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A comparative study of the role of cytopathology, staining for acid-fast bacilli and genexpert MTB/RIF assay in the diagnosis of tubercular lymphadenopathy

Saqib Ahmed^{1,*}, Hena A Ansari¹, Nazish Fatima²

¹Dept. of Pathology, Jawaharlal Nehru Medical College, AMU, Aligarh, Uttar Pradesh, India

²Dept. of Microbiology, Jawaharlal Nehru Medical College, AMU, Aligarh, Uttar Pradesh, India



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ABSTRACT

Introduction: The diagnosis of tubercular lymphadenopathy can be made by FNAC combined with staining for AFB along with newer methods like GeneXpert assay. India carries one of the largest burdens of TB accounting for twenty-seven per cent of worldwide cases.

Objectives: To evaluate the role of cytopathology, AFB staining and GeneXpert assay in the rapid diagnosis of tubercular lymphadenopathy along with the usefulness of GeneXpert assay in the diagnosis of indeterminate aspirates.

Materials and Methods: A total of 372 patients presenting with lymphadenopathy and clinical suspicion of a tubercular aetiology were included in the analysis. 183 patients (56%) were adults (20-59 years) followed by 66 patients (20%) in the paediatric age group. 189 were females (57.3%) and 141 were males (42.7%). The cervical group of lymph nodes were most commonly involved (66%). 69.1% were AFB positive in 201 smears with features favouring tubercular infection (positive cytology) which increased to 183 (91%) when GeneXpert Analysis was done, while 72 cases (55.8%) were AFB positive on cases with indeterminate cytology which showed an increment to 108 cases (83.7%) on the application of GeneXpert.

Conclusion: FNAC combined with ZN staining and GeneXpert has a much higher diagnostic accuracy, GeneXpert further prevents underdiagnosis and enables rapid and accurate diagnosis in cases with indeterminate/inconclusive FNAC and ZN staining.

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1. Introduction

Tuberculosis (TB) is an endemic disease in India amongst which pulmonary tuberculosis accounts for 85% of cases while extrapulmonary tuberculosis (EPTB) accounts for only 15-20% of cases.¹ In India alone, the incidence of TB in the year 2019 was 2.69 million which is equivalent to 199 cases per 100,000 population.² Tubercular lymphadenitis is the most common cause of lymphadenitis in India. Cervical lymphadenopathy is seen in two-thirds of cases.³ The diagnosis of tubercular lymphadenopathy can be made by fine-needle aspiration cytology combined with staining

for acid-fast bacilli, histopathology, mycobacterial culture along with newer methods like PCR- based amplification of mycobacterium DNA. Fine-needle aspiration (FNA) cytology is a minimally invasive and cost-effective, outpatient diagnostic procedure that easily lends itself to supportive tests such as staining for acid-fast bacilli, culture, and more recently, molecular testing by polymerase chain reaction-based systems with variable sensitivity and specificity.⁴ The diagnosis is rendered difficult in those cases where smears lack definitive features such as those showing poorly formed granulomas, or in cases of early tubercular lymphadenopathy with small lymphohistiocytic clusters or neutrophilic infiltrates. Aspirates from such

* Corresponding author.

E-mail address: saqibahmed.mail@gmail.com (S. Ahmed).

