Fine Needle Aspiration Cytology of Cervical Lymph Nodes with Special Emphasis on Different Cytomorphological Presentation of Tuberculous Infection

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ABSTRACT

Aim: Tuberculous lymphadenitis is the most common form of extrapulmonary tuberculosis and constitutes approximately 20-40% of all extrapulmonary tuberculosis. The gold standard for the diagnosis of active tuberculosis is to perform a culture test. With the advance of molecular techniques, various polymerase chain reaction (PCR) methods have been introduced to identify Mycobacterium tuberculosis more easily and quickly. With this background scientific knowledge, the following study was undertaken with the general objective to determine the various causes of cervical lymphadenopathy by fine-needle aspiration cytology (FNAC) and to delineate the pathology of tuberculous lymphadenitis in patients of a government-run medical college of West Bengal by FNAC.

Methodology: Total 100 cases with clinical suspicion of tuberculous lymphadenitis were aspirated amongst which 14 cases has been excluded. The rest 86 cases, amongst which 43 cases were diagnosed as Chronic granulomatous lymphadenitis, 26 cases as tuberculous lymphadenitis and 17 cases as Suppurative lesion. In our study chronic granulomatous lymphadenitis and tuberculous lymphadenitis were regarded as FNAC suggestive of tuberculosis. The suppurative lesion was regarded as FNAC not suggestive of tuberculosis. Cytomorphological features associated with suppurative lesion didn't reliably exclude tuberculous lymphadenitis in our study.

Results: Out of 86 cases, 36% showed AFB positivity, and 69 cases of FNAC suspicious of tuberculous aetiology showed acid-fast bacilli (AFB) in 42% cases. Out of 86 cases, culture was positive in 69 cases (80.2%). TB Culture was positive in 12 (70.58%) cases of the suppurative lesion. The culture was found positive in all the AFB positive cases and out of 55 AFB negative smears, TB culture was positive in 38 (69%) cases.

Conclusion: So to conclude many of the cervical lymph nodes show suppurative lesion without definite granuloma, however they showed AFB positivity in Ziehl Neelsen stain (ZN smear)/ culture positivity. Therefore these cases should be investigated for acid-fast bacilli (AFB) for proper treatment.

Key Words: Tuberculous lymph node, Cytology

INTRODUCTION

Tuberculosis is a common infectious disease associated with high levels of morbidity and mortality especially in developing countries¹. It remains a key challenge to global public health and our ability to tackle this disease has been severely hampered by inadequate diagnostic assays². Lymphadenopathy is a very common clinical manifestation of many diseases including tuberculous lymphadenitis³. Tuberculous lymphadenitis is the most common form of extrapulmonary

tuberculosis and constitutes approximately 20-40% of all extrapulmonary tuberculosis⁴. Cytological criteria for the diagnosis of tuberculous lymphadenitis are defined as epithelioid cell granulomas with or without multinucleated giant cells and caseation necrosis⁵. The diagnosis is confirmed by the presence of acid-fast bacilli (AFB) in the cytological smears and by isolation of Mycobacterium tuberculosis on culture⁵. Detection of AFB by conventional microscopy is simple and rapid but lacks adequate sensitivity⁶, whereas culture is comparatively more sensitive and specific but results become

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available after several weeks^{5,6}. The gold standard for the diagnosis of active tuberculosis is to perform culture test⁶. Conventional culture techniques can take 3-8 weeks on solid media, 1-2 weeks in broth media^{5,6}. With the advance of molecular techniques, various polymerase chain reaction (PCR) methods have been introduced to identify *M. tuberculosis* more easily and quickly⁷. Due to shortcomings of conventional diagnostic tests, cartridge-based nucleic acid amplification test (CBNAAT) have emerged to enable clinicians for early recognition of *M. tuberculosis* from a variety of extra-pulmonary clinical samples, with very good positive predictive value (PPV) (around 99%) and comparatively lesser negative predictive value^{8,9}. Extra-pulmonary tuberculosis is far more complex because of the diversity of clinical sample types, difficulties in obtaining adequate tissue for analysis and in the extraction of *M. tuberculosis* DNA (MTB DNA) from the samples¹⁰. With the improvement of Nucleic Acid Amplification techniques (NAAT) in TB detection, the sensitivity of tests for TB detection has been rising¹⁰.

