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A CORRELATION STUDY OF FINE-NEEDLE ASPIRATION TECHNIQUE WITH ZIEHL–NEELSEN STAINS IN DIAGNOSIS OF TUBERCULOUS LYMPHADENITIS IN SOUTH-EAST RAJASTHAN, INDIA

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ABSTRACT

Background: Tuberculosis (TB) is thought to be one of the oldest human diseases and the history is almost as old as mankind. India has the highest TB burden accounting for one-fifth of the global incidence and nearly 40% of the Indian population is infected with the TB bacillus. In India and other developing countries TB lymphadenitis is the most common form of extrapulmonary TB. **Objectives:** The aim of this study was to compare FNAC and ZN staining findings of clinically suspected tuberculous lymphadenitis cases. This study was undertaken to evaluate cytomorphological patterns of tuberculous lymphadenitis and to correlate the acid-fast bacilli (AFB) positivity. **Methodology:** The present study was conducted in the clinical laboratory of NMCH at Govt. Medical College, Kota from period of 1st January, 2018 to 31th December 2018 to comparative evaluate the detection of Tuberculous lymphadenitis by FNAC and ZN staining. **Result:** Total 463 cases of lymphadenopathy were included in our study. Tuberculous lymphadenitis accounted for 102 (22.03%) cases, among which AFB was present in 68 (66.67%) cases. The maximum incidences of cases were seen in the age range of 21-30 years (39.23%). A slight Female preponderance with a female to male ratio of 1.49:1 was noted. The most common group of lymph nodes affected was cervical (75.49%). Maximum AFB positivity was found in smears with epithelioid granuloma with caseous necrosis (24.51 %). **Conclusion:** FNAC is an assay that has high sensitivities, is optimally selected, efficient, easy to perform, and minimally invasive procedure to diagnose TB lymphadenitis. Supplementation of ZN stain with FNAC increases the rates of diagnosis. Regardless of the presence of granuloma, ZN stain must be employed whenever infective pathology is suspected.

KEYWORDS: Tuberculosis, Lymphadenopathy, Fine Needle Aspiration Cytology, Ziehl Neelsen Stain.

INTRODUCTION

Tuberculosis (TB) is thought to be one of the oldest human diseases and the history is almost as old as mankind.[1] Tuberculosis is the leading infectious cause of morbidity and mortality in adults worldwide, killing about 2 million people every year.[2] India has the highest TB burden accounting for one-fifth of the global incidence andnearly 40% of the Indian population is infected with the TB bacillus.[3] India has the highest burden of tuberculosis in the world as reflected by the World Health Organization statistics for 2011, giving an estimated incidence of 2.2 million cases of tuberculosis for India out of a global incidence of 8.7 million cases.[4] In India, extrapulmonary TB accounting 20% of all TB cases and its prevalence in the country varies between 8.3% and 13.1% in different districts according to cohort analysis by the Central TB Division, Ministry of Health and Family Welfare in 2002.[5] In India and other developing countries, TB lymphadenitis is the most common form of extrapulmonary TB and it comprises 35% of cases.[6] Tuberculosis (TB) is an infectious disease caused by various strains of Mycobacterium, mainly by Mycobacterium tuberculosis in humans.[7] Lymphadenopathy is the most common presentation of extrapulmonary tuberculosis.[8] In extrapulmonary tuberculosis, the most common presentation is cervical lymphadenopathy, especially among the Asian population.[9] Lymph node enlargement could be due to tuberculosis, other inflammatory disease or fungal infection, or some underlying malignancy.[10] There are various diagnostic modalities available for the diagnosis of TB lymphadenitis by fine-needle aspiration cytology (FNAC), histopathological examination excised lymph nodes, culture, Ziehl-Neelsen (ZN) staining for acid fast bacilli (AFB), imaging studies, and molecular tests. Even though culture is considered as gold standard for the diagnosis, FNAC to be used as the initial diagnostic test in suspected cases of TB as it has excellent sensitivity and specificity, and also it is a simple and less expensive

outpatient diagnostic procedure.[11] In general, tuberculous (TB) lymphadenitis is diagnosed using conventional methods such as histopathology on the basis of caseous necrosis and granuloma formation. The chances of acid-fast bacilli (AFB) Identification in tissue section are less because xylene and formalin affect the sensitivity of Ziehl–Neelsen (ZN) method to detect Mycobacterium tuberculosis in histopathology sections.[12] Fine needle aspiration cytology is a simple, quicker, reliable, minimally invasive, and relatively cheap diagnostic modality with minimal risk of complications.[13] The efficacy of FNAC to diagnose TB lymphadenitis is directly proportional to presence of purulent material in samples. AFB commonly found in purulent samples which may not contain granuloma, caseous necrosis or epithelioid cells. In the absence of ZN staining, sample can be wrongly diagnosed as acute suppurative lymphadenitis.[14]

