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To study the differentials of suppurative lymphadenitis by FNAC and their evaluation by Z.N. staining, CB-NAAT, fluorescent microscopy, fungal and bacterial cultures

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Abstract

Introduction: Suppurative lymphadenitis is most commonly unilateral in presentation. It is caused due to transportation of invading microorganisms (initially penetrating from mucosa or skin of the head or neck most commonly) to afferent lymph nodes. The most common bacterial causes of suppurative lymphadenitis are *Staphylococcus aureus* and *Streptococcus pyogenes*, accounting for between 40% and 89% of cases.

AIM: To study the cytomorphological features in fine needle aspiration smears from patients suspected of having suppurative lymphadenitis and to study the differentials of suppurative lymphadenitis by FNAC and their evaluation by Z.N. staining, CB-NAAT, fluorescent microscopy, fungal and bacterial cultures so as to know the exact cause of suppurative lymphadenitis.

Material and Methods: The present study was conducted in 50 patients of clinically suspected cases of suppurative lymphadenitis. Fine needle aspirations were performed and smears from the aspirates were processed for routine cytology (H&E, MGG) N, the conventional ZN method, fluorescent method, CBNAAT, bacterial and fungal culture for evaluation.

Results: Z.N. positivity was seen in 34.14% while with fluorescent stain positivity was increased up to 60.97% and was increased up to 78.05% with CB-NAAT. Fluorescent stain positivity was seen in 60.97% while with CB-NAAT positivity was increased to 78.05%. Out of 9 cases of with bacterial etiology, only 5 cases were positive on bacterial culture. 3 cases showed MRSA and two cases showed *Streptococcus pyogenes*. No case of fungal lymphadenitis was identified in our study as all the cases were negative on PAS staining and fungal culture.

Conclusion: Though cytomorphological appearance and Z.N. staining are commonly used in developing countries as they are economical and convenient alternative to open biopsy of lymph nodes but fluorescent method proves to be more sensitive technique than the Z.N. CB-NAAT proves to be more sensitive than both Z.N. and fluorescent staining for those patients which show cytomorphological appearance of TB. Patients with high risk of tuberculosis in whom AFB smear examination is usually negative are most likely to be benefited from CB-NAAT. Hence for the differentials of suppurative lymphadenitis cytomorphological examination along with various other ancillary techniques (methods) has more diagnostic value.

Keywords: Suppurative lymphadenitis, FNAC, ziehl neelsen, fluorescent microscopy, CBNAAT

Introduction

Lymphadenitis is the inflammation or enlargement of a lymph node. It is a common feature in a variety of diseases and may serve as a focal point for subsequent clinical investigation of diseases of the reticuloendothelial system or regional infection. Suppurative lymphadenitis is most commonly unilateral in presentation. It is caused due to transportation of invading microorganisms (Initially penetrating from mucosa or skin of the head or neck most commonly) to afferent lymph nodes.

The most common bacterial causes of suppurative lymphadenitis are *Staphylococcus aureus* and *Streptococcus pyogenes*, accounting for between 40% and 89% of cases. In contrast to acute suppurative lymphadenitis, infections caused by *Mycobacteria*, fungi and *Bartonellahenselae* can become granulomatous and develop over weeks to months^[1-5].

There are numerous causes of lymphadenopathy which includes reactive lymphadenitis (Secondary to viral and bacterial infections), Tuberculosis, lymphomas, sarcoidosis, metastatic malignancies and uncommon causes like fungal diseases, toxoplasmosis and diseases of the mononuclear phagocyte system among others [6]. In India, however tuberculous lymphadenitis remains one of the most common causes of lymphadenitis.

The precise cause of these enlarged lymph nodes is often difficult to establish by history, physical examination, and radiographic studies alone. Other investigations which can be done are blood tests like WBC count, ESR, and CRP to look for infections. Placing fluid from enlarged lymph nodes into culture to see what type of microorganism grow. Microscopic study of samples taken from lymphadenitis can be useful in many conditions.

