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Original Article

A Comparative Study of Cartridge-based Nucleic Acid Amplification Test, Histopathological Examination, and Culture and Sensitivity in Suspicious Cases of Tubercular Lymphadenopathy

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Abstract

Introduction: Tuberculosis (TB) is one of the most important diseases in the history of mankind and remains as an extraordinary burden to humanity even today. Tuberculous lymphadenitis (TBLN) is the most common extrapulmonary TB which accounts for 25–30% of TB cases. Lymph node (LN) TB is frequent among HIV infected patients. TBLN, abdominal, or cervical is caused largely due to *Mycobacterium tuberculosis* which was once caused by *Mycobacterium bovis*. In this study, we evaluated the performance of cartridge-based Nucleic Acid Amplification Test (CB-NAAT) for the diagnosis of TBLN on histopathological examination (HPE) of excisional LN biopsy and culture and sensitivity.

Aims and Objective: (1) The objective of the study was to evaluate and compare three modalities, that is, CBNAAT, HPE and Culture and Sensitivity for diagnosis of TB in cases of lymphadenopathy, (2) sensitivity and specificity of each modality, (3) to assess efficacy of CBNAAT over other modality, and (4) to identify Rifampicin-resistant cases.

Materials and Methods: Enlarged LN is biopsied. One part of this material was put in a sterile container and sent for CB-NAAT and the other part was smeared on 2–3 slides and send for Lowenstein Jensen culture. Third part of the biopsy sample taken in sterile container with 10% formalin sent for HPE.

Result: CBNAAT is the most sensitive technique. As the rate of drug resistant TB is in increasing trend, it is essential to use a rapid method which detects *M. tuberculosis* and rifampicin resistance simultaneously. Thus, CBNAAT is the best method in the diagnosis of TB. Earlier detection can reduce the death rate and prevent the spread of TB in the community.

Key words: Tuberculosis, Cartridge-based nucleic acid amplification test, Histopathology, Rifampicin, Lowenstein Jensen culture

INTRODUCTION

Tuberculosis (TB) has co-existed with humanity even before recorded history and has been found in the skeletal remains of mummies. Hippocrates described the disease and named it "phthisis" which means to mart or waste away. TB is one of the most important diseases in the history of mankind and remains as an extraordinary burden to humanity even today.

TB remains a major threat to global health, with estimated 10 million people fell ill with TB worldwide. 5.7 million men, 3.2 million women, and 1.1 million children. Cases being recorded in all countries and all age groups, but TB is curable and preventable.

In 2018, 87% of new cases were diagnosed in the 30 highly TB burdened countries. Of which two-third of the total

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from eight countries, with India leading the count, followed by China, Indonesia, the Philippines, Pakistan, Nigeria, Bangladesh, and South Africa.

Extrapulmonary TB is a condition where TB affects any organ other than the lungs. Tuberculous lymphadenitis (TBLN) is the most common extrapulmonary TB which accounts for 25–30% of TB cases. Lymph node (LN) TB is frequent among HIV infected patients. TBLN, abdominal or cervical is caused largely due to mycobacterium TB which was once caused by *Mycobacterium bovis*.

Extrapulmonary TB is generally difficult to diagnose and diagnosis is based mostly on clinical signs and symptoms, radiograph, tuberculin test, and history of contact with known cases of TB. Most often clinical features are non-specific and do not give conclusive evidence for the disease. Demonstration of tubercle bacilli in the tissues and nodes remain the gold standard, but it is not often possible due to paucibacillary nature of the illness. 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.

Diagnosis of extrapulmonary TB is often made on HPE. HPE diagnosis is made when there is caseous necrosis in a granulomatous lymphadenitis. When compared, excisional biopsy has the highest sensitivity. To obtain faster results, nucleic acid amplification test (NAAT) is being increasingly used worldwide for the rapid diagnosis of TB.

Cartridge Based NAAT (CB-NAAT) is an automated DNA test that detects *Mycobacterium tuberculosis* and rifampicin resistance (an indicator of multidrug-resistant TB [MDR-TB]) within 2 h for the investigation of patients who might have TB.