AIMS & OBJECTIVE

The aims and objectives of this study were to describe presentation pattern of TB lymphadenitis and to compare the results of FNAC and ZN stain in the diagnosis of TB lymphadenitis.The present study was conducted to compare the efficacy of detection of tuberculous lymphadenitis by FNAC and ZN staining. This study was undertaken to evaluate cytomorphological patterns of tuberculous lymphadenitis and to correlate the acid-fast bacilli (AFB) positivity.This study was conducted to aid in early detection and treatment of Tuberculosis infections and its prevention in community.

MATERIAL AND METHODS

The present study was conducted in the clinical laboratory of NMCH at Govt. Medical College, Kota from period of 1st January, 2018 to 31th December 2018 to comparative evaluate the detection of tuberculous lymphadenitis by FNAC and ZN staining. A total of 463 samples were collected from the outdoor and indoor patients of Govt. Medical College, Kota and its allied hospitals. A total of 463 Fine needle aspirates were obtained from patients who presented with signs and symptoms of tuberculosis after consent was given. FNA material from lymph nodes was applied to prior labelled slides directly and direct smears prepared for ZN staining.The variables included in the study were age, sex, and site of lesion. Relevant history and examination of nodes were recorded. All patients presenting with lymph node enlargement were included in the study, and they were divided according to the size of lymphadenopathy into following categories: A. <1 cm. B. 1-2 cm. C. >2 cm.The smallest lymph node measured 0.5 cm and the largest measured 4 cm in maximum dimensions. Cytology smears and ZN stain smears were examined. Patient's samples were subjected to following tests for detection of tuberculosis infections.

(1)FNAC method (Fine needle aspiration cytology)[9,10]
(2)Microscopy test method:-Modified Z.N. (Ziehl-Neelsen) staining technique.[13,15]

1. FNAC method

In this technique enlarge nodes and cold abscess were aspirated after all aseptic measures with sterile disposable 23-25 Gauge needle attached with 10 cc disposable syringe. Both air-dried and wet- fixed slides are prepared. Multiple smears were prepared with part of aspirated material, two to three smears were stained with Leishman stain, Giemsa stain and Haematoxylin and Eosin (H & E) stain. Granulomatous lymphadenitis were diagnosed as presence of sheets of epithelioid cells with lymphocytes and plasma cells with or without multinucleated giant cells and eosinophilic granular material containing inflammatory cells and necrotic cell debris was defined as caseous necrosis.[9] A dirty necrotic background would suggest caseation and possibly TB. The TB abscess was described as degenerate caseous necrosis and liquefied necrotic material with marked degenerating and viable inflammatory cell infiltration without epithelioid granuloma.[10] The smears revealing features of tuberculous lymphadenitis were grouped into four categories – (1) Epithelioid cell granuloma with caseous necrosis. (2) Epithelioid cell granuloma without necrosis. (3) Necrosis with poly morpho nuclear (PMN) leukocytosis. (4) Necrosis only without epithelioid granuloma. Figure 1(A, B, C, D) shows above four categories finding with Leishman stain were examined under microscope (40 X)and shows presence of granuloma, necrosis, Langhans giant cells, plasma cells, lymphocytes, macrophages and neutrophils. Figure 2(A, B, C, D) shows above four categories finding which were found in our study with Giemsa stain were examined under microscope (40 X).Figure 3(A, B, C) shows above four categories finding which were found in our study with H & E stain were examined under microscope (40 X).

2. Microscopy test method (Modified Z. N. staining)

FNA material obtained was smeared on two slides and directly stained with strong carbol fuchsine and steamed for 5-10 minutes. The slides were then washed with tap water and decolourised with 20% sulphuric acid until clear. Subsequent rinsing in tap water followed. Counter staining was done with 2% methylene blue for 1-2 minutes and finally rinsed in tap water and left to air dry. Examination was done using high power (100 X) and reported according to RNTCP guideline. The AFB positivity was graded according to the original grading of AFB for the sputum smears and there morphology was assessed broadly. Duplicate slides were examined independently by two experienced laboratory scientists who were blinded to the Microscopy results. In the case of a discrepant result, a third laboratory scientist examined both slides. Results from the third laboratory scientist were considered final. Figure 3(D) shows Acid-fast bacilli with modified ZN stain were examined under oil immersion objectivelens (100 X).