nodes may cause diagnostic dilemmas due to the absence of specific features of tubercular lymphadenopathy. The Ziehl-Neelsen (ZN) staining technique for acid-fast bacilli (AFB) is a simple, cheap and rapid method but has a variable sensitivity.⁵ Therefore it cannot be relied upon to confirm or exclude a diagnosis of tuberculosis with certainty. Fluorescent microscopy has a much higher sensitivity than ZN staining but is expensive and needs specialised equipment.⁶ Mycobacterial culture is considered the gold standard method but has a long turnaround time (around 6 weeks).⁷ Currently, Polymerase Chain Reaction (PCR) is the most sensitive technique for rapid diagnosis of mycobacterium tuberculosis (MTB) although it has its cost implications. Its sensitivity is reported to be 89.5% and specificity to be 86.1%.⁸ The GeneXpert Mycobacterium Rifampicin (MTB/RIF) assay is a real-time polymerase chain reaction (RT PCR) based method for the detection of mycobacterial DNA.⁹ Specimens that can be sent for testing include respiratory specimens as well as extrapulmonary samples like fluid and lymph node aspirates.¹⁰ Taking into consideration its unambiguous, rapid results, and high sensitivity and specificity, the W.H.O 2013 policy recommends the use of the GeneXpert MTB/RIF assay as an initial/add-on test in the rapid diagnosis of pulmonary and extra-pulmonary tuberculosis and detection of Rifampicin resistance.¹

2. Objectives

1. To evaluate the utility of cytopathology, staining for acid-fast bacilli and molecular analysis using GeneXpert Mycobacterium Rifampicin (MTB/RIF) assay in the rapid diagnosis of tubercular lymphadenopathy.
2. To evaluate the usefulness of molecular testing for Mycobacterium tuberculosis using GeneXpert Mycobacterium Rifampicin (MTB/RIF) assay in the diagnosis of indeterminate aspirates.

3. Materials and Methods

The present study was a two-year prospective study conducted in the Department of Pathology in collaboration with the Department of Microbiology, Jawaharlal Nehru Medical College & Hospital, Aligarh Muslim University. During this period, 389 patients presenting with lymphadenopathy and clinical suspicion of a tubercular aetiology, underwent fine-needle aspiration in the Cytopathology laboratory of the Department of Pathology.

3.1. Inclusion criteria

All cases with lymphadenopathy with cytopathological features suspicious for or indicative of tubercular lymphadenitis.

3.2. Exclusive criteria

Cases of lymph node enlargement due to causes other than tubercular infection on cytology.

Relevant history and details of investigations were recorded as available. Smears were prepared from the aspirated material. The residual sample was properly labelled and transported to the molecular testing laboratory in the Department of Microbiology for further analysis by GeneXpert and Culture. The smears thus obtained were thoroughly evaluated microscopically after staining and the observations were compared with results of the GeneXpert MTB/RIF Assay. In the microbiology lab, the received sample was properly labelled and checked for adequacy. The sample was mixed with the standard reagent (NaOH+Isopropanol) in a ratio of 1:2, and then this mixture was shaken and incubated for 10 minutes at room temperature. 2ml of sample- reagent mixture was transferred into the test cartridge and was loaded into the GeneXpert module. Test results were reported as MTB positive/negative and whether Rifampicin sensitive or resistant. The data obtained were compared and statistically analysed by using Odds of diagnosis (No gold standard required).

4. Observations

Out of the total 389 patients with clinical suspicion of tuberculosis, 17 samples were inadequate for cytomorphology and/or molecular analysis. In the remaining 372 samples, 330 cases were rendered a cytopathological diagnosis of Chronic granulomatous lesion (favouring TB) and were included in the final analysis, while 42 cases were diagnosed as conditions other than TB.

Four cytomorphological patterns were recognised on aspirates from cases suspected of tubercular lymphadenopathy. The most common pattern (pattern 1) was that of epithelioid cell granulomas with necrosis seen in 112 cases (33.9%) “(Figure 1)”. The other commonly observed pattern was that of epithelioid cell granulomas without necrosis (89 cases -26.9%)(pattern 2) “(Figure 2)”. 73(22.2%) aspirates showed neutrophilic infiltrates without well defined epithelioid cell granulomas (Pattern 3) “(Figure 3)”. The least common pattern observed was that of poorly formed granulomas or small lymphohistiocytic clusters, seen in 56 aspirates (16.9%), (Pattern 4) “(Figure 4)”. Patterns 1 and 2 were considered to be in favour of chronic granulomatous lesion or tubercular infection so were categorised as showing ‘positive cytology’. Cases with patterns 3 and 4 on cytology were considered suspicious of chronic granulomatous infection. Since a definitive diagnosis could not be derived on cytology alone, these cases were placed under the category of ‘indeterminate cytology’.

