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Clinicopathological Study of Lymph Node Aspirates in Presumptive Cases of Tuberculosis

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ABSTRACT

Clinically known as pulmonary or extra pulmonary (EPTB) tuberculosis, is a chronic granulomatous illness caused by the acid-fast bacillus Mycobacterium tuberculosis. It is a major public health concern, particularly in developing nations like India. Lymph nodes are the most often affected site in EPTBs. The objective is to compare the efficacy of Ziehl Neelsen (ZN) stain, Auramine-O-Rhodamine (AR) stain and cartridge-based nucleic acid amplification test (CBNAAT) for the detection of acid fast bacilli (AFB) in lymph node aspirates. Additionally, the study mainly focused on the cytomorphological features on fine needle aspiration cytology in presumed tuberculous lymphadenitis patients. A prospective study was carried out for 24 months, from 1/10/2019-31/10/2021, in the Pathology Department of ESIC and PGIMSR, Rajajinagar, Bangalore. A total of 116 tuberculosis cases with probable cases were examined. Following FNAC, the aspirated material was placed onto three glass slides for staining with Haematoxylon and Eosin (H and E), ZN and AR. The leftover material was then transferred into a Falcon tube and diluted with 2ml of saline for CBNAAT. The majority of patients (98.25%) had cheesy aspirates, with the cervical area being the most often aspirated site (54%) and most frequently aspirated. In 88.7% of the patients, cytology revealed the presence of tuberculosis and the most common cytomorphological pattern (53.4%) seen in the cases was caseation with epithelioid cells. For ZN and AR stain, the corresponding values were 40.78%, 82.31%, 21.81%, 96.73% and 86.41%, 92.92%, 16.46% and 99.08% for sensitivity, specificity, positive predictive value and negative predictive value, respectively. The CBNAAT exhibited a 92.23% sensitivity and a 96.92% specificity. 17.38% and 99.47%, respectively, were the positive and negative predictive values. At a p value of <0.00001, it was determined that the fluorescence staining and CBNAAT methods for the detection of AFB were statistically significant. Auramine- O-Rhodamine stain is proven to be more efficacious than traditional acid fast stain for fluorescent staining and CBNAAT provides fast results in just two hours while concurrently determining rifampicin resistance. As a result, we advise using CBNAAT and fluorescent staining as an addition to the standard tuberculosis diagnosis process.

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Key Words
Tuberculosis, lymphadenitis, FNAC CBNAAT, fluorescent staining

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INTRODUCTION

Mycobacterium tuberculosis, an acid-fast bacillus, is the chronic granulomatous infection that causes tuberculosis (TB), a serious public health issue that is particularly prevalent in developing nations like India. With three million deaths each year, it is the world's top leading cause of infectious disease. An estimated 8-10 million individuals contract this disease annually[1,2]. Approximately one-third of the world's tuberculosis cases are in India. Over 20,000 people contract tubercle bacillus infection every day[3]. It is now a significant obstacle to socioeconomic advancement[3,4]. Clinically, it presents as extra pulmonary or pulmonary tuberculosis (EPTB)[5]. The prevalence of extrapulmonary TB is rising globally. In India, extrapulmonary tuberculosis may account for 10-20% of newly diagnosed cases in outpatient settings[6]. Peripheral lymphadenitis is a common presenting condition in patients., it may last longer without symptoms and only become ill when the host's resistance is reduced[7]. Over the past 20 years, several studies have demonstrated the value of fine needle aspiration cytology (FNAC) in the diagnosis of tuberculous lymphadenitis[8-10]. Direct smear microscopy for tubercle bacilli of the sample from the lesion is the only practically available approach for diagnosing extra-pulmonary tuberculosis in underdeveloped countries like India. However, when employed in TB control initiatives, its sensitivity ranges from 9-46%, since the estimated minimum number of tubercle bacilli required to obtain a positive smear result is between 5000 and 1000 per millilitre[11]. The standard approach for detecting tubercle bacilli is mycobacterial culture, however it cannot be used frequently due to its time-consuming nature and the need for specific safety precautions in lab settings. The drawback of serologic methods is their low sensitivity and specificity[12]. "Though quicker, more advanced molecular techniques like PCR are expensive to utilise often in developing nations, where the majority of tuberculosis cases arise[13]. Early case detection necessitates the development of a more sensitive AFB identification technique than the ZN approach. The Revised National Tuberculosis Control Programme already uses fluorescent microscopy with Auramine O Rhodamine (AR) staining to identify AFB on sputum smears (RNTCP)[14]. To detect extrapulmonary tuberculosis more quickly, an attempt can be made to apply similar information to lymph node aspirates. The World Health Organisation (WHO) advised in 2014 that patients who are suspected of having EPTB should use the Cartridge Based Nucleic Acid Amplification Test (CBNAAT/GeneXpert) instead of the standard assays for evaluating certain non-respiratory specimens (lymph nodes and other tissues)[15]. However, because of the extremely poor quality of the available data, this suggestion was conditional. Therefore, more research is required, especially in areas where EPTB prevalence is high.The objective of the research was to evaluate the efficiency of ZN stain, fluorescent stain, and CBNAAT in identifying acid fast bacilli (AFB) in lymph node aspirates and to establish a correlation between these findings and cytomorphological characteristics.

