Study of cytomorphological spectrum of tuberculous lymphadenitis and correlation with AFB positivity

Nitika Vashisht[1], Urmi S. Chakravarty Vartak[2], Shailesh Vartak[3,*]

[1]Resident Medical Officer, [2,3]Associate Professor, Dept. of Pathology, Lokmanya Tilak Municipal Medical College and General Hospital, Sion, Mumbai, Maharashtra, India

***Corresponding Author: Shailesh Vartak**
Email: shailvar@gmail.com

Received: 31st July, 2018 **Accepted:** 11th September, 2018

Abstract
A prospective study of lymph node FNAC was done in 500 cases presenting with tuberculous lymphadenitis over a duration of 6 months. Most of the patients were in the age group of 21-40 years, with female to male ratio of 1.9:1. Lymph node enlargement was noted in all the cases, wherein the lymph nodes were multiple, soft to firm and matted in 152 cases (30.4%) and single and discrete in 348 cases (69.6%). The most common group involved was the cervical group of lymph nodes (87.2%), followed by the axillary group (9.4%). Most cases showed whitish aspirates (56%). 21% cases showed cheesy appearance of aspirates. Associated history of contact was found in 97 cases (19.4%). 142 cases (28.4%) had past history of tuberculosis, out of which 52 cases had completed antitubercular treatment (ATT) for 9 months, while 42 cases were defaulters who had not completed ATT course. Epithelioid granuloma with necrosis (63.6%) pattern was the most common followed by epithelioid granuloma without necrosis (22.4%). 9 HIV seropositive cases (1.8%) were seen, out of which, epithelioid granuloma with necrosis and only caseous necrosis were seen in 3 cases (33.33%) each. AFB positive cases were 36%. Necrotizing lymphadenitis showed maximum AFB positivity (95.55%), which was followed by necrotizing and suppurative lymphadenitis which showed 92% AFB positivity. We did not encounter any complications during and after aspiration procedure.

Keywords: Cytomorphological pattern, Tuberculous lymphadenitis, FNAC, AFB positivity.

Introduction
Tuberculosis (TB) is a major health problem with a global mortality ranging from 1.6 to 2.2 million lives per year. It is the developing countries that suffer a major brunt of the disease, where the prevalence is nearly 95%.[1] TB was declared as a global emergency in 1993 by the World Health Organization (WHO).[2] India accounts for nearly one-fifth (20%) of the global burden of the disease.[3] The term extrapulmonary tuberculosis (EPTB) has been used to describe isolated occurrence of tuberculosis at body sites other than the lung.[4,5]

Extrapulmonary tuberculosis (EPTB) comprises 20% of all TB cases in India, with a variable prevalence between 8.3-13.1%.6 Tuberculous lymphadenitis (TBLN) is one of the commonest manifestations of extra-pulmonary tuberculosis.[7] Moreover, it accounts for 30–52% of all cases of lymphadenopathies in the developing countries.[5,8]

The diagnosis of TB cannot be made solely on the basis of clinical findings.[9] Also, serological tests like soluble antigen fluorescent antibodies, indirect hemagglutination,[10] kaolin agglutination[11] and enzyme-linked immunosorbent assay (ELISA)[12] are cumbersome and time-consuming tests. Additionally, antibodies to Mycobacterium tuberculosis (Mtb) are not definitive because false-positive results are seen in high prevalence areas.[12]

TBLN can be diagnosed by using conventional histopathological tools like excisional biopsy, but they lead to considerable morbidity and discomfort to the patient.[13,14] In such a scenario, Fine needle aspiration cytology (FNAC) has assumed an inexpensive, quicker, and safer alternative in the evaluation of peripheral lymphadenopathy.[1,15] By allowing an easy access to lymph nodes, TBLN can be diagnosed by demonstrating different cytomorphological tissue reaction patterns on smear. They can be broadly classified as:[16]

1. Epithelioid granuloma without necrosis
2. Epithelioid granuloma with necrosis
3. Necrosis only

However, the definitive diagnosis depends on demonstration of acid-fast bacilli (AFB) by culture or smear. Detection of AFB by conventional microscopy is rapid and simple.[17] In the absence of AFB, cytological features lack specificity due to difficulty in distinguishing tuberculous lymphadenitis from other granulomatous lesions.[18]