Most cases of tubercular lymphadenopathy were of the adult age group, that is 20-59 years (183-56%). 66 cases (20%) were of the paediatric age group or less than 13 years of age while 62(19%) were adolescents (14-19 years). Only 19 patients were above 60years of age.

Out of a total of 330 cases in the study group, there were 189 females (57.3%) and 141 males (42.7%). The ratio between male and female patients was 0.74:1.

In the present study maximum number of cases showed cervical lymph node involvement (219-66.3%). This was followed by submandibular lymphadenopathy in 51 patients (15.4%), Submental involvement was seen in 24 patients (7.2%) while 20 cases presented with supraclavicular lymphadenopathy (6% of the total). Other lymph node groups involved were axillary (7 cases-2.2%), infraclavicular (5 cases) and inguinal (4 patients).

ZN staining for AFB bacilli showed overall staining for 63.9% with 69.1% in cases with positive cytology and 55.8% in cases with indeterminate cytology (Table 1) "(Figure 5)".while GeneXpert MTB/RIF Assay showed an overall positivity of 88.1, thus showing 21.9% increase in detection rate for positive cytology and 27.8% for indeterminate cytology (Table 2). The above results are compared in Table 3. Odds of diagnosis and non-diagnosis of tubercular lymphadenopathy by FNAC, ZN staining as compared to GeneXpert MTB/RIF assay and their comparison is further depicted in Tables 4, 5, 6 and 7.

5. Results

Out of 330 cases, maximum cases were in the 20-59 age group comprising of 183 patients (56%) followed by 66 patients (20%) in the paediatric age group (<13years). 189 were females(57.3%) and 141were males (42.7%). The male: female ratio was 0.74:1. The cervical group of lymph nodes were most commonly involved as seen in 219 patients (66% of all cases) followed by submandibular lymphadenopathy in 51 patients (15.4%). Four cytomorphological patterns were recognised on aspirates with the most common pattern being epithelioid cell granulomas with necrosis seen in 112 cases (33.9%).

A total of 139 cases (69.1%) were positive for Acid Fast Bacilli (AFB) when Ziehl- Neelsen staining was applied on 201 smears showing features favouring tubercular infection (positive cytology) while a total of 72 cases (55.8%) were AFB positive in the total of 129 cases with indeterminate cytomorphological findings. Out of 330 aspirates, 291 samples (88.1%) were positive for Mycobacterium tuberculosis when analysed by the GeneXpert MTB/RIF Assay out of which 183 cases (91.0%) were positive in 201 cases showing a positive cytomorphology and 108 cases (83.7%) were positive out of 129 cases which showed an indeterminate/inconclusive cytomorphological picture.

Amongst 201 cases with positive cytomorphology favouring tubercular lymphadenopathy, 139(69.1%) were also positive for AFB on the ZN stain. which on analysis by GeneXpert MTB/RIF Assay, the number of positive cases increased to 183 (91%), an increment of 44 cases (21.9%). Among 129 smears with indeterminate/inconclusive cytomorphological findings, 72(55.8%) of cases were positive for AFB on ZN staining which on analysis by GeneXpert MTB/RIF Assay, the number of positive cases increased to 108(83.7%), an increase of 36 cases(27.8%).

The overall rate of a positive diagnosis of FNAC was 60.9% (201 cases out of 330). 129 cases showed inconclusive features (39.1%). As compared to cytology alone, ZN staining yielded a higher overall rate of positivity of 63.9%(211 cases out of 330). The rate of a positive detection on ZN staining amongst cases with a positive cytological picture favouring a chronic granulomatous or tubercular aetiology was 69.1%(139 cases out of 201) while the rate of a negative result was 30.9%(62 aspirates). The positivity rate for ZN staining was lower, 55.8% (72 cases amongst a total of 129). The inference drawn is that Ziehl-Neelsen staining for Acid-fast bacilli has higher diagnostic efficacy than FNAC. The diagnostic efficacy of ZN stain is especially higher as compared to FNAC in cases with positive cytomorphological findings, in contrast to cases with indeterminate/ inconclusive cytology, where ZN stain demonstrates much lower diagnostic utility.

