

Original Article

Comparison Between Auramine-Rhodamine (AR) And Ziehl-Neelsen (ZN) Staining In Cases of Chronic Granulomatous Lymphadenopathy

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ABSTRACT

Study Design: Experimental study.

Place and Duration of Study: This study was conducted at various hospitals of Quetta.

Materials and Methods: This study includes 103 cases of chronic granulomatous lymphadenitis, with 101 cases of tuberculosis lymph nodes amongst a total of 200 cases of non-neoplastic lymphadenopathy. Their ages ranged from 2 to 79 years. Maximum number of cases were in 10-29 years age group. Females (69.31%) were affected more as compared to males. The commonest presenting symptom was fever. Cervical lymph nodes (83 cases) were the commonest site of involvement whereas 18 cases showed multiple site involvement.

Fluorescent staining of histopathological sections from 103 chronic granulomatous lymphadenitis gave positive results in 76 out of 103 (73.78%) cases, however Ziehl-Neelsen staining was positive only in 29 out of 103 (28.15%) cases. The yield of mycobacteria on fluorescent staining was highly significant ($p < 0.001$) as compared to Ziehl-Neelsen staining thereby providing the superiority of fluorescent stain.

Result: In a total of 200 cases of non-neoplastic lymphadenopathy 101 cases showed granulomatous lesions, histologically consistent with tuberculosis. Other causes of lymphadenopathy were chronic non-specific lymphadenitis (n=87) viral lymphadenitis (n=8), fungal lymphadenitis (n=2) and acute bacterial lymphadenitis (n=2). Fluorescent staining of histopathological sections from 103 chronic granulomatous lymphadenitis gave positive results in 76 out of 103 (73.78%) cases, however Ziehl-Neelsen staining was positive only in 29 out of 103 (28.15%) cases.

In 101 cases, the findings were consistent with the diagnosis of tuberculous lymphadenopathy. In our study, significantly greater number of cases, 78 out of 101 ($P < 0.001$) diagnosed as tuberculous lymphadenitis were in age groups 10-29 years. Female (69.31%) were more affected than males (30.69%). The common presenting symptom was fever. Cervical lymph nodes were commonest site of biopsy. Haemoglobin estimation in 98 cases revealed anaemia in 65 out of 101 (66.32%) patients of tuberculous lymphadenopathy. Erythrocyte sedimentation rate was performed in 63 cases and was raised in 52 (82.53%) cases. In a total of 80 cases in whom X-ray chest was performed, 14 (15.5%) cases revealed foci of tuberculosis.

Conclusion: Thus, in conclusion, this study has highlighted the superiority of fluorescent stain over ZN stain.

Key Words: Haematoxylin and Eosin stain, Reticulin stain, Periodic-Acid Schiff stain, Phloxine-Tartrazine stain, Auramine-Rhodamine stain, Ziehl-Neelsen stain, Acid-Fast Bacilli

INTRODUCTION

Tuberculosis is a leading cause of morbidity and mortality not only in developing countries but also in the developed countries due to emergence of human immunodeficiency virus infection^{1, 2}. Peripheral tuberculous lymphadenopathy is the commonest form of extra-pulmonary tuberculosis. Whereas cervical lymphadenitis is the commonest form of the tuberculous lymphadenitis,^{3, 13}. Histological diagnosis of tuberculosis and to be differentiated from simulating granulomatous lesions of lymph nodes caused by other microbial infections.

Bacteriological proof for tuberculosis is always beneficial for making a firm diagnosis either by demonstrating acid-fast bacilli in smear, section or by culture to distinguish it from other granulomatous lesions.⁸ In a review of 23 cases of abdominal tuberculosis in Pakistan Institute of Medical Sciences Islamabad, mesenteric lymphadenitis was present in 5(22%) out of 23 cases.⁴ In another study done in Karachi and Hyderabad during 1971-1991, 133 patients of abdominal tuberculosis were confirmed histologically. At operation, mesenteric lymphadenitis was found in 50(38%) patients.⁴ Sarfaraz⁵ studied 200 resected intestinal specimens at Postgraduate Medical

Institute Lahore, 51 out of 200 cases presented with tuberculous mesenteric lymphadenitis.

The present study was carried out to document the morphological features of tuberculous lymphadenopathy, with comparison between auramine-rhodamine (AR) and ziehl-neelsen (ZN) staining in cases of chronic granulomatous lymphadenopathy.