With this background scientific knowledge, the following study was undertaken with the following general objective:

- i. To determine the various causes of cervical lymphadenopathy by FNAC.
- ii. To determine the different cytological presentations of tuberculous lymphadenitis in patients of a government-run medical college of West Bengal.

MATERIALS AND METHODS

Study design: It is an institution based, cross-sectional observational study. All patients presenting with cervical lymphadenopathy were examined and those who gave consent for participating in the study were included in the study. A detailed history and clinical examination were done. FNAC from the enlarged cervical node was done and cytomorphology studied.

Study setting and timelines: The study was conducted in the Department of Pathology, of one state-run medical college of West Bengal.

The study was conducted as per the following schedule- Preparatory phase-2 months, Data collection period-12 months, Tabulation, analysis of data and report writing-4months

Study population: All patients attending the department of pathology with cervical lymphadenopathy who fulfilled the predetermined(mentioned below) inclusion and exclusion criteria.

Sample size: 100 cases fulfilling the predetermined(below mentioned) inclusion and exclusion criteria will be taken during the study period.

Inclusion criteria: 1) Patients of all age groups and both genders with well palpable and enlarged cervical lymph nodes are included. 2) All those who gave consent for the study are included in this study.

Exclusion criteria: 1) Negative consent for inclusion in the study. 2) Very small or non-palpable lymph nodes. 3) Known cases of tubercular lymphadenitis undergoing treatment.

Study variables: i) Case record form ii) Informed consent form iii) Clinical history: includes patient particulars, contact number, chief complaints, history of fever, weight loss, cough or other symptoms, duration of neck node enlargement, contact history of tuberculosis or another infectious disease, history of tuberculosis, vaccination status, whether is on anti-tubercular treatment, history of any immunodeficiency diseases, any associated co-morbidities, drug history iv) local examination of a cervical lymph node: level, number, size, discrete or matted, consistency, tenderness, temperature, mobility, surface over the cervical lymph node. v) systemic examination vi) laboratory investigation report vii) Tools and stains for cytomorphological examination by FNAC i.e, Leishman-Giemsa stain and Papanicolaou (PAP) stain, ZN (Ziehl-Neelsen) stain.

Laboratory Investigation: Detailed history and clinical examination, Mantoux test, routine blood investigations, serology, Culture, Fine Needle Aspiration Cytology,

RESULTS

Total 100 cases with clinical suspicion of tuberculous lymphadenitis were aspirated amongst which 14 cases has been excluded. Among those 14 cases, 3 cases were metastatic deposits, 1 case of lymphoma and 10 cases of reactive lymphadenitis based on FNAC findings. The rest 86 cases, amongst which 43 cases were diagnosed as Chronic Granulomatous Lymphadenitis, 26 cases as Tuberculous lymphadenitis and 17 cases as Suppurative lesion. In our study Chronic Granulomatous lymphadenitis and tuberculous lymphadenitis was regarded as FNAC suggestive of Tuberculosis. Suppurative lesion regarded as FNAC not suggestive of Tuberculosis. Cytomorphological features associated with suppurative lesion didn't reliably exclude tuberculous lymphadenitis in our study. Out of 86 cases, 31 cases (36%) showed AFB positivity by Ziehl Nelsen stain, and 69 cases of FNAC suspicious of tuberculous aetiology showed AFB in 29 (42%) cases. AFB was found in 2 cases (11.76%) of the suppurative lesion. Out of 86 cases, Culture was positive in 69 cases (80.2%). TB Culture was positive in 12 (70.58%) cases of the suppurative lesion. The culture was found positive in all the AFB positive cases and out of 55 AFB negative smears, TB culture was positive in 38 (69%) cases.

Table 1: Distribution of findings of FNAC

Findings of FNAC	Number	%
Chronic Granulomatous lymphadenitis	43	43.0%
Tuberculous lymphadenitis	26	26.0%
Suppurative lesion	17	17.0%
Reactive lymphadenitis*	10	10.0%
Metastatic deposit*	3	3.0%
Lymphoma*	1	1.0%
Total	100	100.0%

*These 14 are cases not related to tuberculosis. The following tables are based on 86 cases related to tuberculosis

Most of the patients (43.0%) were having Chronic Granulomatous lymphadenitis (43.0%) which was significantly higher ($Z=2.58; p<0.01$).

