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Original research article

Correlation of FNAC of lymphadenopathy cases with Ziehl-Neelsen stain and MTB PCR

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Abstract

Aim: The aim of the present study was to correlate cytomorphologic findings of FNAC with MTB PCR and staining for Mycobacterium tuberculosis by Ziehl-Neelsen stain.

Methods: Patients with lymphadenopathy and a clinical diagnosis of tuberculous lymphadenitis, of different ages, gender, and ethnical groups were enrolled in this prospective longitudinal study. The cases were referred to the lymphadenopathy clinic. A total of 60 clinically suspected patients of tuberculous lymphadenitis are included in the present study.

Results: Out of 60 cases, majority of the patients were between 15 to 30 year age group (33, 55%) followed by 31 to 45 year age group (15, 25%). Out of 60 patients, 36 (60%) patients were females and 24 patients were males (40%) showing female preponderance. Cervical lymph node (51, 85%) was most commonly involved followed by axillary lymph node (6, 10%). Purulent material was found in 21 (35%) aspiration samples, blood mixed material in 18 samples (30%) and blood mixed purulent material was found in 21 samples (35%). 11 cases (18.34%) had microscopic granulomas, other 49 cases (81.66%) were diagnosed as chronic non-specific lymphadenitis. MTB PCR was positive in 3 of these 11 cases. ZN stain was positive in only one of these 3 cases.

Conclusion: MTB-PCR is a sensitive method for the diagnosis of TB in routinely processed FNAC specimens. Overall 10% improved diagnostic yield was achieved. Granuloma formation was not a conclusive method to confirm TB in FNAC. MTB PCR & special stains should be done in all the suspected tubercular lymphadenitis cases seen on FNAC. Although MTB-PCR is more significantly sensitive than ZN stain, cost-effectiveness should be taken into account.

Keywords: FNAC, MTB PCR, Mycobacterium tuberculosis, Ziehl-Neelsen stain, cytomorphologic findings

Introduction

Every day there are more than 23,000 individuals entering TB records worldwide, and around 5000 of them will die from the disease [1]. The incidence of the disease is raised dramatically by the HIV/AIDS pandemic, with an increase in the classical pulmonary tuberculosis as well as the extra-pulmonary forms, especially tuberculous lymphadenitis [2]. Lymph nodes are usually involved in the early stages of the pulmonary disease. However, tuberculous lymphadenitis may arise without a preceding pulmonary involvement [3].

One of the WHO TB strategies after 2015 is global reduction in TB epidemic death and incidence rate of up to 90% and 95%, respectively [4]. TB is caused by a bacterium called Mycobacterium tuberculosis, which is a member of M. tuberculosis complex (M. tuberculosis, M. bovis, M. microti, and M. africanum). There are two forms of clinical TB – pulmonary TB (PTB) which usually attacks the lungs and extrapulmonary TB (EPTB) that attacks other organs such as the kidneys, spine, and brain [5]. However, the major EPTB is Tuberculous lymphadenitis (TBLA). It causes an enlargement of lymph nodes caused by infection or inflammation [6]. Both the diagnosis and therapy for TBLA represent a challenge because it has physical and laboratory findings feature similar to other pathologic processes [7]. It is difficult to diagnose TBLA by routine methods such as the microscopic Ziehl-Neelsen (ZN) stain and microbiology culture in the Lowenstein-Jensen medium. Among the most practical applications for cytological analysis of lymph node aspirates is fine needle aspiration cytology (FNAC) [8, 9]. Fine needle aspiration (FNA) is accepted by most patients as a noninvasive method and is considered by pathologists for evaluating lymphadenopathy and preserving lymph node structure [10]. Enlarged lymph nodes are a prime target for FNA.

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The most prevalent form of extrapulmonary mycobacterial illness is involvement of peripheral lymph nodes. Fine needle aspiration cytology (FNAC) is a less expensive, more efficient, and less hazardous alternative to histopathology for the detection of tuberculosis. In addition to being patient-friendly, this approach provides an accurate examination of cytomorphological features. The presence of an epithelioid granuloma (Fig. 1) is the determining factor in diagnosing tuberculosis. Even in the absence of epithelioid cell granulomas, the aetiology can be conclusively proven by exhibiting acid fast bacilli (AFB) in FNAC smears either directly or through culture [11, 12].
The aim of the present study was to correlate cytomorphologic findings of FNAC with MTB PCR and staining for Mycobacterium tuberculosis by Ziehl-Neelsen stain.

