

Different Cytomorphological Patterns In Tuberculosis In A Tertiary Health Care Centre: 5 Year Study

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Abstract

Background: Tuberculosis (TB) still remains one of the major health concerns of India and accounts for about one fourth of the global TB burden. Extra pulmonary tuberculosis contributes to 20% of the total burden. In cases of extra pulmonary tuberculosis, fine needle aspiration cytology (FNAC) is a very useful and reliable test. Cytomorphology with acid-fast staining can be valuable in diagnosing tuberculosis.

Objective: To identify and describe various cytomorphological patterns of extrapulmonary tuberculosis, and to compare results of fine needle aspiration cytology (FNAC) and Ziehl-Neelsen (ZN) stain in the diagnosis of TB.

Materials and Methods: Total 612 patients suspicious of tuberculosis referred to the Department of Pathology, Jawaharlal Nehru Medical College, Aligarh Muslim University, Aligarh, India, between October 2012 and December 2017. FNAC was performed in all these patients and smears were prepared. Smears were stained with Hematoxylin & Eosin stain, PAP and ZN stain for acid-fast bacilli (AFB).

Results: Maximum number of patients (222; 36.27%) were from age group of 11–20 years. Out of the total 612 cases included in the study, 364 (59.48%) were females and 248 (40.52%) were males. The most common site of aspiration was cervical lymphnode (76.3%) and the most common pattern of tuberculosis was epithelioid cell granuloma with caseous necrosis seen in 464 (75.8%) cases. Overall, AFB positivity was found to be the highest in caseous necrosis without granuloma (78.3%)

Conclusion: FNAC is an optimally selected, efficient, easy to perform, and economical test for initial diagnostic workup in patients with TB lymphadenitis. Supplementation of ZN stain with FNAC increases the rates of diagnosis.

Keywords: Tuberculosis, fine needle aspiration cytology, cytomorphology, Ziehl Neelsen stain

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I. Introduction:

According to the Global Tuberculosis Report by WHO, 2016, India is among the top six countries that contributed to 60% of new cases of tuberculosis in the year 2015. About 2.8 million new cases of tuberculosis were reported in India in 2015¹. Early and correct diagnosis of tuberculosis is essential to start adequate treatment. Demonstration of bacilli by using Ziehl Neelsen (ZN) stain or culture is required to confirm the diagnosis. ZN stain method is the most widely used procedure for the demonstration of Acid Fast Bacilli (AFB). It is highly specific and by far the fastest and cheapest method. However, this method has low sensitivity varying from 50%-80% relative to culture². Culture is considered to be the gold standard for diagnosing tuberculosis. Traditional culture methods like Lowenstein - Jensen culture are sensitive but are time consuming, complex, expensive and need skilled laboratory technicians with appropriate bio-safety conditions³. Extra pulmonary tuberculosis often presents as peripheral lymphadenopathy or palpable intra-abdominal masses, skin nodules or subcutaneous swelling. Fine Needle Aspiration Cytology (FNAC) of such mass lesions is a simple, less expensive, rapid, out-patient diagnostic procedure with high sensitivity and specificity for the diagnosis of EPTB⁴. FNAC is recommended as the initial diagnostic test in a TB suspected accessible mass lesion as the cytology criterion of TB has been well established.³

The aim of the study is to study the different cytomorphological patterns of extra pulmonary tuberculosis on FNAC on H&E and PAP stains over a period of 5 years.

II. Material And Method

The study was conducted on 612 patients, suspected of tuberculosis presenting to the Departments of Pathology, Jawaharlal Nehru Medical College and Hospital, Aligarh Muslim University, Aligarh from October 2012 to November 2017. After a detailed history and thorough physical examination, preliminary investigations were performed. After informed consent from the patients/guardian, FNAC was done using 22 or 23 G needle

and syringe. The varying sites of FNA included lymph nodes, superficial or subcutaneous swelling or any other palpable mass. Aspirates were smeared on glass slides and four smears were prepared. All the cytological smears were fixed with 95% ethanol and stained with Hematoxylin& Eosin (H&E) and Papanicolaou (PAP) stain. Another two slides containing aspirated material were heat fixed and stained with Ziehl Neelson stain for AFB. Aspirates from tuberculous lesion were classified into three major cell patterns: Epithelioid granulomas without necrosis, epithelioid granulomas with necrosis and necrosis without epithelioid granuloma. Acid Fast Bacilli (AFB) appeared as pinkish red, straight or slightly curved rods or beaded, occurring singly or in small groups in a greenish background on ZN stain.

Inclusion Criteria

- (1) Cases diagnosed as granulomatous inflammation consistent with tuberculosis with or without necrosis.
- (2) Cases showing extensive caseous necrosis and degenerated polymorphs suggestive of tubercular abscess.