However, due to paucity of data, more studies are needed to recommend CB-NAAT testing as the initial test in suspected TB lymphadenopathy. In this study, we evaluated the performance of CB-NAAT for the diagnosis of TBLN on HPE of excisional LN biopsy and culture and sensitivity.

This study aimed to assess the applicability of CB-NAAT in early diagnosis of tubercular peripheral lymphadenopathy and early identification of MDR-TB (rifampicin resistance) in LN TB.

MATERIALS AND METHODS

Place of Study

This study was conducted at the Department of Surgery, M.G.M Medical College and M.Y Hospital, Indore.

Source of Data

All suspicious cases of tubercular lymphadenopathy operated in Dept. of Surgery, M.G.M Medical College and M.Y Hospital, Indore.

Study Period

12 months.

Study Design

The study design was prospective and comparative study.

Inclusion Criteria

The following criteria were included in the study:

1. All patients with lymphadenopathy including male and female, of ages 5–70 years
2. Patients who give written informed consent, in pediatric cases consent will be given by parents.

Exclusion Criteria

Patients and parents not willing to give written consent were excluded from the study.

Sample Size

The minimum sample size was 50 cases.

Methodology

A total of 50 patients were taken for this study. Data for the study were collected using convenient sampling techniques after obtaining written consent. The data were collected with face to face interview of the patients, using a pre-designed questionnaire, which included patient's identification data and socioeconomic status (SES).

Patient will be anesthetised locally or systemically accordingly.

The skin of the patient, over the suspected LN/nodes to be excised is marked and cleaned (painting and draping done). An incision is made through the skin and blunt dissection done to identify the enlarged LN/nodes. Part or all of the enlarged LNs are excised out. Wound is closed in layers after achieving hemostasis.

One part of this material was put in a sterile container and sent for CB-NAAT and the other part was smeared on 2–3 slides and send for Lowenstein Jensen culture. One smear slide was heat fixed and stained with carbol fuchsin stain and heated to enable the dye to penetrate the waxy mycobacterial cell wall. After staining, an acid decolorizing solution is applied. This removes the red dye from background cells and any organism in the smear except mycobacteria which retain the dye and are therefore referred to as AFB. The slides were examined under the microscope for visualization of AFB.

Third part of the biopsy sample taken in sterile container with 10% formalin sent for HPE.

Thus, the reports of CBNAAT, bacteriological investigations, and HPE will be compared among themselves and diagnosis will be made.

RESULTS

Table 1 depicts that out of total 50 subjects studied in our study, 62% were males and 38% are females [Figure 1].

Table 2 shows that out of total patients under observation 16% belongs to middle SES and 84% belongs to low SES [Figure 2].

The above graph and table show that 88% of lymphadenopathy on the basis HPE report is tubercular lymphadenitis, 4% of the total cases were neoplastic and 8% shows chronic granulomatous changes (non-tuberculous) [Figure 3 and Table 3].

Above table and graph show more common frequency, that is, 96% of total patients included for excision biopsy for cervical LN. Each 2% frequency is for right inguinal region and diagnostic laparoscopy with mesenteric LN [Figures 4,5 and Tables 4,5].

The above graph helps us to conclude that percentage of lymphadenopathies that resolved after medication is about 94% and 2% in abdominal lymphadenopathies. Percentage of lymphadenopathies that did not resolved after medication is only 2% and also neoplastic cervical lymphadenopathies that were another 2% [Figure 6 and Table 6].

The graph shows that no complication, that is, local wound infection was noticed in 88% of the patients. Local wound infection was present in 12% of the study group [Figure 7 and Table 7].

Table 1: Distribution of study subjects on the basis of gender and age

Gender	Frequency	Percentage
Male	31	62
Female	19	38
Total	50	100
Variable	Mean±SD	Min–Max
Age	31.52±16.20184	6–62

Table 2: Distribution of study groups on the basis of socioeconomic status

Socioeconomic status	Frequency	Percentage
Low	42	84.0
Middle	8	16.0
Total	50	100.0

As per Figure 8 and Table 8, distribution of 50 study subjects according to follow up was done in which 46% patients completed ATT, 44% patients were on ATT, 4% patients completed their course of antibiotics, 4% were started on chemotherapy and in remaining 2% there was no recurrence of lymphadenopathy [Figure 8, Table 8].