Figure 1: Showing distribution of various lymph node lesions.**Table 2: Distribution of age of the patients**

Age Group (in years)	Number	%
<10	4	4.7%
10 - 19	29	33.7%
20 - 29	23	26.7%
30 - 39	14	16.3%
40 - 49	8	9.3%
≥50	8	9.3%
Total	86	100.0%
Mean ± S.D	27.36±14.67	
Median	25	
Range	2 - 65	

The mean age (mean ± standard deviation) of the patients was 27.36±14.67 years with range 2 - 65 years and the median age was 25 years. Test of proportion showed that the proportion of the patients with age between 10 - 29 years (60.4%) were significantly higher than another age group ($Z= 6.41; p<0.01$). Only 4.7% of the patients were with age<10 years. Thus cervical tubercular lymphadenopathy was most prevalent in the age group between 10 – 29 years.

Figure 2: Showing distribution of age of patients(n=86).**Table 3: Distribution of gender of the patients**

Gender	Number	%
Male	29	33.7%
Female	57	66.3%
Total	86	100.0%
Male:Female	1.0:1.9	

The ratio of male and female (Male: Female) was 1.0:1.9. The proportion of males (33.7%) was significantly lower than that of females (66.3%) ($Z= 4.61; p<0.01$). Thus females were at higher risk of having tubercular cervical lymphadenopathy than males.

Figure 3: Showing distribution of gender of patients (n=86).**Table 4: Distribution of size of Lymph Node**

Size of lymph node (in cm)	Number	%
1.0 - 1.5	7	8.1%
2.0 - 2.5	29	33.7%
3.0 - 3.5	24	27.9%
4.0 - 5.0	25	29.1%
>5.0	1	1.2%
Total	86	100.0%
Mean±sd	3.02±1.11	
Median	3	
Range	1 - 6	

The mean size of lymph node size (mean± s.d.) of the patients was 3.02±1.11 cm with range 1- 6 cm and the median was 3 cm. 90.7% of the patients had lymph node size between 2.0 – 5.0 cm which was significantly higher ($Z=11.68$; $p<0.0001$).

Table 5: Distribution of the number of lymph nodes

Number of lymph nodes	Number	%
Single	46	53.5%
Multiple	40	46.5%
Total	86	100.0%

53.5% of the patients had a single lymph node which was higher than multiple lymph nodes (46.5%) but it was not significant ($Z=0.98$; $p=0.76$).

Table 6: Frequency of Involvement of Cervical Lymph Nodes in 86 Cases:

Level	Number	%
I	8	9.3%
I+II	3	3.5%
I+II+III	1	1.2%
I+II+IV+V	1	1.2%
I+IV	3	3.5%
I+V	4	4.7%
II	8	9.3%
II+III+IV	1	1.2%
II+III+IV+V	1	1.2%
II+IV+V	2	2.3%
II+V	12	14.0%
III	1	1.2%
III+IV+V	1	1.2%
IV	20	23.3%
IV+V	3	3.5%
V	17	19.8%
Total	86	100.0%

Most of the lymph nodes at level –IV (28.0%) which was significantly higher than that of other levels ($Z=2.06$; $p=0.042$).

Table 7: Distribution of laterality of lymph nodes

Laterality of lymph Nodes	Number	%
Left	46	53.5%
Right	40	46.5%
Total	86	100.0%

53.5% of the lymph nodes were left-sided which was higher than that of right-sided (46.5%) but it was not significant ($Z=0.98$; $p=0.76$).

Table 8: History of contact with any patients suffering from TB

History of contact	Number	%
Yes	50	58.1%
No	36	41.9%
Total	86	100.0%

58.1% of the patients were having a history of contact with any patients suffering from TB which was significantly higher than of no contact (41.9%) ($Z=2.29$; $p=0.026$).

Table 9: Fever with weight loss of the patients

Fever with weight loss	Number	%
Yes	63	73.3%
No	23	26.7%
Total	86	100.0%

73.3% of the patients were having fever with weight loss which was significantly higher ($Z=6.59$; $p<0.001$).