Fine needle aspiration cytology (FNAC) is the widely practised investigation in the evaluation of lymphadenopathies. It is a safe, simple, rapid and cost-effective procedure used as a investigation in cases of lymphadenopathy. The aspirated material is stained and studied.

However conventional smear microscopy lacks sensitivity due to the paucibacillary nature of fine needle aspirates.⁷ Thus because of its limitations which includes absence of species confirmation and or lack of drug resistance guidance more rapid and reliable methods are needed further confirmation of exact etiology.

Therefore, the present study was aimed to study the differentials of suppurative lymphadenitis by FNAC and their evaluation by Z.N. staining, CB-NAAT, fluorescent microscopy, fungal and bacterial cultures so as to know the exact cause of suppurative lymphadenitis.

Material and Methods

This prospective Comparative study was conducted in 50 patients presenting as clinically suspected cases of suppurative lymphadenitis in the Department of Pathology, Government Medical College, Amritsar, after approval from the institutional thesis and ethics committee. Informed consent of the patient was taken. Relevant history of the patient would be taken as per the proforma attached along with. All clinically suspected cases of suppurative lymphadenitis with well palpable and enlarged peripheral lymph node whether single or multiple were included. While all known cases of non suppurative granulomatous pathology, known cases of metastases and known cases of primary and secondary lymphoid malignancy were excluded from our study.

FNA was performed and the some material was applied to pre labelled slides and stained with H&E, Z.N. staining, Auramine - O staining (fluorescent microscopy) while rest material was used for CBNAAT, fungal and bacterial cultures for further evaluation.

Depending on the cytomorphological features, all the cases were subdivided into six groups as follows:

- Pattern I:** Caseous necrosis & epithelioid cell granulomas
- Pattern II:** Caseous necrosis, epithelioid cell granulomas with inflammatory background (lymphocytes / acute inflammatory infiltrate)

- Pattern III:** Non-necrotic background & epithelioid cell Granulomas
- Pattern IV:** Caseation necrosis only
- Pattern V:** necrosis with inflammatory background
- Pattern VI:** Inflammatory infiltrate only (acute inflammatory cells, lymphocytes)

Staining results: AFB on ZN stained smears were seen as pinkish, thin curved rod-shaped bacterium coloured rods against a blue coloured background on oil immersion (1000X) and in Auramine stained smears as slender bright yellow fluorescent rod shaped bacteria against a dark background on 400X.

Technique for CB-NAAT: Sample was collected & transported whenever possible at 2 to 8°C in pre-sterilized falcon tubes. 2ml of sterile phosphate buffer solution (PBS) is added to sample, from this 0.7 ml of homogenised tissue specimen is collected and mixed with double volume of Gene Expert sample reagent (1.4ml). The processed sample is loaded into Gene Expert MTB/RIF cartridge. The automated generated result were available in 100 minutes.

Culture: A sterile swab is dipped in sample and then rubbed the end of swab onto sterile agar plate. This will give mixed culture of microbes. Then we take a sterile inoculating loop and dip the loop into the culture of microbes and streak this loop in a pattern over the surface of the agar plate and incubated at 37°C overnight. Selected colonies of bacteria can be then picked up from these plates and transferred to separate agar plates to form a pure culture.

Results

The present study was conducted on 50 patients presenting as suspected cases of suppurative lymphadenitis.

Maximum cases in the present study were in the 21-30 years age group (40%) followed by 11-20 years age group (32%). Slight female preponderance was observed with a male: female ratio of 1:1.08. Cervical group of lymph nodes were the most common group involved accounting for 74% of cases. The second most common group was supraclavicular with 12% of cases followed by axillary (6%), submandibular (6%) and submental (2%). (figure 1).