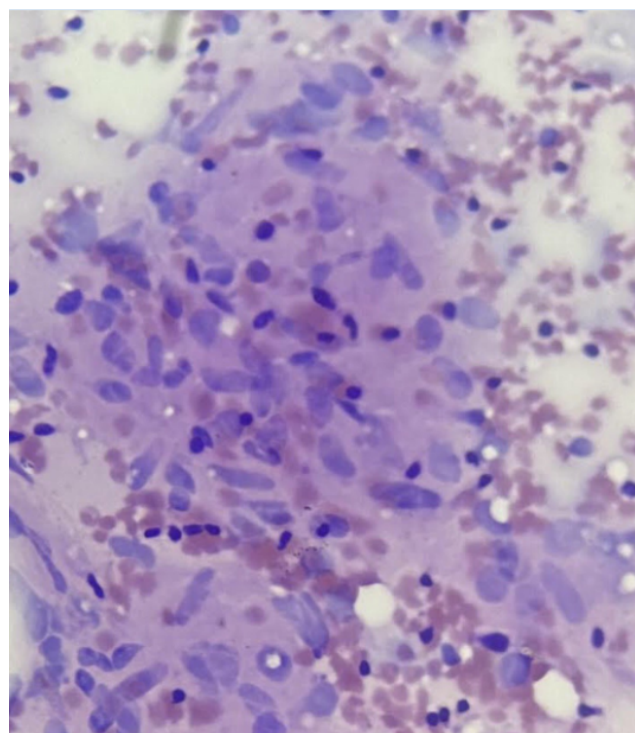


Fig. 1: FNA smear (cervical lymph node) shows well-formed epithelioid cell granuloma mixed with lymphocytes against a necrotic background (Pap X40)

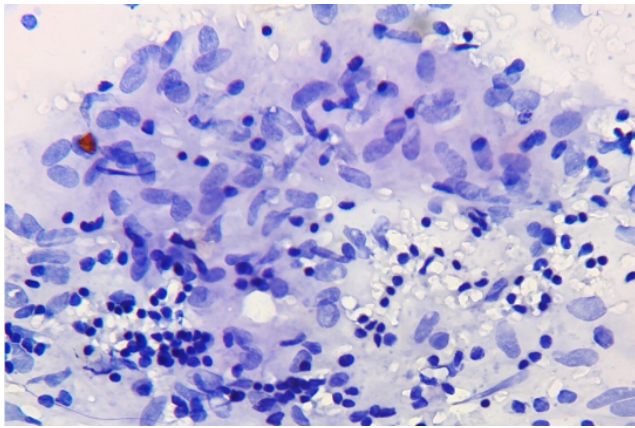


Fig. 2: FNA smear (submandibular lymph node): Chronic granulomatous lesion displaying well-formed epithelioid cell granuloma without necrosis in the background (Pap X40)

