



Research Article

Cytomorphological findings in drug defaulters of tuberculous lymphadenitis

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ABSTRACT

Objectives: Tuberculosis (TB) is an important health problem specially in the developing countries. Although effective chemotherapy is available, compliance of patient is a major challenge. The aim of this study was to describe cytomorphological features of drug defaulters on lymph node on fine-needle aspiration cytology (FNAC) and compare it with newly diagnosed cases.

Material and Methods: A cross-sectional study was done for a period of 18 months, in which all patients having tuberculous lymphadenitis were included after taking detailed history regarding previous anti-TB drug intake. Cytomorphological findings in drug defaulters were evaluated and compared with newly diagnosed cases.

Results: There were a total of 150 cases with 120 newly diagnosed and 30 drug defaulters. Splintered epithelioid cell granuloma was seen in 40% of drug defaulters, while it was absent in case of newly diagnosed cases ($P < 0.0001$). Well-formed epithelioid cell granuloma was most commonly seen in newly diagnosed cases (65.83%) followed by poorly formed granuloma (26.67%). Another significant finding was distribution of eosinophils which were significantly high in case of drug defaulters (56.67%) ($P < 0.0001$).

Conclusion: Cytomorphological findings can help in identification of the drug defaulters on FNAC and thus plan further line of treatment.

Keywords: Defaulters, Fine-needle aspiration cytology, Splintered, Granuloma, Epithelioid

INTRODUCTION

Tuberculosis (TB) has been a global health problem since decades. The World Health Organization has estimated it to affect more than a billion individuals worldwide with 10 million new cases and 1.5 million deaths occurring each year. India has the highest global burden and accounts for nearly 20% cases globally.^[1]

Although effective chemotherapy is available, compliance of the patient is a major challenge. According to a study, around one-quarter of TB patient undergo “Re-treatment” due to drug default. Drug defaulter is defined as the patients who have not taken anti-TB drugs for at least 2 months after starting treatment of at least 1 month.^[2]

Drug defaulter poses a threat to society by spreading disease in community. Treatment outcome observed in them is worse compared to newly diagnosed cases, with increased morbidity and

mortality. Development of drug resistance is seen in them which further increases cost as well as length of treatment.^[3]

Long duration of treatment which needs to be continued even after apparent clinical recovery is one of the main reasons for non-completion of their course of treatment. Other reasons for default include lack of awareness of disease severity, social stigma, financial burden, fear of side effects, older and younger age group, and illiteracy.^[4]

The most common organ involved in TB is lung. Tuberculous lymphadenitis is the most common form of extrapulmonary TB, cervical lymph node being most commonly affected.^[5]

Fine-needle aspiration cytology (FNAC) is increasingly being used for diagnosis of enlarged peripheral lymph nodes. Sensitivity of FNAC ranges from 46% to 87%.^[6] With limited resources and high burden of TB, it has got several advantages of being rapid, simple as well as repeatable, minimally invasive, and cost effective and can be performed on outpatient basis. FNAC of tubercular lymph node shows eosinophilic homogenous caseous necrosis surrounded by inflammatory cells which include lymphocytes, neutrophils, and epithelioid cells which fuse to form giant cells.^[7]

Although several studies have been published regarding socioeconomic conditions and risk factors of defaulters, there are limited data regarding cytomorphological and radiological details in them. Diagnosis of defaulters is solely dependent on history given by patient. Out of fear, the patient hesitates to give history of previous TB treatment as they fail to adhere to full course treatment “as directed” by treating physician. Non-disclosure of TB treatment history can result in erroneous treatment as treatment regime of defaulters is more intensive than standard first-line treatment. The aim of the present study was to evaluate diagnostic findings of defaulters which could help practitioner in suspecting cases of previous default and plan treatment accordingly.

MATERIAL AND METHODS

The study was a cross-sectional study carried out for a period of 18 months at a tertiary care hospital after obtaining clearance from the Institute Ethics Committee and patient consent. All patients referred to the Department of Pathology with tuberculous lymphadenitis diagnosed on the basis of clinical history/culture/ Cartridge based nucleic acid amplification test (CBNAAT) were considered for the study. Clinical details including radiological details along with previous drug compliance were taken to identify drug defaulters.