MATERIALS AND METHODS

This was a 24-month prospective study that was carried out in the Department of Pathology, ESIC and PGIMSR, Rajajinagar, Bangalore, from January 10, 2019, to October 31, 2021. The prevalence of tubercular lymphadenitis among all lymph node patients was determined to be 42% based on prior institutional records. The sample size was determined with a 95% confidence interval, 80% power and 9% relative precision. A sample size of 116 was determined. Patients with palpable swellings suggestive of tuberculosis of the lymph nodes, i.e., patients presenting with enlarged lymph nodes >1cm in diameter, as well as patients presenting with symptoms of fever, weight loss, night sweats and cough lasting longer than 3-4 weeks and not improving with antibiotic treatment, were included in the study. Examples of instances that were not related to tuberculosis, such as malignancies, reactive lymphadenitis, metastatic deposits, etc., cases with known or past TB receiving treatment., individuals unwilling to sign the informed consent form in writing; weakly stained slides or acellular smears with crushed morphology, were not included in the study. Patients who met the study's inclusion criteria were enrolled once the institutional ethics committee gave its approval and clearance. All suspected tubercular lesions underwent fine needle aspiration (FNA) after the demographic information (age, gender, residence, phone number), presenting complaints, symptoms (fever, cough, weight loss and family history) were recorded. The lymph node was palpated and needle was inserted after positioning the patient in supine position. The aspirated material was divided into three glass slides for the H and E, ZN and AR staining and the leftover material was transferred into a Falcon tube containing 2ml of saline. Next, the aspirated material was uniformly distributed on the slide. After letting the smear air dry at room temperature, it was fixed by exposing it to the flame. ZN, AR and hematoxylin and

eosin staining were carried out in accordance with normal procedure protocol. After adding 1ml of saline to the residual aspirate material, it was collected into pre-sterilized Falcon tubes and left to incubate for 25-30 minutes at room temperature. The sample was mixed with buffer in a 1:2 ratio and transferred to an Xpert cartridge using a pasteur pipette. The sample was then sent to K.C. General Hospital, Malleshwaram, Bengaluru, for additional processing, where the cartridge was then loaded onto an Xpert (Cepheid, Dx System Version 4.0c) machine. After then, the outcomes were noted and reports were sent to the RNTCP centre at ESIC Model hospital, Rajajinagar, Bangalore. By comparing the stained organisms to control samples and ZN's Negative for AFB, slides were labelled as positive for AFB using Ziehl-Neelsen stain for acid fast bacilli if the organisms were pink, beaded and rod-shaped after staining, if not it waslabelled as negative. After comparing the results of the acid fast bacilli stain with the control samples, we were able to conclude that the sample tested positive for AFB upon staining with Auramine O Rhodamine stain, by looking at a rod-shaped reddish yellow fluorescence against a black background, on fluorescent microscopy.(Table 1). Based on the identification of M. tuberculosis DNA in the samples, CBNAAT provides a report and a semi-quantitative estimate of the bacilli concentration as determined by the Ct (cycle threshold) range, which is as follows:

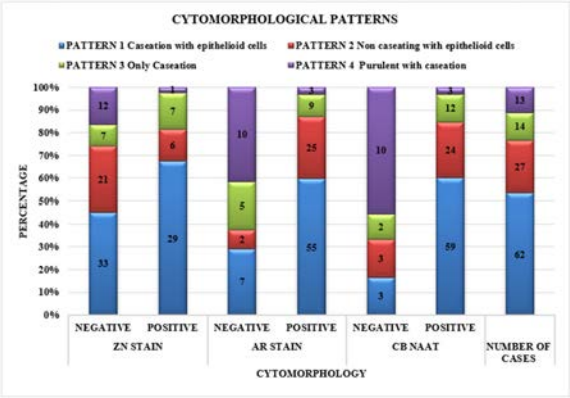
- MTB detected, no Rifampicin resistance detected.
- MTB detected, Rifampicin resistance detected.
- MTB detected, Rifampicin resistance indeterminate.
- MTB not detected.
- Invalid result.