Statistical analysis: Data were analyzed using SPSS 20. Chi Square was used to compare categorical variables. P value of less than 0.05 was considered as statistically significant.

Bio-Safety:-All standard precautions, bio-safety measures & biomedical waste managements in our study according to Biological waste management's Rules 2016 were observed.

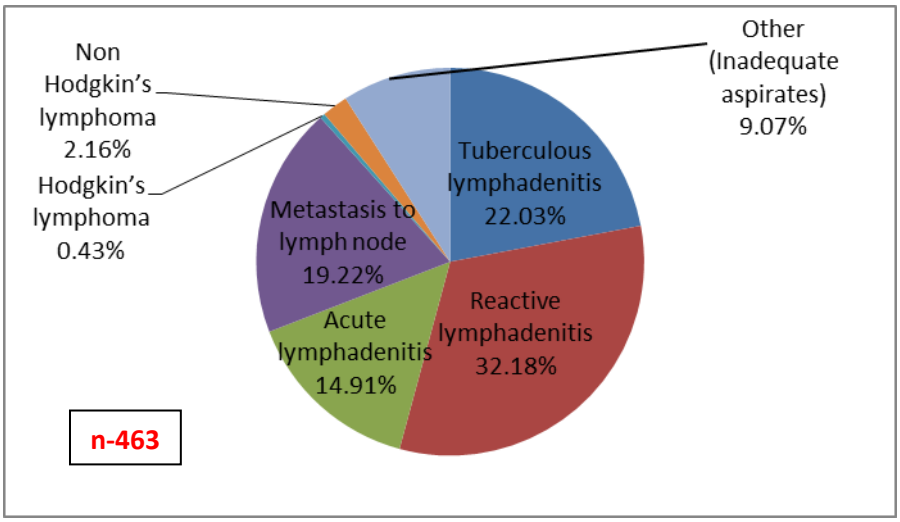
RESULT

In our study, total of 463 samples were collected from the outdoor and indoor suspected TB lymphadenitis patients of Govt. Medical College, Kota and its allied hospitals. Tuberculous lymphadenitis accounted for 102 (22.03%) cases, among which AFB was present in 68 (66.67%) cases. The maximum incidences of cases were seen in the age range of 21-30 years (39.23%). A slight Female preponderance with a female to male ratio of 1.49:1 was noted. The most common group of lymph nodes affected was cervical (75.49%). Maximum AFB positivity was found in smears with epithelioid granuloma with caseous necrosis (24.51 %).

Table 1: Cytomorphological Diagnosis of 463 Cases of Lymphadenopathies.

Type of Lymphadenopathies	Cytomorphological Diagnosis	Total cases (n-463)	Percentage (%)
Inflammatory lymphadenitis (Non-neoplastic)	Tuberculous lymphadenitis	102	22.03 (%)
	Reactive lymphadenitis	149	32.18 (%)
	Acute suppurative lymphadenitis	69	14.91 (%)
Malignant lymphadenopathy (Neoplastic)	Metastasis to lymph node (Metastatic carcinoma)	89	19.22 (%)
	Hodgkin's lymphoma	02	0.43 (%)
	Non-Hodgkin's lymphoma	10	2.16 (%)
Others	Inadequate aspirates	42	9.07 (%)
Total		463	100 (%)

P value <0.001

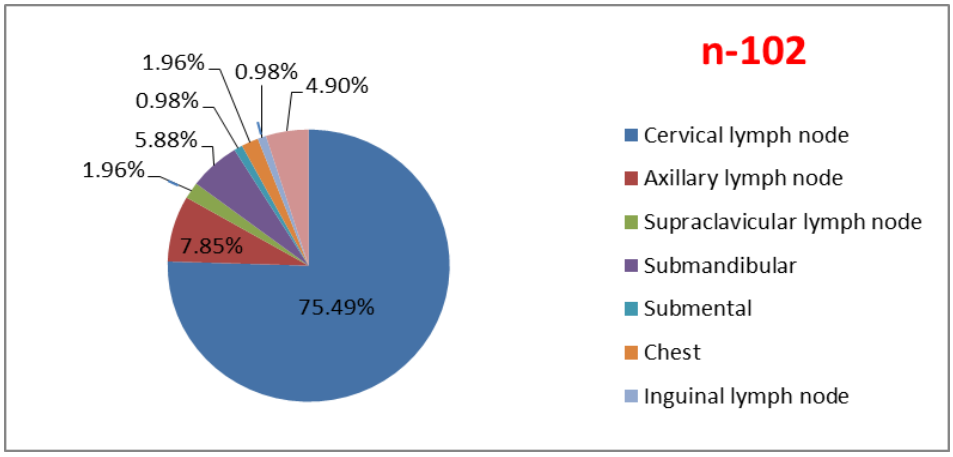


Graph 1: Distribution of Cases of Lymphadenopathies.

