Use of Ziehl-Neelson and Hematoxylin-Eosin Stain in the Diagnosis of Tuberculous Lymphadenitis

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Abstract

Introduction	Tuberculosis is among the top ten causes of global mortality. Higher incidence rate of TB in Nepal with almost 45 percent of the total population infected has led to the need for identification of good, economic, easier and faster diagnostic tools and thus the study was conducted.
Objectives	To determine the sensitivity and specificity of Ziehl-Neelson and Hematoxyline–Eosin stain in the diagnosis of tuberculous lymphadenitis.
Methods	An experimental study was conducted at the department of histopathology, Patan Hospital during September 2002 to March 2003. The biopsy samples that arrived in lab after the excision were further processed and then stained using the standard protocol of H-E and Z-N stains.
Results	All the samples in H&E and only 10 percent samples in Z-N stain were found positive with tuberculous infection.
Conclusion	Tuberculous lymphadenitis being common in country, combination of AFB stain with H-E stain could be useful diagnostic tools and techniques for the diagnosis of tuberculous lymphadenitis.
Key words	Tuberculous, Lymphadenitis

Introduction

Tuberculosis is chronic bacterial infection caused by *Mycobacterium tuberculosis* and characterized by the formation of granuloma in infected tissue as a result of cell-mediated immunity[1]. It has been estimated that approximately one-third of the world's population is infected with the tuberculosis bacillus, and that each year 8 million people develop the disease and 1.8 million die of the disease. Highest incidence rates of TB are found in Africa and South-East Asia[2]. Today the co-epidemic of tuberculosis and HIV is a major problem in the world. HIV increases the risk of getting tuberculosis 30-50 times[3].

Tuberculosis can occur in any part of the body and most common site of infection is lung causing pulmonary tuberculosis. In extra pulmonary cases peripheral lymph nodes are the commonest sites, among them 70 percent to 90 percent occur in cervical lymph nodes[4]. And to define lymphadenitis, it is acute and chronic inflammatory process of lymph node that occurs in response to a variety of pathogenic agents. They may be specific or nonspecific, featuring necrosis, abscess, granulomas and fibrosis in various combinations[5].

Diagnosis of tuberculous lymphadenitis on the basis of clinical finding in combination with Fine Needle Aspiration Cytology (FNAC) of the lymph node aspirate or hematoxylin-eosin staining of the lymph node biopsy is common in practice. And the management of tuberculous lymphadenitis involves appropriate use of antituberculous chemotherapy with the judicious use of surgical excision in a minority of patients[6].

Different staining techniques have been used for the diagnosis of tuberculous lymphadenitis but the use of appropriate diagnostic tool for early, sensitive, specific and affordable method is essential. This study has been thus planned to compare the efficiency of H-E and Z-N staining in the diagnosis of tuberculous lymphadenitis.

Methods

Forty lymph node biopsy samples from clinically suspected cases were collected during September 2002 to March 2003 at the Department of Pathology, Patan Hospital, which were further analyzed and stained. Pre staining of the specimens were done following standard protocol by DAKO[7]. Specimens were then stained by Z-N stain and H-E stain as recommended by the standard protocols [8, 9].

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Results

Tuberculous lymphadenitis cases were observed in age group 1 to 55 years with higher prevalence (35%) in patients of 20-30 years of age with majority of female involvement.

Table 1: Distribution of Tuberculous lymphadenitis according to age and sex.

Age Groups	Hematoxylin-Eosin Stain		Ziehl-Neelson Stain	
	Male	Female	Male	Female
0-10	2	1	0	0
0-20	2	5	1	0
20-30	4	9	0	1
30-40	3	3	0	0
40-50	1	5	0	1
>50	0	5	0	1
Total	12	28	1	3

100 percent of the cases were found positive with typical granuloma and central caseous necrosis surrounded by epethelioid cells and lymphocytes in H-E stain. However, out of 40 biopsy samples only 4 cases (10% of the cases) could be detected positive with presence of beaded, pink coloured acid-fast bacilli in Z-N stain. In Caseous necrosis, no cellular area or deformed cellular structures were observed. The epethelioid cells surrounding the necrosis were elliptical in shape with clear visualization of plasma with central nucleus, where as lymphocytes were circular darkly stained cells scattered heavily around the outer part of a typical granuloma. Giant cells were present in only 95 percent of the cases where as in 5 percent it was lacking.