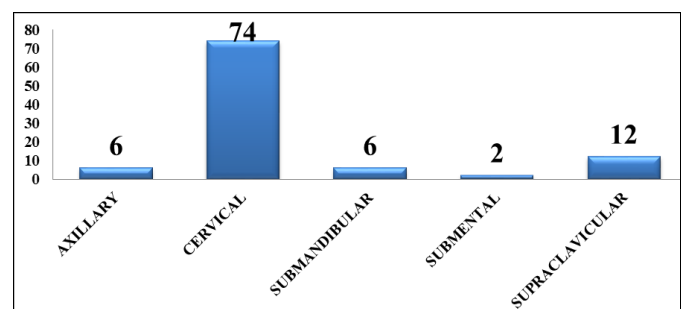


Fig 1: Cases distribution according to the group of lymph nodes involved

In the present study tubercular lymphadenitis was the most common encountered cause of lymphadenitis (82%) in our study followed by bacterial lymphadenitis (18%). (Figure 2)

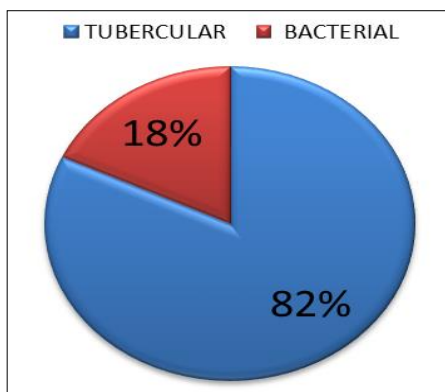


Fig 2: Distribution of cases according to causes of suppurative lymphadenitis in the present study.

In our study, majority of the cases showed pattern I constituting 38% of the total cases. This was followed by pattern VI (22%), pattern II (18%), pattern III and pattern IV (8% each) and least was pattern V (6%).

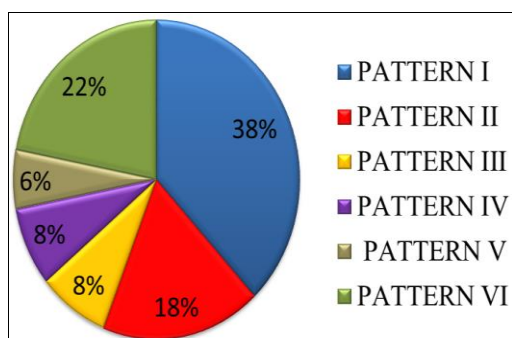


Fig 3: Graphical representation of case distribution according to different cytomorphological patterns studied

On Zeil Nelson (ZN) staining, 34.14% of cases demonstrated presence of AFB and 65.85% of cases were negative. One of the HIV positive patient was Z.N. positive in our study. Cytomorphological features of tuberculosis were present in 39 cases and 11 cases demonstrated suppurative pathology on cytology. Both ZN stain and cytological diagnosis was positive in 13 cases and were negative in 10 cases. Out of 11 cases which were negative on cytology, only one showed Z.N. positivity. Both Z.N. staining and cytological diagnosis were negative in 10 cases for tuberculosis. Therefore sensitivity and specificity for ZN stain came to be 33.33% and 90.91%.

On fluorescent staining (AO) staining, 60.97% of cases demonstrated presence of positive AO staining while 39.03% of cases were negative. One of the HIV positive patient was positive on fluorescent stain. Cytomorphological features of tuberculosis were present in 39 cases and 11 cases demonstrated suppurative pathology on cytology. Both AO stain and cytological diagnosis were positive in 24 cases while both were negative in 10 cases. Out of 11 cases which were negative on cytology, only one showed fluorescent positivity. Therefore sensitivity and specificity for AO stain came to be 61.54% and 90.91%.

Hence, overall on comparing, ZN positivity was seen in

28% cases while with fluorescent stain positivity was increased upto 50%. (Table 1). In cases of TB lymphadenitis, Z.N. positivity was seen 34.15% while with fluorescent stain positivity was increased upto 60.98%. Fluorescent stain proves to be more sensitive than ZN stain while both are equally specific.