Table 2: Various Site Distribution of Tubercular Lymph Node.

Site	Suspected cases (n-463)	Total positive (n-102)	Percentage (%)
Cervical lymph node	352 (76.02%)	77	75.49(%)
Axillary lymph node	12 (2.59%)	8	7.85(%)
Supraclavicular lymph node	8 (1.72%)	2	1.96(%)
Submandibular	24 (5.18%)	6	5.88(%)
Submental	23 (4.96%)	1	0.98(%)
Chest	2 (0.43%)	2	1.96(%
Inguinal lymph node	12 (2.59%)	1	0.98(%)
Other	30 (6.47%)	5	4.90(%)
Total	463	102	100(%)

P value <0.001

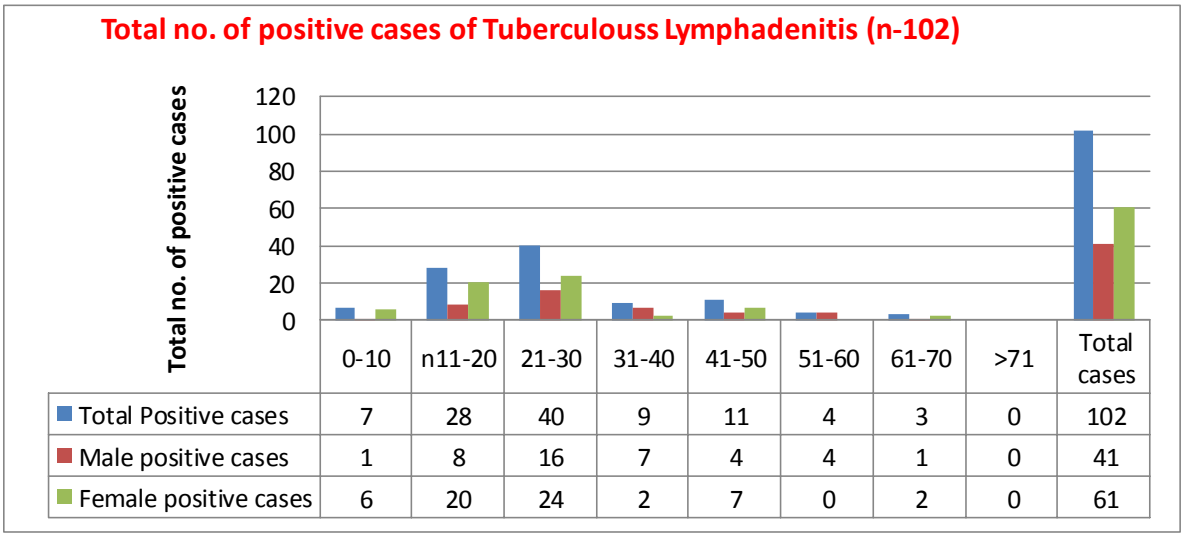


Graph 2: Distribution of Lymph Node Involvement in Positive Cases of Tuberculous Lymphadenitis.

Table 3: Age-wise and Sex-wise Distribution of 463 Cases of Lymphadenopathies.

Age (years)	Male		Female		Total no. of cases	
	Total cases	Positive cases	Total cases	Positive cases	Total cases (n-463)	Total Positive Cases (n-102)
0-10	48 (10.37%)	1 (0.98%)	29 (6.26%)	6 (5.88%)	77(16.63 %)	7 (6.86%)
11-20	67 (14.47%)	8 (7.84%)	57 (12.31%)	20 (19.61%)	124 (26.78%)	28 (27.45%)
21-30	54 (11.66%)	16 (15.69%)	64 (13.83%)	24 (23.5%3)	118 (25.48%)	40 (39.23%)
31-40	25 (5.40%)	7 (6.87%)	27 (5.83%)	2 (1.96%)	52 (11.23%)	9 (8.82%)
41-50	18 (3.88%)	4 (3.92%)	19 (4.11%)	7 (6.86%)	37 (7.99%)	11 (10.78%)
51-60	22 (4.75%)	4 (3.92%)	10 (2.16%)	0 (0%)	32 (6.91%)	4 (3.92%)
61-70	11 (2.38%)	1 (0.98%)	9 (1.94%)	2 (1.96%)	20 (4.31%)	3 (2.94%)
>71	3 (0.65%)	0 (0%)	0 (0%)	0 (0%)	3 (0.64%)	0 (0%)
TOTAL	248 (53.56%)	41 (40.20%)	215 (46.44%)	61 (59.80%)	463 (100%)	102 (100%)

P value <0.001



Graph 3: Age-wise and Sex-wise Distribution of Positive Cases of Tuberculous Lymphadenitis.

