Original Article

DETECTION OF MYCOBACTERIAL LOAD AND LOCATION OF PERSISTING AFB IN CASES OF NON RESPONDERS ON CONVENTIONAL ANTITUBERCULOSIS DRUGS; A HISTOMORPHOLOGICAL STUDY

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ABSTRACT

Objective: To examine the patterns of infection in terms of tissue pathology, bacillary load and bacterial location seen in non responders to routine antituberculosis drugs.

Design: Cross sectional descriptive study

Place and duration: Department of Histopathology, Army Medical College Rawalpindi, National University of Sciences & Technology (NUST) Islamabad, and Military Hospital Rawalpindi, Pakistan from October 2009 to February 2011.

Patients and Methods: The patients receiving supervised multidrug therapy for tuberculosis and revealing evidence of tuberculosis on microscopic examination were included in the study. The tissue pathology on H&E staining was categorized as mild, moderate or severe. Histomorphological patterns for all granulomas were also assessed. Ziehl-Neelsen (ZN) stain was used to visualize acid fast bacilli.

Results: Twenty nine cases examined comprised of 16 lung biopsies and 13 extrapulmonary tissues. Mild inflammation was found in 14 (48.3%) cases out of 29 while 11 (37.9%) cases exhibited moderate and 4 (13.8%) cases severe pathology. Large coalescing granulomas were found in 17 (58.6%), multifocal lesions in 9 (31.0%), and necrotic granulomas in 9 (31.0%) cases. Regarding cellular composition of granulomas, 23 (79.3%) cases revealed lymphocytic cuff while giant cells were seen in 55.2% of cases. Three cases (10.3%) had foamy macrophages. On ZN stain, scanty AFB were seen predominantly (48.3%) within necrotic area of the granulomatous tissue.

Conclusion: The TB cases resistant to conventional antimycobacterial drugs show distinct tissue pathology. The findings highlight mild to moderate chronic inflammatory changes in the form of large coalescing granulomas with few persisting mycobacterium mainly within the necrotic foci of granulomatous tissue.

Keywords: Caseous necrosis, Drug resistance, Mycobacterium tuberculosis, Non responders.

INTRODUCTION

Mycobacterium tuberculosis is responsible for global epidemic of the disease, with close to 9 million new tuberculosis cases each year and nearly 3 million deaths[1,2]. In addition, half a million cases of drug resistance are seen per year[3–6]. The problem of multi-drug resistance (MDR-TB) contributes to compromise the tuberculosis control programmes. In WHO's new report it is estimated that 4,40,000 people had MDR-TB worldwide in 2008 and that a third of them died. In sheer numbers, Asia bears most of the burden. Almost 50% of MDR-TB cases worldwide are estimated to occur in China and India[7]. The exact prevalence and burden of MDR-TB disease is not known in Pakistan. According to the data collected by the Armed Forces Institute of Pathology, Rawalpindi in 2004, the MDR-TB prevalence was found to be 28%[8] while 47% by the Aga Khan University Hospital, Karachi in 2006[9].

The lengthy course of treatment of tuberculosis (TB) enables a small subpopulation of bacteria to persist in infected individuals. These persistent organisms are responsible for increasing burden of nonresponders to routine anti tuberculosis treatment and are associated with significant morbidity and mortality. To date, the exact location of these persisting bacteria is not known[10].

Few bacilli are found in hypoxic caseous necrotic lesions of non responders[11]. In this environment, it appears that the bacteria are less responsive to current chemotherapy[12]; thus,

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increasing attention has been given to understand the histology of necrotic granulomas and the residing tubercle bacilli within them. Recent animal studies have identified that within these caseous necrotic lesions, the bacteria may have acquired a nonreplicating state along with resistance to antimycobacterial drugs[13,14]. Therefore, the tissue pathology, load of resistant *Mycobacterium tuberculosis* and its location for new drug selection needs to be investigated.

In the present study, we examined the patterns of infection in terms of tissue pathology, bacillary load and bacterial location seen in non responders to routine antituberculosis (anti TB) drugs.

MATERIALS AND METHODS

This cross sectional descriptive study was carried out in the department of Histopathology, Army Medical College Rawalpindi, National University of Sciences and Technology (NUST) Islamabad and Military Hospital Rawalpindi, Pakistan from October 2009 to February 2011.