Exclusion Criteria

- (1) Pyogenic inflammation seen on microscopy as extensive neutrophilic infiltration.
- (2) Acellular smears/smears with crushed morphology or poorly stained slides will be excluded.
- (3) Previously diagnosed cases and cases already getting ATT.
- (4) Malignancy (primary or metastatic)

The diagnosis of tuberculosis was made when the following criteria were met:

- (i) presence of epithelioid cell granuloma with or without multinucleate giant cells and caseous necrosis on H&E and PAP
- (ii) ZN smear positivity for Acid-Fast Bacilli⁵.

Granulomatous inflammation was defined by the presence of aggregates of epithelioid histiocytes forming a granuloma with or without necrosis. Sometimes multinucleated giant cells are also seen. Eosinophilic granular material containing inflammatory cells and necrotic cell debris was defined as caseous necrosis⁶. The TB abscess was described as degenerate caseous necrosis and/or liquefied necrotic material with marked degenerating and viable inflammatory cell infiltration without epithelioid granuloma.⁷

III. Result

Aspirates from 612 patients clinically suspicious of tuberculosis were enrolled in this study. 65.5% of cases were from patients below the age of 30 years. A majority of which (222; 36.27%) were from 11–20 years age group (Table 1). Females were affected more than males (59.48 %, 40.92% respectively) and the male to female ratio was approximately 0.7:1.

TABLE 1: DISTRIBUTION OF CASES ACCORDING TO AGE

AGE(IN YEARS)	NUMBER OF CASES	PERCENTAGE
0-10	44	7.19
11-20	222	36.27
21-30	179	29.25
31-40	98	16.01
41-50	32	5.23
51-60	19	3.10
>60	18	2.95
TOTAL	612	100

The most common site of FNA cytology was from cervical lymph node which comprised of 467 (76.3%) cases, followed by 49 (8.0%) cases from axillary lymph nodes, 32 (5.2%) cases from supraclavicular lymph nodes, 16 (2.6%) cases from breast, 12 (2%) cases of swelling on the back, 9 (1.5%) cases from chest wall swelling, 7 (1.1%) cases from inguinal lymph nodes, 5 (0.8%) cases from post auricular lymph nodes, 4 (0.7%) cases from abdominal wall swelling, 3 (0.5%) cases each from parotid, thyroid and pre-auricular lymph nodes and 2 (0.3%) cases from iliac fossa (Table 2).

TABLE 2: DISTRIBUTION OF CASES ACCORDING TO THE SITE OF ASPIRATION

SITE OF FNA SAMPLE	NUMBER OF CASES	PERCENTAGE
CERVICAL LYMPH NODE	467	76.30
AXILLARY LYMPH NODE	49	8.0
SUPRACLAVICULAR LYMPH NODE	32	5.2
PREAURICULAR LYMPH NODE	03	0.5
POST AURICULAR LYMPH NODE	05	0.8
INGUINAL LYMPH NODE	07	1.1
PAROTID SWELLING	03	0.5
BREAST	16	2.6
CHEST WALL SWELLING	09	1.5
SWELLING ON THE BACK	12	02
THYROID	03	0.5
ILIAC FOSSA	02	0.3
ABDOMEN	04	0.7
TOTAL	612	100

The most common cytomorphological pattern on FNAC was that of epithelioid cell granuloma with caseous necrosis seen in 464 (75.8%) cases. This pattern was characterised by well- formed epithelioid granuloma consisting of epithelioid cells, multinucleated giant cell of langhan’s type, chronic inflammatory cells and caseous necrosis in the background. The second most common pattern was that of caseous necrosis without granuloma as seen in 106 (17.3%) cases. Microscopy showed caseous necrosis with degenerated lymphocytes, foamy macrophages and numerous polymorphs. Rarely epithelioid cells could be seen. Epithelioid cell granuloma without necrosis was seen in 42 (6.9%) cases on cytology. Mainly well-defined granulomas were seen without caseous necrosis in the background (Table 3).

On cytomorphological correlation, maximum AFB positivity (78.3%) was seen in caseous necrosis without granuloma, which was followed by 30.4% positivity in epithelioid cell granuloma with caseous necrosis and least positivity (16.7%) was seen in epithelioid cell granuloma without necrosis (Table 4).

