International Journal of Clinical and Diagnostic Pathology

ISSN (P): 2617-7226
ISSN (E): 2617-7234
www.patholjournal.com
2020; 3(1): 475-479
Received: 15-11-2019
Accepted: 19-12-2019

Kagathara Pooja D
Senior Resident, Department of Pathology, P.D.U. Government Medical College and Hospital, Rajkot, Gujarat, India

Gandhi Shilpa H
Associate Professor, Department of Pathology, P.D.U. Government Medical College and Hospital, Rajkot, Gujarat, India

Dhruva Gauravi A
Dean, Professor & Head of Department, Department of Pathology, P.D.U. Government Medical College and Hospital, Rajkot, Gujarat, India

Corresponding Author:
Kagathara Pooja D
Senior Resident, Department of Pathology, P.D.U. Government Medical College and Hospital, Rajkot, Gujarat, India

Cytomorphological patterns of tuberculous inflammation and its comparison with positivity for tubecle bacilli by fluorescent staining

Kagathara Pooja D, Gandhi Shilpa H and Dhruva Gauravi A

DOI: https://doi.org/10.33545/pathol.2020.v3.i1g.214

Abstract
Background: Tuberculosis is a contagious, infectious disease caused by Mycobacterium Tuberculosis. Due to its complex immunological response, chronic progression and the need for long-term treatment, tuberculosis has always been a major health burden.
Aims: To study cytomorphological features of all cases of tuberculosis diagnosed on FNA smears, and to compare the positivity by fluorescent staining for mycobacterium Tuberculosis with different cytomorphological features**.**
Material & methods: The study was carried out at the Department of Pathology, P.D.U. Medical College, Rajkot, during the period of two years from September 2016 to August 2018. Total 461 cases diagnosed cytomorphologically as tuberculousis were included in the study. Cases were divided into three patterns based on Cytomorphologic features, namely Granuloma only, Granuloma with caseous Necrosis and Caseous Necrosis without Granuloma, with or without other inflammatory cells. Each case was also studied by fluorescent staining for Tubercle bacillilli. The positivity in different patterns of cytomorphologic features was compared.
Results: Among 461 cases studied, age of patients ranged from 9 months to 85 years with a mean age of 37 years and there was male predominance. Most common presentation of was cervical lymphadenopathy. The commonest cytomorphological feature was granuloma with caseous necrosis. Overall positivity for Tubercule Bacilli was 46.4%. Maximum positivity was seen in caseous necrosis without granuloma pattern, indicating increased bacterial load in such cases.
Conclusion: Fine needle aspiration cytology (FNAC) is a simple, effective and safe modality for obtaining a representative material from any palpable/non palpable lesions (under imaging guidance). Cytomorphological features of tuberculosis are well established, so it can be easily diagnosed on FNA smears. Fluorescent staining of Tubercle Bacilli is well established technique and has got advantage that bacilli are detected readily on low power objective due to sharp contrast & fluorescence. Cases in which granulomas are not seen and only caseous necrosis with or without other inflammatory cells are seen are sometimes difficult to diagnose as tuberculosis cytomorphologically. Such cases show maximum positivity for tuberculous bacilli thus helping in diagnosis in many cases.

Keywords: Cytomorphology, fluorescent, tuberculosis

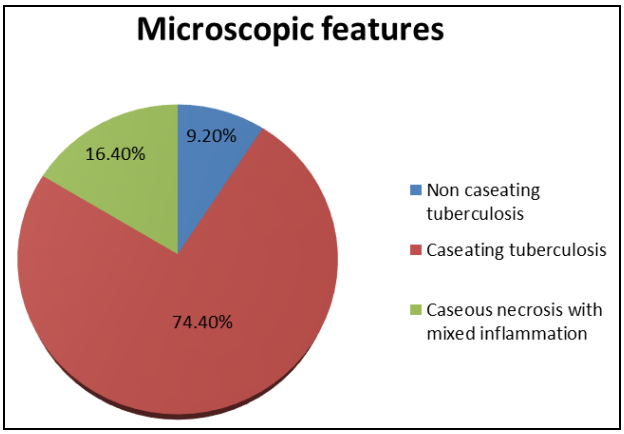
Introduction
Tuberculosis remains a major health problem faced by mankind, particularly in developing countries. Fine needle aspiration cytology (FNAC) is a simple, effective and safe modality for diagnosis of tuberculous inflammation particularly lymphadenitis and other palpable lesions. Cytomorphology of Tuberculosis is well characterized and the diagnosis can be confirmed by utilizing a number of other modalities including stains for tubercle bacilli, culture and molecular studies on aspirated material, if required. Unfortunately, culture takes weeks for identification, and its sensitivity is also relatively low in paucibacillary conditions. Newer molecular techniques such as CB-NAAT, although rapid, are costly [1]. The Ziehl-Neelsen method for demonstration of acid-fast bacilli plays a key role in the diagnosis but as it requires use of 100x oil immersion lens has got disadvantage of being cumbersome & becomes comparatively less sensitive [2, 3]. Auramine-Rhodamine staining of tubercle bacilli & visualization of bacilli by fluorescent microscopy is easier way of detection of Mycobacteria because bacilli are readily identified at lower magnifications and large areas on smear can be screened [4, 5]. The method is quick and comparatively inexpensive.