There is considerable variability in the rate of AFB positivity in cytological smears in different studies. It is found to be highest in purulent aspirates, followed by caseous aspirates, and least often in aspirates mixed with blood.[19]

AFB demonstration is most commonly done by the conventional Ziehl-Neelsen (ZN) method. Alternatively, other stains like auramine and rhodamine can be used either separately or in combination.[20]

Nevertheless, with limited resources in our country, the presence of epithelioid cell granuloma is still considered as an evidence of TBLN.[6,7] With this background, the present study was done to evaluate cytomorphological patterns of tuberculous lymphadenitis along with the overall AFB positivity and correlation of cytomorphological patterns with AFB positivity.[5]

Materials and Methods
This study was conducted in the Department of Cytopathology at a tertiary care hospital in Mumbai from 1st

January 2015 to 30th June 2015. Institutional ethical clearance was obtained before the start of this study. Cytologically proven 500 cases of tuberculous lymphadenitis were studied. All FNAC smears diagnosed as acute suppurative inflammation, reactive nodular hyperplasia, metastasis in lymph nodes, primary lymphoid malignancies and haemorrhage were excluded from the study. Informed consent of patient was taken and in cases of minor, consent of parent /guardian was obtained before fine needle aspiration procedure. A detailed clinical history was taken and physical examination was done along with laboratory findings. FNAC was done by 5-10 cc disposable syringe with 23 G needle under strict aseptic precaution. Care was taken not to aspirate through dependant area of swelling to prevent sinus formation. Three or four punctures were done and ultra-sonic guided aspirations were taken as and when required. Smears were drawn in clean glass slides. Slides were fixed with a spray fixative and used for Papanicolaou (PAP) stain. Remaining slides were air dried for Zeihl-Neelsen (ZN) stain.
Papanicolaou staining procedure –

1. Fix slide with 95% alcohol for 15 min.
2. Stain in Hematoxylene for 4 min.
3. Rinse under running tap water.
4. Bluing done using alcoholic ammonia for 10 seconds.
5. Rinse under running tap water.
6. Dip in 75% alcohol and 95% alcohol for 10 seconds each.
7. Stain with Orange G for 15 seconds.
8. Dip in 95% alcohol for 20 seconds.
9. Stain with Eosin Azure for 10 seconds.
10. Dip in 95% alcohol for 10 seconds.
11. Clear using acetone or xylene. Ziehl-Neelsen staining procedure –

i. Cover the slide with Ziehl's carbol fuschin working solution.
ii. Heat the slide from underneath with the flame of Bunsen burner until vapour start to rise.
iii. Wash the slide gently with water to remove excess carbol fuschin. Drain excess water.
iv. Cover the slide with 25% sulfuric acid and allow to stand for 3 minutes.
v. Wash the slide with water to remove excess sulfuric acid. Drain excess water.
vi. Cover the slide with 0.3% methylene blue solution and allow to stand for 1 minute.
vii. Wash the slide gently with water. Drain excess water.

Detailed cytomorphological features were studied on FNAC and they were correlated with AFB positivity on ZN stain. The data was represented in numbers and percentages. For the statistical analysis, Chi-square test was applied. A probability level lesser than 0.05 ($p<0.05$) was used to indicate statistical significance.

Results and Discussion

A total of 500 cases of tuberculous lymphadenitis were evaluated in the present study. The age of the patients ranged from 3 years to 75 years with majority of the cases in the age group of 21–40 years (52%), followed by 0–20 years (36.4%). Table 1 shows detailed age and sex distribution of the cases. Similar age distribution was observed in many studies[23-25,27,28] except those done by Rana et al. (10-19 years),[21] and Majeed and Bukhare (11-20 years).[22] In our study, 328 cases were reported in females and 172 were reported in males with a female:male ratio of 1.9:1. Paliwal et al.,[23] Chaudhari et al.,[24] Majeed and Bukhare[22] and Rana et al.[21] also noted female preponderance, in contrast to studies done by Jagtap et al.,[25] Mistry et al.[26] and Giri et al.[27] which showed male preponderance.

