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COMPARATIVE PROSPECTIVE STUDY OF CARTRIDGE-BASED NUCLEIC ACID AMPLIFICATION TEST (CBNAAT) WITH FINE NEEDLE ASPIRATION CYTOLOGY AND HISTOPATHOLOGY EXAMINATION ON CASES OF SUSPECTED PALPABLE TUBERCULAR LYMPHADENOPATHY

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ABSTRACT

Introduction: In developing countries the detection of TBLN is a major challenge. The clinical parameters for the diagnosis of TB in lymph nodes are not specific for some cases and the absence of lymphadenitis does not rule out TB involvement. **Aim:**Diagnosis of tubercular lymph node by CBNAAT on lymph node tissue taken by excisional biopsy in case of suspected palpable tubercular lymphadenopathy and comparison of CBNAAT with FNAC and histopathology examination. **Methods:**This was a Prospective observational study, conducted on 50 suspected and confirmed case of tubercular lymphadenopathy, attending outpatient department or admitted during January 2021– December 2022 in the department of surgery JLN Hospital Ajmer. **Results:** Mean age of study population was 29 ± 15.64 yearrs. The most common presenting complaint was swelling (100%) followed by loss of weight (50%) and loss of appetite (40%). Mean ESR was 43 ± 24.01 per hr. 54% of the patients had positive mantoux test for TB. On histopathology, 60% cases were diagnosed as Tubercular. 2 (4%) male and 3 (6%) female had rifampicin resistance on CBNAAT. CBNAAT the true positive were 23 whereas true negative were 20. FNAC the true positive were 27 whereas true negative were 18. CBNAAT, PPV (66.66), NPV(100), Sensitivity= 100%, and Specificity= 74.07%. FNAC, Positive Predictive Value = 90%, Negative Predictive Value = 90%, Sensitivity= 93.1%, and Specificity= 85.71%.**Conclusion:** With high sensitivity and average specificity of CBNAAT and FNAC they are a good tool for diagnosis in combination with other testing modality like culture and histopathology examination.

Keywords: CBNAAT, FNAC, Histopathology, tubercular lymphadenopathy.

INTRODUCTION

Tuberculosis (TB) is a communicable disease that is a major cause of ill health, one of the top 10 causes of death worldwide and the leading cause of death from a single infectious agent (ranking above HIV/AIDS). In 2019, about 10 million people developed TB and 1.4 million died. [4]

India is the home of world's largest tuberculosis (TB) burden, accounting for around 21% of the TB incidence globally.Extra-pulmonary tuberculosis comprises 10-50% of all TB in human immunodeficiency virus (HIV) negative patients and approximately 35-80% in HIV infected patients. TB lymphadenitis is seen in nearly up to 40% of extra-pulmonary TB which constitutes approximately 15-20% of all cases of TB in India. [5-6]

Tubercular lymphadenopathy comprises only <5% of all cases of tuberculosis. It is more common among children, women as well as in immunosuppressed patients (like HIV etc.). The cervical lymph nodes are most frequently involved followed by mediastinal, supra-clavicular, mesenteric, axillary and inguinal groups of LN according to the order of frequency. It is a chronic granulomatous inflammation of the lymph node with the presence of caseous necrosis. It is caused by infection of Mycobacterium tuberculosis.

Tuberculous lymphadenitis usually presents as a slowly progressive, painless swelling of a single group of lymph nodes.[8] The duration of symptoms at the time of presentation is typically 1–2 months, varying from 3 weeks to 8 months.[9]

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In developing countries the detection of TBLN is a major challenge. The clinical parameters for the diagnosis of TB in lymph nodes are not specific for some cases and the absence of lymphadenitis does not rule out TB involvement. Clinical features, though indicative of tuberculous etiology, are not adequate for making a definitive diagnosis.[10] Various diagnostic methods are available for detection of tubercular bacilli. Only definative criteria for diagnosis of TB is to demonstrate the bacilli from affected tissue. It is well known that acid fast bacilli can be demonstrated in only 25-30% cases showing tubercular granuloma.However, Zeihl-Neelsen (ZN) staining have low sensitivity for detection of acid-fast bacilli. Improvement in detection rate of AFB has been reported by various techniques.[12,13]