Table 1: Comparison of ZN and fluorescent positivity in TB lymphadenitis

		ZN Stain		Total Cases
		0	1	
Fluorescent Stain	0	22	3	25
	1	14	11	25
Total		36	14	

On CBNAAT, 78.05% of cases of tubercular lymphadenitis demonstrated CBNAAT positivity while 21.95% cases were negative. One of the HIV positive patient was positive on CB-NAAT. Cytomorphological features of tuberculosis were present in 39 cases and 11 cases demonstrated suppurative pathology on cytology Both CBNAAT and cytological diagnosis were positive in 60% cases while both were negative in 18% cases. Out of 11 cases which were negative on cytology, two showed CBNAAT positivity. Also out of total cases with CB-NAAT positivity, 2 were found to positive for CBNAAT which were rifampicin resistant. Therefore sensitivity and specificity for CB-NAAT came to be 76.92% and 81.82%.

Overall Z.N. positivity was seen in 14/50 cases (28%) while with CB-NAAT positivity was increased upto 32/50 cases (64%). (Table 2). Therefore on comparison, CB-NAAT is more sensitive as compared to Z.N. stain.

Table 2: Comparison of Z.N. and CB-NAAT positivity.

		ZN Stain		Total Cases
		0	1	
CB-Naat	0	16	2	20
	1	20	12	32
Total		36	14	50

Overall fluorescent stain positivity was seen in 25/50 cases (50%) while with CB-NAAT positivity was increased upto 32/50 cases (64%). (Table 3) Therefore On comparison, CB-NAAT is more sensitive in comparison to fluorescent stain.

Table 3: Comparison of fluorescent stain and CB-NAAT positivity.

		Fluorescent Stain		Total Cases
		0	1	
CB-Naat	0	17	1	18
	1	8	24	32
Total		25	25	50

Further in our study on bacterial culture, all cases of tubercular lymphadenitis (41 cases) were negative on bacterial culture. Out of 9 cases with bacterial etiology, only 5 cases were positive on bacterial culture. 3 out of 5 cases showed MRSA (Methicillin resistant staphylococcus aureus) and 2 out of 5 cases showed streptococcus pyogenes colonies on bacterial culture. All the cases were negative on PAS stain and showed negative results on fungal culture.

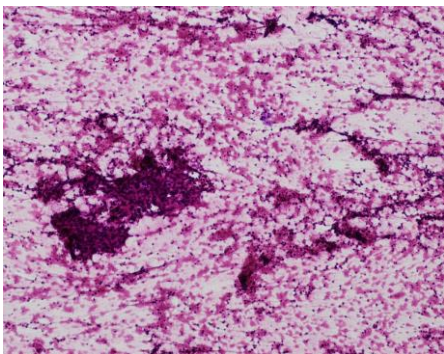


Fig 1: Photomicrograph showing epitheloid cell granuloma with inflammatory infiltrate (H&E, 100X)

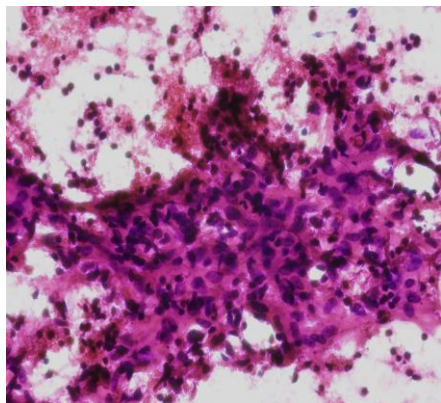


Fig 2: Photomicrograph showing epitheloid cell granuloma, caseous necrosis and inflammation in the background (H&E, 400X)

