Original Research Article

Correlation of histopathological and cytological features in proved tubercular lymphadenitis

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Abstract

Background: Changes in pattern and incidence of Tuberculosis have strikingly altered the etiology of mycobacterial lymphadenitis as children are usually more affected by atypical form while adults and geriatrics are mostly infected by *M.tuberculosis*.[5] Diagnosis of tuberculous lymphadenitis is mostly clinical and histopathological but in some cases where microscopic appearances are not exactly typical so diagnosis becomes difficult. **Material and method**: The study protocol included 150 patients more than 15years belonging to both sexes. Detailed history of selected patients was taken, after this clinical examination and routine investigation were carried out. Patients below 15 years, with any chronic illness, pregnant woman, with any hepatic and renal failure were excluded from study.Result: Necrotizing granulomatous lymphadenitis was most common (66.67%) cytological diagnosis followed by granulomatous lymphadenitis (18%) and necrotizing lynmphadenitis (14.66%). Out of 150 cases 78 (52%) were positive for AFB on FNAC smears while 72 (48%) were smear negative for AFB. Lymph node biopsy was done in 42 cases. Those who were not willing common most was histopathological feature (71.4%).**Conclusion:** FNAC smear confirmed the diagnosis bacteriologically in 52% cases subsequent FNAC culture for AFB contributed in 11 (7.3%) cases more as an additional yield over FNAC smear.

Keywords: FNAC, Necrotizing, Histopathological

Introduction

Tuberculosis, a mycobacterial disease, is still a biggest infectious health problem that records about millions of new case per year[1]. It can involve other systems beside lungs like peripheral lymph nodes, bone and joints, genito-urinary tract and central nervous system[2]. In 1882 Robert Koch was first to describe the tubercle bacilli and Bollinger reported the presence of *M.bovis* in cow milk that cause scrofula in human[3]. " Scrofula" is extra-pulmonary tuberculosis that involves cervical lymph nodes. It is usually caused by *M.tuberculosis, M.bovis, M. africanum* and recently it has been reported that it may be caused by *M.bohemicum*[4]. Changes in pattern and incidence of Tuberculosis have strikingly altered the etiology of mycobacterial lymphadenitis as children are usually more affected by atypical form while adults and geriatrics are mostly infected by *M.tuberculosis*[5]. Diagnosis of tuberculous lymphadenitis is mostly clinical and histopathological but in some cases where microscopic appearances are not exactly typical so diagnosis becomes difficult[6]. It is necessary to isolate and identify the causative agents for clinching the diagnosis for proper management of such cases. In India, the infection with tubercle bacilli of human type is still widespread[7]. However, studies in higher institutes of India reported the presence of atypical mycobacteria as causative agent in cervical lymphadentitis.

The cytological diagnosis of the tuberculous lymphadenitis was based on any one of the following features:

1. Moderately cellular smears with epithelioid cells lying singly or in clusters. Epithelioid cells were usually elongated, often semilunar with royal blue eosinophilic cytoplasm in Leishman stain / H & E stain.

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These features were classified as granulomatous lymphadenitis.

2. In addition to the findings of reactive lymphadenitis, presence of variable amount of necrotic material and clusters of epithelioid cells scattered throughout the lymphoid cells. These were classified as necrotising granulomatous lymphadenitis.
3. Presence of caseous necrotic material i.e. pink, amorphous, acellular debris with or without cellular components like lymphoblasts, lymphocytes, histiocytes and foreign body or Langhans type of giant cells. These were classified as necrotising lymphadenitis.

All the above mentioned cytological criteria were not present in a single smear but the presence of any of them suggested the diagnosis of tuberculosis[8].

Therefore, bacteriological study of tuberculous lymphadenitis seems to be significant. Considering this, the aim of present study was to investigate the microflora in histopathologically approved tubercular cervical lymphadenitis in adults.

Material and Methods

The present study conducted in Department of Tuberculosis and Chest Disease in collaboration with Department of Pathology, PGIMR, Rohtak. The study protocol included 150 patients more than 15years belonging to both sexes. Detailed history of selected patients was taken, after this clinical examination and routine investigation were carried out. Patients below 15 years, with any chronic illness, pregnant woman, with any hepatic and renal failure were excluded from study. Informed consent was taken from each included patients. Fine needle aspiration (FNAC) of lymph nodes was done and after this cytological examination of lymph node aspirate for mycobacteria was done. Lymph node aspirate was subjected to direct and concentrated smear examination followed by culture one part stained with ZN stain for AFB and other part was inoculated on two tube of LJ medium with one has antibiotics , both were incubated for 2-8weeks at 37°C. For Culturing and isolation of aerobic, blood agar and Mac Conkey agar and for anaerobic microorganism Robertson's cooked meat medium was used. Modified Stoke's Disc diffusion

technique was used for antibiotic sensitivity test for aerobes and facultative anaerobes.

