work and analysed these data and necessary inputs were given towards the designing of the manuscript. All authors discussed the methodology and results and contributed to the final manuscript.

8. CONFLICT OF INTEREST

Conflict of interest declared none.

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