Material and method

The present study was carried out in the cytopathology laboratory of Pathology Department and Tuberculosis laboratory of P.D.U. Medical College, Rajkot, from September 2016 to August 2018- two year duration. All cases diagnosed as Tuberculosis on Cytomorphology were included in study. FNAC was done by using 10 ml syringe with 22 – 23 gauge needles using aseptic standard technique. Smears were wet fixed and air dried and stained with Hematoxyline and Eosin and Giemsa stain respectively. One or two air dried smears were heat fixed and used for fluorescent stain. Cytology smears were examined for morphology under light microscopy, to reach a diagnosis. Smears stained by fluorescent stain, were observed under fluorescent lens at low power magnification. Bacilli appear as bright yellow to orange against a dark background. Smears were either labeled as Positive – showing presence of bacilli or labeled as Negative – showing absence of bacilli.

Results

The present study was conducted to study various cytomorphological patterns of tuberculous inflammation diagnosed on FNA smears and to compare positivity of tubercle bacilli by fluorescent staining in various patterns. Total 461 cases diagnosed cytomorphologically as tuberculousis were included in the study. The age of the patients ranged from 9 months to 85 years**.** The maximum numbers of patients were in the age group of 16 to 30 years (235 cases i.e. 51%). The least affected age groups were those above 60 years, comprising of 10 cases (2.16%). There was male preponderance in this study with Male to Female ratio being 1.16:1. Cervical group lymphadenopathy, was the commonest presenting symptom seen in 387 (84%) of patients followed by Axillary lymphadenopathy in 42 (9.1%) patients and Inguinal lymphadenopathy in 13 cases (2.8%). Extranodal involvement like breast mass and chest swelling were seen in 19 cases (4.1%). Nature of aspirate was Greyish-white and granular (Cheesy aspirate) in 189 cases (41.0%) constituting the most common group followed by purulent aspirate in 143 cases (31.0%), while hemorrhagic aspirate was the least common, constituting of 129 cases (28.0%)**.** The categorization of cytomorphological findings was done into three patterns: First consisting of epithelioid granuloma and Langhan's giant cells, without caseous necrosis. Second pattern consisting of both epithelioid granuloma & typical granular eosinophilic caseous necrosis and third showing caseous necrosis only without Epithelioid Granuloma with or without presence of other inflammatory cells including Polymorhs & Lymphocytes (Fig 1, 2, 3). In present study, maximum no. of patients, showed pattern 2 i.e. both caseation with epithelioid granuloma on cytomorphology, consisting of 346 (74.4%) cases. While Caseous Necrosis without Granuloma was seen in 74 (16.4%) cases and Granuloma pattern only without necrosis was seen in least i.e. 41 (9.2%) no. of cases (Graph 1).



Graph 1: Pie diagramme-Microscopic features of tuberculous inflammation

On fluorescent staining of smears, Bacilli were demonstrated in total 214 (46.4%) of cases. Purulent aspirate showed positivity for bacilli in 106(74.1%) cases, while grey- white, cheesy aspirate showed positivity in 93 cases (49.2%) and blood mixed aspirate showed positivity in 15 cases (11.6%) for tubercle bacilli.