- Sensitivity = 31/(31+13) = 70.45%
- Specificity = 5/(5+1) = 83.33%
- Positive predictive value (PPV) = 31/(1+31) = 96.88%
- Negative predictive value (NPV) = 5/(13+5) = 27.78% [Figure 9 and Table 9].

On comparing the sensitivity and specificity of CBNAAT and HPE, $P < 0.05$ is significant. This concludes that CBNAAT is better than HPE.

- Specificity = 6/(6+0) = 100%
- Sensitivity= 17/(17+27) = 38.6%
- PPV = 17/(17+0) = 100%
- NPV = 6/(27+6) = 18.2% [Figure 10 and Table10]

On comparing, the sensitivity and specificity of smear AFB and HPE, $P > 0.06$ is insignificant [Figure 10 and Table 10]. This concludes that HPE is better than smear AFB.

- Specificity = 6/(6+0) = 100%
- Sensitivity = 15/(15+29) = 34.09%
- PPV = 15/(15+0) = 100%
- NPV = 6/(29+6) = 17.14% [Figure 11 and Table 11].

On comparing, the sensitivity and specificity of LJ culture and HPE, $P > 0.05$ is insignificant. This concludes that HPE is better than LJ culture.

On comparing sensitivity, specificity, PPV, NPV, and accuracy, we hereby conclude that CBNAAT is a better test that can be used for screening of lymphadenopathy [Figure 12 and Table 12].

DISCUSSION

Despite the discovery of the tubercle bacillus more than a 100 years ago and all the advances in our knowledge of the disease made then, TB still remains one of the major health problems facing mankind particularly in developing countries. Early diagnosis of TB and initiation of optimal treatment would not only enable a cure of an individual patient but will also curb the transmission of infection and disease to others in the community.[1]

Tuberculous lymphadenitis being the most common extrapulmonary tuberculous infection is often diagnosed

on clinical evidence only. TBLN often presents a diagnostic challenge especially when clinical presentation is suggestive but bacteriological proof is lacking.

The culture isolation of tubercle bacilli from LN biopsy specimen remains the gold standard confirmatory test for the diagnosis of the disease.

However, here in the present study, which is being conducted in a tertiary care hospital, various diagnostic techniques, including CBNAAT, were done from excisional LN biopsy to confirm the diagnosis of TBLN and the results are compared, to find out which of the techniques are more sensitive and specific and gives earlier results.

The sex distribution in this study showed that among the clinically suspected TBLN cases, there was a slight male preponderance. The percentage of male patient being 62 and female. However, no causing factor has been identified for which there can be any striking discrepancy in the sex distribution of TB. A similar Madurai Study conducted by TB Research Centre (TRC) where there were equal number of male and female.[2] Globally, the prevalence of infection with *M. tuberculosis* is similar in males and females until adolescence, after which it is higher in males. However, there is possibility that cases of TB among women are under-reported in developing countries. This is supported by the results of a study comparing active and passive case finding in which women with TB were under-notified to public health authorities when relying on passive case-finding.[3]

This study showed that TBLN is most frequently found in the age group between 15 and 40 years, 54%, which consists of the productive age group. In the recent past, numerous studies have shown a peak age range of 20–40 years. This shift in age probably reflects the falling incidence of childhood TB in the developed countries. In India, the disease is still common in children and young adults.[4,5] In a large clinical trial on LN TB conducted by TRC in Madurai, 35% of patients were aged 12 years or less and 87% were aged 30 years or below. More than 50% of patients were still in the age group of 13–30 years in a similar study in Chennai.[6] Overall, the age distribution of TB diagnosed incident cases shows a predominance in the adolescent and young adult age groups between 15 and 30, indicating ongoing disease transmission.[7]

In our study, out of 50 patients, 42, that is, 84% belonged to low socio-economic status and eight patients that is 16% belonged to middle socio-economic status. The association between poverty and health is well documented.[8] Exactly how poverty may lead to TB remains unclear. Poor SES with its attendant poor education is associated with poor knowledge of TB, risks of infection and dissemination, and with inadequate and/or delayed availability of health care. Poverty also results in poor nutrition and low body weight, which are likely to render the immune system more vulnerable to the invading organisms.[9] There is a significant SES-health gradient in TB prevalence; TB risk increases with lowering of socio-economic status.[10]