Preparation of smear for cytological examination

Aspirate containing fluid or blood was spread over the slides just like blood smear. If the content were tissue fragments or thick cellular material, the smears were prepared by pressing two slides to each other and then sliding down. Atleast one air dried smear was kept from each aspirate to be stained subsequently with Leishman stain. Rest of the smears were fixed while still wet in 95% ethyl alcohol for 15 minutes and then smears were subjected to various staining procedures.

Staining methods were used

1. Leishman stain
2. Haematoxylin and eosin staining
3. Ziehl-Neelsen stain

After staining, smears were mounted in DPX. Stained smears were examined microscopically and cytological observations were recorded.

Clinical staging in tuberculous lymphadenitis

Stage 1 Enlarged, firm, mobile discrete nodes showing non-specific reactive hyperplasia.
Stage 2 Larger rubbery nodes fixed to surrounding tissue owing to periadenitis.
Stage 3 - Central softening due to abscess formation.
Stage 4 Collar-stud abscess formation.
Stage 5 - Sinus tract formation.

Treatment and Follow-up

These patients were put on antitubercular treatment and were followed-up every 15 days on the date of collection of antitubercular treatment for 6-9 months. During follow-up each case was subjected to thorough clinical examination. Any complication such as re-appearance of swelling, any newer site of involvement, increase in size, sinus formation, cold abscess formation, any other system involvement or any other complication was noted during this period. In these subjects fine needle aspiration was repeated to see the microbial flora and histopathological changes and the cases were managed accordingly.

Any adverse reaction occurring during chemotherapy were noticed, recorded and were managed accordingly.

Observation and results

The present study was conducted on 150 cases of proved tubercular cervical lymphadenitis

Table 1: Cytological, ZN smear examination and Histological findings of lymph node specimen

1.	**Cytological feature on FNAC lymph nodes**	**Number of patients**	**Percentage (%)**
	i. Necrotizing lymphadenitis	100	66.67
	ii. Granulomatous lymphadenitis	27	18
	iii. Necrotizing granulomatous lymphadenitis	22	14.66
	iv. Inconclusive	01	.67
	Total	150	100
2.	ZN smear examination in FNAC smear-		
	Lymph node aspirate a) Positive for AFB b) Negative for AFB	 78 72	 52 48
	Total	150	100
3.	Histological finding of lymph node biopsy specimen		
	a) Necrotizing granulomatous lymphadenitis	30	71.4
	b) Granulomatous lymphadenitis	10	23.8
	Non-specific lymphadenitis with dystrophic calcification	01	2.4
	d) Inconclusive	01	2.4
	Total	42	100

Table 2: Correlation of ZN smear results on FNAC with Histological specimen (n=42)

ZN stained smear for AFB (n=42)	**FNAC smear results**	
Lymph node Biopsy smear results	Smear positive for AFB	Smear negative for AFB
ZN smear positive for AFB (n= 15)	5 (33.33%)	10 (66.67%)
ZN smear negative for AFB (n= 27)	14(51.85%)	13 (48.15%)

Table 3: Correlation of AFB smear positivity with cytological features (n=150)

	Number of patient	**Percentage**
Necrotizing granulomatous lymphadenitis (n=100) (66.67%) ZN smear positive ZN smear negative	 58 42	 58 42
Necrotizing lymphadenitis (n=22) (14.66%) ZN smear positive ZN smear negative	 17 5	 77.27 22.73
Granulomatous lymphadenitis (n=27) (18.0%) ZN smear positive ZN smear negative	 03 24	 11.11 88.89

Table 4:Correlation of AFB smear positivity with histological features

	Number of patient	**Percentage**
Necrotizing granulomatous lymphadenitis (n=30) (71.4%) ZN smear positive ZN smear negative	 10 20	 33.33 66.67
Granulomatous lymphadenitis (n=10) (23.80%) ZN smear positive ZN smear negative	 5 5	 50 50
Non specific lymphadenitis with dystrophic calcification (n= 1) (2.4%) ZN smear positive ZN smear negative	 00 01	 00 100