Table 10: Presented with a cough by the patients

Cough	Number	%
Yes	39	45.3%
No	47	54.7%
Total	86	100.0%

45.3% of the patients presented with a cough but it was not significant ($Z=1.48$; $p=0.29$).

Table 11: Duration of cervical lymphadenopathy

Duration (in months)	Number	%
<1	18	20.9%
1 - 6	64	74.4%
>6	4	4.7%
Total	86	100.0%
Mean±sd	2.31±5.71	
Median	2	
Range	0.23 - 12	

The mean duration (mean± s.d.) of the patients was 2.31±5.71 months with range 0.23 -12 months and the median was 2 months

In the case of 74.4% of the patients, duration was for 1 - 6 months which was significantly higher than other durations ($Z=7.57$, $p<0.01$). Only 4.7% of the patient had a duration greater than 6 months. Thus most of the patients reported to the hospital within 6 months from the date of initiation of cervical lymphadenopathy.

Table 12: Nature of aspirate

Type of Aspirate	Number	%
Caseous	40	46.5%
Purulent	27	31.4%
Blood mixed	15	17.5%
Blood mixed pus	2	2.3%
Serous	2	2.3%
Total	86	100.00%

40.0% of the aspirates were caseous aspirate which was significantly higher than other Aspirate ($Z=2.18$, $p=0.037$).

Table 13: Presence of AFB

AFB	Number	%
Found	31	36.0%
Not found	55	64.0%
Total	86	100.0%

AFB was found in 36.0% of the patients. Thus the prevalence of AFB was 36.0%. But in most of the cases (64.0%) AFB was not found which was significantly higher ($Z=3.95$; $p<0.001$).

Table 14: Frequency of Differentcytomorphologies (n=86)

	Number
Granuloma with necrosis	37 (43.02%)
Granuloma without necrosis	26 (30.23%)
Necrosis without granuloma	13 (15.11%)
Polymorphs with necrosis	10 (11.62%)
Total	86 (100%)

43% of the cases were having the cytomorphology of Granuloma with caseous necrosis which was slightly higher than other cytomorphology

DISCUSSION

Tuberculous lymphadenopathy is most commonly found in young adults but can be seen in any age group¹¹. In the current study, we found that the age group of 10-29 years (60.4%) were more commonly affected than other age groups. According to Mittal et al.¹², most of the patients (36%) were in the third decade of life. Similar age distributions were found in studies by Natraj et al.¹³, Target and Bekele¹⁴, Purohit et al.¹⁵ and Dandapat et al.¹⁶

In our study, ratio of male and female (M: F) was 1:1.9 means, females (66%) were more commonly affected than males (40%). Female predilection was seen in many other

studies including a study by Nataraj et al.¹³ with a male: female ratio of 1:1.3. A slight female predominance with 1:1.2 sex ratios was seen in a study conducted by Nidhi et al.¹⁷. Similarly, female predominance was noted by Pamra et al.¹⁸, Mittal et al.¹², Ergete and Bekele¹⁴ and Purohit et al.¹⁵ while male predominance was noted by Rajsekaran et al.¹⁹ and Bezabih et al.²⁰.

In the current study, we found single lymph nodal involvement (53.5%) was slightly higher than multiple lymph nodal involvement (46.5%). Paliwal et al.²¹ and Chand²² also reported a single palpable lymph node as the most common presentation. Sharma et al.²³ observed a similar pattern of findings among a study conducted among paediatrics age groups.

In the current study, we found that the history of fever with weight loss (73.3%) was higher than cough (45.3%). However, Avashia et al.²⁴ found that most common symptoms were cough (72.2%) followed by fever (69.4%). In a similar study from France, Le Palud et al.²⁵ in 2014 found cough (51.9%) as the main symptom followed by general symptoms (45.1%).

FNAC is the most important diagnostic tool to aid in the diagnosis of lymph node lesions. It is inexpensive, safe and quick and reduces the need for surgical biopsy²⁶.

Most of the patients in our study were having chronic granulomatous lymphadenitis (43%) on FNAC which was significantly higher. This finding is corroborating with a study by More et al.²⁷ where Granulomatous lymphadenitis was diagnosed in 48.88 % of cases whereas in a study done by Anuradha S et al.²⁸ it was found that 22% nodes showed granulomatous lymphadenitis.