The anatomical distribution of the enlarged lymph nodes of the cases in our study are shown in Table 2. The cervical group of lymph node was most commonly involved (87.2%), followed by the axillary group (9.4%), and inguinal lymph nodes in 1.6% cases. These findings are in concordance with the other similar studies.[21-25,27,28,30,31]

Table 3 shows gross appearance of aspirates in the present study. Out of 500 cases of tuberculous lymphadenitis, 280 (56%) yielded whitish aspirate and 75 (26.78%) of these cases demonstrated AFB on ZN staining. This was followed by cheesy aspirate which was seen in 105 cases (21%), which demonstrated AFB in 71 (67.61%) cases. Associated history of contact was found in 97 cases (19.4%). 142 cases had past history of tuberculosis, out of which 52 cases had completed anti-tubercular treatment (ATT) for 9 months, while 42 cases were defaulters who had not completed ATT course. Constitutional symptoms like fever, cough and weight loss were present in 161 cases.

In our study, the lesions were categorised into 4 cytological patterns as shown in Table 4:

1. Epithelioid granuloma with caseous necrosis – 318 cases (63.6%) showing epithelioid granuloma, caseous necrosis with or without giant cells.
2. Epithelioid granuloma without caseous necrosis – 112 cases (22.4%) showing only epithelioid granuloma without necrosis, with or without giant cells.
3. Necrotizing lymphadenitis – 45 cases (9%) which showed only caseous necrosis.
4. Necrotizing and suppurative lymphadenitis – 25 cases (5%) which showed necrosis and polymorphonuclear cells.

Chaudhari et al.,[24] Jagtap et al.,[25] Giri et al.,[27] Paliwal et al.,[23] Hemalatha et al.,[28] Narayanamurthy et al.,[29] also described four cytological patterns of tuberculous cytology. In our study, the most common pattern observed was epithelioid granuloma with caseous necrosis (63.6%). Similar pattern was also noted by Chaudhari et al. (51.6%),[24] Giri et al. (53.79%),[27] Hemalatha et al. (56%),[28] Khanna et al. (50.5%),[30] Masilamani et al. (48.1%),[31] Rana et al. (66%),[21] Mistry et al. (63.82%),[26] Majeed and Bukhare (69%).[22] However, Paliwal et al.[23] noted most common pattern as caseous necrosis only (39.2%) and Jagtap et al.[25] and Narayanamurthy et al.[29] found most common pattern as epithelioid granuloma without necrosis (48.07% and 37.61% respectively). Although granulomas without caseous necrosis may be seen in other conditions like leprosy, actinomycetes and sarcoidosis etc., in India, however, the overall prevalence

and incidence of tuberculosis being very high, epithelioid cell granulomas is highly indicative of tuberculosis.

In our study, a definitive diagnosis of tuberculous lymphadenitis was considered in the smears in the first two patterns, while third and fourth were diagnosed as acute suppurative lymphadenitis in the absence of a positive ZN stain. Total AFB positivity in our study was 36%. Maximum AFB positivity was seen in necrotizing lymphadenitis (95.55%), followed by necrotizing and suppurative lymphadenitis (92%). The other two patterns, i.e. epithelioid granuloma with caseous necrosis and epithelioid granuloma without caseous necrosis, had AFB positivity in 30.81% and 16.96% cases respectively. In comparison, frequency of AFB positivity in various other studies ranges from 10 to 70%. Our findings were similar to other studies which showed maximum AFB positivity in cases with only caseous necrosis except where Jagtap et al.,[25] Afrose et al.,[16] and Majeed and Bukhare[22] found maximum AFB positivity in cases with epithelioid granulomas with caseous necrosis.

An inverse relationship was observed between granuloma and presence of AFB. Low AFB positivity can be explained by the following facts:

1. Maximum cases of extra pulmonary tuberculosis are paucibacillary.
2. Early stages of disease and immunological status of patients. It can be presumed that patients with low immunological status would show high number of AFB positivity.
3. Number of AFB has to be 103 to 106/ml of the material to be detected by light microscopy.
4. Treatment with anti-tuberculous drugs.