Necrotizing granulomatous lymphadenitis was most common (66.67%) cytological diagnosis followed by granulomatous lymphadenitis (18%) and necrotizing lynmphadenitis (14.66%). Out of 150 cases 78 (52%) were positive for AFB on FNAC smears while 72 (48%) were smear negative for AFB. Lymph node biopsy was done in 42 cases. Those who were not willing common most was histopathological feature (71.4%) followed by granulomatous lymphadenitis (23.80%). Out of these, 15 (35.7%) patients were smear positive for AFB on biopsy specimen. Histopathology confirmed 10 additional cases bacteriologically who were smear negative for AFB on FNAC. Overall histopathology contributed to the diagnosis in 23 cases whose FNAC smear were negative for AFB. Among FNAC smear positive group, the maximum yield for AFB positivity was observed among patients who were reported as necrotizing granulomatous lymphadenitis (58%) or necrotizing lymphadenitis (77.27%).Necrotic pus was found to be best material for bacteriological confirmation. The same observation was made for AFB smear positivity in biopsy were reported as necrotizing granulomatous.

Discussion

Tuberculous lymphadenitis is the commonest disease affecting peripheral lymph nodes and is the most frequent form of extra-pulmonary tuberculosis[9]. Extra-pulmonary tuberculosis is much more common in HIV positive patients (nearly 20% to 70%) 34, as compared to nearly 10% to 20% in HIV Sero-negative patients[10]. In India, the incidence of extra-pulmonary tuberculosis in HIV positive patients has been reported between 19.8% to 70% and tubercular lymphadenitis was the commonest manifestation (46.5% to 70.6%)[11]. A high frequency of tuberculous lymphadenitis has been reported among Asians, Hispanics Afro-Asians and Native Americans. No satisfactory immunological, bacteriological or epidemiological explanation for this high incidence among Asian ethnic group was found. However, socio-economic factors such as living in crowded inner cities, immigration from high incidence areas, differences in host-parasite relationships, differences in balance between endogenous and exogenous disease in different ethnic groups, hereditary factors and a high suspician with close supervision of these easily identifiable high risk groups have been suggested as possible explanation for this racial difference. Tuberculosis nodes were observed in the present study too. 85% of patients were having cervical lymphadenopathy alone. It is claimed that preponderance of cervical node tuberculosis could be the result of the tonsils, adenoids and Waldeyer's ring providing an easy portal of entry to mycobacteria[12]. Majority of patients (67.33%) noticed swelling accidently while in 27.33% patients, it was noticed because of pain. Majority of patients (63.33%) presented to us within 6 months of onset of disease followed by 6 months to one year (16.67%). Thus 80% of patients presented within one year of onset of disease. Aggarwal et al[13] and Jha et al[1] also observed similar presentation. The present study, diagnosis of tubercular lymphadenitis was established in all except one case by lymph node aspiration and subsequent cytological examination. Cytological criteria for the diagnosis of tubercular lymphadenitis have been clearly defined as being epithelioid cell granuloma with or without multinucleated giant cells and caseation necrosis. The present study, FNAC confirmed the diagnosis of tubercule lymphadenitis showing epithelioid granuloma with or without necrosis in 84.67% cases while 14.67% cases showed only caseous necrotic mate. In the present study, all 150 FNAC smears were stained for AFB by ZN staining technique. FNAC smears confirmed the diagnosis bacteriologically in 52% cases. Necrotic pus was found to be the best material for bacteriological confirmation as maximum yield (77.27%) was obtained amongst patients who were reported as having necrotising lymphadenitis. Similar observation was made for AFB positivity in biopsy specimens reported as granulomatous lymphadenitis with (33.33%) or without necrosis (50%). Several others workers have also found similar results. Arora and Aroras reported highest AFB smear positivity (96%) in FNAC smears showing necrosis only[14]. Gupta et al reported 75.6% AFB smear positivity in FNAC smears showing only necrosis without epithelioid clusters or Langhans giant cells[15]. Das Gupta et al reported highest AFB smear positivity (83.3%) in FNAC smears showing caseous necrosis only whereas AFB smear positivity was much lower in FNAC smears showing epithelioid cell granuloma with caseation (33.3%) and epithelioid cell granuloma without caseation (27.7%)[16]. Dua et al also observed increase in AFB positivity in the presence of increasing degree of necrosis. They reported AFB positivity of 0.0%, 36.5% and 100% respectively in FNAC smears showing granuloma only, granuloma and necrosis, and necrosis only[17]. Metre and Jaya Ram reported highest AFB smear positivity (66%) in purulent aspirates followed by 64% AFB positivity in cheesy aspirates[18]. Das et al found in a multivariate regression analysis that necrosis was the only independent contributing factor in AFB positivity[19]. Liquefaction of necrotic foci has been shown to be associated with marked proliferation of tubercle bacilli and infiltration of polymorphonuclear cells, whereas lymphocytes, epithelioid cells and multinucleate giant cells are likely to have some role in limiting the proliferation of AFB. Findings in our series also commensurate with that of Das et al[19] and Sen et al[20] in their study found that unless AFB are demonstrated, the diagnosis of tuberculosis is difficult, (i) when the smears are richly cellular with an occasional cluster of epithelioid cells but no necrosis; in such cases other granulomatous conditions have to be taken into consideration, and (ii) when only necrotic material is seen with just a few leucocytes. Several authors have stressed the importance of AFB demonstration in cases where only necrotic material and cells are seen in FNAC smears. The epithelioid granuloma forms the basis of making the diagnosis of tuberculosis. However, demonstration of AFB by culture or smear finally settles the etiology, even if the granuloma is not seen. Lymph node biopsy is still not a very acceptable procedure due to residual scarring in the neck area which may have its own social impact especially among young females. Thus only in 42 (28%) patients lymph node biopsy could be done. Among lymphadenitis, necrotizing granulomatous was most common histopathological feature (71.4%) followed by granulomatous lymphadenitis (23.80%). One case (2.4%) revealed non-specific lymphadenitis with dystrophic calcification suggestive of tuberculosis while in other one (2.4%) biopsy was inconclusive. Krishnaswami et al also reported typical histopathological picture of necrotizing granulomatous lymphadenitis in 92.2% cases while 10 (7.8%) cases showed only granulomatous lesions without caseation[12]. Margileth et al reported caseous granulomas in 75% cases and non-caseous granuloma in 25% cases of tuberculous lymphadenitis[21]. Hawkins et als reported caseating granuloma in 76.2% cases and non-caseating granulomas in 21.43% cases. Several other workers reported similar results in their series[22].