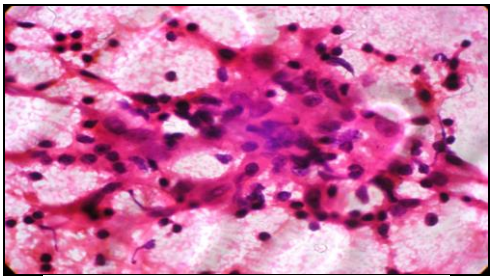


Fig 1: Epithelioid granuloma with caseous necrosis

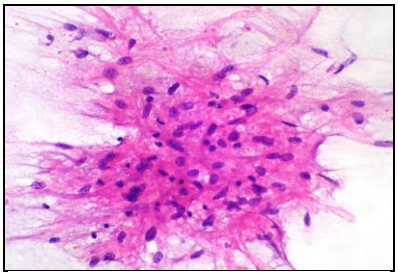


Fig 2: Epitheloid granuloma only without caseous necrosis

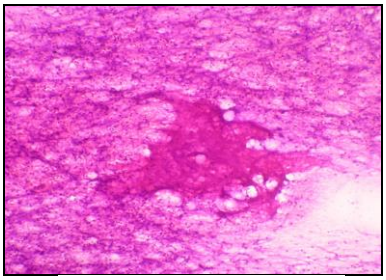


Fig 3: Caseous necrosis with inflammation

The positivity for bacilli in various cytomprphological patterns was compared (Table 1). Maximum positivity was seen in caseous necrosis without granuloma pattern consisting of 64 (86.4%) cases. Many of these cases showed presence of mixed inflammation, including many polymorphs. Least positivity (4.8%) was seen in seen in granuloma only pattern, without caseous necrosis. Cases showing both caseous Necrosis and granuloma showed 42.7% positivity for tubercle bacilli.

Table 1: Comparision of cytomorphology of tuberculosis with Bacilli demonstration

Microscopic feature	Total No. of cases	No. of cases positive for bacilli	Percentage of positivity
Non caseating tuberculosis	41	2	4.8%
Caseating tuberculosis	346	148	42.7%
Caseous necrosis with mixed inflammation	74	64	86.4%
Total	461	214	46.4%

Discussion

Tuberculosis is a common infectious disease caused by Mycobacterium Tuberculosis. It is an airborne disease and most commonly affects lungs, causing pulmonary tuberculosis. One-third of the world's population is currently infected with tuberculous bacilli, out of which 5 to 10% will develop active disease in life time. Approximately 0.2 million people die each year due to tuberculosis. Among all patients 15-20% of cases are reported as having only extrapulmonary involvement when the infection occurs in other parts of the body and is not clinically manifested in the lungs [6]. Extrapulmonary tuberculosis continues to be major health problem in developing countries and lymphadenopathy is the most common form of extrapulmonary tuberculosis [7, 8]. The present study highlights fine needle aspiration cytology (FNAC) as an inexpensive, less invasive procedure for early diagnosis of such tuberculosis and timely initiation of specific therapy [6]. The role of fine needle aspiration cytology in the evaluation of lymphadenopathies is well known [9, 10, 11, 12]. The present study involves 461 cases diagnosed cytomorphologic ally as tuberculosis, to know percentage of cases showing positivity for tubercle bacilli by fluorescent stain and to compare positivity in different cytomorphologic features.

In the present study, patient's age ranged from 9 months to 85 years, with highest no. of patients in the age range of 16-30 years. In other studies also, maximum number of patients were in age range of 16 to 30 years. The least affected age was more than 60 years, which is also comparable with other studies. (Table 2)

Table 2: Comparison of age distribution

Age group	Present study	Rajesh Singh Lashram *et al.* (2002-2007) [13]	Raghunath Rao (2010)	Geetha. J.P (2007)	Baligar S S (2014) [14]
0-15	12.36%	33.96%	20.18%	20%	15.0%
16-30	51.0%	39.00%	37.16%	50.91%	34.0%
31-45	26.03%	21.3%	28.44%	23.64%	24.0%
46-60	8.45%	3.14%	11.00%	5.45%	19.0%
> 60	2.16%	1.73%	0.92%	0%	8.0%

In the present study male preponderance was seen, suggesting that tuberculousis is more common in male. Slight discrepancy in sex ratio in different studies was due to ethnic, geographical and racial differences in study population (Table 3).