RESULTS AND DISCUSSIONS

Every lymph node aspirate suspected of having tuberculosis was examined cytomorphologically. Research was done comparing AFB ZN stain positivity with AFB AR stain positivity and CBNAAT. The results were tallied and the significance of the data was ascertained by computing the p-value, which is dependent on several criteria. Next, the test under review's sensitivity, specificity and positive predictive value were contrasted with the gold standard. The age range of the study's participants was 01 year-80 years. The greatest number of patients seen (32 instances) was within the age group of 21-30. The age group that saw the fewest patients (02 instances) was 71-80 years old. A clear majority of women were present, accounting for 62.06% of the total, or 72 out of 97 cases. The male to female ratio, which was found to be 1:1.69. Patients were divided into two groups: those who presented with swelling alone and those who had a history of fever, weight loss, appetite loss, evening fever spike, or cough with expectoration. A total of 69/116 cases, or 59.58%, were found to be symptomatic., these cases primarily came with a history of fever and edema.On the other hand, 47 out of 116 cases (40.51%) had no symptoms. 23 out of 116 cases (19.82%) had positive family history with known cases of tuberculosis in family members and 11 cases (9.48%) were with HIV positive status. Of the 23 cases, 18 showed positivity for AFB on ZN stain, 20 showed positivity for AFB on AR stain and all the 23 cases had M.TB detected on CBNAAT. All of the 11 cases with HIV status, were positive for TB on CBNAAT. (Table 2). It was discovered that the cervical group of lymph nodes (61.8%) was the most often aspirated group, followed by the supraclavicular (14.5%), axillary (11.4%), submandibular (7.2%), submental (2.1%), preauricular (1%), subcoastal (1%) and inguinal (1%). In 12 cases, the cervical lymph node had bilateral involvement., in the remaining cases, the laterality of involvement did not differ significantly. The anterior, lateral and posterior cervical lymph nodes were part of the cervical group of lymph nodes. (Table 3). The majority of patients (77.58%) had lymph nodes that measured 1-2cm when they first appeared. Of the patients, 22.41% had lymph nodes larger than 2 cm. The aspirate was purulent/grey white granular in 13 cases, cheesy/frank pus-like in 62 cases and hemorrhagic in 27 cases. Patterns 1 and 3 were primarily associated with cheesy aspirates, while Patterns 2 and 4 showed purulent and hemorrhagic aspirates, respectively. The 116 lymph node aspirates from presumptive cases of tuberculosis were further divided into four patterns on the basis of cytomorphological analysis.,

- **Pattern 1:** Caseating with epithelioid cells 62/116 (53.4%).
- **Pattern 2:** Non caseating with epithelioid cells 27/116 (23.27%).
- **Pattern 3:** only caseation-14/116 (12.06%).
- **Pattern 4:** Purulent with caseation-13/116 (11.20%).

In the present study, maximum number of cases were of Pattern 1 and least number of cases were of Pattern 4. (Table 4). (Table 5) compares the positive for bacilli in different cytomorphological patterns. Pattern 1-Caseation with epithelioid cells had the highest level of ZN stain positivity for AFB, accounting for 29 out of 43 ZN positive cases. Up to 55 out of 92 AR stain positive cases and up to 59 out of 98 CBNAAT positive cases had improved positivity with AR stain for Pattern 1.