Table 4: Correlation between FNA Cytology and ZN staining of 102 Cases of Tubercular Lymphadenitis.

Tubercular lymphadenitis cases	Total positive cases	Percentage (%)
Cytology suggestive of tuberculous lymphadenitis	102	100(%)
ZN staining demonstrating AFB	68	66.67(%)
Total Cases	102	102(%)

P value <0.001

Table 5: Cytological findings of 102 cases of Tubercular Lymphadenitis and correlation with ZN stain.

Cytomorphological Picture	Total Cases	Percentage (%)	AFB Positive Cases	AFB Negative Cases
Epithelioid granuloma with caseous necrosis	35	34.31(%)	25 (24.51%)	10 (9.80%)
Epithelioid granuloma without caseous necrosis	31	30.39(%)	14 (13.73%)	17 (16.67%)
Necrosis with poly mopho nuclear (PMN) leukocytosis	25	24.51(%)	21 (20.59%)	4 (3.92%)
Necrosis only	11	10.79(%)	8 (7.84%)	3 (2.94%)
Total	102	100(%)	68 (66.67%)	34 (33.33%)

P value <0.001

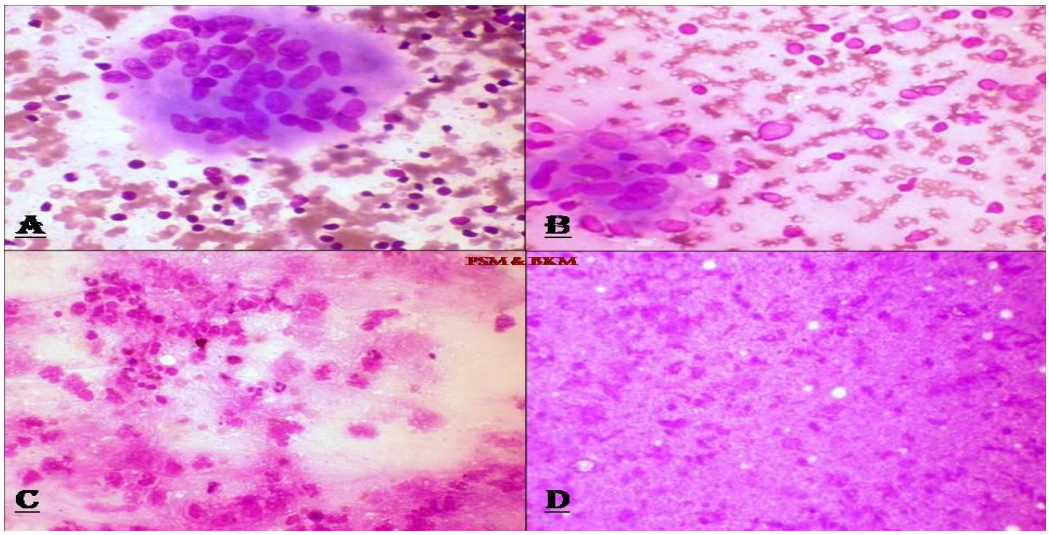


Figure 1: (A) FNAC smear showing Epithelioid cell granuloma with necrosis (Leishman stain ×400). (B) FNAC smear showing Epithelioid cell granuloma without necrosis (Leishman stain ×400). (C) FNAC smear showing Necrosis with poly morpho nuclear (PMN) leukocytes (Leishman stain ×400). (D) FNAC smear showing caseous necrosis only (Leishman stain ×400).

