Pathology

Tuberculous Lymphadenitis: Analysis of Cytomorphological Features with Utility of Genexpert MTB/RIF and Other Microbiological Test as an Adjunct to Cytology

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Abstract	Original Research Article

Objectives: This study was undertaken to categorize various cytomorphological patterns of tuberculous lymphadenitis and with utility of Xpert MTB/RIF as an adjunct to cytology. ***Materials and methods:*** Cases based on various cytomorphological features were categorized into 3 patterns as 1. Granulomatous lymphadenitis (GL), 2. Necrotizing granulomatous lymphadenitis (NGL) 3. Necrotizing lymphadenitis (NL). The utility of microbiological test on cytosmears and histopathological examination were assessed in these categories wherever available. ***Results:*** Overall cytology had 86% sensitivity, 94% specificity, 92% positive and negative predictive value for diagnosis of tuberculosis. All these categories were correlated with microbiological test and gene Xpert wherever available. NGL had higher positive predictive value indicating that this group when subjected to microbiological methods provide definite diagnosis of tuberculosis. GL had higher specificity; hence negative microbiological test should be subjected to histopathological examination to rule out other causes of granulomatous lymphadenitis. GeneXpert when combined with cytologically suspicious smears, has high sensitivity of 85% and the agreement with culture is moderate as indicated by the kappa value of 0.4%. ***Conclusion:*** A synchronous analysis of cytomorphological features and GeneXpert and other microbiological test increases the diagnostic yield of Tuberculosis. Excisional biopsy for diagnosis of tuberculosis should be recommended only for patients in whom Fine Needle Aspiration Cytology PCR is negative or there is discrepancy with the clinical impression.
Keywords: FNAC, lymph node, tuberculosis, Gene Xpert, Extrapulmonary.

INTRODUCTION

Tuberculosis (TB) is a communicable disease causing major ill health (Christof *et al.,* 2020). India accounts for more than one-fourth of the world's Tuberculosis (TB) cases (Jain *et al.,* 2020). The WHO has recognized TB as a global problem, this applies to both pulmonary and extrapulmonary TB(EPTB) (Kulchavenya, 2014). The term extrapulmonary TB has been used to describe isolated occurrence of tuberculosis at body sites other than the lung (M U *et al.,* 2020). Diagnosis of EPTB is difficult due to clinical presentation with vague symptoms and signs (M U *et al.*, 2020). Tuberculous lymphadenitis(TBLN) is the most common form of extrapulmonary with higher incidence of 30 – 52% (Singh *et al.,* 2000) (Mitra *et al.,* 2017; Handa *et al.,* 2012). Paucibacillary character of EPTB and wide differential diagnosis poses a big challenge in the diagnosis of EPTB (M U *et al.,* 2020).

Fine needle aspirate cytology has been proved to be very useful, safe, cost effective, highly sensitive, and first line investigation has assumed an important role in the evaluation of EPTB especially for tuberculous lymphadenitis as a possible non-invasive alternative to excisional biopsy. The cytological criteria for diagnosis of tuberculous lymphadenitis have been clearly defined as being epithelioid cell granulomas with or without multinucleate giant cells and caseation necrosis (Singh *et al.,* 2000) Based on that they can be broadly classified as: Granulomatous lymphadenitis (GL), Necrotizing granulomatous lymphadenitis (NGL) and Necrotizing lymphadenitis (NL) (Vashisht *et al.,* 2019).

The conventional methods to diagnose EPTB includes fine needle aspiration cytology (FNAC) and demonstration of acid fast bacilli (AFB) using Zeihl-Neelson (ZN) staining and culture (M U *et al.,* 2020). However, Positive smear requires more than 5,000 to

10,000 bacilli/ml and hence it has limited diagnostic value in majority of EPTB samples (M U *et al.,* 2020) and has poor sensitivity (Mitra *et al.*, 2017). AFB cultures were positive in 35% to 65% of patients. However, culture takes six to eight weeks and causes an inordinate delay in the initiation of treatment (Singh *et al.,* 2000). More recently, Cartridge Based-Nucleic Acid Amplification Test (CBNAAT)/ Gene Xpert is a real time polymerase chain reaction system of molecular technology that can simultaneously detect rifampicin resistance from clinical specimens in less than 2 h (Biadglegne *et al.,* 2014). Gene Xpert endorsed by World Health Organization (WHO) as the most sensitive rapid test for TB diagnosis in paucibacillary samples. Many studies reported successful use of the Xpert MTB/RIF test on extrapulmonary samples, with overall sensitivities of over 80% and specificity reaching 100% (Kulchavenya, 2014) .