Table 2: Effeciency of stain in the diagnosis of Tuberculous lymphadenitis

	Stain used					
	Z-N			H-E		
	Male	Female	Total (%)	Male	Female	Total(%)
Positive	1	3	4 (10)	12	28	40(100)
Negative	11	25	36 (90)	0	0	0(0)
Total	12	28	40 (100)	12	28	40(100)

Involvement of cervical lymph nodes was highest followed by Axillary, submandibular, supraclavicular, inguinal and extensive respectively. Of which, right cervical node involved was higher than left node and bilateral nodes involved.

Table 3: Site and number of lymph nodes involved in Tuberculous lymphadenitis.

Site	Single	Multiple	Total	%
R Cer	2	14	16	40
L Cer	2	4	6	15
B/L Cer	0	1	1	2.5
Axillary	2	6	8	20
Submandi	2	2	4	10
Supraclavi	0	2	2	5
Linguinal B/L	0	2	2	5
Extensive	0	1	1	2.5
Total	8	32	40	100

Discussion

Higher disease frequency between the ages of 20-30 was in agreement with a study done at Tribhuvan University Teaching Hospital [10]. The maximum numbers of patients infected were found to be female (70%) and then male (30%) which agrees with a similar study conducted by Huhti[11].

Involvement of cervical lymph nodes was observed in most of the cases with higher right node involvement than left and bilateral nodes. the result was similar to studies done by Huhti, Brander, *et al.* (1975). The cause for higher right-sided infection might be due to wider, shorter and vertical right sided bronchus. Axillary lymph nodes followed by submandibular, supraclavicular, inguinal and extensive nodes infection was also observed. Principally, one or more of the lymph node sites may be involved in infection by tubercle bacilli depending upon the immunity of an individual. In 80 percent of the cases, multiple nodes were found to be affected which was higher than 20 percent of the cases that involved single node. This study agrees with studies done by different authors [11, 4, 12].

Only 10 percent slides were found positive for AFB stain, which is higher two cases of AFB positive slides out of 349 slides in a study done by Shrestha at Tribhuvan University Teaching Hospital during 1989[10]. Out of four positive cases, three had multiple cervical nodes involved where as remaining one had single axillary node involvement. Detection of low percentage of tubercle bacilli in lymph nodes may be due to reasons like, specimen sections of 3-5um in which, organisms in only one plane can be observed. Absence of the Mycobacterium in slides may be due to the fact that the bacillus lies within the macrophages, which could not be stained. In patients with low immunity like in HIV infected individuals, who has lower cell mediated immunity, formation of granulomas cannot be seen as a defense mechanism. These conditions have increased neutrophil activities and high bacteremia in turn, which was observed in one case.

Among 40 clinical cases of tuberculous lymphadenitis, in all cases, epitheloid cell granuloma along with caseous necrosis and with or without giant cells in Haematoxylin-Eosin stain could be observed, which was considered as the diagnostic criteria for tuberculous lymphadenitis[13]. However a study reports that, the finding of epithelioid cell granuloma or the epithelioid cell clusters only cannot be considered as the diagnostic tool for tuberculosis, which can also be found in other granulomatous inflamations like sarcoidosis, fungal infections, cat scratch fever and parasitic infection [14]. In this study, typical granuloma was seen in all cases with central necrosis which is the late manifestation induced by the phospholipid containing cell wall of Mycobacteria.

The sensitivity of H-E stain in this study was found to be 100 percent where as that of Z-N stain was only 10 percent for the diagnosis of tuberculous lymphadenitis. However the positive predictive values were 100 percent in both the staining techniques.

Conclusion

In a country like Nepal where tuberculosis is highly epidemic, use of appropriate diagnostic tool for early, sensitive, specific and affordable diagnosis is essential.

Tuberculous lymphadenitis being common in country, H-E can be considered as a gold standard for the diagnosis of tuberculous lymphadenitis. Though, presence of AFB in Z-N stain showed the diagnostic accuracy of 100 percent, combination of Z-N stain with H-E stain could be a useful diagnostic tool for the diagnosis of tuberculous lymphadenitis.

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