MATERIALS AND METHODS

The present study included 103 patients of granulomatous lymphadenopathy, with 101 patients of tuberculous lymphadenopathy from various hospitals of Quetta. They were taken from total of 200 patients of non-neoplastic lymphadenopathy seen during this period. Patients of all ages and both sexes were included in the study. The specimens of the lymph nodes were collected irrespective of any specific sites for morphological study. Clinical information regarding history, physical examination and relevant investigations were obtained from the patients and doctor incharge, after detailed gross examination of each specimens, all the sections were stained with Haematoxylin and Eosin (H&E) and reticulin stains. Whereas granulomatous lesions were stained with Ziehl-Neelsen, Auramine-Rhodamine, periodic acid-Schiff, whereas methenamine silver and Giemsa stains were used when required. In non-granulomatous lesions, Gram stain, Congo red and phyloxin-tartrazine stains were used. The sections stained with fluorescent stain were examined under the Zeiss Axioplan fluorescent microscope.

RESULTS

In a total of 200 cases of non-neoplastic lymphadenopathy 101 cases showed granulomatous lesions, histologically consistent with tuberculosis. Other causes of lymphadenopathy were chronic non-specific lymphadenitis (n=87) viral lymphadenitis (n=8), fungal lymphadenitis (n=2) and acute bacterial lymphadenitis (n=2)(Table No.5). Fluorescent staining of histopathological sections from 103 chronic granulomatous lymphadenitis gave positive results in 76 out of 103 (73.78%) cases (Table No.1), however Ziehl-Neelsen staining was positive only in 29 out of 103 (28.15%) cases (Table No.2).

In 101 cases, the finding were consistent with the diagnosis of tuberculous lymphadenopathy. In our study, significantly greater number of cases, 78 out of 101 (P<0.001) diagnosed as tuberculous lymphadenitis were in age groups 10-29 years (Table No.3). Female (69.31%) were more affected than males (30.69%) Figure No.1. The common presenting symptom was fever (Figure No-2). Cervical lymph nodes were commonest site of biopsy (Table-4) Haemoglobin

Table No.1: Comparison of fluorescent (auramine-rhodamine) staining in cases of chronic granulomatous lymphadenopathy

Histopathological findings	Fluorescent positive	Fluorescent negative	No. of cases
Caseous granulomatous Lymphadenitis	61	19	80
Non-caseous granulomatous Lymphadenitis	15	08	23
Total	76	27	103

P = Not significant

Table No.2: Comparison of ziehl-neelsen staining in cases of chronic granulomatous lymphadenopathy

Histopathological findings	Ziehl-Neelsen positive	Ziehl-Neelsen negative	No. of cases
Caseous granulomatous lymphadenitis	20	60	80
Non-caseous granulomatous lymphadenitis	09	14	23
Total	29	74	103

P = Not significant

Table No.3: Distribution of age in cases of tuberculous lymphadenopathy

Age(Years)	No. of cases	Percentage
0-9	7	6.93
10-29	78*	77.23
30 and above	16	15.84
Total	101	100.0

*P<0.001 as compared to 0 – 9 and 30 and above age groups

Table No.4: Site of lymph node biopsy of patients with lymphadenopathy

Site of biopsy	No. of cases	Percentage
Cervical	83	82.18
Axillary	7	6.93
Submandibular	4	3.96
Inguinal	2	1.98
Mediastinal	2	1.98
Mesenteric	1	0.99
Anterior chest.	1	0.99
Pre-auricular	1	0.99
Total	101	100.0

Table No.5: Histopathological diagnosis of patients with lymphadenopathy

Dignosis	No. of cases	Peren -tage
I Chronic-specific lymphadenitis		
1 Bacterial		
a) Granulomatous	101	50.5
b) Non-granulomatous	2	1.0
2 Viral lymphadenitis	8	4.0
3 Fungal lymphadenitis	2	1.0
II Chronic non-specific Lymphadenitis	87	43.5
Total	200	100.0

estimation in 98 cases revealed anaemia in 65 out of 101 (66.32%) patients of tuberculous lymphadenopathy. Erythrocyte sedimentation rate was performed in 63 cases and was raised in 52 (82.53%) cases . In a total of 80 cases in whom X-ray chest was performed, 14 (15.5%) cases revealed foci of tuberculosis (Figure No.3).