MATERIALS AND METHODS

This study was conducted in the Department of pathology at a multispecialty hospital in Mumbai. The population in this study comes under the urban middle-class population. Tuberculosis being a socioeconomically dependent disease, observations of this study vary from those of general population. Institutional ethical clearance was obtained before the start of this study. Retrospective analysis of clinically suspected tuberculous lymphadenitis cases that underwent FNAC over the period of 2 years (Jan 2019-Dec 2020) was performed. 100 cases were suggestive of tuberculosis on cytology were studied. All FNAC smears diagnosed other than TBLN, TBLN without microbiological correlation and inadequate material were excluded. Cases suggestive of tuberculosis on cytology were categorized into 3 patterns based on of various cytomorphological features like epithelioid cells, giant cells, granuloma, neutrophils, necrosis, and macrophages as follows:
1. Granulomatous lymphadenitis (GL-Group 1)
2. Necrotizing granulomatous lymphadenitis (NGL-Group 2)
3. Necrotizing lymphadenitis (NL- Group 3).

The results of bacillary detection methods and histopathological examination wherever available were analyzed and correlated in each of these categories. The sensitivity, specificity, positive and negative predictive value of cytology for diagnosis of tuberculosis were determined for each category. Agreement between cytomorphological categories and bacillary detection methods was determined. The utility of Gene Xpert in each cytomorphological category was studied in comparison with gold standard culture test wherever available. For statistical analysis, Chi-Square test used for calculation of accuracy, sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) of FNAC. Agreement between cytomorphological categories and Gene Xpert was determined using Cohen's kappa coefficient.

RESULTS

In this study, 252 clinically suspected tuberculosis lymphadenopathy cases were considered. 100/252 (39.68%) cases of lymphadenopathy had cytological findings suggestive of TBLN. The age of the patients ranged from 10 years to 78 years with the mean age of 39.78. Most common age group affected was between 21-30 years, 3rd decade. There were 46 males and 54 females. Male: Female ratio-1.78. All the patients presented with the chief complaint of swelling in different regions of the body. The other complaints were fever, cough, anorexia, and weight loss. Maximum number of patients presented with lymphadenopathy in the head and neck region. 63 cases (63%) in cervical lymph nodes followed by 22 cases (22%) in supraclavicular lymph nodes followed by 10% axillary lymph nodes and 5% were other sites involved. We had majority of the cases aspirated under ultrasonography guidance 86% and the aspirates were mostly cheesy (45%), blood mixed (30%) and pus (25%).

Out of 100 cytologically suggested TB, 86 cases (86%) were confirmed as TB either by or combining the 3 modalities, i.e., ZN stain, culture, and PCR. Out of these 86 cases, 84.4% were positive for tuberculosis through CBNNAT, 34% were positive by ZN staining and 74.1% were positive by culture method. Remaining 14 Cases,7 cases histopathology proved other diseases, 4 lost to follow up and 3 started on empirical AKT. Distribution of microbiological tests amongst cytologically suggested tuberculosis and non-tuberculosis were described in table 1.

Table 1: Distribution of microbiological tests amongst cytologically suggested tuberculosis and non-tuberculosis

Microbiology test	Cytologically -tuberculosis	Cytologically -non tuberculosis
AFB smear + Xpert	11	0
Culture +Xpert	23	0
Xpert alone	31	8
AFB smear alone	6	0
Culture alone	4	6
Total	86	14

Considering total 252 cases, overall cytology had 86% sensitivity, 91% specificity, 86% positive and 91% negative predictive value for diagnosis of tuberculosis (Table 2 and 3). These values indicate that FNAC is a useful test for diagnosis of TB lymphadenitis.

Table 2: Predictive validity of FNAC microbiologically proven tuberculosis(n=252)

	Microbiologically positive tuberculosis (n=100)	Microbiologically negative tuberculosis (n=152)
Cytologically positive tuberculosis (n=100)	86	14
Cytologically negative tuberculosis (n=152)	14	138

Table 3: Predictive validity of FNAC microbiologically proven tuberculosis(n=252)

Statistic	Value (%)
Sensitivity	86.00
Specificity	90.79
Positive Predictive value	86.00
Negative Predictive Value	90.79