Jones and Campbell described tuberculous lymphadenitis (TBLN) into five stages:[32]

Stage 1 – Enlarged, firm, mobile, discrete nodes with non-specific reactive hyperplasia

Stage 2 – Larger rubbery nodes fixed to surrounding tissue due to peri-adenitis.

Stage 3 – Central softening due to caseation necrosis and abscess formation.

Stage 4 – Collar-stud abscess formation

Stage 5 – Sinus tract formation

The aspirates from stage 1 or 2 TBLN usually provide inflammatory cells similar to those seen in reactive lymphadenitis. Thus, FNAC of these stages can only be non-specific reactive. Typical necrotic materials or tubercular bacilli can be seen in advanced stages in which an abscess is readily formed in the core of lymph node. Thus, aspirates from an early stage of lymph node are the main cause of low AFB sensitivity. If lymph node aspiration is done in the early stage, the diagnosis is likely to be dismissed as a reactive node.

Table 1: Age & sex distribution

Age group (yrs)	No of cases (n=500)				Total (n=500)	%
	Males (n=172)		Females (n=328)			
	No	%	No	%		
0 – 20	62	36.05	120	36.58	182	36.4
21 – 40	89	51.74	171	52.14	260	52
41 – 60	20	11.63	32	9.75	52	10.4
61 – 80	1	0.58	5	1.53	6	1.2

Table 2: Anatomical distribution of enlarged lymph node in tuberculous lymphadenitis

Anatomic site	No of cases (n=500)	%
Cervical	436	87.2
Infraclavicular	1	0.2
Inguinal	8	1.6
Axillary	47	9.4
Infra-auricular	1	0.2
Post-auricular	5	1
Pre-auricular	2	0.4

Table 3: Gross appearance of aspirates

Gross appearance	No of cases (n=500) (%)	No of AFB positive (n=183)
Blood stained	68 (13.6)	15
Cheesy	105 (21)	71
Whitish	280 (56)	75
Purulent	47 (9.4)	22

Table 4: Cytological patterns in patients with tuberculous lymphadenitis

Cyto-morphological pattern	No of cases (n=500) (%)	AFB positive cases (n=183) (%)	p value
Epithelioid granuloma with necrosis	318 (63.6)	98 (30.81)	$p < 0.0001^{****}$ Highly significant
Epithelioid granuloma without necrosis	112 (22.4)	19 (16.96)	
Only caseous necrosis (without epithelioid granuloma)	45 (9)	43 (95.55)	
Only caseous necrosis (without epithelioid granuloma), with neutrophils	25 (5)	23 (92)	

**** Chi square test was applied

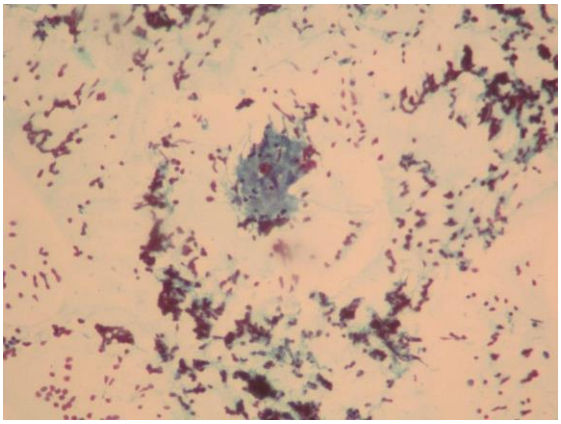

Fig. 1: PAP stained smear showing epithelioid cell clusters (100x)