Table 3: Percentage of various possible diagnosis of lymphadenopathy on the basis of histopathology report

HPE	Frequency	Percentage
Chronic granulomatous change present	4	8.0
Neoplastic Change	2	4.0
Tubercular lymphadenitis	44	88.0
Total	50	100.0

HPE: Histopathological examination

Table 4: Distribution of study subject according to type of surgery

Type of surgery	Suspected Tubercular lymphadenitis	Percentage
Diagnostic laparoscopy with excision of mesenteric LN	1	2.0
Excision biopsy of cervical lymph node	48	96.0
Excision biopsy of Rt inguinal lymph node	1	2.0
Total	50	100.0

Table 5: Distribution of study subject according to diagnosis

Diagnosis	Frequency	Percentage
Neoplastic Lymphadenopathy	1	2.0
Non tuberculous lymphadenopathy	4	8.0
Tubercular lymphadenitis	45	90.0
Total	50	100.0

Table 6: Percentage of lymphadenopathies that resolved after treatment

Outcome	Frequency	Percentage
Abdominal lymphadenopathy resolved	1	2.0
Lymphadenopathy not resolved	1	2.0
Lymphadenopathy Resolved	47	94.0
Neoplastic cervical ln	1	2.0
Total	50	100.0

Table 7: Table depicting percentage of complications after excision biopsy and treatment

Complication	Frequency	Percentage
Local wound infection	6	12.0
Nil	44	88.0
Total	50	100.0

Table 8: Distribution of study subject according to follow-up

Follow-up	Frequency	Percentage
Completed antibiotics	2	4.0
completed ATT	23	46.0
no recurrence of lymphadenopathy	1	2.0
patient on ATT	22	44.0
Started chemotherapy	2	4.0
Total	50	100.0

Table 9: Comparison between sensitivity and specificity of CBNAAT and HPE

CBNAAT	HPE		Total	*P*-value
	Positive	Negative		
Positive	31	1	32 (64%)	0.01
Negative	13	5	18 (36.0%)	
Total	44	6	50 (100%)	

CB-NAAT: Cartridge-based nucleic acid amplification test, HPE: Histopathological examination

Table 10: Comparison between sensitivity and specificity of smear AFB and HPE

Smear AFB	HPE		Total	*P*-value
	Positive	Negative		
Positive	17	0	17 (34%)	0.06
Negative	27	6	33 (66%)	
Total	44 (88%)	6 (12%)	50 (100%)	

HPE: Histopathological examination, AFB: Acid fast bacilli

Table 11: Comparison between sensitivity and specificity of LJ culture and HPE

LJ culture	HPE gold		Total	*P*-value
	Positive	Negative		
Positive	15	0	15 (30%)	0.08
Negative	29	6	35 (70%)	
Total	44	6	50 (100%)	

HPE: Histopathological examination

Table 12: Comparing sensitivity, specificity, PPV, NPV and accuracy of CBNAAT, Smear AFB and LJ Culture

Diagnostic parameters	CBNAAT	Smear AFB	LJ Culture
Sensitivity	70.45%	38.64%	34.09%
Specificity	83.33%	100%	100%
PPV	96.88%	100%	100%
NPV	27.78%	18.18%	17.14%
Accuracy	72.00%	46.00%	42.00%