The cytosmears were assessed for various cytomorphological features and categorized into 3 groups (Figure 1):
1. Granulomatous lymphadenitis (GL-Group 1)-20 cases
2. Necrotizing granulomatous lymphadenitis (NGL-Group 2) -54cases
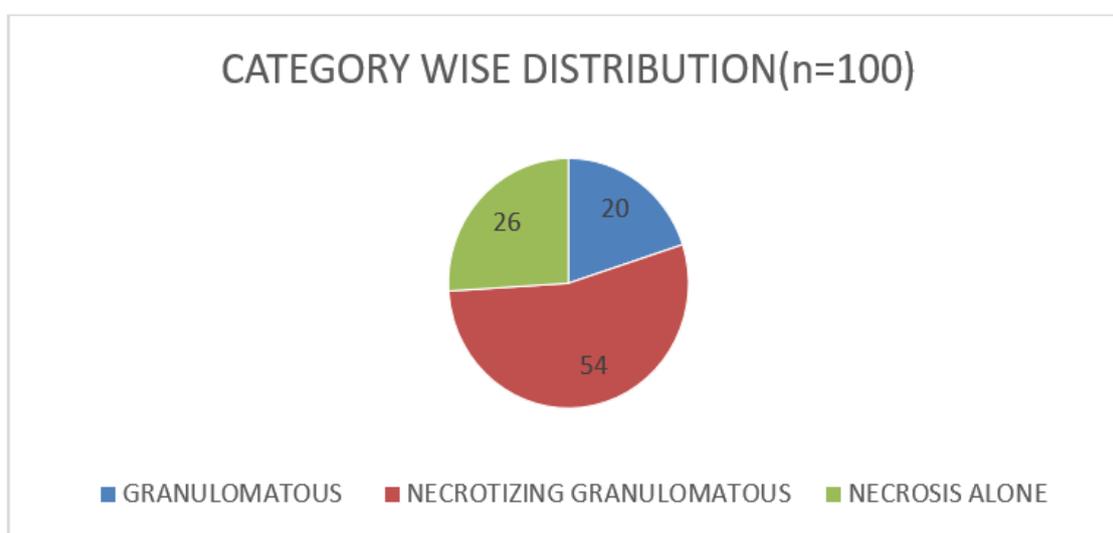3. Necrotizing lymphadenitis (NL- Group 3) – 26cases

Figure 1: Category wise distribution(n=100)

Group 1: Out of 20 cases, 11 cases were microbiologically proven tuberculosis, 5 were histopathologically proven non-TB along with 1 lost to follow up. This group when subjected to microbiological test has specificity of 94%, sensitivity of 11%, positive predictive value of 55%and negative predictive value of 62%.

Group 2: Out of 54 cases, 50 cases were microbiologically proven tuberculosis, 1 was histopathologically proven Non-TB and 3 were started on empirical AKT. This group when subjected to microbiological test has specificity of 97%, sensitivity of 50%, positive predictive value of 93%and negative predictive value of75 %.

Group 3: Out of 26 cases, 25 cases were microbiologically proven tuberculosis, 1 was histopathologically proven non-TB and 3 lost to follow up. This group when subjected to microbiological test has specificity of 99%, sensitivity of 25%, positive predictive value of 96%and negative predictive value of 66 % (table 5).

Cases were proven non-tuberculous had a greater number of granulomatous pattern (Group1). Group 2 when subjected to microbiological methods provide definite diagnosis of tuberculosis and likely to be negative in the absence of the disease (Figure 2).
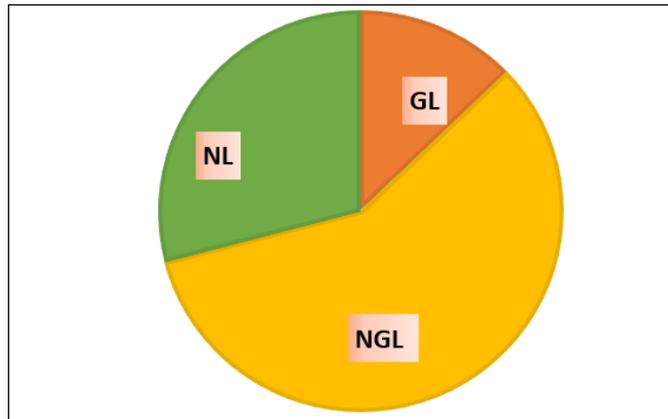
Figure 2: Category wise distribution of true positives. Cases that were confirmed by microbiological methods also showed necrosis with granuloma as predominant pattern. Percentage of granulomatous lymphadenitis was lower