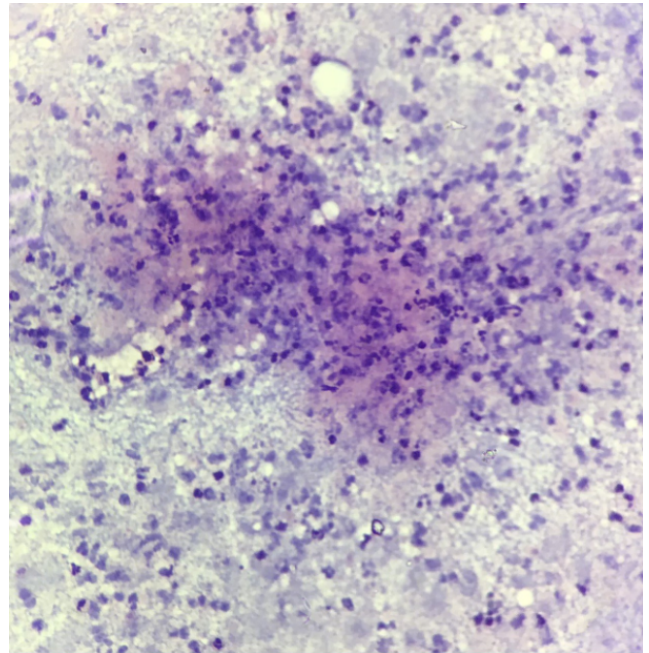


Fig. 4: FNA smear (submental region): early/poorly formed granuloma with degenerated cells (H and E X10)

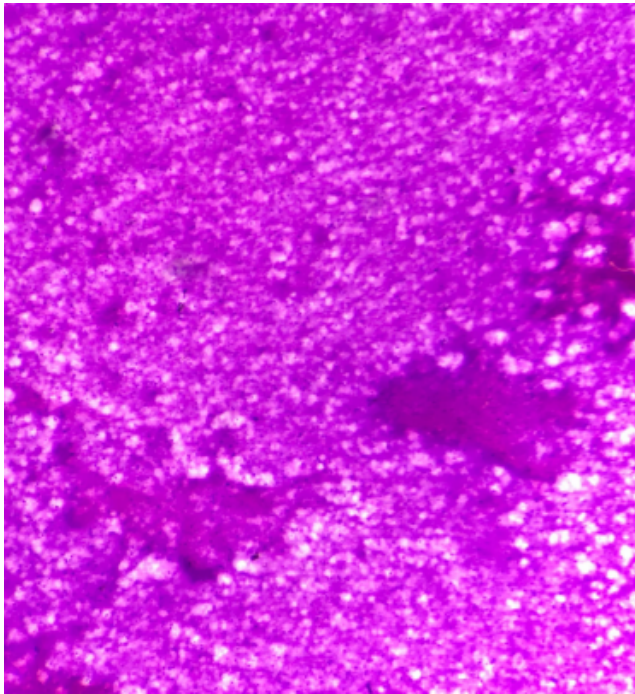


Fig. 3: FNA smear (cervical lymph node): Extensive amorphous necrosis without granuloma (PapX10)

The overall rate of a positive result by GeneXpert MTB/RIF Assay amongst all 330 cases was 88.2% (291 positive results). In Cases with positive cytology, a very high positivity rate of 91% was seen in this group (183 samples), while in cases with indeterminate cytology positivity rate was 83.7% in this group (108 positive results) which was an increase of 27.8% as compared to ZN staining. It can be inferred that GeneXpert MTB/RIF Assay has a superior diagnostic efficacy as compared to both FNAC and Ziehl-Neelsen staining for Acid-fast bacilli, in the diagnosis of aspirates from cases of tubercular lymphadenopathy.

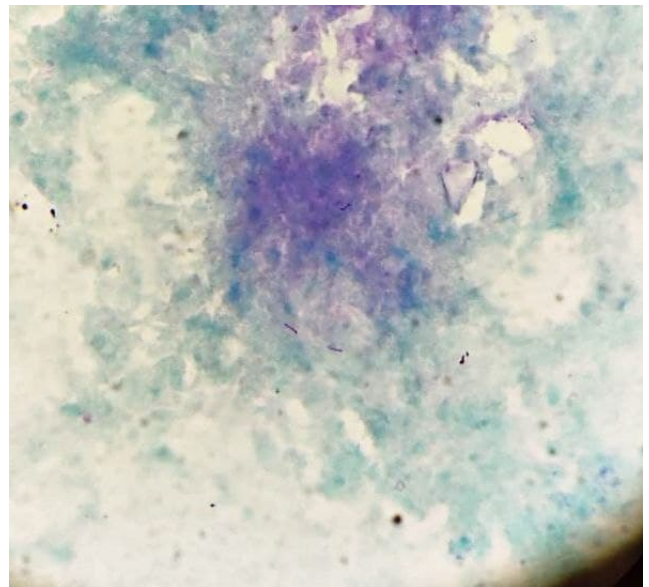


Fig. 5: FNA smear of lymph node (Pattern 1) –ZN staining for acid-fast bacilli showing several thin elongated red coloured bacilli against a bluish background (X100, oil immersion)