The histopathological examination (HPE) of LN biopsy for macroscopic caseation, typical tuberculous granulation, examination of direct smear from the cut surface for acid fast bacilli (AFB), and culture specimen can make the diagnosis.Gold standard for diagnosis of TB is culture but it takes 4-6 weeks, hence other modalities are needed for rapid diagnosis. Fine-needle aspiration (FNA) has become a widely used diagnostic tool and it remains one of the most rapid and cost-effective diagnostic methods of tuberculous lymphadenitis but is characterized by low specificity. FNAC is an efficient primary diagnostic tool but Mycobacterial confirmation by CBNAAT. To obtain faster results, nucleic acid amplification test (NAAT) is being increasingly used worldwide for the rapid diagnosis of TB.[14]This is a prospective observational study conducted for the diagnosis of tubercular lymph node by CBNAAT on lymph node tissue taken by excisional biopsy in case of suspected tubercular lymphadenopathy and its comparision with FNAC and histopathological examination.

MARERIAL &METHODS

This was a Prospective observational study, conducted on 50 suspected and confirmed case of tubercular lymphadenopathy, attending outpatient department or admitted during January 2021– December 2022 in the department of surgery at JLN Hospital Ajmer. Patients of all age group, suspected as peripheral tubercular lymphadenopathy and who gave consent were included. Patients with acute lymphadenitis other than tubercular lymphadenopathy, not given consent for study and patient with peripheral lymphadenopathy who were known case of malignancy or suspected case of malignancy were excluded from study.

After obtaining permission from Ethical Committee and informed written consent of study population selected through analyzing inclusion and exclusion criteria and with help of consecutive sampling, the questionnaire was administered to study subjects.

All patients undergo general physical examination and base line investigations which include CBC, ESR, BT, CT, RBS, RFT, LFT, HIV, HbsAg, Chest X-RAY, Mantoux test done. The suspected patients of lymph node tuberculosis after proper informed and written consent were subjected to FNAC and sent for cytology examination to pathology department. All patients irrespective of FNAC report underwent excisional biopsy. Excised biopsy tissue divided into two samples. One sample was crushed and sent in normal saline falcon tube for CBNAAT and other sample sent in formalin jar for histopathology examination.

Statistical analysis

Statistical analysis was done by using Epi info software of CDC. The results consisted of sensitivity, specificity, predictive values and diagnostic accuracy. In order to find out the agreement in diagnosis between two modalities kappa statistics were employed. A *p* value of less than 0.05 was taken as statistically significant result.

RESULTS

Most of the patients were belong to 21 – 40 yr age group (40%) with mean age of study population was 29±15.64 yrs. The male and female patient having equal contribution (50%) in this study. Most of the patients (60%) were from rural areas.Mean BMI of study population was 44±13.75kg/m^2. Most of the cases (84%) were belong to lower socioeconomic class.