Table 3: Comparison of sex ratio

No.	Study	Year	Sex ratio (M:F)
1	Present study	2016 - 2018	1.16:1
2	Yogesh Mistry *et al.* [15]	2012	1.25:1
3	Rajesh Singh Lashram *et. al*	2002-2007	1.78:1
4	Raghunath Rao	2010	1.28:1
5	Deepjyoti V.Garde *et al.* [16]	1990	1.083:1
6	Baligar S S	2014	1:1.08

Table 4: Comparison of site of lesion

Location of lymphadenopathy	Present study	Rajesh Singh Lashram *et al. (*2002-2007)	Ajay Kochhar *et al.* (2012)	Raghunath Rao (2010)	Geetha. J.P (2007)	Baligar S S (2014)
1)Cervical group	84.0%	50.49%	85%	68.1%	69.09%	72.4%
3)Axillary	9.11%	24.13%	3.8%	9.17%	7.27%	1.14%
5)Inguinal	2.81%	9.66%	7.2%	7.34%	1.82%	1.14%
6) others	4.1%	1.9%	2.0%	1.8%	1.82%	4.59%

In the present study, cheesy white and granular aspirate was most common followed by purulent and haemorrhagic aspirates respectively. Studies done by Raghunath Rao (2010) and Goswmi H.M (2012) are in accordance with our study. While in other studies there were variations in nature of aspirates. This might be due to difference in immunity of

patients in various study groups, including immunosupression.

In present study commonest (74.4%) microscopic pattern was epithelioid granuloma with caseous necrosis, followed by caseous necrosis only without granuloma with/without mixed inflammation and Epithelioid granuloma only pattern seen in 16.4% and 9.2% of cases respectively. Our study was comparable with Geetha JP *et al*. Variation in frequency of microscopic patterns in other studies can again be explained by difference in immune status of patients studied (Table 5).

Table 5: Comparison of microscopic pattern

Microscopic features	Present study	Rajesh Singh Lashram *et al*. (2002-2007)	Goswami. H.M (2012)	Dev Prasoon *et al.* (1999) [17]	Geetha J. P *et al.* (2007)	Baligar S S (2014)
Epithelioid granuloma with caseuos necrosis	74.4%	51%	50%	44.13%	69%	35.0%
Epitheliod Granuloma only	9.2%	29.25%	29.17%	23.94%	14.54%	31.0%
Caseous Necrosis only without granuloma with/without mixed inflammation	16.4%	15.38%	20.33%	31.9%	16.36%	34.0%

In present study, positivity for tubercle bacilli was 46.4%, which was somewhat more than other studies. This might be due to use of Fluorescent staining in present study, while in other studies (Dev Prasoon *et al.*, Tarun Dua *et al.* and Rajesh Singh Lashram *et al.).* conventional Zn (AFB) staining was used to demonstrate the bacilli. Baligar S S (2014) demonsrated AFB positivity by fluorescent staining which was 44.0%, which is comparable with our study (Table 6). Thus Fluorescent staining can be considered somewhat more sensitive in detecting Tubercle bacilli as compared to conventional Zn stain.

Table 6: Comparison of positivity for Tubercle Bacilli

AFB status	Present study	Dev Prasoon *et al.* (1999)	Tarun Dua *et al.* (1996) [18]	Rajesh Singh Lashram *et al.* (2002-2007)	Baligar S S (2014)
AFB Positive	46.4%	40.8%	39.4%	42.2%	44.0%

Table 7: Comparison of microscopic features with bacilli positivity

Study	AFB% in cases showing both Granuloma & Caseation	AFB% in cases showing non caseating granuloma	AFB% in caseous necrosis only with/without mixed inflammation
Present study	42.7%	4.8%	86.4%
Geetha J.P(2007)	13.16%	0%	88.88%
Dev Prasoon *et al*. (1999)	20.58%	1.16%	78.6%
Paliwal Nidhi *et al*. (2010) [19]	68.9%	2%	82.78%
Baligar SS (2014)	88.20%	28.57%	93.50%

Predominantly granulomatous reaction with little or no co-existing necrosis was associated, with minimum positivity, while many of cases showing caseous necrosis with no granulomas showed positivity for bacilli (Table 7). Thus demonstration of bacilli is directly proportional to presence of caseous necrosis, and inversely proportional to presence of epithelioid cell granuloma. The findings are comparable with all the studies, as shown in table. Some of the cases showing caseous necrosis without Epitheliod granuloma, showed other inflammatory cells including polymorhs and it was difficult to render clear cut diagnosis of Tuberculosis morphologically. Most of such cases, showed positivity for bacilli and thus helped to reach final diagnosis.