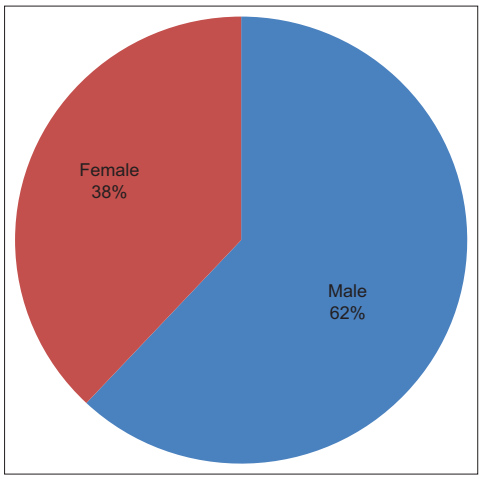

Figure 1: Distribution of study subject according to the gender

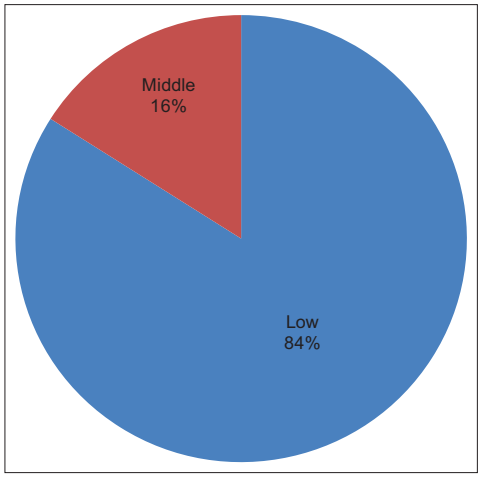

Figure 2: Distribution of study subject according to the socio-economic status

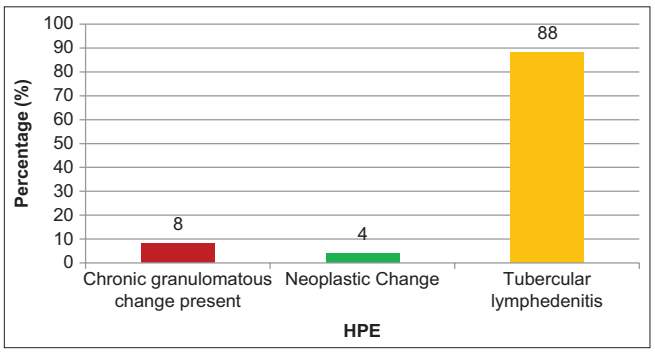

Figure 3: Distribution of study subject according to the histopathological examination

In the present study, excision biopsy has been taken from suspected cases of tubercular lymphadenopathy for confirming the diagnosis. Of these 50 cases, excision biopsies were taken mostly from cervical nodes, 46 cases accounting for 96% of cases, 2% from inguinal LN, and 2% from mesenteric LN by diagnostic laparoscopy and excision of mesenteric LN [Figure 5 and Table 5].

Histopathological diagnosis of TB depends on demonstration of epithelioid cells and Langerhans's giant cells in smears. However, epithelioid granulomas can be seen in non-tuberculous lesions such as Sarcoidosis,

