Research Article

Comparison of fine-needle aspiration technique with Ziehl–Neelsen stains in diagnosis of tuberculous lymphadenitis

Jignasha M Patel[1], Kamini R Patel[1], Kamlesh Shah[1], Niraj U Patel[2], Hinal Baria[3], Prashant D Patel[4]

[1]Department of Pathology, GMERS Medical College & Hospital, Vadodara, Gujarat, India.
[2]Department of Biochemistry, GMERS Medical College & Hospital, Vadodara, Gujarat, India.
[3]Department of Community Medicine, GMERS Medical College & Hospital, Vadodara, Gujarat, India.
[4]Department of Dentistry, SSG Hospital, Vadodara, Gujarat, India.
Correspondence to: Jignasha M Patel, E-mail: dr.jignashapatel@gmail.com

Received September 11, 2014. Accepted November 23, 2014

Abstract

Background: Lymphadenopathy is one of the most common clinical presentation among patients. In developing countries such as India, tuberculous (TB) lymphadenitis is one of the common causes of lymphadenopathy. Cytomorphology with acid-fast staining is a valuable diagnostic tool in cases of tuberculosis.

Objective: To describe pattern of TB lymphadenitis presentation, and to compare results of fine needle aspiration cytology (FNAC) and Ziehl–Neelsen (ZN) stain in the diagnosis of TB lymphadenitis.

Materials and Methods: Total 351 patients of lymphadenopathy referred to the Department of Pathology, GMERS Medical College & Hospital, Vadodara, Gujarat, India, between March 2011 and December 2013included. FNAC was performed in all these patients and smears were prepared. Smears were stained with hematoxylin and eosin stain. ZN staining for acid-fast bacilli (AFB) was carried out on separate slide.

Results: Maximum number of patients (162; 46.15%) were from age group of 11–30 years. Total 351 cases [179 (50.99%) females and 172 (49.00%) males] were included in the study. Of 351 cases, 173 (49.28%) had tuberculosis, 141 (40.17%) had lymphadenitis other than tuberculosis, and 37 (10.54%) had malignant lymphadenopathy, including 2 (0.56%) cases of primary malignancy (i.e., lymphoma) and 35 (09.97%) of metastasis to lymph nodes. Overall, 119 (33.90%) cases were found to be positive for AFB on ZN staining.

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.

KEY WORDS: Tuberculosis, lymphadenopathy, fine-needle aspiration cytology, Ziehl-Neelsen stain

Introduction

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 of a global incidence of 8.7 million cases.[1]

Access this article online	
Website: http://www.ijmsph.com	Quick Response Code:
DOI: 10.5455/ijmsph.2015.1109201482	

In extrapulmonary tuberculosis, the most common presentation is cervical lymphadenopathy, especially among the Asian populations.[2,3] Lymph node enlargement could be due to tuberculosis, other inflammatory disease or fungal infection, or some underlying malignancy.[4] In general, tuberculous (TB) lymphadenitis is diagnosed using conventional methods such as histopathology on 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.[5]

Fine-needle aspiration cytology (FNAC) is a simple, quicker, reliable, minimally invasive, and relatively cheap diagnostic modality with minimal risk of complications.[6] The efficacy of FNAC to diagnose TB lymphadenitis is directly

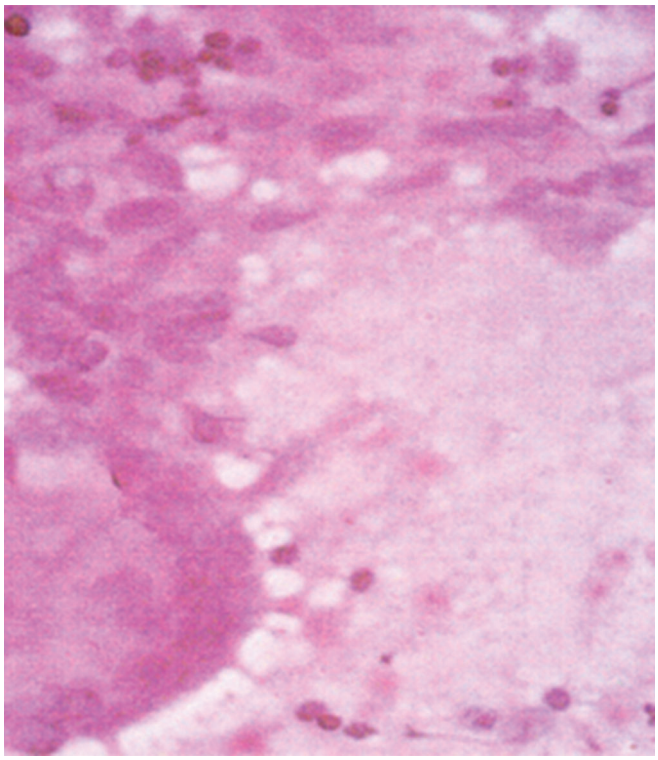

Figure 1: Granuloma formation, H&E stain, 40× .

proportional to presence of purulent material in sample.[7] AFB are commonly seen 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.[3]

The aims and objective of this prospective study were (1) to describe presentation pattern of TB lymphadenitis and (2) to compare results of FNAC and ZN stain in the diagnosis of TB lymphadenitis.