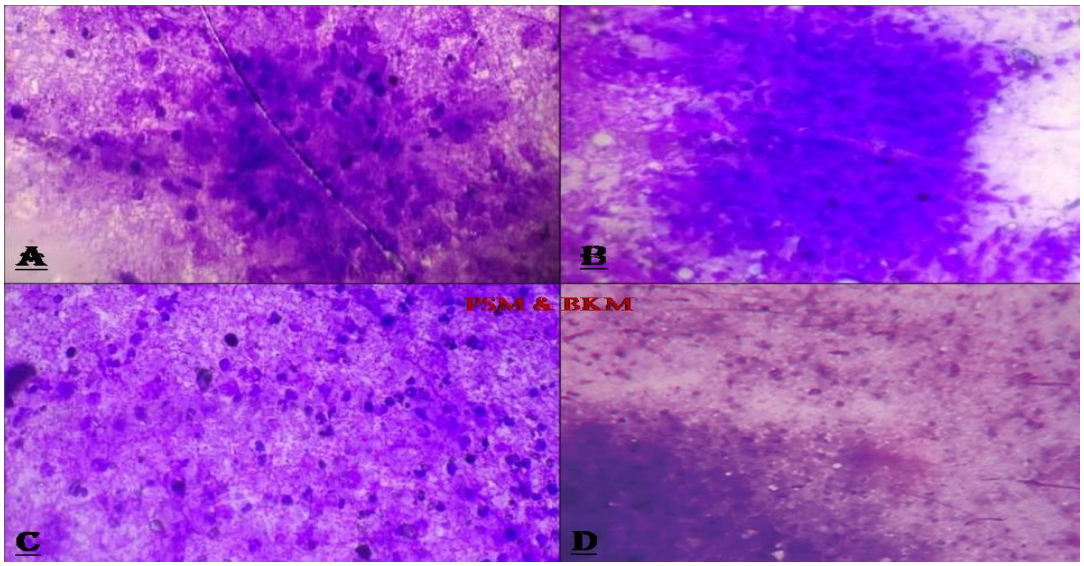


Figure 2: (A) FNAC smear showing Epithelioid cell granuloma with necrosis and mixed inflammatory cells polymorphs, collection of epithelioid histiocytes and lymphocytes (Giemsa stain ×400). (B) FNAC smear showing Epithelioid cell granuloma without necrosis (Giemsa stain ×400). (C)FNAC smear showing Necrosis and poly morpho nuclear (PMN) leukocytes (Giemsa stain ×400). (D) FNAC smear showing caseous necrosis only (Giemsa stain ×400).

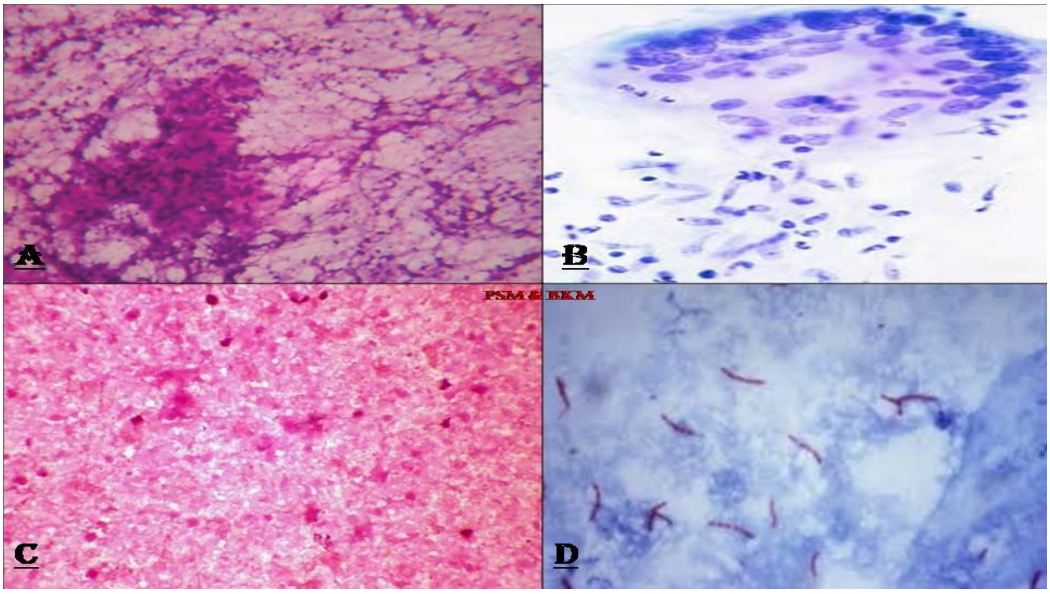


Figure 3: (A) FNAC smear showing Epithelioid cell granuloma with caseous necrosis (Haematoxylin and Eosin (H & E) stain ×400). (B) FNAC smear showing Epithelioid cell granuloma without necrosis with a group of epithelioid cells and Langhan's giant cell (H & Estain ×400). (C) FNAC smear showing caseous necrosis only (H & E stain ×400). (D) Acid-fast bacilli in ZN stain (Ziehl-Neelsen stain ×1000, oil immersion).