Finally, the results of cytomorphology with each microbiological method were compared. ZN staining for AFB was positive in 34% cases. Utility of Gene Xpert as adjunct to cytology was calculated using kappa co-efficient test considering culture as gold standard. Gene X pert when combined with cytologically suspicious smears, has high sensitivity and the agreement with culture is moderate as indicated by the kappa value (Figure 3). However, when Gene X pert is specifically combined with smears showing necrosis with or without granuloma, the sensitivity and positive predictive value of the test increases significantly also the agreement with culture increases as indicated by increase in the kappa value (Figure 4).
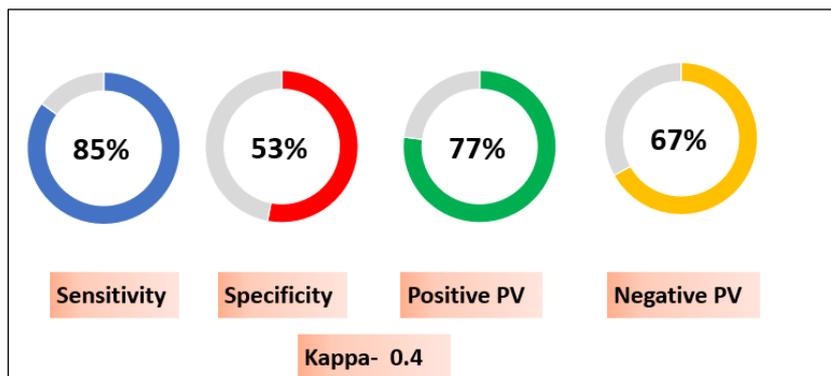

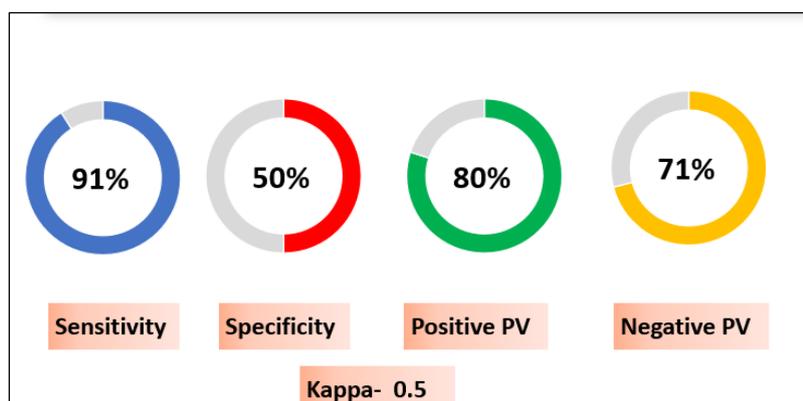**Figure 3: Utility of GeneXpert when combined with cytologically suspicious smears**

Figure 4: Utility of GeneXpert when combined with cytologically suspicious smears

DISCUSSION

TB remained the most common cause of death from a single infectious pathogen (Nur *et al.,* 2019). In developing countries tuberculosis is a major cause of morbidity and mortality (Nur *et al.,* 2019). Therefore early diagnosis and treatment without much delay decreases the infectivity of exposure (Singh *et al.,* 2000). Fine needle aspiration cytology (FNAC) plays a vital role in solving these issues, nowadays being recognized as a rapid diagnostic technique because of its simplicity, cost effective, early availability of results, accuracy, minimal invasion and can replace excision biopsy (Mitra *et al.*,

2017). In addition, when FNAC aspirate when subjected to CBNNAT can have better diagnostic yield with further no delay in the initiation of treatment.

In our study, common age group affected was between 3rd decade with slight preponderance to female (Male: Female ratio-1.78). Similar pattern of age and sex distribution was reported by Mitra *et al.,* (2017); Vashisht *et al.,* (2019) Vimal *et al.,* (2016). Cervical region (66%) being the commonest site which is similar to that of studies done by Masilamani *et al.,* (2015) Nitika *et al.,* (2019) Vimal *et al.,* (2016) Most of the aspirates were guided and yielded cheesy material.