Inclusion criteria

The following criteria were included in the study:

- Newly diagnosed patients were those who have never been treated for TB or taken any anti-TB drugs for <1 month

- Drug defaulters were defined as the patients who had not taken anti-TB drugs for at least 2 months after starting treatment of at least 1 month.^[8]

Exclusion criteria

The following criteria were excluded from the study:

- Known cases of nephrotic syndrome, malignancy, acquired immunodeficiency syndrome or autoimmune disease
- Individual on immune suppressive drugs.

FNAC was carried out using 23 Gauge 10 cc disposable syringes. Aspirated material was stained with May-Grunwald-Giemsa, Papanicolaou, Ziehl-Neelsen, and Van Gieson stain. The stained smears were examined under microscope and interpreted.

Overall cell population and predominant pattern was assessed under low power. The individual cell morphology, type of giant cell, caseous necrosis, epithelioid cell, type of cell infiltration, thin or thick fibrotic tissue, and leaching of degenerated lymphoid and epithelioid cells were evaluated under high magnification.

The cytomorphologic pattern of granuloma^[6] was categorized into following patterns:

1. Well-composed epithelioid cell granuloma
2. Poorly formed epithelioid cell granuloma
3. Splintered epithelioid cell granuloma
4. Occasional degenerated lymphoid cells.

Well-composed epithelioid cell granuloma was defined as epithelioid cells along with caseous necrosis, lymphocytes, and giant cells. Poorly formed granuloma was defined as a group of epithelioid cells along with caseous necrosis or lymphocytic infiltrate. Splintered epithelioid cell granulomas consisted of dispersed epithelioid cells with or without lymphocytic infiltration. Other cytological parameters evaluated included-Langhans and foreign body type of giant cells, necrosis, degenerating epithelioid cells, thick/thin fibrotic bands, leaching of degenerating lymphoid and epithelioid cells, and eosinophilic and neutrophilic infiltrate.

ZN staining was done and interpretation of the result was done based on the criteria of number of bacilli in varied number of oil immersion fields – “scanty” for 1–9 acid-fast bacilli (AFB)/100 oil immersion fields, 1+ for 10 to 99 AFB/100 oil immersion field, 2+ for 1 to 10 AFB/oil immersion field, and 3+ for >10/oil immersion field.^[9]

Statistical analysis

The analysis was done with the Statistical Package for the Social Sciences software, IBM manufacturer, Chicago, USA, version 25.0. Mann-Whitney test and Chi-square

test/Fischer's exact test were used for quantitative and qualitative variables, respectively.

RESULTS

A total of 120 newly diagnosed cases and 30 drug defaulters were included in study after detailed clinical history and drug intake history. The age of the patients in this study ranged from 5 years to 74 years with a mean age of 24.87 ± 11.84 years with no significant difference between newly diagnosed cases and drug defaulters.

The distribution of clinical features – fever (23.33% vs. 26.67%), TB contact (25% vs. 16.67%), and matted lymph nodes (15.83% vs. 13.33%) was comparable between newly diagnosed cases and drug defaulters. However, proportion of patients with pulmonary TB was significantly lower in newly diagnosed cases as compared to drug defaulters (7.50% vs. 20%, respectively) ($P = 0.041$).

Majority of newly diagnosed patients showed well-formed granulomas as compared to splintered granulomas in the drug defaulter patients ($P < 0.0001$) [Figure 1]. Other cytological parameters such as giant cells, neutrophils, presence of fibrotic tissue, necrosis, and leaching were comparable between the two groups [Figure 2]. However, eosinophilic infiltrate was significantly lower in the newly diagnosed cases as compared to the drug defaulters ($P < 0.0001$).

Proportion of patients with AFB grading was comparable in newly diagnosed cases and drug defaulters (Scanty:- 81.67% vs. 90%, respectively, 1:-17.50% vs. 10% respectively).

The clinical findings, granuloma type, other FNAC findings, and AFB grading are tabulated in [Tables 1-4], respectively.

DISCUSSION

Despite availability of treatment, TB remains a major health concern especially in developing nation like India. Around 19.3–39.3% patients present with extrapulmonary TB with or without pulmonary TB.