Materials and Methods

Patients with lymphadenopathy and a clinical diagnosis of tuberculous lymphadenitis, of different ages, gender, and ethnical groups were enrolled in this prospective longitudinal study. The cases were referred to the lymphadenopathy clinic. A total of 60 clinically suspected patients of tuberculous lymphadenitis are included in the present study. The work up of lymphadenopathy cases includes clinical examination, routine laboratory investigation, HIV testing, Mantoux test, FNAC and surgical biopsy, when deemed necessary. Patients were treated and followed up in the same clinic for an average period of 24 months. FNA was performed by 21 G needle. Two air-dried smears were prepared, one stained by Leishman-Giemsa (LG) for cytological evaluation another stained by ZN for AFB (Fig. 2). The remainder of the aspirate was inoculated in LJ egg medium and incubated in 37 °C for at least 4 weeks. The left over material in the needle was washed in sodium dodecyl sulphate-based lyses buffer for DNA extraction and PCR. The smears were classified as either tuberculous, reactive or malignant. The diagnosis of tuberculous lymphadenitis was issued if the smears reveal one of the three following patterns: epithelioid cell granuloma, epithelioid cell granuloma with caseous necrosis and necrosis without epithelioid cell granuloma. The PCR assay in this study used two sets of primers for the genus Mycobacteria and for species M. tuberculosis and a single primer for M. bovis [17, 19]. The cytological diagnosis was correlated with the MTB PCR and ZN stain.
The following data was correlated

- Presence or absence of granulomas
- Results of TB-PCR
- Results of ZN stain
- Calculated the sensitivity and specificity of granulomatous detection by using the positive results of MTB-PCR as the criterion standard

The selection of patients for the anti-tuberculosis therapy was based on a strong clinical suspicion along with microbiological and FNA results. Clinicians were blinded to the PCR results. The anti-tuberculosis therapy was initiated in the absence of a positive microbiological or FNAC result if the patient continued to have nocturnal fever, enlarging and fluctuant lymph nodes, a Mantoux test of more than 10 mm and Erythrocyte Sedimentation Rate (ESR) of more than 60 mm per hour. The results of FNAC, microbiological examinations and PCR correlated with the clinical outcome, which is considered the gold standard. Subsequently, sensitivity and specificity of the three modalities were calculated and compared.

Results

Table 1: Basic characteristics

Characteristics	No. of Cases (%)
Age Group (years)	
15-30 years	33 (55%)
31-45 years	15 (25%)
45-60 years	9 (15%)
> 60 Years	3 (5%)
Gender	
Female	36 (60%)
Male	24 (40%)
Site	
Cervical lymph node	51 (85%)
Axillary Lymph Node	6 (10%)
Inguinal lymph node	2 (3.34%)
Soft tissue swellings	1 (1.66%)
Aspiration Material	
Only purulent material	21 (35%)
Blood mixed material	18 (30%)
Blood mixed purulent material	21 (35%)

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Total cases on FNAC	
Microscopic granulomas	11 (18.34)
Chronic non-specific lymphadenitis	49 (81.66)

Out of 60 cases, majority of the patients were between 15 to 30 year age group (33, 55%) followed by 31 to 45 year age group (15, 25%). Out of 60 patients, 36 (60%) patients were females and 24 patients were males (40%) showing female preponderance. Cervical lymph node (51, 85%) was most commonly involved followed by axillary lymph node (6, 10%). Purulent material was found in 21 (35%) aspiration samples, blood mixed material in 18 samples (30%) and blood mixed purulent material was found in 21 samples (35%). 11 cases (18.34%) had microscopic granulomas other 49 cases (81.66%) were diagnosed as chronic non-specific lymphadenitis.

Table 2: Correlation between granuloma on FNAC & MTB-PCR

	MTB PCR positive	MTB PCR negative	Total
Granulomatous	03(27%)	08(73%)	11
Chronic non-specific Lymphadenitis	05(10%)	44(90%)	49
Total	08(13%)	52(87%)	60

Taking MTB-PCR as criterion standard

- Sensitivity of finding granulomas on FNAC = 3/3+5 (37%)
- Specificity of finding granulomas on FNAC = 44/44+8 (84%)
- Positive predictive value was 27% (3/3+8)
- Negative predictive value was 89% (44/5+44)

Table 3: Correlation between MTB-PCR and ZN stain

	MTB PCR positive	MTB PCR negative	Total
ZN stain positive	01	0	01
ZN stain negative	07	52	59
Total	08	52	60

Taking MTB-PCR as criterion standard

- Sensitivity of finding AFB on FNAC = 1/1+7 (12.5%)
- Specificity of finding AFB on FNAC = 52/52(100%)
- Positive predictive value for AFB = 52/52 (100%)
- Negative predictive value = 52/52+7 (88%)

Table 4: Correlation between granuloma & ZN stain

	ZN stain positive	ZN stain negative	Total
Granulomatous lympadenitis	01	10*	11
Chronic nonspecific lymphadenitis	0	49	49
Total	01	59	60

*MTB PCR was positive in 2 of these 10 cases.