Table 1: Distribution of cases according to the results of ziehl neelsen staining and genexpert mtb/rif assay

Results	Cases With Positive Cytology	Cases With Indeterminate Cytology	Total
For ZN staining			
Positive	139 (69.1%)	72 (55.8%)	211(63.9%)
Negative	62 (30.9%)	57(44.2%)	119(36.1%)
Total	201(100%)	129 (100%)	330 (100%)
For GeneXpert			
Positive	183(91%)	108(83.7%)	291(88.1%)
Negative	18(9.0%)	21(16.3%)	39(11.9%)
Total	201(100%)	129(100%)	330(100%)

Table 2: Comparison of results of zn staining and genexpert mtb/rif assay

Results of test	ZN Staining	GeneXpert MTB/RIF Assay
For cases with Positive Cytomorphology		
Positive	139 (69.1%)	183 (91.0%) (↑ by 21.9%)
Negative	62(30.9%)	18 (9.0%) (↓ by 21.9%)
Total	201(100%)	201 (100%)
For cases with Indeterminate Cytomorphology		
Positive	72(55.8%)	108 (83.7%)(↑ by 27.8%)
Negative	57 (44.2%)	21 (16.3%) (↓ by 27.8%)
Total	129 (100%)	129 (100%)

Table 3: Comparison of rates of detection for fnac, zn stain and genexpert mtb/rif assay

Rate of detection	FNAC	Ziehl-Neelsen Stain	GeneXpert Assay
1.)For total cases (330)			
Positive	60.9%	63.9%	88.2%
Negative	39.1% (indeterminate aspirate)	36.1%	11.8%
2.)For cases with positive cytomorphology (201)			
Positive		69.1%	91%
Negative		30.9%	9.0%
3.) For cases with indeterminate cytomorphology (129)			
Positive		55.8%	83.7
Negative		44.2%	16.3%

Table 4: Odds of diagnosis of tubercular lymphadenopathy by fnac as compared to genexpert mtb/rif assay on all cases(n=330)

Parameter	Value	Confidence Interval (95%)
Odds of diagnosis (cytomorphology favouring TB)	0.90	0.85-0.93
Odds of non-diagnosis (cytomorphology favouring TB)	0.09	0.06-0.14
Odds of diagnosis (Indeterminate cytomorphology)	0.48	0.37-0.58
Odds of non-diagnosis (Indeterminate cytomorphology)	0.51	0.41-0.62

Table 5: Odds of diagnosis of tubercular lymphadenopathy by ZN staining as compared to genexpert MTB/RIF assay on all cases (n=330)

Parameter	Value	Confidence Interval (95%)
Odds of diagnosis (AFB- positive)	0.99	0.96-0.99
Odds of non-diagnosis (AFB – positive)	0.09	0.06-0.14
Odds of diagnosis (AFB -negative)	0.625	0.53-0.70
Odds of non-diagnosis (AFB -negative)	0.375	0.29-0.46

Table 6: Odds of diagnosis of tubercular lymphadenopathy by ZN staining as compared to Genexpert MTB/RIF assay on cases with indeterminate cytomorphology (n=129)

Parameter	Value	Confidence Interval (95%)
Odds of diagnosis (AFB -positive)	0.66	0.59-0.72
Odds of non-diagnosis (AFB-positive)	0.33	0.27-0.35
Odds of diagnosis (AFB -negative)	0.19	0.12-.0.23
Odds of non-diagnosis (AFB-negative)	0.81	0.75-0.86