Table 1 : Sociodemography

Age (yrs)	**Number**	**Percentage**
Up to 20	18	36
21 – 40	23	46

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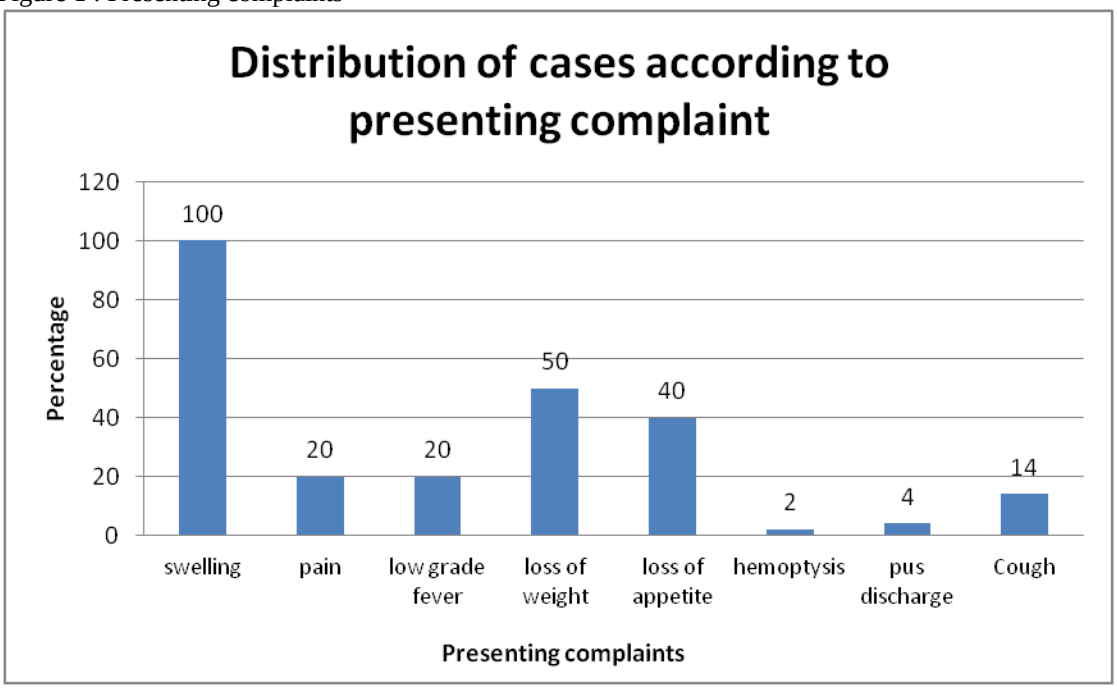
41 – 60	5	10
61 – 80	4	8
Residence		
Rural	30	60
Urban	20	40
BMI		
<18.5	13	26.0
18.5 – 24.99	17	34.0
25.0 – 29.9	15	30.0
≥ 30	5	10.0
Socio-Economic-Class		
(V) Lower class	25	50
(IV) Lower middle class	17	34
(III) Middle class	5	10
(II) Upper middle class	3	6
(I) Upper class	0	0

Only 30% of the patients having history of contact with TB case, 8% of the patients had past history of TB and 18% of the patients had positive family history of TB. Most common presenting complain was swelling (100%) followed by loss of weight (50%) and loss of appetite (40%).

Figure 1 : Presenting complaints



Most of the patients (80%) were presented with cervical lymphadenopathy followed by axillary (10%), inguinal (4%), submandibular (4%) and supraclavicular lymphadenopathy (2%). Most of the patientspresented with firm lymph nodes(88%) followed bymatted (60%) and fluctuation (10%)whereas only 2% of the cases had sinus.

Table 2 :Site and clinical nature of presenting lymph nodes

Site of lymphadenopathy	**Number**	**Percentage**

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Cervical	40	80
Axillary	5	10
Inguinal	2	4
Submandibular	2	4
Supraclavicular	1	2
Clinical feature		
Firm	44	88.0
Fluctuation	5	10.0
Sinus	1	2.0
Matted	30	60

54% of the patients had positive mantoux test for TB, Mean ESR was 43±24.01 per hr, 14% of the patients having parenchymal lesion suggestive of tuberculosis on their chest X ray. On histopathology, 60% cases were diagnosed as tuberculosis whereas 58% of the patientsdiagnosed as tubercular on FNAC and 46% of the patients were found to be microbiologically confirmed for mycobacterium tuberculosis on CBNAAT. In our study, 10% of the patients had microbiologically confirmed case on CBNAAT with rifampicin resistance. The refampicin resistance was found in 2 male and 3 female.

In our study, histopathology was taken as gold standard diagnostic modality. When compared histopathology to CBNAAT, the true positive were 23 whereas true negative were 20.So Positive Predictive Value = 66.66, Negative Predictive Value = 100, Sensitivity = 100%, and Specificity= 74.07% of CBNAAT. Difference between two groups were found to be statistically significant. ($p<0.05$)

When compared histopathology to FNAC, the true positive were 27 whereas true negative were 18.So Positive Predictive Value = 90%, Negative Predictive Value = 90%, Sensitivity = 93.1%, and Specificity= 85.71% of FNAC. Difference between two groups were found to be statistically significant. ($p<0.05$)

Table 3 : Comparision of Histopathology, FNAC and CBNAAT

Histopathology	**CBNAAT**		
	Positive	**Negative**	**Pvalue**
Positive	23	7	0.001*
Negative	0	20	
Total	23	27	
Histopathology	**FNAC**		
Positive	27	3	0.001*
Negative	2	18	
Total	29	21	

On histopathology, most of the patientshad giant cell and caseation(80%), followed by epitheloid cell (70%) whereas only 3.3% cases had histiocytes.