Conclusion

Tuberculosis remains a major health problem faced by mankind, particularly in developing countries. Extrapulmonary manifestations of tuberculosis with peripheral lymphadenopathy form an important aspect of the disease, which can be easily diagnosed by FNAC. The morphological spectrum of tuberculosis varies widely depending on the stage of the disease and the immunity of the host. Predominently granulomatous reaction with little or no co-existing necrosis is associated with few or no AFB, while a predominantly necrotic material with mixed inflammation is associated with positivity for bacteria in most of the cases, denoting increased bacterial load. Fluorescent microscope is much the same as a conventional light microscope with added features to enhance its capabilities. The advantage of using fluorescent stain and microscopy to detect Tubercle bacilli lies in rapidity and increased sensitivity of detection, as low power screening would allow many more fields to be examined. When only caseation with or without mixed inflammation is present, chances of AFB positivity by fluorescent microscopy is highest, which sometimes helps in final diagnosis of tuberculosis, which may be otherwise difficult on cytomorphology only, making it a useful adjunct in diagnosis of Tuberculosis.

References

1. Savic B, Sjobring U, Alugupalli S, Larsson L, Miorner H. Evaluation of polymerase chain reaction, tuberculostearic acid analysis, and direct microscopy for the detection of Mycobacterium tuberculosis in sputum. J Infect Dis. 1992; 166(5):1177-80.
2. Daniel TM. Rapid diagnosis of tuberculosis: Laboratory techniques applicable in developing countries. Rev Infect Dis. 1989; 2(Suppl 2):471-8.
3. Balows A, Hausler WJ, Hermann KL, Shadomy HJ. Manual of clinical Microbiology. 5th ed. Washington: D.C: American society for Microbiology, 1991, 308-11.

4. Jain A, Bhargava A, Agarwal SK. A comparative study of two commonly used staining techniques for acid fast bacilli in clinical specimens. Indian Journal of Tuberculosis. 2002; 49:161-2.
5. Wright CA, van Zyl Y, Burgess SM, Blumberg L, Leiman G. Mycobacterial autofluorescence in papanicolau stained lymph node aspirates: a glimmer in the dark? Diagnostic Cytopathology. 2004; 30(4):257-60.
6. Roopa AN, Suma MN, Shariff S. Role of fine needle aspiration cytology and fluid cytology in Extrapulmonary tuberculosis. Diagnostic Cytopathology. 2012; 41:392-398.
7. Nagolimath SJ, Deodhar KP, Talib VH. Textbook of Pathology of Respiratory system. 1st edition, 1998, 386p.
8. Sheik MM, Zeenat A, Parvin A, Tyagi SP. Tuberculous lymphadenopathy in children. Indian Pediatr. 1981; 18:293-297.
9. Das DK. Lymphnodes. In: Comprehensive Cytology. M Bibbo (Ed.). Philadelphia WB Saunders, 1991, 671-702.
10. Reid AH, Milled RF, Kogan GI. Diagnostic utility of fine needle aspiration cytology in HIV-infected patients with lymphadenopathy. Cytopathology. 1998; 9:230-239.
11. Kline TS, Kannan V, Kline IK. Lymphadenopathy and aspiration biopsy cytology: review of 376 superficial nodes. Cancer lymphnodes HIVinfected individuals. Acta Cytologica. 2000; 44:60-66.
12. Gita J, Chew MC. Fine needle aspiration cytology of lymphnodes in HIV-infected individuals. Acta Cytologica. 2000; 44:60-66.
13. Rajesh Singh Laishram *et al*, Aspiration cytology for the diagnosis of tuberculous lymphadenitis-a five year study, Journal, Indian academy of clinical medicine. 2010; 21(1).
14. Baligar SS. Correlation of cytomorphological changes at FNAC with presence of acid fast bacilli detected by ZN and fluorescent stain in tuberculous lymphadenitis – a 100 case study. M V J Medical college & Research center, Bangalore, 2010.
15. Yogesh Mistry *et al.* Efficacy of FNAC and Zeihl Neelson staining in diagnosis of Tuberculous lymphadenitis, National journal of medical research. 2012; 2(1).
16. Deepjyoti V Garde *et al*., Diagnosis of tubercular cervical lymphadenitis by FNAC, microscopy and culture. Ind. J. Tub. 1991; 38:25.
17. Dev Prasoon. *et al.* Acid- Fast bacilli in FNA smears from Tuberculous lymph nodes, where to look for them Acta Cytologica. 2000; 44:3.
18. Tarun Dua *et al.* Correlation of cytomorphology with AFB positivity by smear and culture in tuberculous lymphadenitis, Indian Journal of Tuberculosis. 1996; 43:81.
19. Paliwal Nidhi *et al.*, FNAC in Tuberculous lymphadenitis: Experience from a tertiary level referral centre, Indian Journal of Tuberculosis, 2011; 58:102-107