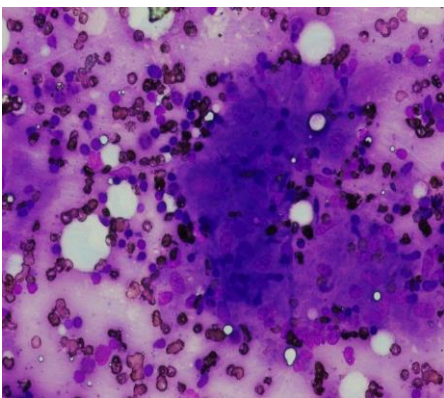


Fig 3: Photomicrograph showing epitheloid cell granuloma, caseous necrosis and inflammation in the background (MGG, 400X)

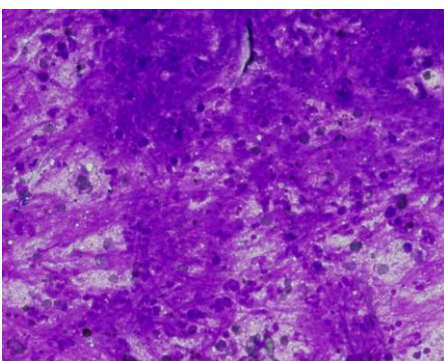


Fig 4: Photomicrograph showing caseous necrosis with inflammatory background (MGG, 400X)

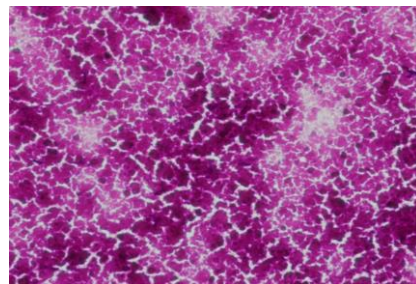


Fig 5: Photomicrograph showing caseous necrosis (H&E, 400X)

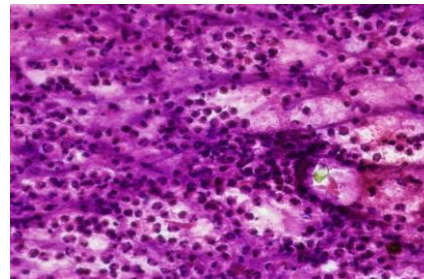


Fig 6: Photomicrograph showing inflammatory infiltrate composed of neutrophils and lymphocytes (H&E, 400X)

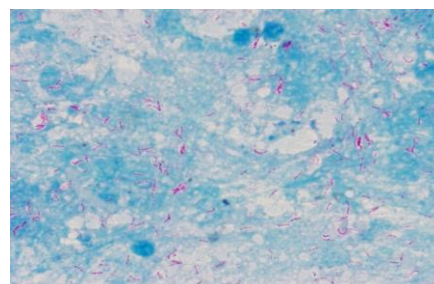


Fig 7: Photomicrograph of Ziehl Neelsen (ZN) staining showing many acid fast bacilli (1000X)

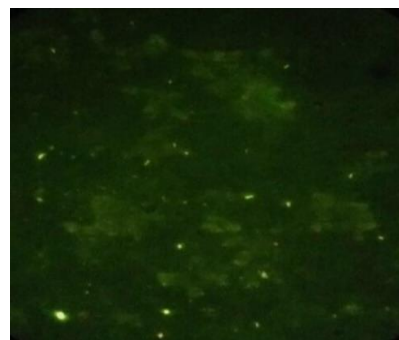


Fig 8: Photomicrograph of fluorescent stain showing acid fast bacilli (400X)

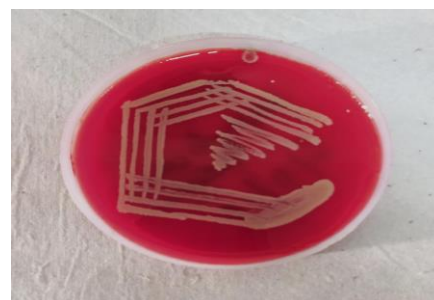


Fig 9: Photograph showing growth of Staphylococcus aureus on blood agar

Discussion

The present study was conducted on 50 patients presenting as suspected cases of suppurative lymphadenitis in the Department of Pathology, Government Medical College, and Amritsar.