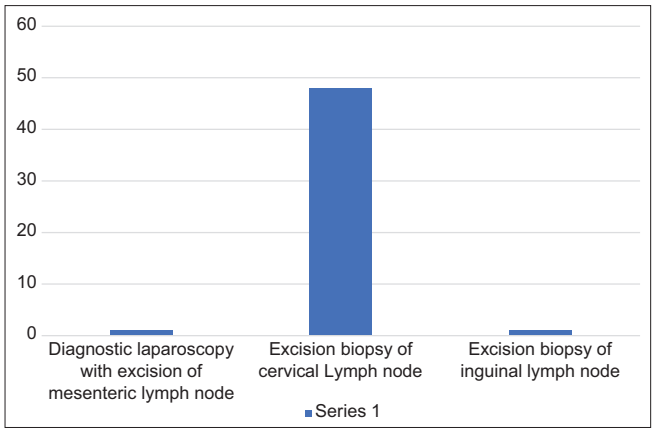

Figure 4: Distribution of study subject according to the type of surgery

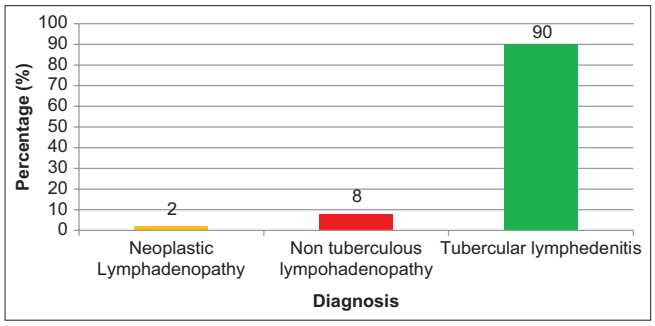

Figure 5: Distribution of study subject according to the diagnosis

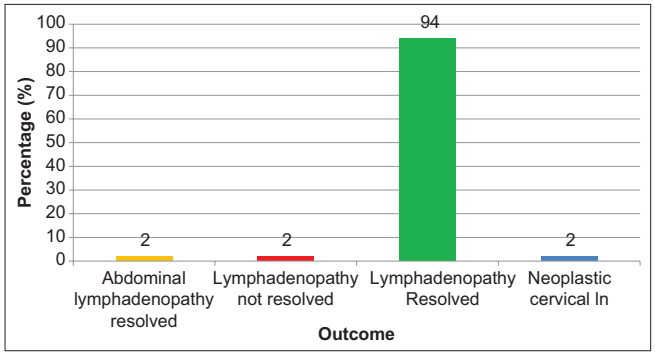

Figure 6: Distribution of study subject according to the outcome

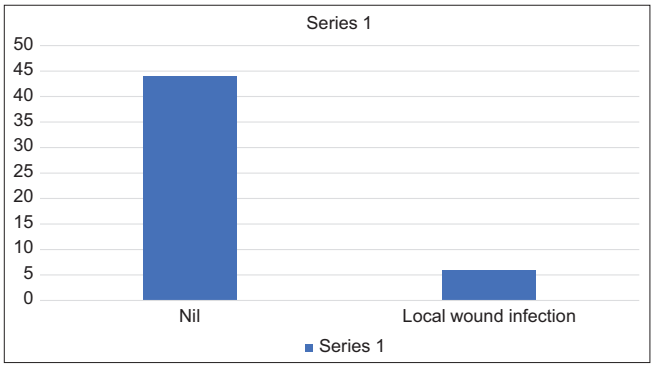

Figure 7: Distribution of study subject according to the complication

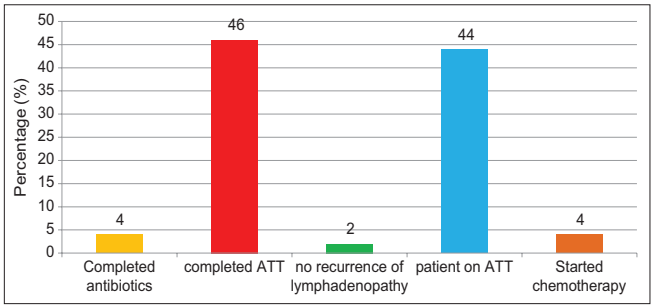

Figure 8: Distribution of study subject according to follow-up

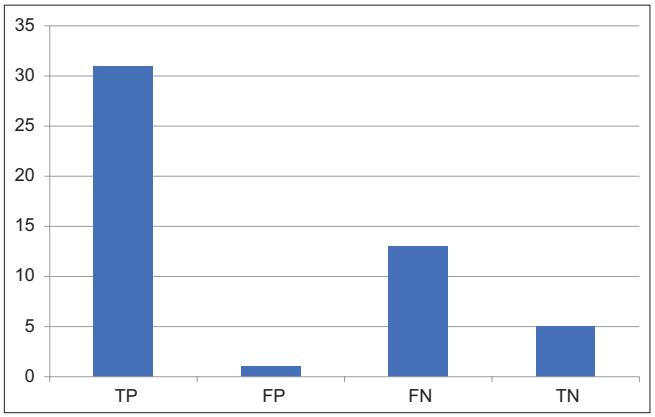

Figure 9: Comparison between sensitivity and specificity of cartridge-based nucleic acid amplification test and histopathological examination

Brucellosis, Cat Scratch disease, Leprosy, and occasionally malignancies such as Hodgkin's disease and metastatic lesions also. The presence of epithelioid cells is the first feature suggestive of diagnosis of TBLN while further data on morphological, microbiological and clinical features can be of additional help.