Graph 1: Distribution of Cases with Different Cytomorphological Patterns and Detection of AFB Using ZN Stain, AR Stain and CBNAAT

Together, patterns 1, 2 and 3 were thought to be cytologically diagnostic of tuberculosis in a total of 103/116 cases (88.79%), while patterns 4 were thought to be non-diagnostic in 13/116 instances. The identification of M. TB on CBNAAT as well as the smear positivity for AFB on ZN and AR stain were compared to the cytomorphological positivity for tuberculosis. (Refer to Table 5 and Graph 1). Next, the fluorescent staining technique and CBNAAT were used to compare the outcomes of traditional ZN stained smears (Table 6). Out of the 43 ZN positive cases, it was discovered that 40 cases had M. tuberculosis identified on CBNAAT and 41 cases were also positive for AFB on AR stain. 51 out of 61 cases had positive results for AFB on AR staining, while 58 out of 73 ZN negative individuals had M. TB present on the CBNAAT test. (Table 7). The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of ZN stain with respect to AR stain (Table 8) was found to be 44.57%, 91.67%, 21.96% and 96.92% respectively. The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of ZN stain with respect to CBNAAT (Table 9)was found to be 40.82%, 83.33%, 11.42% and 96.40% respectively. On comparison of positivity for AFB on AR stain, with that of CBNAAT, it was found that out of 92 AR positive cases, 88 cases had M.TB detected on CBNAAT. 10 out of 24 AR negative cases, showed presence of M.TB on CBNAAT test. (Table 10). The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of AR stain with respect to CBNAAT (Table 11) was found to be 89.80%, 77.78%, 17.54% and 99.31% respectively.

Interpretation: The CBNAAT has a high sensitivity rate of 92.23%. As a result, there is a probability of predicting the disease because the positive rate is higher than that of ZN and AR stains. The accuracy of the CBNAAT is 77.69%, which is higher than the chance of accurately classifying a patient in the other two tests.

Interpretation: Chi-Square analysis, which is used to determine the relationship between test results and the diagnostic outcome of tuberculosis, found a significant link in all tests, particularly with AR stain and CBNAAT. According to the results, there is more proof of the diagnostic outcome in the AR stain and CBNAAT.

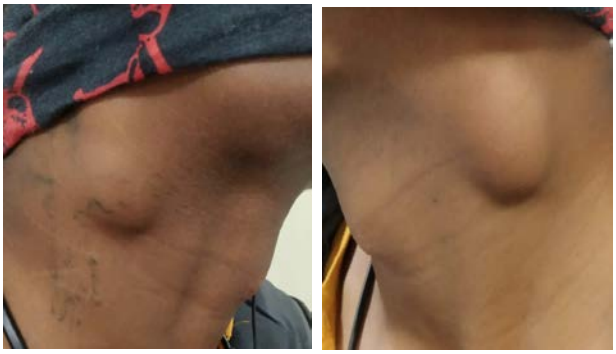


Fig 1a and 1b: Clinical Images Showing Bilateral Cervical Lymphadenopathy

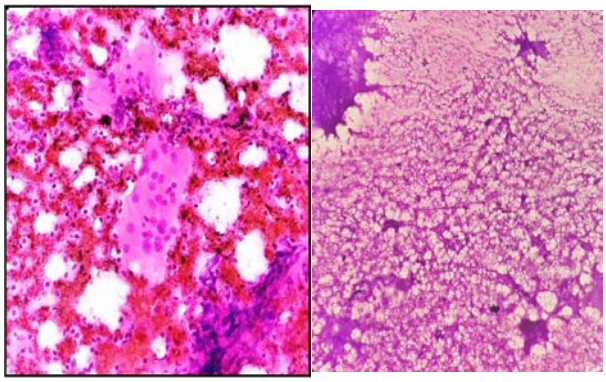


Fig. 2a: Photomicrographs Showing Langhan's Type Giant Cells (H and E, 40X)
2b: Photomicrograph Showing Caseous Necrosis (H and E, 40X)

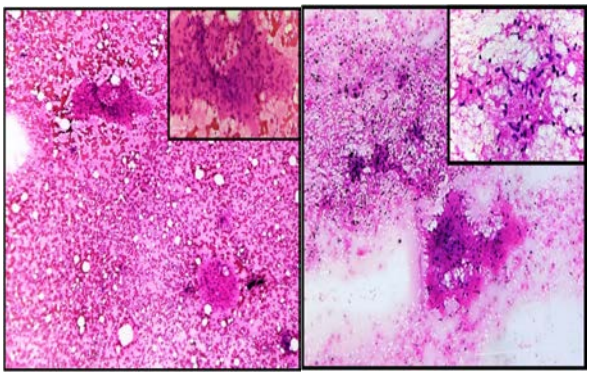


Fig. 3a and 3b: Photomicrograph Showing Epithelioid Cell Granulomas in a Non Caseating Background. Inlet Showing Granulomas on Higher Power. (H and E, 10X)