Figure 2 : Histopathological findings

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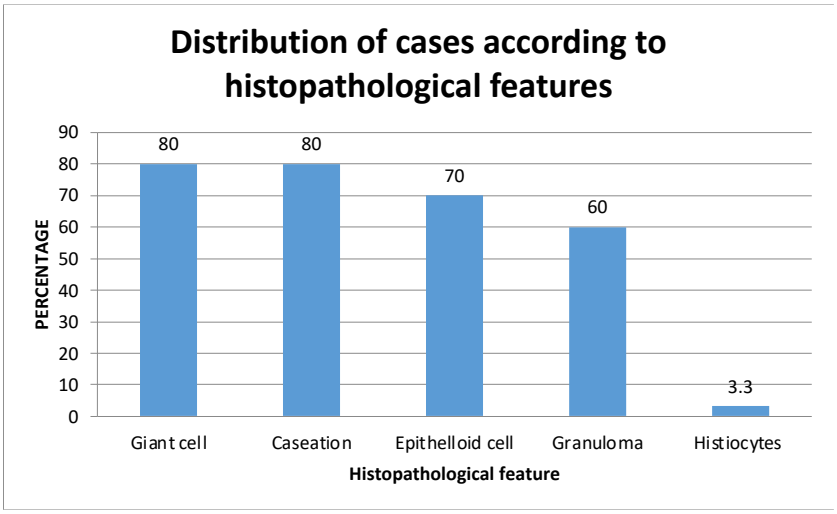

There was no association with age, sex or ESR with any one of the investigation.

DISCUSSION

In our study, about 46% of the patients were belong to 21 – 40year of age group and mean age of study population was 29±15.64 years. Similar study was done by Saurabh Kumar Singh et al. (2016) found that mean age of the patients were 28.45±12.83. Also Manju R. Purohit et al. (2009) found that 75% of the patients were aged between 14 and 35 years.

In our study, about half (50%) of the patients were male. Similar study was done by Moumita Adhikary et al. (2022) found that out of 79 patients, 47 (59.49%) patients were male. Also Manju R. Purohit et al. (2009) found that in their study a male to female ratio of 1:2.1. On contrary female predominance was seen by Saurabh Kumar Singh et al. (2016) in their study found that 84 (41.2%) male and 120 (58.8%) females were diagnosed as the cases of tubercular lymphadenopathy.

In our study, 58% of the patients were diagnosed tubercular lymph node on FNAC. Similar study was done by Bhaskar Thakkar et al. (2022) FNAC has detected tuberculosis in 281 (59.15%) patients.

In our study, 46% of the patients were found microbiologically confirmed for mycobacterium tuberculosis on CBNAAT. Similar study was done by Moumita Adhikary et al. (2022), out of 79 suspected EPTB patients, CBNAAT confirmed 62 patients (78.48%) as having TB. Also Manju M D et al. (2020) found that thirty one patients (51.67%) had mycobacterium detected in CBNAAT. Also Vishnu Kumar Goyal et al. (2019) Among 51 samples tested, CBNAAT detected DNA of TB in 37 samples (72.5%). Lower detection rates were found in Bhaskar Thakkar et al. (2022) and 20.84% cases and Samreen Sarfaraz et al. (2018) found 32.6%.

On histopathology 60% cases were diagnosed as Tubercular whereas 40% were non tubercular. Similar study was done by Samreen Sarfaraz et al in 2018 found that out of 297 samples tubercular lymph node was diagnosed on histopathology in 89.6% of cases. According to Vishnu Kumar Goyal et al. (2019) highest yield was found with cytopathology.