Table 7: Odds of diagnosis of tubercular lymphadenopathy by FNAC and ZN staining as compared to Genexpert MTB/RIF assay on cases with indeterminate cytomorphology(n=129)

Test	Value	Confidence Interval (95%)
FNAC	0.48	0.37-0.58
ZN stain (AFB-positive)	0.66	0.27-0.35
ZN stain (AFB-negative)	0.19	0.12-.0.23

GeneXpert MTB/RIF Assay has greater diagnostic utility in cases with inconclusive cytomorphology and AFB -negative aspirates.

The odds of diagnosis by FNAC as compared to GeneXpert MTB/RIF Assay were calculated for 330 samples, at 95% confidence interval (calculated for true proportions) and the diagnostic efficacy of FNAC was determined to be 10% less as compared to GeneXpert Assay, for cases with positive cytological findings. For cases with Indeterminate cytomorphology, it was 0.48(48%, 0.37-0.58), while odds of non-diagnosis were 0.51(51%, 0.41-0.62). FNAC had a low diagnostic efficacy for Tubercular lymphadenopathy, in cases with indeterminate cytomorphology as compared to Genexpert Assay (52% less). It can be inferred that GeneXpert MTB/RIF Assay has higher diagnostic utility as compared to FNAC alone in the diagnosis of tubercular lymphadenopathy especially in cases with indeterminate/inconclusive findings.

The odds of diagnosis by ZN stain as compared to GeneXpert for cases showing positive cytology were calculated and the diagnostic efficacy was inferred to be nearly equivalent to that of GeneXpert Assay, for AFB-positive samples while it was much higher as for AFB -negative samples. In cases showing indeterminate cytology ZN stain had a lower rate of diagnosis as compared to GeneXpert Assay. Specifically, the diagnostic efficacy was 34% lower for AFB positive smears and 81% lower for AFB negative aspirates. Thus GeneXpert MTB/RIF Assay has a higher diagnostic utility as compared to Ziehl-Neelsen staining especially for cases of tubercular lymphadenopathy that have inconclusive cytological features and are AFB negative.

6. Discussion

In the present study, the most common cytomorphological pattern observed on FNAC was that of well-formed epithelioid cell granulomas with caseous necrosis (34%). This was in agreement with the studies done by Laishram

et al.,¹¹ Shirish et al.,¹² Khanna et al.,¹³ Thakur et al.,¹⁴ Mitra et al.,¹⁵ and Naaz et al.,¹⁶

Most cases of tubercular lymphadenopathy were observed to occur between 20-59 years of age in the present study, this was comparable with the study by Muluye et al.,¹⁷ who documented 54.6% cases in the adult age group (25-60 years) while Singh et al.,¹⁸ observed that the maximum number of the patients were between 20-40 years. Similar observations were made by Gupta et al.,⁴ and Jasim et al.¹⁹

Out of the total of 330 cases, females were more frequently diagnosed as suffering from tubercular lymphadenopathy (189 cases, 57.3% of all). These observations are in agreement with the data published by Khan et al.,²⁰ and Mohapatra et al.,²¹ showed female preponderance with the female: male ratio being 2:1. Our findings are also in agreement with those of Biadlegne et al.,²² and Naaz et al.,¹⁶ However, a study conducted by Somaiah et al.,²³ on cases with tubercular lymphadenopathy found a greater involvement of males (71.5%) as compared to females(28.4%).

In the present study, the most common site affected by tuberculosis was found to be the cervical group of lymph nodes followed by the submandibular lymph node. This was comparable with the study done by Parveen et al.,⁽²⁴⁾ and Muluye et al.¹⁷

Out of a total of 330 cases, FNAC displayed positive cytomorphological findings favouring a diagnosis of chronic granulomatous lesion/tubercular lymphadenopathy in 201 smears (60.9%). In 129 aspirates (39.1%), the findings were suspicious but insufficient for a favourable diagnosis (indeterminate cytology). Lakhey et al.,²⁴ reported that out of 122 suspected cases of tubercular lymphadenopathy, cytology was diagnostic in 48.2% of cases. In a study by Vimal et al.,²⁵ 117 smears showed diagnostic features of tuberculosis (68.8%).