By far the most reliable study has been the histological analysis of biopsy material. In the present study, histopathology showed caseating or non-caseating granulomas consistent with tuberculosis in 40 (95.23%) of 42 cases subjected to lymph node biopsy. Results of other studies revealed positive histology in 88% 123 to 100%.[10] Weiler et al reported histological confirmation in only 69% of patients with tuberculous mphadenitis in their series[23]. Of the 40 cases who subsequently had a histologically confirmed tuberculous lymphadenitis, 23 had no evidence of AFB in the cytological smears. Thus histopathology contributed to the diagnosis in 23 (15.33%) additional cases whose FNAC smears were negative for AFB. The histological and cytological appearance of tuberculosis is so similar to other granulomatous lesions that bacteriological studies are often necessary to confirm the diagnosis. The typical granulomatous pattern of epithelioid cells, necrosis and giant cells are found with both tuberculous and non-tuberculous mycobacterial and other infections involving the nodes. This is helpful but not pathognomonic in ruling out other granulomatous diseases including sarcoidosis, carcinoma, lymphoma, viral or bacterial adenitis, fungal diseases, syphilis, leprosy, tularemia, brucellosis, collagen vascular diseases and diseases of reticulo-endothelial system[24]. Isolation of M.tuberculosis in culture is the best method to confirm the diagnosis, although these facilities are often not available and it takes a

minimum period of 6-9 weeks[14]. In the present study, culture for mycobacteria was positive in only 37(24.67%) of 150 cases subjected to microbial culture. Various studies have reported culture positivity rate for mycobacteria from lymph node aspirates or biopsy specimens varying from 10%" to 62%[15]. Shikhani et al[25] also reported culture positivity for mycobacteria in 25% of cases while Gandhi and Deshmukh[26] reported positive culture for mycobateria in only 35(28.45%) of 123 histopathologically confirmed cases. Similar results were reported by Chakrabarti et al[27] (32.3%). Results of cultures for mycobacteria in our study are comparable to these workers. Other workers have found very low positive cultures for mycobacteria in their series. Kim et al reported mycobacterial culture positivity in only 19% cases of cytologically confirmed cases of tuberculous lymphadenitis[28]. All patients in our series responded well to the treatment and none of the patient showed treatment failure, thus again confirming the presence of typical mycobacterial infection which usually responds well to the primary anti-tubercular treatment.

Conclusion

FNAC smear confirmed the diagnosis bacteriologically in 52% cases subsequent FNAC culture for AFB contributed in 11 (7.3%) cases more as an additional yield over FNAC smear. The diagnosis was confirmed in all cases except for 2 cases who were subjected to lymph node biopsy. Biopsy smear and culture contributed to the diagnosis in 10 more cases who could not be confirmed by FNAC smear. All the culture who were positive didn't show any atypical mycobacterium.

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