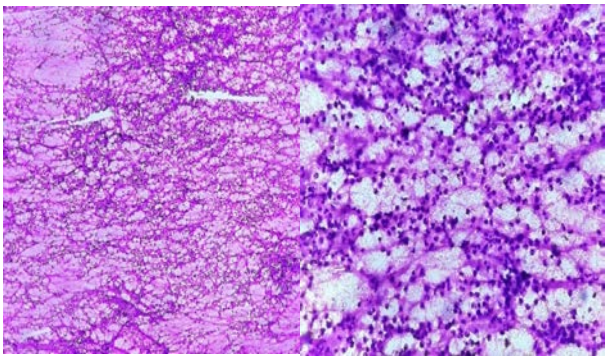


Fig. 4a and 4b:Photomicrographs in 10X and 40X Showing Dense Infiltrate of Neutrophils with a Necrotic Background, a Purulent with Caseation Pattern Seen. (H and E)

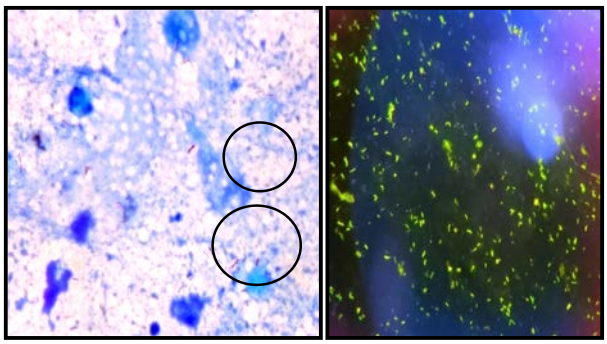


Fig. 5a and 5b: Photomicrographs of Ziehl Neelsen (ZN) Staining, Showing Beaded, Rod Shaped Acid Fast Bacilli (Marked with Black Circle, 100X)., Photomicrograph Showing Numerous (3+) Rod Shaped Acid FAST Bacilli on Auramine-O-Rhodamine Stain. (Florescent Microscopy)

This study examines the cytomorphological characteristics of tuberculous lymphadenitis on FNAC and is cross-sectional, hospital-based. In order to determine which method was more sensitive, specific, and produced earlier results, we also compared the efficacy of ZN stain, fluorescent stain and CBNAAT for the detection of acid fast bacilli (AFB) in lymph node aspirates and correlated the same with cytomorphological features. Our ability to provide quicker recovery is made possible by earlier results, and this is crucial in halting its spread. The study's sex distribution among cases of clinically diagnosed tuberculous lymphadenitis revealed a slight female preponderance, with a male to female ratio of 1.69:1. This was comparable to research conducted by Supreet[5] Komanapalli[16] and Vikas[14] that similarly revealed a female preponderance. (Table 14). Similar to studies by Supreet[5] Vikas[14], Komanapalli[16], Suwarna[17] and Heena Gupta[18], this study revealed that tubercular lymphadenitis is most commonly found in the age group between 21 and 30 years, which consisted of the productive age group. This study shows that 49 of the 116 patients had symptoms, which included fever, weight loss and coughing up expectoration. It was discovered that among these patients, fever with swelling was the most typical presenting symptom. Furthermore, it was discovered that 23 of the 49 patients had positive ZN stain for AFB. Of the 116 individuals, 67 (57.7%) had swelling or no constitutional symptoms., 20 of these patients had positive ZN stain results. The study revealed that the cervical group of lymph nodes accounted for 60.31% of all aspirations. This finding was consistent with research conducted by Vikas[14], Komanapalli[16], Supreet[5], Suwarna[17] and Heena Gupta[18], which found that 81.4%, 94.1%, 65%, 63% and 74%, respectively. (Table 15). The nature of the aspirate was found to be cheesy in majority of the cases, i,e., 65.5%, which was similar to the study done by Kagathara[19], having 41% cases with cheesy aspirate. 55.17% of cases with Cheesy aspirate, 21.55% of cases with hemorrhagic aspirate and 2.5% cases with purulent aspirate showed positivity for AFB on AR stain. This was found to be similar to the study done by Kagathara[19], showing positivity for AFB on AR stain with 74.1%, 49.2% and 11.6% cases for cheesy, hemorrhagic and purulent aspirates respectively. (Table 16). Cytomorphological, the most common pattern in the present study was caseation with epithelioid cells (53.4%), followed by non caseating with epithelioid cells (23.27%), smears with only caseation (12.06%) and lastly purulent with caseation (11.2%) morphology.This predominance was similarly seen with the studies done by Hemalatha[21], Supreet[5] and Heena[18] (Table 17). In our study, the overall positivity with ZN stain for AFB was found to be 37.06%, which increased upto 79.31% on AR stain for AFB. Thus, use of fluorescent stain greatly improves the diagnostic value of the smears and is more sensitive than conventional Z.N. staining method as it allows diagnosis with low density of bacilli (104 bacilli/ml in comparison to 10 bacilli/ml required for Z.N. stain) which are likely to be missed on Z.N. stained smears. (Table 18). Lokeshwaran[22] reported that Auramine-O staining method is more efficient and advantageous than conventional Z.N. staining method particularly in paucibacillary cases. They explained that as AR stained smears are scanned under lower magnification (40x) than Z.N stained smears (100x), a greater area is screened per field which makes the process less time-consuming and reduces observer fatigue. But due to disadvantages like, a high equipment cost AO staining cannot be easily available in every setup, fading of slides with no possibility for permanent preparations, requirement of a trained personnel, positive and negative control required every time and background staining. Therefore use of fluorescent stain alone could not be an alternative to conventional Ziehl Neelsen staining. Hence, it would be