Materials and Methods

Total 351 clinically diagnosed patients of lymphadenopathy referred to the Department of Pathology, GMERS Medical College & Hospital, Vadodara, Gujarat, India, between March 2011 and December 2013 were included in this study. The variables included in the study were age, sex, and site of lesion. Relevant history and examination of nodes were recorded. Nodes were aspirated after all aseptic measures with sterile disposable 23-G needle attached with 10 cc disposable syringe. Multiple smears were prepared with part of aspirated material; two to three smears were stained with hematoxylin and eosin (H&E) stain and ZN staining was performed on separate slide. All data were grouped and analyzed. Smears stained with H&E stain were examined under microscope for the presence of granuloma, necrosis, Langhans giant cells, plasma cells, lymphocytes, macrophages, and neutrophils. Smears stained with ZN stain were examined under oil immersion objective for AFB. Presence of sheets of epithelioid cells with lymphocytes and plasma cells with or without multinucleated giant cells were diagnosed as granulomatous lymphadenitis, and eosinophilic granular material containing inflammatory cells and necrotic cell debris was defined as caseous necrosis.[7] 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.[8]

Results

Aspirates from 351 patients were enrolled in this study with clinically diagnosed cases of lymphadenopathy. A majority of patients (162; 46.15%) were from 11–30 years age group [Table 1]. There were 179 (50.99%) female and 172 (49.00%) male patients with female/male ratio approximately 1.04:1. The most common site involved in lymphadenopathy was cervical in 339 (96.58%) cases followed by axillary in 8 (02.27%) and inguinal in 4 (01.13%) [Table 2]. TB lymphadenitis was found in 173 (49.28%) cases, inflammatory lymphadenitis other than tuberculosis in 141 (40.17%), and malignant lymphadenopathy in remaining 37 (10.54%) cases, consisting 2 (00.56%) cases of primary malignancy (i.e., lymphoma) and 35 (09.97%) of metastasis to lymph node [Table 3]. Of 314 cases of lymphadenitis, ZN stain was found to be positive for AFB in 119 (33.90%) cases [Table 4].

Table 1: Age- and sex-wise distribution of cases of lymphadenopathy

Age (in years)	Male	Female	Total	Percentage
0–10	17	12	29	08.26
11–20	29	49	78	22.22
21–30	34	50	84	23.93
31–40	34	29	63	17.94
41–50	26	18	44	12.53
51–60	22	13	35	09.97
61–70	08	08	16	04.55
>70	02	00	02	00.56
Total	172	179	351	100
Percentage	49.00	50.99	100	-

Table 2: Site of lymph node involvement

Site	No. of cases	Percentage
Cervical lymph nodes	339	96.58
Axillary lymph nodes	08	02.27
Inguinal lymph nodes	04	01.13
Total	351	100

Table 3: Various cytomorphological picture in cases of lymphadenopathy

Type of lesion	Cytomorphological diagnosis	No. of cases	Percentage
Nonneoplastic (inflammatory lymphadenitis)	Tuberculous lymphadenitis	173	49.28
	Chronic nonspecific lymphadenitis	105	29.91
Acute lymphadenitis	36	10.25	
Neoplastic (malignant lymphadenopathy)	Metastasis to lymph node	35	09.97
	Hodgkin's lymphoma	01	00.28
Non-Hodgkin's lymphoma	01	00.28	
Total	351	100	

Table 4: AFB positivity in various cytomorphological subpatterns in cases of lymphadenitis

Cytomorphological picture	AFB positive cases	AFB negative cases	Total	Percentage
Epithelioid granuloma with caseous necrosis	71	54	125	35.61
Necrosis only without inflammatory cells	08	00	08	02.27
Necrosis with polymorphs	40	36	76	21.65
Neither necrosis nor granuloma	00	105	105	29.91
Total	119	195	314	89.45
Percentage	33.90	55.55	-	89.45