In the present study, ZN stain was showed an overall positivity of 63.9%. Balaji et al.,^{2009,}²⁶ in a similar study reported a positivity of 32.5% cases while Lakhey et al.,²⁴

Mittal et al.,⁵ found a positivity of 58.1% and 76.5% respectively.

GeneXpert Assay yielded a very high rate of detection of 91% for Mycobacterium tuberculosis, as compared to 69.1% for ZN stain as was able to increase the number of positive results in this category by a margin of 21.9%. GeneXpert Assay also demonstrated a high rate of a positive result (83.7%) even in aspirates with indeterminate/inconclusive findings on cytology, as compared to 55.8% for ZN stain. Similar observations were also shown in a similar study done by Denkinger et al.,²⁷ in which GeneXpert MTB/RIF Assay was able to detect 81.2% of the total cases. A study conducted by Manju et al.,²⁸ documented the positivity of Genexpert Assay on fine-needle aspirates of tubercular lymphadenopathy to be 79.49%, while Lighthelm et al.,²⁹ noted that the positivity rate was 96.7%. Hilleman et al.,³⁰ conducted a similar study and observed a slightly lower rate of detection of 77.3%.

The odds of diagnosis by ZN stain for Acid-fast bacilli were higher than FNAC for AFB positive smears 0.99 (99%, 0.96-0.99) which correlates with the high specificity of this technique when acid-fast bacilli are present. However, ZN staining demonstrated lower odds of a diagnosis of 0.625(62.55, 0.53-0.70) for AFB negative smears as compared to FNAC. In the case of aspirates with indeterminate cytomorphology, FNAC had lower odds of a diagnosis of 0.48(48%, 0.37-0.58), in comparison to ZN staining which demonstrated odds of a diagnosis of 0.66 (66%, 0.59-0.72) for AFB positive samples. However, for aspirates with inconclusive/indeterminate cytology and AFB- negative results, ZN stain had very low odds of a diagnosis of only 0.19(19%, 0.12-0.23). This again is in agreement with the low sensitivity of ZN stain for paucibacillary samples.

7. Conclusion

The findings of the present study indicate that the diagnostic efficacy of both techniques is lower in comparison to GeneXpert Assay especially in cases with indeterminate cytomorphology and for AFB -negative aspirates. For AFB -positive samples, the diagnostic efficacy of ZN stain is comparable to GeneXpert Assay but that of FNAC is still lower.

Hence, it is comprehensible that GeneXpert plays an immense role in diagnosing the cases of suspected tubercular lymphadenopathy which are difficult to diagnose on FNAC and are missed by ZN staining. Thus GeneXpert is recommended for the diagnosis of tubercular lymphadenopathy to increase the diagnostic accuracy and enable rapid diagnosis. This is especially useful in cases with indeterminate/ inconclusive cytological findings and/or AFB- negative aspirates where the diagnostic utility of FNAC and ZN staining is much lower. The incorporation of the GeneXpert MTB/RIF Assay into diagnostic protocols

in laboratories with prioritization for high-burden regions would help in enabling timely and accurate diagnosis, thus reducing patient morbidity, and decreasing unnecessary expenditure on repeated investigations. This would in turn contribute towards reducing the overall burden of the disease and help in furthering the aims and objectives of the END TB Program.

8. Conflict of Interest

The authors declare that there is no conflict of interest.

9. Source of Funding


None.

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Author biography

Saqib Ahmed, Junior Resident  <https://orcid.org/0000-0002-7256-7175>

Hena A Ansari, Associate Professor

Nazish Fatima, Associate Professor

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