Table 1: Auramine Rhodymenia Reporting System

IUATLD/WHO Scale (1000x field=HPF) RESULT	FLORESCENCE (400X MAGNIFICATION: 1length=40 fields=200 HPF)
Negative	Zero AFB/ 1length
Scanty	1-19 AFB/ 1length
1+	20-199 AFB/ 1length
2+	5-50 AFB/1 field
3+	>50 AFB/ 1 field on average

Table 2: Distribution of Cases with Family History of TB and Detection of AFB

Family History	ZN Stain		AR Stain		Cbnaat	
	Positive	Negative	Positive	Negative	Positive	Negative
Present (23)	18	05	20	03	23	00
Absent (93)	25	68	72	21	75	18
Total	43	73	92	24	98	18

Table 3: Distribution of Cases According to site

Site	Number of Cases	%
Axilla	15	13%
Cervical	63	54%
Inguinal	1	1%
Preauricular	1	1%
Subcoastal	1	1%
Submandibular	8	7%
Sub mental	8	7%
Supra clavicular	19	16%
Grand Total	116	100%

Table 4: Distribution of Cases According to the Cytomorphological Patterns

Patterns	Cytomorphology	Number of cases	Percentage (%)
Pattern 1	Caseation with epithelioid cells	62	53.44
Pattern 2	Non caseating with epithelioid cells	27	23.27
Pattern 3	Only Caseation	14	12.06
Pattern 4	Purulent with caseation	13	11.20
Grand Total		116	100

Table 5: Comparison of Cytomorphological Patterns with Detection of AFB Using ZN Stain, AR Stain and CBNAAT

Patterzns	Cytomorphology	ZN Stain for AFB		R Stain for AFB		B NAAT		
		Negative	Positive	Negative	Positive	Negative	Positive	No of cases
Pattern 1	Caseation with epithelioid cells	33	29	7	55	3	59	62
Pattern 2	Non caseating with epithelioid cells	21	6	2	25	3	24	27
Pattern 3	Only Caseation	7	7	5	9	2	12	14
Pattern 4	Purulent with caseation	12	1	10	3	10	3	13
Grand Total		73	43	24	92	18	98	116

Table 6:Distribution of Cases Based on Detection of M.TB

Result	ZN STAIN		AR STAIN		CB NAAT		
	Negative	Positive	Negative	Positive	Negative	Positive	No of cases
Diagnostic of TB	61	42	14	89	8	95	103
Non Diagnostic of TB	12	1	10	3	10	3	13
Grand total	73	43	24	92	18	98	116

Table 7: Comparison of Detection of AFB on ZN Stain, with Detection on AR Stain and CBNAAT

ZN Stain	AR Stain		CBNAAT	
	Positive	Negative	M.TB Detected	M.TB Not Detected
	92	24	98	18
Positive (43)	41	2	40	3
Negative (73)	51	22	58	15