Variable AFB positivity by ZN smear technique has been observed by different authors, ranging from as low as 0%²⁹ to 77.8%³⁰. In our study AFB was found in only 36% of the patients. AFB positivity was 47% in a study by Pahwa et al.³¹, 42% by Mirza et al.³² which is comparable with our study findings. AFB positivity was observed in 71.7% of cases by Ergete and Bekele¹⁴, 59.4% cases by Bezabih et al.²⁰, 45.6% cases by Dasgupta et al.¹³ and 19.6% cases by Aggarwal et al.³⁴. In our study out of 86 cases, 31 cases (36%) showed AFB positivity by Ziehl Nelsen stain, and 69 cases of FNAC suspicious of tuberculous aetiology showed AFB in 29 (42%) cases. AFB was found in 2 cases (11.76%) of the suppurative lesion.

The most common cytomorphological pattern in our study was epithelioid granulomas with caseous necrosis in 43% cases. Gupta et al.³⁵ also reported epithelioid granulomas with caseous necrosis as the most common cytomorphological pattern (52–55%)³⁵. Singh S et al.³⁶ in their study found that epithelioid granuloma with necrosis was a most common pattern forming 52.4%, epithelioid granuloma without necrosis was the next common cytomorphological pattern,

forming 40.5%. A similar study was done in the past. Malhotra et al.³⁷ also had similar observations. According to their study, epithelioid granuloma with necrosis had the highest incidence with 47 cases (45.63%), followed by epithelioid granuloma without necrosis with 29 cases (28.16%). However, Paliwal et al. reported necrosis only without epithelioid granuloma the most common cytomorphological pattern in 39.2% patients²¹. In the current study, the highest AFB positivity of 56.75% was found in epithelioid granuloma with necrosis. Mitra et al.³⁸ found that the most common cytomorphological pattern in their study was epithelioid granulomas with caseous necrosis in 40% cases. Gupta et al. also reported epithelioid granulomas with caseous necrosis as the most common cytomorphological pattern (52–55%)³⁵. Bezabih et al.²⁰ found the highest AFB positivity in cases showing necrosis only without epithelioid granulomas (69.7%) and the lowest in cases showing epithelioid granulomas without necrosis (20.0%). Mittal et al.¹² found that highest AFB positivity of 88.2% was seen in smears showing both granulomas and necrosis, followed by smears having necrosis only (76.9%). However, highest AFB positivity was seen in smears revealing necrosis only without epithelioid granulomas (85.5%) and polymorphs with necrosis with or without epithelioid granulomas (79.2%) while the lowest was seen in smears showing epithelioid granulomas without necrosis (3.2%). Gupta et al. reported overall AFB positivity of 65% with maximum positivity (75%) in necrosis with polymorphs and with or without epithelioid granulomas³⁵. Aggarwal et al. reported AFB positivity in 19.6% among 138 cases. However, studies by various authors reported maximum AFB positivity in necrosis only without epithelioid granulomas³⁴.

CONCLUSION

From this study, we conclude the following results:

1. In this study, the age group of 10–29 years was most commonly affected by tubercular cervical lymphadenopathy with a slight female predominance.
2. Many of the cervical lymph nodes showing suppurative lesion without definite granuloma, however, they showed AFB positivity in ZN smear/ culture positivity. Therefore these cases should be investigated for AFB for proper treatment.
3. Most commonly observed cytomorphology is granuloma with necrosis (43%). Highest AFB positivity of 56.75% is found in granuloma with necrosis.
4. Out of 69 cases of FNAC suggestive of tuberculosis, AFB was found in 29 (42.02%) cases and out of 17 cases of FNAC not suggestive of tuberculosis, AFB was found in 2 (11.76%) cases.
5. Out of 86 cases, Culture was positive in 69 cases (80.2%). TB Culture was positive in 12 (70.58%) cases of the suppurative lesion. The culture was found

positive in all the AFB positive cases and out of 55 AFB negative smears, TB culture was positive in 38 (69%) cases.

6. 53.5% of the patients had a single lymph node which was higher than multiple lymph nodes (46.5%).
7. 40.0% of the FNAC aspirates were caseous.

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