In our study, 2 (4%) male and 3 (6%) female patients had rifampicin resistanceand the difference was not statistically significant. ($p>0.05$) Similarly Moumita Adhikary et al. (2022) found three cases were detected as rifampicin resistant. Also Samreen Sarfaraz et al. (2018) found drug resistance was displayed by 8.2% of GeneXpert-positive cases

In our study, the most of the cases had left side lymphadenopathy (56%), followed byright side (42%) whereas in 12% of the cases bilateral lymphadenopathy was found. The most of the cases had cervical lymphadenopathy (80%),

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followed byaxillary (10%) and supraclavicular (2%). Similar study was done by Bhaskar Thakkar et al. (2022) majority of the aspirates are from posterior triangle of neck lymph node accounting for 56.42% (268 cases). Also Moumita Adhikary et al. (2022) found that cervical lymph node was the most common site of aspiration (42, 52.16%). Our study was consistant with Saurabh Kumar Singh et al. (2016) found cervical lymphadenopathy (92.6%) was the most common presentation of peripheral tubercular lymphadenopathy.

Most of the cases had firm lymph nodes (88%), followed by matted lymph node (60%), fluctuation in 10% and sinus tract in 2% cases.. Similar study was done by Saurabh Kumar Singh et al. (2016) found that Palpable mass (100%) was the most common presenting feature.

On histopathology, most of the caseshad giant cell and caseation necrosis (80%), followed byepitheloid cell (70%) and histiocytes (3.3%). Similar study was done by Suwarna B. Patil et al. (2020) observed that out of 439 cases, presumptive tuberculosis was diagnosed in 192 cases showing either epithelioid cell granulomas or caseous necrosis or both upon morphology. Also Moumita Adhikary et al. (2022) found Granuloma with necrosis (38, 48.10%) followed by necrosis only (14, 17.72%) were the common findings on FNAC. Also Saurabh Kumar Singh et al. (2016) found that most common cytological pattern seen was epithelioid granuloma with caseous necrosis (32.84%) followed by epithelioid granuloma without caseous necrosis on fine needle aspiration cytology (FNAC).

A high index of suspicion is needed for the diagnosis of tubercular cervical lymphadenitis. A thorough history and physical examination, tuberculin test, staining for acid-fast bacilli, radiologic examination, and fine-needle aspiration cytology (FNAC) will help to arrive at an early diagnosis of mycobacterial lymphadenitis which will allow early institution of treatment before a final diagnosis can be made by biopsy and culture.

In our study histopathology was taken as gold standard diagnostic modality. When compared histopathology to FNAC the true positive were 27 whereas true negative were 18 so Positive Predictive Value = 90%, Negative Predictive Value = 90%, Sensitivity= 93.1%, and Specificity = 85.71% of FNAC. Similar study was done by Bhaskar Thakkar et al. (2022) found that the sensitivity of FNAC in our study was 95.9% and specificity was 100% Also Singh KG, Tandon S.et al. (2017) this study showed that diagnostic yield of FNAC was 47/57 i.e. 82.4%.

In our study histopathology was taken as gold standard. When compared histopathology to CBNAAT the true positive were 23 whereas true negative were 20 so Positive Predictive Value = 66.66, Negative Predictive Value = 100, Sensitivity = 100%, and Specificity = 74.07% of CBNAAT. Similar study was done by Vishnu Kumar Goyal et al. (2019) in their study the sensitivity and specificity of CBNAAT were 77.27% and 57.1% respectively. Also Bhaskar Thakkar et al. (2022) the sensitivity of XPERT was 29.53% and specificity was 93.4%. our study was consistent with Suwarna B. Patil et al. (2020) found that 146 cases were CBNAAT positive with the sensitivity of 84.04% and specificity of 80.57%. Also Singh KG, Tandon S.et al. (2017) this study showed that diagnostic yield of CB-NAAT (44/57 i.e. 77.19%) in tubercular peripheral lymph node sample and Samreen Sarfaraz et al. (2018) found the sensitivity of GeneXpert was 65.7%

CONCLUSION

Tuberculosis is a systemic disease and lymphadenitis is the most common extrapulmonary manifestation of the disease. Their diagnosis and distinction need a high index of suspicion, and application of a variety of diagnostic modalities. The approach to diagnosis should be individualized depending on the location of the disease and the clinical evaluation.

With high sensitivity and average specificity of CBNAAT and FNAC they are a good tool for diagnosis in combination with other testing modality like culture and Histopathology examination.

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