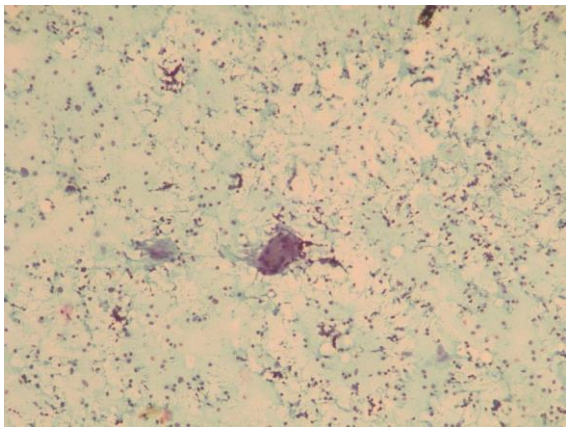

Fig. 2: PAP stained smear showing scattered epithelioid cells along with single giant cell (100x)

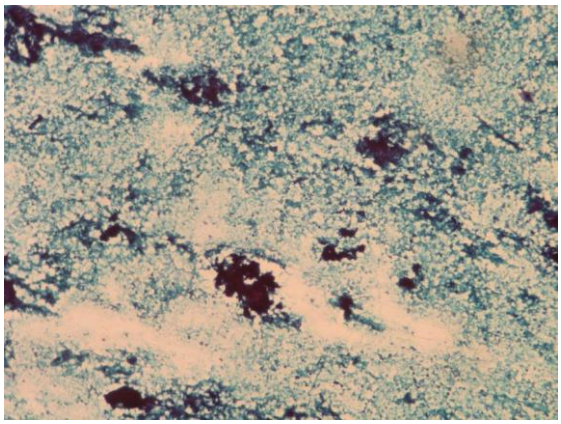

Fig. 3: PAP stained smear showing caseous necrosis (100x)

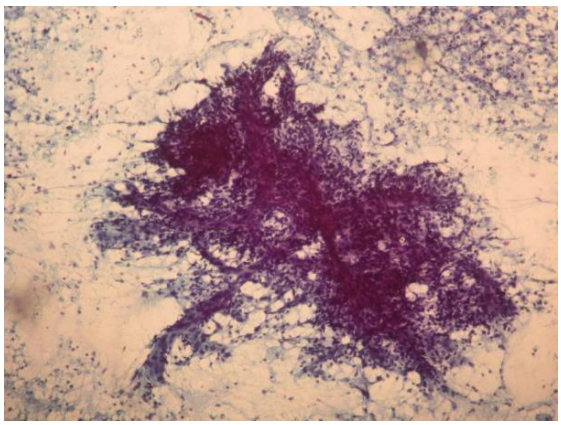

Fig. 4: PAP stained smear showing capillary proliferation in the background of polymorphonuclear lymphocytes, occasional histiocytes (100x)

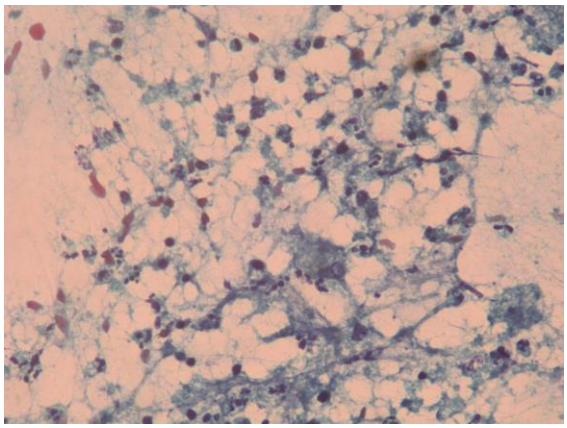

Fig. 5: PAP stained smear showing scattered epithelioid cells, polymorphonuclear cells, lymphocytes, histiocytes (100x)

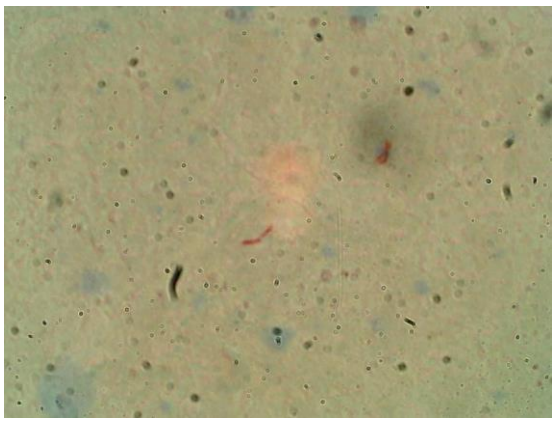

Fig. 6: ZN stained smear showing acid fast bacilli (100x)