TABLE 3: DISTRIBUTION OF CASES ACCORDING TO MORPHOLOGY ON CYTOLOGY

MICROSCOPIC FINDINGS	CYTOLOGY	
	NO. OF CASES	PERCENTAGE
Epithelioid cell granuloma with necrosis	464	75.8
Epithelioid cell granuloma without necrosis	42	6.9
Caseous necrosis without granuloma	106	17.3
Total	612	100

TABLE 4: CORRELATION BETWEEN CYTOMORPHOLOGY ON H&E AND AFB POSITIVITY ON ZN STAIN

CYTOMORPHOLOGY	TOTAL NO. OF CASES ON H&E	AFB POSITIVITY	
		NO. OF CASES	PERCENTAGE
Epithelioid cell granuloma with necrosis	464	141	30.4
Epithelioid cell granuloma without necrosis	42	07	16.7
Caseous necrosis without granuloma	106	83	78.3
Total	612	231	37.8

IV. Discussion

Like pulmonary tuberculosis, Extrapulmonary Tuberculosis is a major health problem in both developing and developed countries. The number and proportion of the case reports of EPTB have increased over the last several years⁸. According to the annual reports from Revised National Tuberculosis Control Programme, Extrapulmonary Tuberculosis accounts for about one-fifth of all TB cases⁹. It often presents as peripheral lymphadenopathy or palpable intra-abdominal masses. To access such mass lesions FNAC is an excellent technique as it is simple, less expensive, rapid, out-patient diagnostic procedure with high sensitivity and specificity for the diagnosis of EPTB⁴. It is recommended as the initial diagnostic test in a TB suspected accessible mass lesion as the cytology criterion of TB has been well established³. Granulomatous inflammation is the most common cytological and histological presentation of tuberculosis. However, there are many other diseases that can result in granuloma formation such as fungal infection, sarcoidosis, actinomycosis and other inflammatory conditions¹⁰. The diagnosis of tuberculosis can be confirmed by using special stain such as ZN stain or by culture. However, ZN stain has low sensitivity and the conventional culture techniques despite being gold standard are time consuming and need skilled technical staff¹¹. Therefore, demonstration of Mycobacterium tuberculosis fine needle aspirates becomes necessary for an early and accurate treatment.

In our study, aspirates from 612 patients clinically suspicious of tuberculosis were taken. Majority of the patients, 401(65.5%) were from the age group 11- 30 years. Our study was in concordance with studies conducted by Bezabih et al.,¹² Lobo et al.,¹³ Majeed et al.,¹⁰ Patel et al.,¹⁴ and Krishna et al.,¹⁵, who also reported the maximum prevalence of the disease between the ages of 11- 30 years. Our study showed a female preponderance, with male: female ratio of 0.7:1, which was in concordance with studies by Patel et al.,¹⁶ Paliwal et al.,¹⁷ Majeed et al.,¹⁰ and Natraj et al.,¹⁸. But, other studies of Gupta et al.,¹⁹ Kivihiya- Ndugga et al.,²⁰ and Annam et al.,²¹ have shown a male preponderance. The most common site of aspiration was cervical lymph node in 467 cases (76.3%) followed by axillary lymph node in 49 cases (8.0%) which correlates with the findings of Patel et al.,¹⁴ Ahmad et al.,²² Egea et al.,²³ Bezabih et al.,¹², Majeed et al.,¹⁰ and Krishna et al.,¹⁵, who have also reported the cervical lymph node as the most common site of aspiration followed by axillary lymph nodes. The most common cytomorphological pattern was epithelioid cell granuloma with caseous necrosis seen in 464 cases (75.8%).

Our finding was in concordance with the all of the studies below that showed epithelioid cell granuloma with caseous necrosis as the most common cytomorphological pattern of tuberculosis:

STUDY	Epithelioid cell granuloma with caseous necrosis (%)	Epithelioid cell granuloma only without necrosis (%)	Caseous necrosis only (+/- inflammatory cells) (%)
Our study	81.6	7.2	11.2
Patel et al, 2015 ¹⁴	35.61	-	23.92
Krishna et al, 2016 ¹⁵	85.23	79.55	-
Thakur et al, 2013 ²⁴	57.8	-	31.1
Joshi et al, 2012 ²⁵	62.5	37.5	-
Majeed et al, 2011 ¹⁰	69	31	-
Ahmad et al, 2005 ²²	45.8	28.9	25.3
Bezabih et al, 2002 ¹²	32.8	15.6	51.2
Gupta et al, 2002 ¹⁹	50.35	32.14	14.65

Highest AFB positivity by ZN stain was 78.3%, seen in caseous necrosis without granuloma followed by 30.4% in epithelioid cell granuloma with necrosis and least AFB positivity was seen in epithelioid cell granuloma without necrosis in 16.7% of cases. Our study was in concordance with Majeed et al.,¹⁰ Patel et al.,¹⁴ Gupta et al.,²⁰⁰²¹⁹ and Ahmad et al²².

V. Conclusion

Cytomorphology obtained by FNAC is useful in diagnosing tuberculosis. FNA is a safe and reliable approach for obtaining samples from superficial lesions, including lymph nodes, skin, and soft tissue nodules to study cytomorphology. However, granulomatous inflammation which is commonly seen in tuberculosis on cytology is also caused by a number of other infectious and non-infectious diseases. Demonstration of bacilli in the aspirates by using special stains such as ZN stain increases the diagnostic accuracy of FNA. Moreover, aspirates can also be subjected to culture for confirming the diagnosis.

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