Table 1 and Graph 1 show the distribution cytomorphological diagnosis of 463 cases of lymphadenopathies. A total of 463 patients were examined, 320 (69.12%) were non-neoplastic lymphadenopathy, 101 (21.81%) cases were malignant lymphadenopathy and 42(9.07%) were others (inadequate aspirates). Among these cases, 102 (22.03%) were of tubercular, 149 (32.18%) were reactive nature, 69 (14.91%) were acute lymphadenitis, 89 (19.22%) cases were metastasis to lymph nodes, 12(2.59%) were lymphomas (out of which 2(0.43%) were Hodgkin's lymphoma and 10(2.16%) were Non-Hodgkin's lymphoma). Table 2 and Graph 2 shows site distribution of tubercular lymph node. The cervical group of lymph node was most commonly involved in the present study 77(75.49%). 85% of cases of reactive lymphadenopathy showed discrete lymph nodes as compared to tubercular lymphadenopathy which showed matted lymph nodes in 73%. Other commonly lymph node involved was Axillary (7.85%) and Submandibular (5.88%) lymph node. The lymph node size was less than 1 cm in 72% of reactive lymphadenopathy where as it was over 1 cm in 85% of tubercular lymphadenopathy. Table 3 and Figure 3 shows results age-wise, sex-wise and total no. of positive cases wise distribution of 463 cases of lymphadenopathies. Out of which 248(53.56%) were males while 215 (46.44%) were females and the majority of the cases being in the 11-20 and 21-30 years age groups 26.78% and 25.48% respectively. Out of the 102 cases of tuberculosis, 61(59.80%) were reported in females and 41(40.20%) were reported in males, with a female to male ratio of 1.49:1. The age of the patients with tubercular lymphadenitis ranged from 2 to 80 years, the majority of the cases being positive in the 21-30 and 11-20 years age groups 39.23% and 27.45% respectively. Other age group affected were 41-50 years, 31-40 years, 1-10 year, 51-60 years, 61-70 years, and > 70 years with 10.78%, 8.82%, 6.86%, 3.92%, 2.94% and 0% respectively in decreasing order. Table 4 shows the correlation between FNAC and ZN staining of 102 cases of tubercular lymphadenitis. Out of the 102 cases of tubercular lymphadenitis with FNAC, AFB was found in 68 cases (66.67%). The AFB positivity was graded according to the original grading of AFB for the sputum smears and there morphology was assessed broadly. AFB on ZN stain was seen as short and long beaded rods. Table 5 shows cytomorphological pictures of 102 cases of tubercular lymphadenitis and correlation with ZN stain. Tuberculous lymphadenitis accounted for 102 (22.03%) cases, among which AFB was present in 68 (66.67%) cases. Maximum AFB positivity was found in smears with epithelioid granulomas with caseous necrosis (24.51 %). The aspirates from lymph nodes were diagnosed as tubercular lymphadenopathy based on the smears revealing features were grouped into four categories in the present study: 34.31 % were Epithelioid cell granulomas with caseous necrosis, 30.39 % were Epithelioid cell granulomas without necrosis, 24.51 % were Necrosis with poly morpho nuclear (PMN) leukocytosis and 10.79 % were Necrosis only without epithelioid granulomas and AFB positivity was found 24.51%, 13.73%, 20.59% and 7.84% in above four categories respectively. Figure 1, 2, and 3 shows above four categories finding which were found in our study with Leishman stain, Giemsa stain, H & E stain and ZN stain were examined under microscope.

DISCUSSION

India has the highest TB burden as shown in the 2008 World Health Organization (WHO) statistics.[1] In India, 1000 people a day or one per minute die of tuberculosis.[16] Tubercular lymphadenopathy remains the

commonest extrapulmonary manifestation of tuberculosis in children and adults. The high rate of tubercular lymphadenitis in our country is due to low socioeconomic status, illiteracy, incomplete treatment, resistance and increased incidence of HIV. Although histopathology remains the gold standard for diagnosis of lymphadenopathy, its feasibility is limited as it is time consuming and an invasive procedure. Now it has been replaced by FNAC which is a simple, safe, minimally invasive and rapid diagnostic technique. This study thus emphasizes the need for continuous epidemiological surveillance for the timely formulation and implementations of effective TB control programme, So TB infection detected in early phase of disease and prevent morbidity, mortality and future complications.

Table 6: Comparison of Cytomorphological Diagnosis of Various Lymphadenopathies in the present study with other studies (n-463).