Our results showed that maximum cases in the present study were in the 21-30 years age group (40%), while minimum number of cases belonged to 41-50 years of age. Youngest patient in our study was 2 years old while the oldest was 69 years old. Our results were in accordance with various authors like Ahmad *et al.* [8], Kalra SK *et al.* [9] and Lokeshwaran RS *et al.* [10] who also reported 2nd decade to be the most common age group in their studies.

Female population predominated in our study with a male: female ratio of 1:1.08. Among both males and females the highest number of cases were in the age range of 21-30 years.

In similarity authors like Bharathi K *et al.* [11], Kalra SK *et al.* [9] and Lokeshwaran RS *et al.* [10] also reported female predominance in their studies while on contrary Prakashan M *et al.* [12] and Ishar T *et al.* [13] reported male predominance in their studies.

In our study, cervical group of lymph nodes were the most common group involved accounting for 74% of cases. These results are very well correlated with the studies conducted by Khan *et al.* [14], Chau *et al.* [15], Bezabih *et al.* [16], Lee *et al.* [17], Bharathi K *et al.* [11], Hema Arora [16], Kalra SK *et al.* [9] and Lokeshwaran RS *et al.* [10].

Tubercular lymphadenitis was the most common cause of suppurative lymphadenitis i.e. (82%) while only 18% cases were of bacterial etiology. Further. The predominant cytomorphological pattern in our study was Pattern I as 38% of the smears cytologically presented as epithelioid cell granulomas in a background of caseous necrosis, which are

characteristic of TB lymphadenitis. Whereas, 22% of cases presented with only inflammatory infiltrate (acute inflammatory cells, lymphocytes) which was suggestive of suppurative lymphadenitis. This predominance of pattern I was similarly seen by other authors like Chandrasekhar B *et al.* [19] Ergete W *et al.* [20], Kalra SK *et al.* [9] and Lokeshwaran RS *et al.* [10].

In our study, Z.N. positivity was seen in 14/50 cases (28%) while with fluorescent stain positivity was increased upto 25/50 cases (50%). 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 10⁵ bacilli/ml required for Z.N. stain) which are likely to be missed on Z.N. stained smears.

Lokeshwaran RS *et al.* [10] 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 AO 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 beneficial if we use it as an adjuvant along with routine cytology for the early diagnosis of tuberculous lymphadenitis.

Table 4: Comparison of ziehl neelsen (ZN) and fluorescent (AO) stain smear positivity

Various Authors	Smear Positivity With Z.N. Staining	Smear Positivity With Ao Staining
Kumar N <i>et al.</i> [21]	33.5%	45.4%
A. Jain <i>et al.</i> [22]	22%	52%
Laifangbam <i>et al.</i> [23]	25%	71.6%
Annam V <i>et al.</i> [7]	44.1%	81.3%
Thakur B <i>et al.</i> [24]	26.67%	34.44%
Kalra SK <i>et al.</i> [9]	37%	73%
Lokeshwaran RS <i>et al.</i> [10]	44%	88%
Present Study	28%	50%

Table 5: Comparison of Sensitivity and specificity of Z.N. staining and fluorescent staining given by various authors

Various Authors	Z.N. Staining		Fluorescent Staining	
	Sensitivity	Specificity	Sensitivity	Specificity
Githui W <i>et al.</i> [25]	65%	96%	80%	97%
Hooja S <i>et al.</i> [26]	55.55%	99.19%	71.85%	99.19%
Thakur B <i>et al.</i> [24]	80.00%	93.85%	88.00%	86.15%
Abdissa K <i>et al.</i> [27]	25%	93.8%	45.8%	89.6%
Kalra SK <i>et al.</i> [9]	36.73%	50%	73.47%	50%
Present Study	33.3%	90.91%	61.54%	90.91%

Next in our study, we observed that 78.05% of cases demonstrated CB-NAAT positivity while 21.95% of cases were negative. Like previous techniques, cytologically presence of epithelioid cell granulomas and necrosis was most common pattern observed in this technique as well, while 2 cases presented with inflammatory infiltrate only