Tubercular lymphadenitis can be seen in patients in the early to advanced age. The age of the patients in this study ranged

from 5 years to 74 years with a mean age of 24.87 ± 11.84 years. Age distribution was in accordance with the study done by Ratnesh, Bhadke *et al.*, and Chand *et al.*^[8,10,11] More than half of the patients belonged to age group 11–30 years accounting for 70.67%. This explains that TB most commonly affects the middle age group individuals. The more rampant spread and infection in this population could be due to congregate working conditions and crowded spaces. Distribution of age was comparable between newly diagnosed cases and drug defaulters with no significant difference between them ($P = 0.489$). Result was in concordance to the study done by Bhadke *et al.*^[8]

A slight female preponderance was seen in this study with male-to-female ratio of 1:1.6. Paliwal *et al.* and Chand *et al.* also reported similar results.^[5,11] Higher incidence in females, specially belonging to households of lower strata of society, may be attributed to poor nutritional status, contact with cattle, ignorance, reluctance to move out of homes, and overall lower standard of living mainly in developing countries. Distribution of gender was comparable between newly diagnosed cases and drug defaulters with no significant difference ($P = 0.933$) which is comparable to the results of Ratnesh.^[10]

Most of the patients presented with slowly enlarging cervical lymph nodes. According to the study by Purohit *et al.*, only 56.6% of the patients had constitutional symptoms. Constitutional symptoms can be fever, cough, night sweats, loss of weight, and weakness. Fever was the most prevalent constitutional symptom.^[12]

Fever in TB is usually low grade and associated with evening rise of temperature. In the present study, fever was observed in 24% of cases. Incidence of fever is variable in different studies. Wei *et al.* and Mukherjee and Ramamurthy reported lower incidence of fever (10.3% and 18%, respectively) in their study.^[13,14] Absence of fever may be due to previous history of TB. However, in the present study, distribution of fever was equal among both the groups ($P = 0.702$).

History of TB contact was seen in 23.33% of cases (comparable between newly diagnosed cases and drug

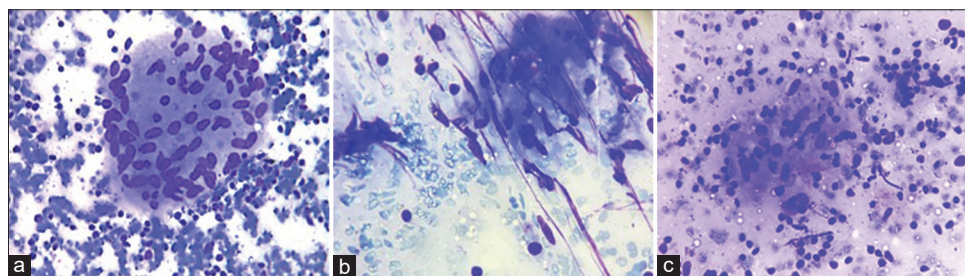


Figure 1: Well-formed granuloma (a) and splintered epithelioid cell granuloma (b and c) in a newly diagnosed case and drug defaulter, respectively (May–Grunwald–Giemsa, $\times 400$).

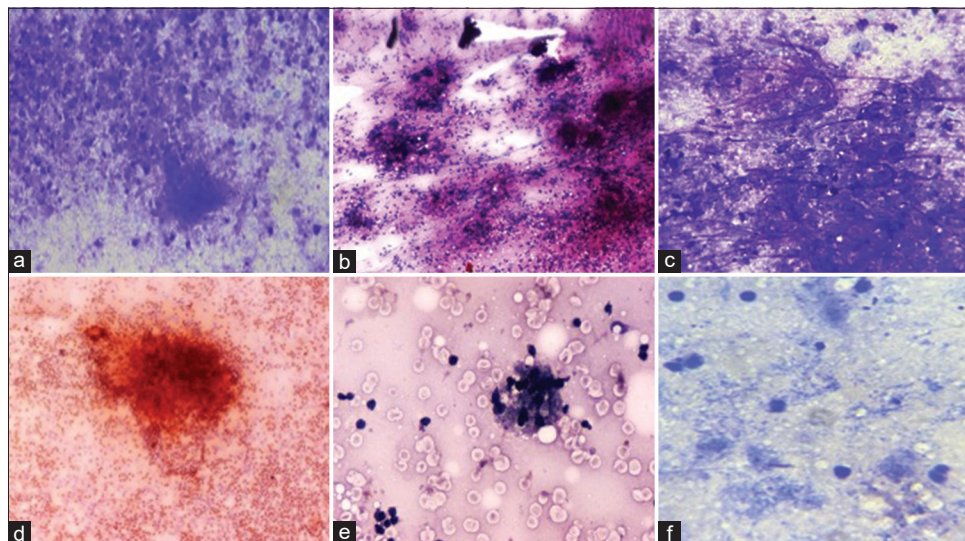


Figure 2: Cytological findings in a case of drug defaulter-Caseous necrosis (a), splintered granuloma enmeshed in fibrous tissue (b-d), occasional degenerated epithelioid and lymphoid cell (e) and acid-fast bacilli 1+ (f) (May–Grunwald–Giemsa, Papanicolaou, Ziehl–Neelsen, and Van Gieson; $\times 400$, $\times 1000$).