Conclusion

The advantage of FNAC of tuberculous lymph node can be illustrated as being a safer, simpler and more conclusive outdoor procedure, helping in prompt diagnosis and early treatment of tuberculous lymphadenitis. Thus, it also helps to reduce significant morbidity associated with tuberculosis by avoiding unnecessary biopsy. FNAC & Z-N staining, along with other supportive laboratory tests can be considered to for early diagnosis. In our study AFB positivity was seen in 36.6% cases. However, the cases devoid of AFB positivity, did show a cytomorphological pattern suggestive of tuberculosis as seen on smear. Thus, FNAC appears to be more efficient in diagnosis of tubercular lymphadenitis. Moreover, being relatively cheaper, FNAC can be repeated for multiple lesions. This is of particular significance in countries with a high prevalence of tuberculosis, where FNAC along with Z-N staining should be kept as the first line of investigation in tuberculous lymphadenitis.

Conflict of Interest: None.

References

1. Mittal P, Handa U, Mohan H, Gupta V. Comparative evaluation of fine needle aspiration cytology, culture, and PCR in diagnosis of tuberculous lymphadenitis. *Diagn Cytopathol* 2010;39(11):822-826.
2. Harries A, Maher D, Graham S. TB/HIV: A clinical manual. 2nd ed. Geneva: World Health Organization, 2004:329.
3. Park K. Textbook of Preventive and Social Medicine. 20th ed. New Delhi: Banarsidas Bhanot, 2009:160.
4. Sharma SK, Mohan A. Extrapulmonary tuberculosis. I*ndian J Med Res* 2004;120:316-353.
5. Ozvaran MK, Baran R, Tor M, Dilek I, Demiryontar D, Arinc S et al. Extrapulmonary tuberculosis in non-human immunodeficiency virus-infected adults in an endemic region. *Ann Thorac Med* 2007;2:118-121.
6. Fraser W, Balasubramanian R, Mohan A, Sharma SK. Extrapulmonary tuberculosis: Management and control. Tuberculosis Control in India. In: Agarwal SP, Chauhan LS, editors. New Delhi: Elsevier;2005:95-114.
7. Gopinathan VP. Tuberculosis in the Indian scene. From a clinician's angle. *J Assoc Physicians India* 1989;37:525-528.
8. Singh KK, Muralidhar M, Kumar A, Chattopadhyaya TK, Kapila K, Singh MK, et al. Comparison of in house polymerase chain reaction with conventional techniques for the detection of Mycobacterium tuberculosis DNA in granulomatous lymphadenopathy. *J Clin Pathol* 2000;53:355-361.
9. Shrinivas MR, Dewan M. Etiology of chronic cervical lymphadenopathy in infancy and childhood. *Indian J Med Microbiol* 1988;32:65-80.
10. 10. Sharma UK. Significance of soluble antigen fluorescent antibody test in the serodiagnosis of tuberculosis. *Indian J Tuberc* 1985;32:65-80.
11. Sarnaik RM. Evaluation of kaolin agglutination test as serodiagnostic test for tuberculosis. *Indian J Tuberc* 1989;36:81-93.
12. Agarwal A, Moudgil KD. Immunodiagnosis of tuberculosis: Problems, progress and future projections. *Indian J Tuberc* 1989;36:3-14.
13. Pahwa R, Hedau S, Jain S, Jain N, Arora VM, Kumar N, et al. Assessment of possible tuberculous lymphadenopathy by PCR compared to non-molecular methods. *J Med Microbiol* 2005;54:873-878.
14. Baek CH, Kim SI, Ko YH, Chu KC. Polymerase chain reaction detection of Mycobacterium tuberculosis from fine- needle aspirate for the diagnosis of cervical tuberculous lymphadenitis. *Laryngoscope* 2000:110:30-34.
15. Rajwanshi A, Bhambhani S, das DK. Fine needle aspiration cytology in diagnosis of tuberculosis. *Diagn Cytopathol* 1987;3:13-16.
16. Afrose R, Singh N, Bhatia A, Arora VK. Cytomorphological tissue reaction patterns in lymph node tuberculosis and their correlation with bacterial density. *Ann Trop Med Public Health* 2014;7:255-262.
17. Banavaliker JN, Bhalotra B, Sharma DC. Identification of Mycobacterium tuberculosis by polymerase chain reaction in clinical specimens. *Indian J Tuberc* 1998;45:15-18.
18. Mirza S, Restrepo BI, McCormick JB, Fisher-Hoch SP. Diagnosis of tuberculosis lymphadenitis using a polymerase chain reaction on peripheral blood mononuclear cells. *Am J Trop Med Hyg* 2003;69:461-465.
19. Metre MS, Jayaram G. acid-fast bacilli in aspiration smears from tuberculous lymph nodes. An analysis of 255 cases. *Acta Cytol* 987;31:17-19.
20. Mudduwa LK, Nagahawatte Ade S. diagnosis of tuberculous lymphadenitis: Combining cytomorphology, microbiology and molecular techniques – a study from Sri Lanka. *Indian J Pathol Microbiol* 2008;51:195-197.