(acute inflammatory cells, lymphocytes) in comparison to previous techniques. On correlating both, we observed that CB-NAAT and cytological diagnosis were positive in 30/50 cases (60%) while both tests were negative in 9/50 cases (18%). CB-NAAT was negative in 9/50 cases (18%) which were positive on cytological diagnosis while cytological features of tuberculosis were negative for 2/50 cases (4%) in which CB-NAAT was positive.(Table 6)

Table 6: Sensitivity and specificity of CB-NAAT given by various authors

Various Authors	Sensitivity	Specificity
Singh KG <i>et al.</i> [28]	91%	90%
Ligthelm <i>et al.</i> [29]	96.7%	88.9%
Komanapalli SK <i>et al.</i> [30]	85.71%	96.8%
Present Study	76.92%	81.82%

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^[30].

Overall Z.N. positivity was seen in 28% while with CB-NAAT positivity was increased up to cases 64%. Both Z.N. and CB-NAAT were positive in 12/50 cases and negative in 16/50 cases. CB-NAAT positivity was seen in 20/50 cases in which Z.N. was negative. 2 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. Also, 2 cases of total CB-NAAT positivity, were found to be rifampicin resistant. Hence as CB-NAAT is helpful in patients with high risk of tuberculosis in whom AFB smear examination is usually negative, even patients with rifampicin resistance are most likely to be benefited from CB-NAAT.

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^[31]. Because of low bacillary load and its detection limit of 131cfu/ml might be the reason for CB-NAAT negativity in these patients^[32].

Komanapalli SK *et al.*^[30]. 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 50% while with CB-NAAT positivity was increased to 64%. Both fluorescent stain and CB-NAAT were positive in 24/50 cases and negative in 17/50 cases. CB-NAAT positivity was seen in 8/50 cases in which fluorescent stain was negative. 1 case was positive for fluorescent stain which was negative for CB-NAAT. Therefore, on comparison, CB-NAAT is more sensitive than fluorescent stain.

Lastly on bacterial culture, all cases of tubercular lymphadenitis (41 cases) were negative on bacterial culture. Out of 9 cases with bacterial etiology, only 5/50 cases were positive on bacterial culture. 3 out of 5 cases showed MRSA (Methicillin resistant *Staphylococcus aureus*) and 2 out of 5 cases showed *Streptococcus pyogenes* colonies on bacterial culture. No case of fungal lymphadenitis was seen in our study as all the cases were negative on PAS staining and fungal culture.

Conclusion

From our study we conclude that though cytomorphological appearance and Z.N. staining are commonly used in developing countries as they are economical and convenient alternative to open biopsy of lymph nodes but fluorescent method proves to be more sensitive technique than the Z.N. method, as the slides can be examined for much larger areas of the smear to be screened in a short period of time. Also, it greatly improves the diagnostic value especially in paucibacillary patients that are likely to be missed on Z.N. stained smears. Hence as CB-NAAT is helpful in patients with high risk of tuberculosis in whom AFB smear examination is usually negative, even patients with

refampicin resistance are most likely to be benefited from CB-NAAT.

Although culture is considered as a gold standard method but it is time consuming as it takes days to come positive. CB-NAAT proves to be more sensitive than both Z.N. and fluorescent staining for those patients which show cytomorphological appearance of TB. Patients with high risk of tuberculosis in whom AFB smear examination is usually negative and even rifampicin resistant cases are most likely to be benefited from CB-NAAT. But as every method as its own limitations, these methods does not replace histological examination and microscopy but should be used as an adjunct in diagnosis of suspected cases along with clinical history, haematological investigations and cytological features in lymph node aspirates specially in developing countries. In addition, an important feature highlighted in our study is that, for the differentials of suppurative lymphadenitis cytomorphological examination along with various other ancillary techniques (methods) has more diagnostic value.

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