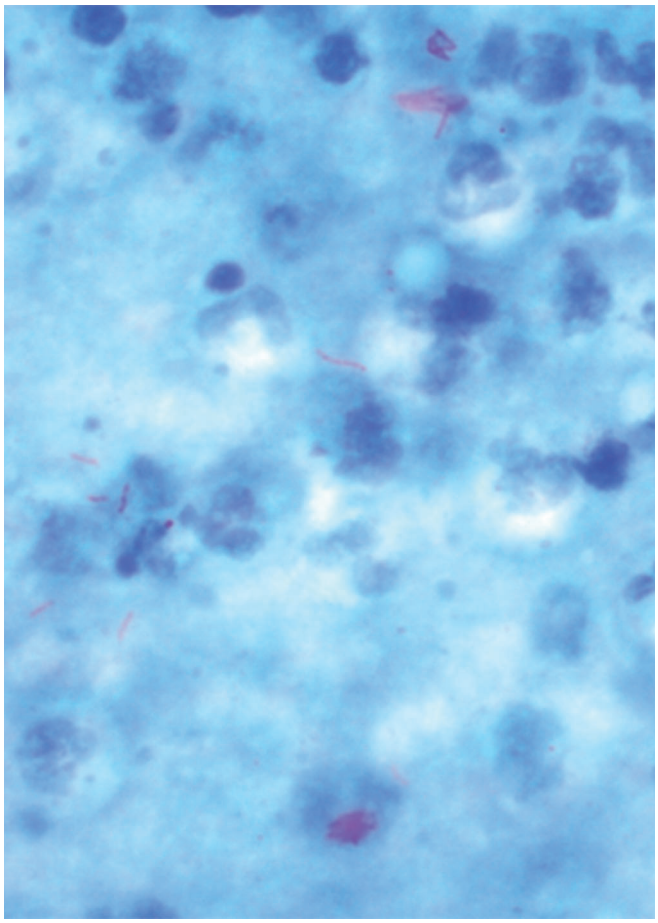

Figure 2: Acid-fast bacilli in ZN stain, ZN stain, 100×.

Discussion

India has the highest TB burden as shown in the 2011 World Health Organization (WHO) statistics.[1] The diagnosis of extrapulmonary tuberculosis still remains to be more of a clinical decision. Not many clinically sensitive tests are available in India to assist the treating physician. For accurate diagnosis of *M. tuberculosis*, isolation and culture of organism is gold standard, but as *M. tuberculosis* is slow growing organism, culture on conventional Lowenstein–Jensen medium takes 6–8 weeks. Middlebrook medium isolates growth of organism comparatively more rapidly. Mean duration to yield positive culture is about 3 weeks. But for the disease such as tuberculosis, this is too long to wait for results of culture as it is necessary to start treatment at the earliest. Therefore, comparatively rapid diagnostic strategies need to be established for diagnosis of TB lymphadenitis.[9] FNAC is a well-established diagnostic technique for lymphadenopathy evaluation. It is cost effective, safe, minimally invasive, and rapid method of diagnosing not only TB lymphadenitis but also other pathologies. It also avoids the possible physical and psychological complications of an excision biopsy.[9,10] In this prospective study, we have examined 351 cases of lymphadenopathy referred to the Department of Pathology. The finding that the majority of the patients (162; 46.15%) were from 11–30 years age group correlates with those of the other studies conducted by Bezabih and Mariam,[4] Lobo et al.,[12] Teklu et al.,[13] Hart et al.,[14] and Majeed and Bukhari.[15] Most common site involved was cervical region in 339 (96.58%) cases, which also correlates with the findings of other studies carried out by Bezabih et al.,[4] Lau et al.,[7] and Chen et al.[16] Tuberculosis was the most common finding in 173 (49.28%) cases, followed by other inflammatory

lymphadenitis in 141 (40.17%). ZN stain was found to be positive for AFB in 119 (33.90%) cases, which correlates with the findings of other studies conducted by Majeed and Bukhari,[15] Kheiry and Ahmed,[17] and Rajwanshi et al.,[18] which reported ZN positivity of 37.4%, 59.4%, and 40% respectively. Most common cytological pattern observed was epithelioid granuloma with caseous necrosis and with or without Langhans giant cells in 125 (35.61%) cases, which is similar to the study conducted by Gupta et al.[19] Highest AFB positivity was seen in 119 (33.90%) cases with necrosis with or without granuloma and inflammatory cells. Few cases (54; 15.38%) with necrosis and granuloma showed AFB negativity whereas 36 (10.25%) smears that showed necrosis and polymorphs were reported as suppurative lymphadenopathy and 105 (29.62%) cases that did not show necrosis or granuloma and also were negative for AFB were reported as chronic nonspecific lymphadenitis, which is also similar to the study conducted by Gupta et al.[19] 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.[20]

Conclusion

Cytomorphological features of FNAC on H&E stain have significant diagnostic yield. 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 diagnostic yield. AFB were mostly seen in purulent aspirate whether acellular or accompanied by granuloma. In the absence of ZN staining, case can be misinterpreted as an acute lymphadenitis.

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How to cite this article: Patel JM, Patel KR, Shah K, Patel NU, Baria H, Patel PD. Comparison of fine-needle aspiration technique with Ziehl–Neelsen stains in diagnosis of tuberculous lymphadenitis. Int J Med Sci Public Health 2015;4:400-403

Source of Support: Nil, **Conflict of Interest:** None declared.