



## CYTOLOGICAL STUDY AND ZN STAINING OF ASPIRATES FROM PRESUMPTIVE TUBERCULAR LYMPHADENOPATHY IN A TERTIARY CENTRE

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### ABSTRACT

**Background:** The most common manifestation of extrapulmonary tuberculosis (EPTB) is peripheral lymphadenopathy [1,2]. Fine needle aspiration cytology (FNAC) being patient friendly, cost effective and conclusive method for diagnosis of EPTB, is therefore commonly used [3]. Ziehl-Neelsen (ZN) staining is used to look for acid fast bacilli (AFB) in the aspirates from lymph nodes.

**Methods And Material:** In the Department of pathology, RIMS, Ranchi, a prospective study was done from August 2019 to April 2020. Smears prepared from fine needle aspiration of suspected TBLN of 229 patients was used for cytomorphological and bacteriological study.

**Results:** In our study, we found that cervical lymph nodes were the most common lymph nodes involved. Cytomorphologically, 141 out of 229 patients i.e. 61.57% were diagnosed with TBLN. Among these 141 patients, 62 patients (43.97%) were positive for AFB on ZN staining. Most common cytomorphological finding was necrosis without epithelioid cell granuloma with highest AFB positivity.

**Conclusion:** FNAC along with ZN staining should be the 1st line of investigation for the diagnosis of tubercular lymphadenopathy.

**KEYWORDS :** TBLN, FNAC and ZN staining

### INTRODUCTION :

Lymph nodes are the 2<sup>nd</sup> most common site of infection by mycobacterium tuberculosis after lungs. According to the WHO TB Statistics for India for 2015, the incidence of TB in India was 2.2 million out of 9.6 million globally [4]. As most of the available techniques are low either in terms of specificity or sensitivity as compared to culture, definite diagnosis of tuberculous lymphadenitis is quite difficult. Even the clinical features, although indicative of tuberculous etiology, are not sufficient enough to make a definitive diagnosis [5]. For the diagnosis of pulmonary TB in developing countries, direct smear microscopy plays an very important role as it is rapid, specific, inexpensive and sensitive. But in case of EPTB, its sensitivity is limited to 20 to 43% [6,7,8].

TB Lymphadenitis being the most common form of extra pulmonary TB, and usually involving superficial lymph nodes, makes FNAC an important diagnostic test for EPTB [9]. FNAC is an outpatient procedure which is cheap, rapid, and patient friendly. Cytomorphological features along with ZN staining for AFB are used to establish a diagnosis [10].

**AIM:** To study the relative frequency of different cytomorphological findings of presumptive tubercular lymphadenopathies (TBLN) and assess the association between FNAC and (ZN) staining as diagnostic tools for TBLN.

### MATERIAL AND METHODS:

This was a prospective study undertaken in Department of Pathology, RIMS, Ranchi from August 2019 to April 2020. All patients suspected clinically of having tuberculosis with well palpable peripheral lymph nodes were included in the study. A detailed history of the patients along with clinical examination was done. Duration and site of the lymphadenopathy, its consistency, size, tenderness, mobile or fixed, temperature were some of the important points that were sought for. Patients were asked if the swelling is associated with symptoms like evening rise of temperature, night sweats, malaise, loss of weight etc. Laboratory investigations were thoroughly studied and checked for any

abnormalities. Informed consent of the patients was taken after explaining the procedure of FNAC.

Under available aseptic precaution, aspiration was done by inserting 22 G needle attached with 10 ml disposable plastic syringe into the lymph node. After developing a negative pressure in the syringe, it was inserted in the lymph node. The needle was passed in and out multiple times, without exiting the node. The aspiration was repeated, if found insufficient. The gross appearance of the aspirate was described, as cheese like caseous, purulent / non caseous or admixed with blood. The aspirates were used to make 5 to 6 smears, one for Leishman & Geimsa (L&G) stain which was air dried, one for Hematoxylin & Eosin (H&E) stain which was fixed immediately using alcohol. The smears prepared for ZN stains were sent to Department of Microbiology, RIMS, Ranchi.

Cytological diagnosis of tuberculosis was applied to those with granulomatous lymphadenitis with or without caseous necrosis and polymorphs with necrosis, and / or bacilli positive cases on ZN stain.

H & E and L & G stained smears were examined under microscope and diagnosed as either of the following 5 groups:

1. Granulomatous lymphadenitis
2. Necrotising lymphadenitis
3. Necrotising granulomatous lymphadenitis
4. Reactive lymphadenitis
5. Others which included metastatic carcinoma, lymphomas, epidermal cysts etc.

Granulomatous, necrotising, both necrotising and granulomatous or polymorphs with necrosis are considered diagnostic of TBLN [11].

ZN stained slides were labelled positive for acid fast bacilli (AFB), if pink, beaded and rod shaped bacilli were present under oil immersion.

### RESULTS:

A total of 229 patients were recruited in our study .There

were 107 male and 122 female patients .

This highlights that the cervical lymph nodes were the most common site of lymph node involvement and inguinal lymph node being the least involved.