21. Rana S, Sharma P, Kalhan S, Singh P, Gill M, Kumar A. Cytomorphological patterns of tuberculous lymphadenitis: Experience from a tertiary centre in rural Haryana. *Scholars J App Med Sci* 2015;3(3G):1547-1552.
22. Majeed M, Bukhari M. Evaluation for granulomatous inflammation on fine needle aspiration cytology using special stains. *Pathol Res Int* 2011;2011:1-8.
23. Paliwal N, Thakur S, Mullick S, Gupta K. FNAC in tuberculous lymphadenitis: Experience from a tertiary level referral centre. *Indian J Tuberc* 2011;58:102-107.
24. Chaudhari S, Batra N, Halwal D, Bhat S. FNAC of tubercular lymph node – An alternative to excision biopsy. *Indian J Pathol Oncol* 2016;3(2):237-241.
25. Jagtap S, Jagtap S, Aher V. Fine needle aspiration cytology in diagnosis of lymphadenopathy associated with tuberculosis. *J Evidence Based Med Healthc* 2015;2(45):8127-8130.
26. Mistry Y, Ninama G, Mistry K, Rajat R, Parmar R, Godhani A. Efficacy of fine needle aspiration cytology, Ziehl-Neelsen stain and culture (Bactec) in diagnosis of tuberculous lymphadenitis. *Natl J Med Res* 2012;2(1):77-80.
27. Giri Singh K. Fine needle aspiration cytology for the diagnosis of tuberculous lymphadenitis. *Int J Cur Res Rev* 2012;4(24):124-130.
28. Hemalatha A, Shruti PS, Kumar MU, Bhaskaran A. Cytomorphological patterns of tuberculous lymphadenitis revisited. *Ann Med Health Sci Res* 2014;4:393-396.
29. Narayanamurthy C, Kodanda Swamy C. Study of cytological pattern of tubercular lymphadenitis. *Glob J Med Res* 2012;12(1):1-3.
30. Khanna A, Khanna M, Manjari M. Cytomorphological patterns in the diagnosis of tuberculous lymphadenitis. *Int J Med and Dent Sci* 2013;2(2):182-188.
31. Masilamani S, Arul P, Akshatha C. Correlation of cytomorphological patterns and acid-fast bacilli positivity in tuberculous lymphadenitis in a rural population of southern India. *J Natl Sci Biol Med.* 2015;6:S134-138.
32. Kumar A. Chapter 26. Lymph node tuberculosis. In Sharma SK (Ed). Tuberculosis, 2nd Edition. New Delhi: Jaypee Brothers Medical Publishers (P) Ltd. 2009:397-409.

How to cite this article: Vashisht N, Vartak USC, Vartak S. Study of cytomorphological spectrum of tuberculous lymphadenitis and correlation with AFB positivity. *Indian J Pathol Oncol* 2019;6(1):84-89.