Table 8: Statistical Analysis of ZN Stain with AR Stain

Statistic	Value	95% CI
Sensitivity	44.57%	34.19-55.30%
Specificity	91.67%	73.00-98.97%
Positive likelihood Ratio	5.35	1.39-20.55
Negative Likelihood Ratio	0.60	0.49-0.75
Disease Prevalence (*)	5.00%	
Positive Predictive Value (*)	21.96%	6.82-51.96%
Negative Predictive Value(*)	96.92%	96.19-97.51%
Accurancy(*)	89.31%	82.22-94.28%

Table 9: Statistical Analysis of ZN Stain with CBNAAT

Statistic	Value	95% CI
Sensitivity	40.82%	30.99-51.21%
Specificity	83.33%	58.58-96.42%
Positive likelihood Ratio	2.45	0.85-7.07
Negative Likelihood Ratio	0.71	0.55-0.92
Disease Prevalence (*)	5.00%	
Positive Predictive Value (*)	11.42%	4.27-27.12%
Negative Predictive Value(*)	96.40%	95.36-97.21%
Accurancy(*)	81.21%	72.90-87.86%

Table 10: Comparison of Detection of AFB on AR Stain and CBNAAT

AR Stain	CBNAAT	
	Positive	Negative
	98	18
Positive (92)	88	4
Negative (24)	10	14

Table 11: Statistical Analysis of AR Stain and CBNAAT

Statistic	Value	95% CI
Sensitivity	89.80%	82.03-95.00%
Specificity	77.78%	52.36-93.00%
Positive likelihood Ratio	4.04	1.70-9.61
Negative Likelihood Ratio	0.13	0.07-0.25
Disease Prevalence (*)	5.00%	
Positive Predictive Value (*)	17.54%	8.20-33.60%
Negative Predictive Value(*)	99.31%	98.71-99.64%
Accurancy(*)	78.38%	69.77-85.48%

Table 12: Comparision of Statistical Analysis of Various Methods of Detection of M.TB with Cytomorphology

	ZN Stain			AR Stain			CB NAAT		
	Diagnostic of TB	Non Diagnostic of TB	Total	Diagnostic of TB	Non Diagnostic of TB	Total	Diagnostic of TB	Non Diagnostic of TB	Total
Positive	42	1	43	89	3	92	95	3	98
Negative	61	12	73	14	10	24	8	10	18
Sensitivity	40.78%			86.41%			92.23%		
Specificity	82.31%			92.92%			96.92%		
PPV	21.81%			16.46%			17.38%		
NPV	96.73%			99.08%			99.47%		

Table 13: Chi-Square Analysis

RESULTS	ZN STAIN		TOTAL	Chi-Square(df=1)	P-Value
	Diagnostic of TB	Non Diagnostic of TB			
Positive	42	1	**43**	**5.41**	**0.019**
Negative	61	12	**73**		
Results	**AR STAIN**		**TOTAL**	**Chi-Square (df=1)**	**P-Value**
	Diagnostic of TB	**Non Diagnostic of TB**			
Positive	89	3	**92**	**28.21**	**<0.00001**
Negative	14	10	**24**		
Results	**CB NAAT**		**TOTAL**	**Chi-Square (df=1)**	**P-Value**
	Diagnostic of TB	**Non Diagnostic of TB**			
Positive	95	3	**98**	**42.11**	**<0.00001**
Negative	8	10	**18**		

Table 14: Comparison of Sex Distribution with Other Studies

Studies	Male	Female	Ratio M:F
Present study	32.9%	62%	1:1.64
Supreet[5]	44%	56%	1:1.3
Vikas[14]	38.2%	61.7%	1:1.6
Komanapalli[16]	48%	51%	1:1.06

Table 15: Comparison of Most Common Site of Aspirate with Other Studies

Studies	Percentage
Present study	60.31%
Supreet[5]	65%
Vikas[14]	81.4%
Komanapalli[16]	94.1%
Suwarna[17]	63%
Heena Gupta[18]	74%

Table 16: Comparison of Nature of Aspirate with Other Studies

Studies	Cheesy	Hemorrhagic	Purulent
Present study	65.5%	23.2%	11.2%
Kagathara[19]	41%	28%	31%
Komanapalli[16]	4%	41%	55%
Brijesh[20]	31.1%	51.1%	15.6%

Table 17: Comparison of Cytomorphological Feature of Tubercular Lymphadenitis with Other Studies

Patterns	Present Study	Hemalatha[21]	Supreet[5]	Heena[18]
Caseation with epithelioid cells	53.44%	84.56%	63%	56%
Non caseating with epithelioid cells	23.27%	19.3%	06%	08%
Only Caseation	12.06%	22.6%	06%	14%
Purulent with caseation	11.20%	2.6%	25%	22%