Table 1: Comparison of clinical findings between newly diagnosed cases and drug defaulters.

Clinical features	Newly diagnosed cases ($n=120$) (%)	Drug defaulters ($n=30$) (%)	Total ($n=150$) (%)	P-value
Fever	28 (23.33)	8 (26.67)	36 (24)	0.702
TB contact	30 (25)	5 (16.67)	35 (23.33)	0.334
Matted lymph node	19 (15.83)	4 (13.33)	23 (15.33)	1
Pulmonary tuberculosis	9 (7.50)	6 (20)	135 (90)	0.041

TB: Tuberculosis

Table 2: Comparison of granuloma type between newly diagnosed cases and drug defaulters.

Granuloma type	Newly diagnosed cases ($n=120$) (%)	Drug defaulters ($n=30$) (%)	Total ($n=150$) (%)	P-value
Occasional degenerated epithelioid cell	15 (12.50)	10 (33.33)	25 (16.67)	0.014 [†]
Poorly formed epithelioid cell granuloma	26 (21.67)	8 (26.67)	34 (22.67)	0.733 [†]
Splintered epithelioid cell granuloma	0 (0)	12 (40)	12 (8)	<0.0001*
Well-formed epithelioid cell granuloma	79 (65.83)	0 (0)	79 (52.66)	<0.0001*
Total	120 (100)	30 (100)	150 (100)	-

defaulters with $P = 0.334$) which is similar to the data of Mukherjee and Ramamurthy, where a positive contact history was present in 22.7% cases. Wei *et al.*, showed that 37% subjects had history of contact exposure.^[13]

Characteristic features of tuberculous lymphadenitis are multiplicity, matting, and caseation. In the present study, matted lymph node was seen in only 15.33% of patients. Incidence as high as 68.18% of matted lymph nodes was reported in a study done Mukherjee and Ramamurthy.^[14]

Fine-needle aspiration cytology is the first-line investigation in patient with enlarged lymphadenopathy. Well-formed epithelioid cell granuloma was most commonly seen in newly diagnosed cases in 65.83% cases followed by poorly formed epithelioid cell granuloma (26.67%). None of the drug defaulter showed a well-formed granuloma. Findings were similar to the study done by Zaheer *et al.*, where well-formed granuloma is seen in 60% of newly diagnosed cases.^[6]

Table 3: Comparison of FNAC findings between newly diagnosed cases and drug defaulters.

FNAC findings	Newly diagnosed cases (n=120) (%)	Drug defaulters (n=30) (%)	Total (n=150) (%)	P-value
Caseous necrosis	83 (69.17)	22 (73.33)	105 (70)	0.656
Giant cells	14 (11.67)	2 (6.67)	16 (10.67)	0.740
Eosinophilic infiltrate	21 (17.50)	17 (56.67)	38 (25.33)	<0.0001
Neutrophilic infiltrate	24 (20)	10 (33.33)	34 (22.67)	0.119
Thin fibrotic tissue	75 (62.50)	19 (63.33)	94 (62.67)	1
Thick fibrotic tissue	31 (25.83)	8 (26.67)	39 (26)	1

FNAC: Fine-needle aspiration cytology

Table 4: Comparison of AFB grading between newly diagnosed cases and drug defaulters.

AFB grading	Newly diagnosed cases (n=120) (%)	Drug defaulters (n=20) (%)	Total (%)
Scanty	98 (81.67)	18 (90)	116 (82.85)
1	21 (17.50)	2 (10)	23 (16.42)
2	1 (0.83)	0 (0)	1 (0.71)
Total	120 (100)	20 (100)	140 (100)

AFB: Acid-fast bacilli

Splintered epithelioid cell granuloma was seen in 40% of drug defaulters and while it was absent in case of newly diagnosed cases ($P < 0.0001$). Hence, splintered epithelioid cell granuloma was the characteristic feature found in drug defaulters. Proportion of poorly formed granuloma was comparable between newly diagnosed cases and drug defaulters ($P = 0.733$). Occasional degenerated epithelioid cells were seen more commonly on drug defaulters (33.33%) compared to newly diagnosed cases (12.50%), which may have resulted from previous drug intake.