In the present study, out of 50 excisional biopsies, histopathological diagnosis of TBLN was made on 45 samples (90%), non-tuberculous lymphadenopathy in 8% cases and neoplastic lymphadenopathy in 2% cases. In a study conducted by Arora and Arora *et al.*, at the Department of Pathology and Microbiology, Medical College, Rohtak, out of 200 clinically suspected TBLN cases, HPE showed positivity of 62% for tubercular lymphadenitis.[11] Similar results were obtained by Nataraj *et al.*, at Mumbai.[12]

In the present study in 50 samples, the direct smears by Neelsen method were positive in 15 cases. On comparing with HPE as gold standard, its sensitivity was 38.6% and specificity was 100%. The detection rate of AFB from biopsy materials in extrapulmonary TB is usually low because of the paucibacillary nature of the disease and direct smear could be positive only if the number of AFB

is more than 10^4/ml in the specimen.[13] Positive predictive value being 100% and negative predictive value 18.2% in case of direct smear test by Ziehl Neelson method for diagnosing TB. On comparing sensitivity and specificity of smear AFB and HPE, $P = 0.06$ which is >0.05 are insignificant. This concludes that HPE is better than smear for AFB (ZN staining).

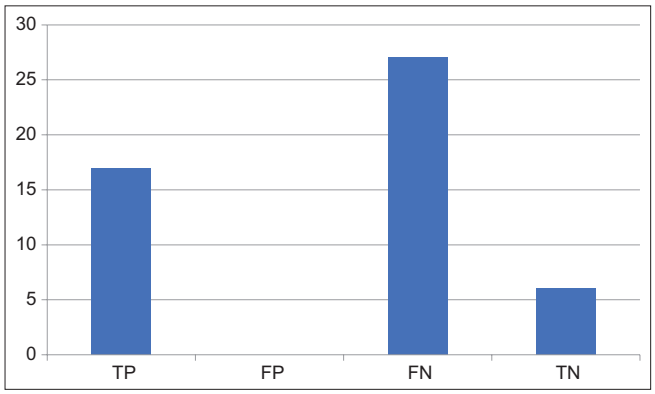

Figure 10: Comparison between sensitivity and specificity of smear acid fast bacilli and histopathological examination

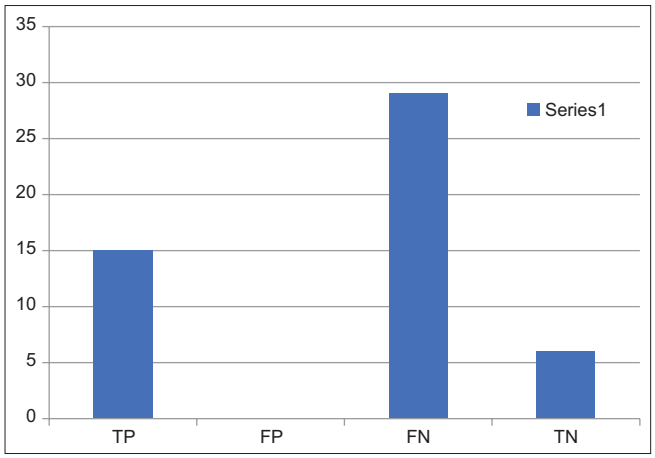

Figure 11: Comparison between sensitivity and specificity of LJ culture and histopathological examination

In the present study, culture isolation of *M. tuberculosis* and the HPE reports is compared. 15 samples out of 44 with tuberculous cytomorphology grew *M. tuberculosis*. Failure to obtain growth of tubercle bacilli is certainly not a conclusive evidence of their absence in the lesions (Middle Brook 1965). The natural healing process, previous antituberculosis treatment and unrepresentative specimens of LNs used for culture can all account for negative cultures (Braunstien and Adriaro: 1961, Kubica and Diji: 1967). Otherwise, the culture negative cytology positive samples may be due to smears, which are richly cellular with occasional clusters of epithelioid cells but no necrosis. In such cases, other granulomatous conditions have to be taken into consideration.[14] Thus, in the present study, sensitivity and specificity were 34.09% and 100%, respectively, when HPE report kept as gold standard here. On comparing, the sensitivity and specificity of LJ culture and HPE, $P = 0.08$, that is, >0.05 is insignificant [Tables 11, 12 and Figure 12].

As a part of analysis in this study, CBNAAT results were compared with the results of any other conventional method such as HPE, Direct Smear or Culture and it was observed that, out of 44 samples positive by any method, CBNAAT was positive in 31 samples. The sensitivity of CBNAAT against any other method was 70.45% and specificity was 83.33%. This finding suggests that CBNAAT can be used as an effective screening and confirmative test in the diagnosis of tuberculous lymphadenopathy from the excisional biopsy samples, which can be obtained with ease from the clinically suspected patients of tubercular lymphadenitis.