Figure No.1: Sex distribution of patients with lymphadenopathy

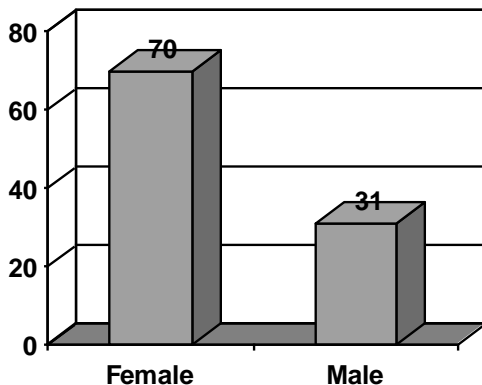


Figure No.2: Presenting complaints of patients with lymphadenopathy

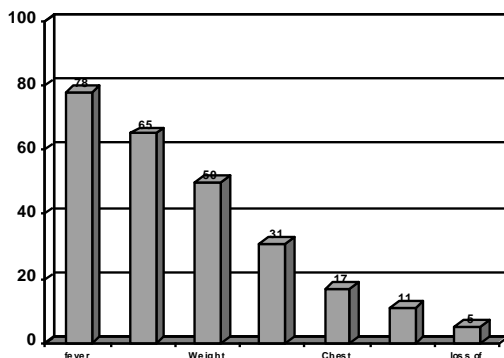
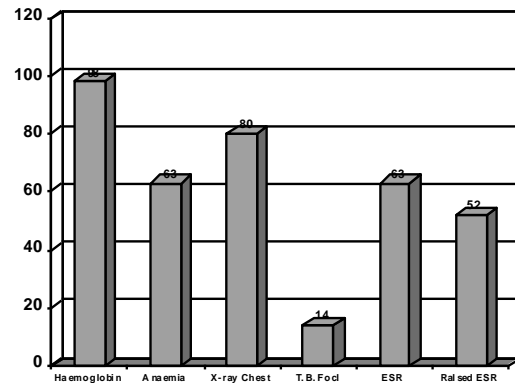


Figure No.3: Investigation of patients with lymphadenopathy



DISCUSSION

Tuberculous lymphadenopathy has been the commonest form of extra pulmonary tuberculosis both in India and Pakistan ^{3,6,7,14}. In the present study in which 101 cases of tuberculosis formed the major group in a total of 200 (51.5%) cases of non-neoplastic lymphadenopathy were the commonest. The same were the findings of Krishnaswami et al ⁸ and Dandapat et al ⁷.

Maximum numbers of cases of tuberculous lymphadenitis in the present study were of 10-29 years age group. Ahmed ⁶ has also reported the same age group from Lahore. In India the commonest age group of tuberculous lymphadenitis has been reported to be 21-30 years ^{7,8} . In United States of America the reported age group was 20-70 years ¹⁶.

In the present study females out numbered the males. This is similar to the findings of other workers in the Sub-Continent ^{6,8} . This could be due to the fact that females in our male dominant society have low nutritional status and are exposed to overcrowding more than the males ^{6,8}.

In this study a large majority of the lymph nodes (83 out of 101) belonged to the cervical group of lymph nodes. The predominance of cervical lymph node involvement in tuberculosis has already been established ^{3,6,7} . It has been suggested that tonsils, adenoids and waldayers ring provide an easy portal of entry of Mycobacterium lymph node involvements ⁷.

In this study X-Ray chest was available in 80 cases. Out of these only 14 revealed foci of tuberculosis. Our findings are in accordance with those of Ahmed^{6,15} who reported tuberculous lesions in X-Ray Chest in only 3 out of 43 cases. This also suggests that X-Ray Chest has a very limited value in the diagnosis of tuberculous lymphadenitis. Moreover it is also concluded that a normal chest radiography does not rule out the possibility of patient suffering from tuberculous lymphadenitis.

In this study 80 cases showed caseating granulomas whereas 23 had non-caseating granulomatous inflammation. Zeihl-Neelsen staining for acid-fast bacilli was positive in 29 out of 103(28.15%) cases. Different studies have quoted frequency of demonstration of acid-fast bacilli on Ziehl-Neelsen staining varying from (16.97%) to (37.5%)^{9,10} However yield of Mycobacterium tuberculosis on fluorescent staining is significantly more (P<0.001) as it was seen in 76 out of 103 (73.78%) cases, there by proving the superiority of fluorescent stain over Zeihl-Neelsen stain has already been reported in many studies.^{11,12}

CONCLUSION

Thus, this study has highlights the superiority of fluorescent stain over Zeihl-Neelsen stain, it is noted that tuberculosis is an important differential diagnosis of cervical lymphadenopathy and cervical lymph node biopsies should be sent for histological as well as for microbiological examination.

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