In our study, only 16% of subjects showed presence of giant cells. A study by Khajuria and Singh reported giant cells in 22.8% of cases.^[15] Giant cells were seen associated with epithelioid cell clusters and completely absent in smears having occasional degenerated epithelioid cells. About 11.67% of newly diagnosed cases and 6.67% of drug defaulters had giant cells in the smears examined. Langhan type as well as foreign body type giant cells were seen. There was no significant difference in the presence of giant cells in smears examined from the both groups ($P = 0.740$).

Eosinophils were seen in around 25.33% cases. Distribution of eosinophils was significantly lower in case of newly diagnosed cases (17.50%) as compared to drug defaulters (56.67%) ($P < 0.0001$). Thus, eosinophils could be used as a marker of previous drug intake. The findings are similar to a study by Zaheer *et al.*, in which 67.5% of drug defaulters had eosinophilic infiltration.^[6]

In our study, necrosis was present in 70% of cases. Similar results were seen in study by Khajuria and Singh, in which necrosis was seen in 89.9% of cases.^[15] While, a study done by Paliwal *et al.* reported necrosis in only 39.2% of cases.^[5] Proportion of distribution of necrosis was comparable between newly diagnosed cases (69.17%) and drug defaulters (73.33%) ($P = 0.656$).

Distribution of fibrotic tissue was comparable between newly diagnosed cases and drug defaulter ($P = 1$). Results were in contrast to study done by Zaheer *et al.*, where fibrotic tissue was more commonly seen in drug defaulters compared to newly diagnosed cases.^[6]

Coupling FNA with ZN staining increases diagnostic accuracy. No bacilli were seen in 10 (33.3%) cases of drug defaulters, so they were excluded from grading. In newly diagnosed cases, AFB grade was scanty in 81.67%, 1+ in 17.50%, and 2+ in 0.83% cases, respectively. A study by Masilamani *et al.* has grading of scanty in 29.7%, 1+ in 28.8%, and 2+ in 41.5% cases. This difference in grading might be due to inclusion of immunodeficient individuals.^[16,17] Higher grade in newly diagnosed cases was seen in cases having well-formed granuloma in contrast to the previous studies. AFB positivity in drug defaulters was seen in 66.6% of cases which are in contrast to previous studies where all drug defaulters were positive for AFB.^[18] Distribution of AFB grade was similar in drug defaulters having scanty in 90% and 1+ in 10%. No drug defaulter had 3+ AFB grading. Proportion of AFB grading between newly diagnosed cases and drug defaulters was comparable. Hence, incomplete treatment does not offer any additional benefit in decreasing the burden of disease. In contrast, it might lead to development of multidrug resistant TB.

In the present study, pulmonary lesion on X-ray was present in 10% of the cases. Similar results were seen in the study done by Mukherjee and Ramamurthy, in which chest X-ray positivity was seen in 9.09% cases, while majority (90.9%) had normal chest X-ray.^[14] In contrast, a study by Wei *et al.* showed higher proportion (33%) of association of pulmonary lesion along with tubercular lymphadenitis.^[15] Proportion of patients with pulmonary lesion was significantly lower

in newly diagnosed cases (7.50%) as compared to drug defaulters (20%) ($P = 0.041$).

SUMMARY

Thus, we can conclude that cytological findings of splintered epithelioid cell granuloma and eosinophilic infiltrate were seen characteristically in drug defaulters. Suspected drug defaulters on cytomorphology can alert treating physician to take detail drug intake history along with drug resistance testing and monitor and treat the patient accordingly.

COMPETING INTEREST STATEMENT BY ALL AUTHORS

The authors declare no potential conflicts of interest and no source of financial support.

AUTHORSHIP STATEMENT BY ALL AUTHORS

All authors state that they contributed to this publication according to the guidelines of the journal and no part of this manuscript was plagiarized.

ETHICS STATEMENT BY ALL AUTHORS

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LIST OF ABBREVIATIONS (In alphabetic order)

AFB- Acid fast bacilli
 CBNAAT- Cartridge based nucleic acid amplification test
 FNAC- Fine needle aspiration cytology
 SPSS- Statistical package for social sciences
 TB- Tuberculosis
 USA- United States of America
 ZN- Ziehl Neelson

EDITORIAL/PEER-REVIEW STATEMENT

To ensure the integrity and highest quality of CytoJournal publications, the review process of this manuscript was

conducted under a **double-blind model** (authors are blinded for reviewers and vice versa) through automatic online system.

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