This shows that CBNAAT is the most sensitive single technique available to date for the demonstration of

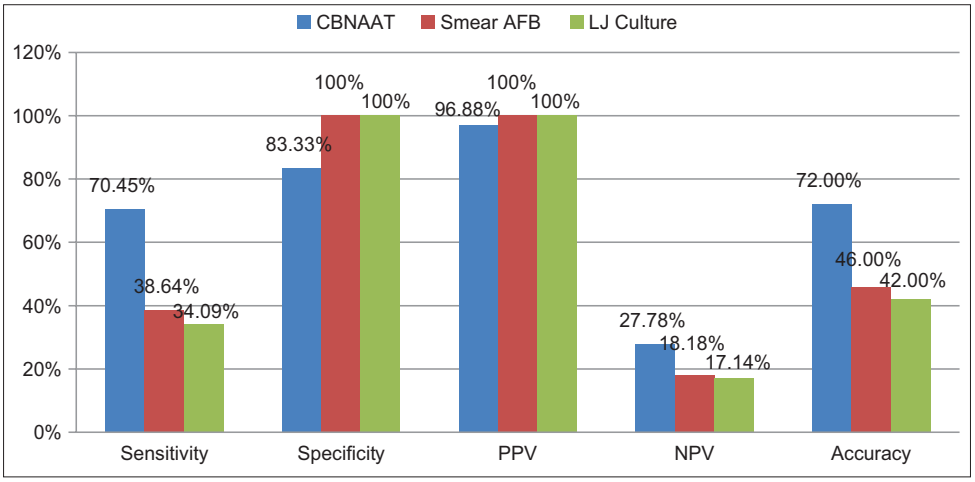

Figure 12: Comparing sensitivity, specificity, positive predictive values, negative predictive values, and accuracy of cartridge-based nucleic acid amplification test, smear acid fast bacilli, and LJ culture

M. tuberculosis in specimen derived from patients with a clinical suspicion of TBLN. Furthermore, the value of CBNAAT lies in its use as a supplementary test in the diagnosis of TBLN, particularly in those patients where conventional methods fail.

CBNAAT has more significant statistical association with HPE ($P = 0.01$ which is <0.05), in comparison with other conventional test such as culture and direct smear and also it is found that CBNAAT is a highly sensitive tool. For the above reasons, CBNAAT has to be considered as an ideal test alone or along with other conventional techniques.

In the present study, it is also seen that no local wound infection was noticed in 88% of the patients and local wound infection was present in 12% of patients which were subsequently treated with antibiotics.

In the present study, after diagnosis being made as tubercular lymphadenopathy, non-tubercular lymphadenopathy, and neoplastic lymphadenopathy, treatment was started accordingly and 4% cases were found where lymphadenopathy did not resolve; whereas in 96% cases of lymphadenopathy got resolved.

In the present study, when CBNAAT, smear AFB, and LJ culture are compared with their sensitivity, specificity, PPV, NPV, and accuracy, CBNAAT found to have the best sensitivity 70.45%, best NPV 27.78%, and best accuracy 70%. Thus, CBNAAT can be used as a single best modality to detect TB cases and can also detect rifampicin resistance. CBNAAT detected *M. tuberculosis* in 1 day rather 2 h compared to an average of 24 days required to detect by culture. This is supported by earlier studies by Pahwa *et al.*[14]

CONCLUSION

Diagnosis of TBLN based on clinical finding alone gives false positive results.

Single diagnostic parameter alone is not sufficient for correct diagnosis.

Among Direct smear (AFB stain), Lowenstein Jensen culture and CBNAAT, CBNAAT ($P < 0.05$, i.e. $P = 0.01$) is most significantly associated with HPE.

CBNAAT is the most sensitive technique. As the rate of drug resistant TB is in increasing trend, it is essential to use a rapid method which detects *M. tuberculosis* and rifampicin resistance simultaneously. Thus, CBNAAT is the best method in the diagnosis of TB. Earlier detection can reduce the death rate and prevent the spread of TB in the community.

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