Study	Year	Total no. of cases	Tuberculous lymphadenitis (%)	Reactive lymphadenitis (%)	Acute lymphadenitis (%)	Metastasis to lymph node (%)	Lymphoma (%)	Other (Inadequate aspirates) (%)
Khajuria *et al.*[17]	2006	656	52.2(%)	37.1(%)	1.06(%)	1.98(%)	3.8(%)	1.5(%)
Annam *et al.*[18]	2008	336	29.01(%)	7.10(%)	4.62(%)	4.01(%)	5.55(%)	8.91(%)
Vimal et al.[19]	2014	187	28.09(%)	33.69(%)	5.88(%)	2.67(%)	17.61(%)	10.1(%)
Present study	2018	463	22.09 (%)	32.18 (%)	14.91(%)	19.22 (%)	2.59 (%)	9.07 (%)

Table 7 Comparison of Cytomorphological Patterns of Tuberculous Lymphadenitis and AFB positivity with other studies (n-102).

Study	Year	Epithelioid granuloma with necrosis n (%)	Epithelioid granuloma without necrosis n (%)	Necrosis with PMN leukocytes n (%)	Necrosis only n (%)	Overall AFB positivity (%)
Nidhi et al.[20]	2010	29 (16.4%)	25 (14.3%)	-	69 (39.2%)	71(%)
Chand et al.[21]	2013	120 (21.8%)	156 (28.4%)	-	85 (15.4%)	44.54(%)
Hemalatha et al.[22]	2014	84 (56%)	29 (19.3%)	-	34 (22.7%)	54(%)
Masilamani et al.[23]	2015	102 (48.1%)	40 (18.9%)	-	70 (33%)	55.7(%)
Present study	2018	35 (34.31 %)	31 (30.39%)	25 (24.37%)	11 (10.79%)	66.67(%)

In the present study FNAC was compared with the ZN staining for the screening of tubercular lymphadenitis. Table 6 shows comparison of cytomorphological diagnosis of various types of lymphadenopathies in the present study with other studies. In our study, tubercular lymphadenopathy with 102 (22.09%) cases was the second most common cause of peripheral lymphadenopathy, reactive lymphadenopathy with 149 (32.18%) cases being the commonest. Our finding is supported by various other studies done in other parts of the India. Table 7 shows comparison of cytomorphological patterns of tuberculous lymphadenitis and AFB positivity with other studies. The cytological diagnosis of tubercular lymphadenitis is usually based on the presence of epithelioid cell granulomas with or without Langhan's giant cells and with or without necrosis. Even in the absence of epithelioid cells, necrotic material has proved to be useful as it yields the highest yield of AFB positivity.[24] In our study, AFB staining in cases of tuberculosis showed a positivity rate of 66.67%. Acid fast bacilli were usually seen in areas of necrosis and degeneration away from the epithelioid cells. The bacilli were identified as short, stumpy or long beaded rods. These findings correlated with the observation by Rajshekaran et al.[25] AFB were mostly visible in purulent aspirate whether acellular or accompanied by granuloma, and in the absence of ZN staining, case can be misinterpreted as an acute lymphadenitis. The diagnostic difficulties encountered were parallel to those experienced by different authors.[26, 27] In our study, tubercular lymphadenitis was seen most frequently in the 21-30 and 11-20 years age group with a female / male ratio of (1.49:1). The cervical lymph node was the most common site involved (75.49%) followed by axilla (7.85%) which is similar to observations by other workers. [28] Comparison of studies conducted by other researchers showed slight variations in results. Results were varies with geographical distribution and social characteristic of population groups. Our study is a step ahead in this direction with the purpose of providing authentic scientific data based on the affected population.

SUMMARY AND CONCLUSION

FNAC is an optimally selected, efficient, easy to perform, and economical test for initial diagnostic work upon patients with TB lymphadenitis, So patients can be put to treatment immediately without much delay, thus decreasing the infectivity of exposure. Compared with AFB smear, FNAC assay has high sensitivities and cytomorphological features of FNAC on Leishman stain, Giemsa stain & H&E stain have significant diagnostic yield. It also avoids the possible physical and psychological complications of an excision biopsy.Supplementation of ZN Stain with FNAC increases the diagnostic yield. AFB were mostly seen in purulent aspirate whether acellular or accompanied by granuloma. In the absence ZN staining, case can be misinterpreted as an acute lymphadenitis. Regardless of the presence of granuloma, ZN stain must be employed whenever infective pathology is suspected. Diagnostic accuracy can be further increased by submitting material obtained by FNAC for culture. The present study suggests that FNAC coupled with ZN staining for AFB is an excellent method for diagnosing tubercular lymphadenitis. In conclusion, findings from the present study emphasize a definite need for a combination of tests for diagnosing TB lymphadenitis.

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Nil.

CONFLICTS OF INTEREST

There are no conflicts of interest.

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