In this study, 252 clinically suspected tuberculosis lymphadenopathy cases were considered. 100/252 (39.68%) cases of lymphadenopathy had cytological findings suggestive of TBLN. Masilamani *et al.,* (2015) and Mitra *et al.,* reported 38% and 45% incidences, respectively, in their study. Out of 100 cytologically suggested TB, 86 cases (86%) were confirmed as TB either by or combining the 3 modalities, i.e., ZN stain, culture, and PCR. Considering total 252 cases, overall cytology had 86% sensitivity, 91% specificity, 86% positive and 91% negative predictive value for diagnosis of tuberculosis. These values indicate that FNAC is a useful test for diagnosis of TB lymphadenitis. Apart from cytologically diagnosed 100 cases, on strong clinical grounds of TB suspicion in 252 total cases, 22 lymph nodes were subjected to re-aspirated under guidance and few were excised. Out of those 22, 14 cases were proven to be tuberculosis by microbiological methods.

In present study, we classified cytology smears into 3 patterns based on cytomorphological features such as epithelioid cells, giant cells, granuloma, necrosis, PMN and macrophages. Granulomatous lymphadenitis (GL-Group 1), Necrotizing granulomatous lymphadenitis (NGL-Group 2) and Necrotizing lymphadenitis (NL- Group 3). Nitika *et al.,* Mitra *et al.,* (2017) Ruchi *et al.,* (Khajuria & Singh, 2016) also described similar classification. Majority of our cases were in Necrotizing granulomatous lymphadenitis (NGL-Group 2). Majority of other studies by Mittal *et al.,* (Handa *et al.,* 2012) Ruchi *et al.,* (Khajuria & Singh, 2016) Nitika *et al.,* (Vashisht *et al.,* 2019).

Cases were proven non-tuberculous had a greater number of granulomatous pattern (Group1). Group 1 when subjected to microbiological test has specificity of 94% and less sensitivity of 11% when compared to other groups. These values indicate that probability that these group are less likely to be positive when subjected to microbiological test. This is because granulomatous lymphadenitis has various differential diagnosis caused by other conditions, like atypical mycobacterial lymphadenitis, fungal lymphadenitis, sarcoidosis, toxoplasmosis, and cat scratch fever.

Henceforth making it important to arrive at a definitive diagnosis.

Out of 54 cases, 50 cases were microbiologically proven tuberculosis in Group 2. This group when subjected to microbiological test has specificity of 97%, sensitivity of 50%, positive predictive value of 93% and negative predictive value of 75. This group had higher specificity, Positive predictive value and higher sensitivity compared to other groups. This indicates that smears showing necrosis along with granuloma if subjected to microbiological methods provide definite diagnosis of tuberculosis and likely to be negative in the absence of the disease. Out of 26 cases, 25 cases were microbiologically proven tuberculosis. This pattern was seen relatively lesser in our population due to population difference, lower number of immunocompromised patients and early access to diagnosis. Presence of necrosis in the smears have a very high specificity and positive predictive value. This group when subjected to microbiological test has specificity of 99%, sensitivity of 25%, positive predictive value of 96% and negative predictive value of 66 %. From the values obtained from NL it indicates that microbiological test result is likely to be negative when the disease is not present, which is indicated by high specificity. True to our knowledge this is the first study comparing each cytomorphological category with bacillary detection methods to give precise analysis.

The results of overall cytomorphology with each microbiological method were compared. ZN staining for AFB was positive in 20% cases. The study by Mitra *et al.,* (Mitra *et al.,* 2017) reported AFB positivity more in group 3. In our study the positivity was almost equal in all 3 cytomorphological patterns. This can be attributed to the lesser percentage of necrotizing lesions in our study. Gene X pert positivity was seen in 55% cases of granulomatous (GL), 85% of necrotizing granulomatous (NGL) and 96% of necrotizing lymphadenitis (NL). Gene X pert detected resistant MTB maximum in necrotizing granulomatous lymphadenitis. Thereby, presence of necrosis in the smears have a remarkably high sensitivity, specificity, and positive predictive value. Utility of Gene Xpert as adjunct to cytology was calculated using kappa co-efficient test considering culture as gold standard. Percentage of agreement of FNAC smears with Gene Xpert was 0.4 (Kappa test) implying moderate agreement similar to that of the study by Tamanna-E-Nur *et al.,* (2019).

CONCLUSION
FNAC serves as sensitive tool in detection of tuberculosis lymphadenitis, A synchronous analysis of cytomorphological features and microbiological test increases the diagnostic yield of Tuberculosis. Thereby patients can be treated immediately without much delay, thus decreasing the infectivity of exposure. Necrotic smears should be subjected to GeneXpert as there is high probability of diagnosis tuberculosis is high. Excisional

biopsy for diagnosis of tuberculosis should be recommended only for patients in whom Fine Needle Aspiration Cytology PCR is negative or there is discrepancy with the clinical impression.

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