Table 18: Comparison of Ziehl Neelsen (ZN) and Fluorescent (AR) Stain Smear Positivity

Studies	Smear Positivity For ZN Stain	Smear Positivity for AR Stain
Present study	37%	79%
Supreet[5]	37%	73%
Vikas[14]	36.5%	51.3%
Heena[18]	28%	50%
Brijesh[20]	26.67%	34.44%
Lokeshwaran[22]	44%	88%

Table 19: Sensitivity and Specificity of CB-NAAT Given by Various Authors

Various Authors	Sensitivity	Specificity
Present study	96.9%	55.5%
Komanapalli[16]	85.71%	96.8%
Heena[18]	76.92%	81.82%
Singh[23]	91%	90%
Ligthelm[24]	96.7%	88.9%

be beneficial if we use it as an adjuvant along with routine cytology for the early diagnosis of tuberculous lymphadenitis. Subsequently, we found that 15.51% of patients in our study were negative and 84.48% of cases showed CB-NAAT positivity. We found that in 95 out of 116 instances (81.89%), the cytological diagnosis and CB-NAAT tested positive, while in 10 out of 116 cases (8.62%), both tests came out negative. Cytological characteristics of tuberculosis were negative in 3/116 cases (2.58%) in which CB-NAAT was positive, but CB-NAAT was negative in 8/116 cases (6.89%) that had a positive cytological diagnosis. (Table 19). According to WHO Xpert guidelines those patients who were cytologically positive and clinically suspicious of tuberculosis should receive TB treatment. So, this explains that CB-NAAT negative result can still have TB[16]. Overall ZN. Positivity was seen in 37% while with CB-NAAT positivity was increased up to cases 84.4%. Both ZN. and CB-NAAT were positive in 40/116 cases and negative in 15/116 cases. CB-NAAT positivity was seen in 58/116 cases in which ZN. was negative and 03/116 cases were positive for Z.N. which were negative for CB-NAAT. Therefore on comparison CB-NAAT proves to be more sensitive than Z.N stain. The possible cause for CB-NAAT negativity in above cases may be due to less fluidity of aspirated material which may be solid/cheesy material which usually have very low bacillary load compared to liquid caseous material which have high bacillary load or blood mixed aspirate in some cases. Because of low bacillary load and its detection limit of 131cfu/ml might be the reason for CB-NAAT negativity in these patients. Komanapalli[16] reported that their 23 samples were FNAC+CB-NAAT-ve. Majority of these cases were blood mixed and mostly these aspirates were from the children. It was thus possible that in these cases representative sample might not be obtained as aspirations from the children is difficult or bacterial load may have been too low for the Gene Expert to detect the DNA. Further on comparison fluorescent stain positivity was seen in 79.3% while with CBNAAT positivity was increased to 84.4%. Both fluorescent stain and CB-NAAT were positive in 88/116 cases and negative in 14/116 cases. CB-NAAT positivity was seen in 10/116 cases in which fluorescent stain was negative. 04 cases which were positive for fluorescent stain was negative for CB-NAAT. Therefore, on comparison, CB-NAAT is more sensitive than fluorescent stain.

CONCLUSION

Our research leads us to the conclusion that, while cytomorphological appearance and ZN staining are frequently employed in developing nations as less expensive and more practical alternatives to open lymph node biopsies, the fluorescence method is a more sensitive technique because it allows for the rapid screening of much larger areas of the smear on the slides than the ZN method. Additionally, it significantly raises the diagnostic value, particularly in paucibacillary individuals whose smears with Z.N. staining are likely to miss. Patients with a high risk of tuberculosis, for whom an AFB smear screening is typically negative, benefit from CB-NAAT. Culture is regarded as the gold standard approach, but it takes days for the results, thus it is time-consuming. For patients who exhibit the cytomorphological look of tuberculosis, CB-NAAT turns out to be more sensitive than both Z.N. and fluorescent staining. CB-NAAT is most likely to be beneficial for patients with a high risk of tuberculosis in whom the results of an AFB smear examination are often negative, including those that are resistant to rifampicin. However, since each method has its own limitations, these techniques should only be used as a supplement to clinical history, haematological investigations, cytological features in lymph node aspirates and histological examination and microscopy when diagnosing suspected cases. This is especially true in developing nations.

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