**Table 1: Correlation Of Cytological Diagnosis And AFB Positivity :**

Cytological Diagnosis	Total no. of cases	Total no. of ZN positive cases	Percentage of ZN positive cases among each group of cytological diagnosis
1.Granulomatous lymphadenitis	42 (18.34%)	12	28.57%
2.Necrotising lymphadenitis	67 (29.26%)	34	50.75%
3.Necrotising granulomatous lymphadenitis	32 (13.97%)	16	50%
4.Reactive lymphadenitis	37 (16.16%)	3	8.10%
5.Others which included metastatic carcinoma, lymphomas, epidermal cysts, abscess etc.	51 (22.27%)	4	7.84%
<b>Total</b>	<b>229</b>	<b>69</b>	

As already mentioned, granulomatous, necrotising, both necrotising and granulomatous or polymorphs with necrosis are considered diagnostic of TBLN [11]. So, from the above table, we can conclude that cytomorphologically, 141 out of 229 patients i.e. 61.57% were diagnosed with TBLN. Among these 141 patients, 62 patients (43.97%) were positive for

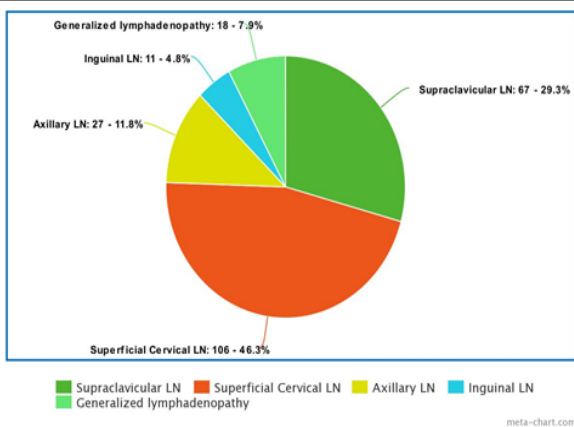
Gross appearance of the aspirate was cheesy caseous material in 88 cases (38.86%) followed by 73 cases with purulent aspirate while the blood mixed aspirates were of 68 cases.

AFB on ZN staining. Overall AFB positive cases was 30.13 %.

Most common cytomorphological finding was necrosis without epithelioid cell granuloma with highest AFB positivity.

**Table 2: Relative Frequency Of Cases Diagnosed Under 5<sup>th</sup> Group :**

Lymph node lesions diagnosed under group 5 (others)	No. of cases	AFB Positive Cases
Metastatic deposits	16	2
Abscess	15	1
Lymphomas	5	1
Infected epidermal cysts	3	0
Acute infection	8	0
Round cell tumor	1	0
Lymphangioma	1	0
Thyroglossal cyst	1	0
Submandibular sialadenitis	1	0
<b>TOTAL</b>	<b>51</b>	<b>4</b>



**Fig 1 : Distribution Of Site Of Lymph Nodes Involved**

**DISCUSSION:**

Lymphadenopathy is a common day to day finding in clinical practice. Most common lymph nodes involved are superficial cervical, supraclavicular, axillary and inguinal. The etiology for the lymphadenopathies varies from reactive to tubercular lymphadenopathy and metastatic lesions. Granulomatous lesion with or without necrosis and polymorphs with necrosis are diagnosed as TBLN.

FNA cytology and ZN staining are the conventional diagnostic tools for TBLN. FNAC has limited specificity as the cytomorphological picture of TBLN which shows necrosis, epithelioid cells forming granuloma and multinucleated giant cells are also present in sarcoidosis, fungal infection, and inflammatory conditions [12,13].

Moreover, highly trained pathologists are required to make a reliable diagnosis. But being a simple, rapid and patient friendly method, FNAC is a commonly used for diagnosis of TBLN. Moreover, it is an alternative method to excision biopsy for lymph nodes and materials for cytological and bacteriological examination can be easily collected [14].

As already discussed, superficial cervical lymph nodes was the most common lymph node involved in our study. Similar were the results of studies done by Kusum Verma et al and Rajshekaran et al [15,16].

According to the study of Bedi et al and Ahmed et al, lymph nodes of smaller size have reduced chance of being tubercular and added that lymph nodes of <1cm were mostly reactive [17]. Even the present study follows the same trend.

The presence of AFB on ZN staining is diagnostic of tuberculosis. But, in our study, we found that the overall ZN positive cases were low. The reasons may be the immune status of different patients, clinical stages of the disease, nature of aspirate, and the requirement of a minimum of 5 to 10000 bacilli/ml of the aspirate are required for a positive outcome on ZN staining [18]. To overcome the issues related with scanty bacilli count, studies suggest that the sensitivity of the direct smear microscopy can be improved by first decontaminating and digesting the FNA specimen with 1% N-acetyl-L cystiene (NALC), sodium hydroxide (NaOH)- 2.9% and sodium citrate solution. Thereafter it is concentrated by centrifugation before ZN stainin [19]. The higher density of bacilli per microscopic field and depletion of debris, provide a clear field for microscopy, increasing its sensitivity.

AFB positivity and epithelioid cell granulomas share an inverse relation, whereas its relation with necrotic material is direct one. The reason being that liquefaction of necrotic focus is associated with an enhanced proliferation of AFB [20].

In India, according to Singh et al, the basis of diagnosis of tuberculous lymphadenitis is the presence of epithelioid cell granulomas [21].

### CONCLUSION:

In the present study, tuberculous lymphadenitis was one of the most common types of lymphadenopathies. 61.57% of cases were diagnosed as TBLN on cytomorphological examination. Out of which 43.97% were AFB positive. Epithelioid cell granulomas were sufficient to establish the diagnosis of tuberculosis on FNAC. So, we can conclude that FNAC along with ZN staining should be the 1<sup>st</sup> line of investigation for the diagnosis of tubercular lymphadenopathy. As India is a developing country and these diagnostic tools are cost effective, they remarkably contribute in reducing the morbidity and mortality due to tuberculosis.

In case, we have patients in which there is strong clinical suspicion with equivocal results from above two diagnostic tools, ancillary investigations like culture, PCR, ELISA and other molecular techniques should be used.

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