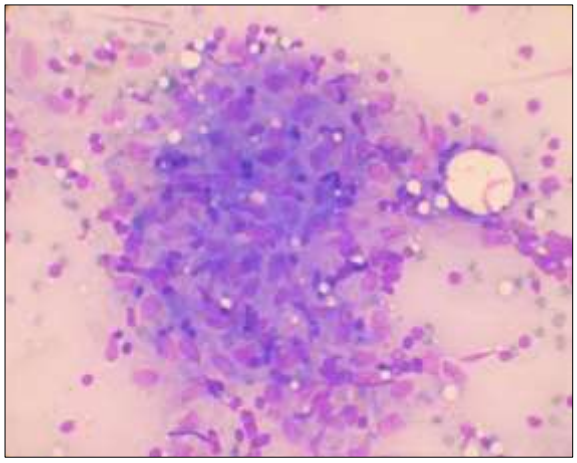

Fig 1: Epithelioid Granulomas (LG Stain)

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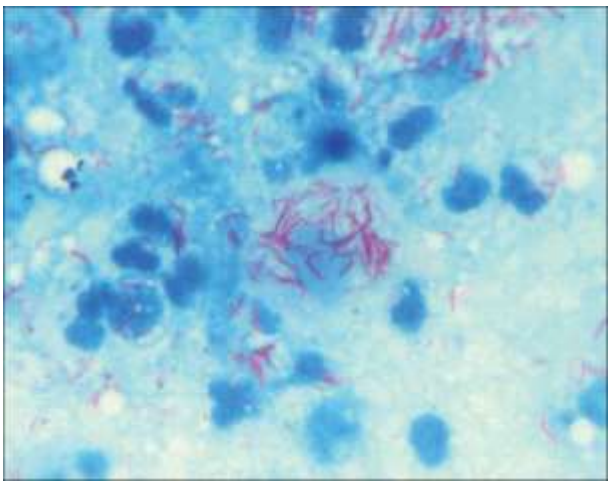

Fig 2: Acid Fast Bacilli (ZN Stain)

Discussion

Granulomatous lymphadenitis is characteristic of several diseases, such as mycotic, viral, bacterial infections (tuberculosis, leprosy, and syphilis), sarcoidosis and toxoplasmosis, as well as a secondary response in lymph nodes draining carcinomas or lymphomas [13]. In developing nations, TB poses a substantial hazard to public health. Per day in India, one thousand people die from tuberculosis, which translates to one death every minute [11, 14, 15].

Out of 60 cases, majority of the patients were between 15 to 30 year age group (33, 55%) followed by 31 to 45 year age group (15, 25%). Similar to the study conducted by Dhawan I *et al.* [16] revealed that TB lymphadenitis is most prevalent in the age range of 15 to 30 years, which is the productive age group. Out of 60 samples, 36 (60%) patients were females and 24 patients were males (40%) showing female preponderance. Cervical lymph node (51, 85%) was most commonly involved followed by axillary lymph node (6, 10%). In this study, the posterior cervical lymph nodes were most frequently affected by lymphadenopathy, followed by the anterior cervical lymph nodes, supraclavicular lymph nodes, and submandibular lymph nodes. This observation parallels the research conducted by Bibhuti Das *et al.* [17].

Purulent material was found in 21 (35%) aspiration samples, blood mixed material in 18 samples (30%) and blood mixed purulent material was found in 21 samples (35%). 11 cases (18.34%) had microscopic granulomas other 49 cases (81.66%) were diagnosed as chronic non-specific lymphadenitis. MTB PCR was positive in 3 of these 11 cases. FNA cytology demonstrating epithelioid cells, granulomas with or without multinucleated giant cells and caseation necrosis were diagnostic criteria for tuberculosis. Cytology demonstrating necrosis only/non-caseating granulomas/acute suppurative lymphadenitis in the absence of AFB was suggestive for tuberculosis. Cytomorphological findings of tuberculous lymphadenitis was described by various authors with minor modifications with different names *viz.*, patterns or category [18, 19].

Previous studies for detection of AFB from various clinical specimens, comprising sputum, CSF, fine needle aspirate, pus, and miscellaneous body fluids which were examined by ZN and Auramine Rhodamine (AR) staining techniques, showed that AR was 86.6% sensitive as compared to ZN 67.3% sensitive with more marked difference in extrapulmonary samples [20]. In other studies on FNA smears of lymph nodes, autofluorescence was found to be more sensitive than ZN staining [21, 22], but as compared to cytodiagnosis, it was less sensitive [23]. However, some organisms exhibiting spontaneous emission spectra following excitation at specific wavelengths may pose problem in the detection of Mycobacteria. These organisms such as spore-forming like Bacillus subtilis, non-spore forming bacteria like Staphylococcus aureus, Nocardia, budding yeast may produce fluorescence [24]. Even air-drying artifacts in Papanicolaou stained smears may produce problems in identifying Mycobacteria by fluorescent microscopy.

Conclusion

MTB-PCR is a sensitive method for the diagnosis of TB in routinely processed FNAC specimens. Overall 10% improved diagnostic yield was achieved. Granuloma formation was not a conclusive method to confirm TB in FNAC. MTB PCR & special stains should be done in all the suspected tubercular lymphadenitis cases seen on FNAC. Although MTB-PCR is more significantly sensitive than ZN stain, cost-effectiveness should be taken into account.

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