Twenty nine cases were included in the study. The slides examined were comprised of 16 lung biopsies and 13 extrapulmonary tissues with differing extents of pathology. The patients who were receiving supervised multidrug therapy for tuberculosis and revealed evidence of tuberculosis on microscopic examination were included in the study.

Tissue samples of the patients who were non responders to anti TB drugs were selected for histological examination. The tissues were placed in 10% normal buffered formalin and paraffin embedded. Sections (5 µm thick) were cut and stained with hematoxylin and eosin. Histology was reviewed by a histopathologist. The tissue pathology was categorized as mild, moderate or severe on visual estimation[10]. Histomorphological patterns for all granulomas were assessed by focusing on the size, type of granuloma (caseous, solid, suppurative, or mixed), distribution pattern (focal, multifocal and coalescing), and cellular composition (absence or presence of lymphocytic cuff, fibrosis, multinucleated giant cells, and macrophages)[15].

All tissue sections were stained with ZN stain (Ziehl-Neelsen) to visualize acid fast bacilli (AFB). Ten random fields were selected in each section for semiquantitative enumeration of AFB. The number of mycobacteriam in each area of the section was oil immerlens graded as none, scanty (individual bacilli found in each granuloma), moderate (1 to 10 bacilli in each granuloma), or numerous (>10 bacilli in clumps found in each field examined)[16].

Data was analyzed using SPSS version 15. Descriptive statistics were used to describe mean and standard deviation for quantitative variables like age and frequency along with percentages for qualitative variables like patterns of granulomas.

RESULTS

Twenty nine patients were included in the study, 19 were males and 10 were females. The mean age of the non responders to anti tuberculosis treatment was 36.6±14.78 years.

Twenty nine cases examined comprised 16 lung biopsies and 13 extrapulmonary tissues with differing extents of pathology. Mild inflammation was found in 14 (48.3%) cases out of twenty nine while 11 (37.9 %) cases exhibited moderate and 4 (13.8 %) cases severe pathology. The various areas of the examined tissue contained diverse lesions, including maximum percentage of large coalescing granulomas (58.6%), multifocal lesions (31.0%) and necrotic granulomas (31.0%) (Table-1).

Regarding cellular composition of granulomas, sections of the various lesions revealed heterogeneous cellular architecture consisting of numerous mononuclear cells surrounded by a layer of acellular caseous necrotic material. Next to the acellular necrotic layer there was granulomatous fibrotic tissue with a mixed mononuclear cell infiltrate consisting of Langhan-type giant cells, sheets of epithelioid macrophages and many scattered lymphocytes.

Localization of macrophages and AFB: Out of 29 cases, 23 revealed bacilli on ZN staining. In the lesions containing AFB, bacteria were seen predominantly within necrotic area of the granulomatous tissue (11 cases) but in small numbers (Fig.1) (Table-2).

The distinct acellular rim of the granuloma also harbored large numbers of acid fast staining bacteria (31.0%). All were extracellular and primarily dispersed throughout this region. The granulomatous fibrotic layer with abundant macrophages and giant cells was essentially devoid of visible AFB except two cases where numerous bacilli were detected in foamy type of macrophages in this area (Figure-2). The macrophages were giving the appearance of globi.

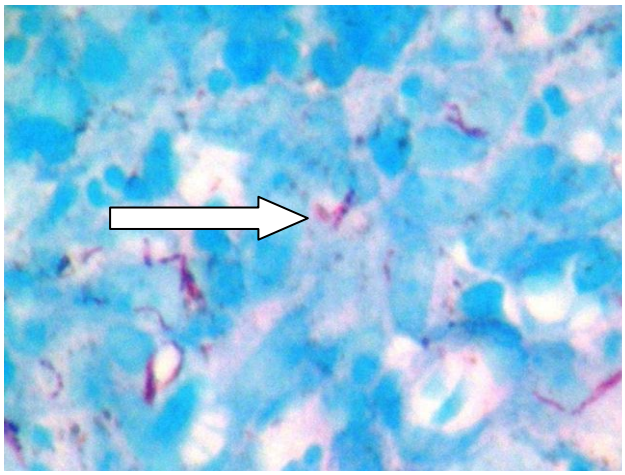

Figure 1: Scanty AFB in caseous necrotic material in lung tissue in a case of non responder (ZN stain x 3200)

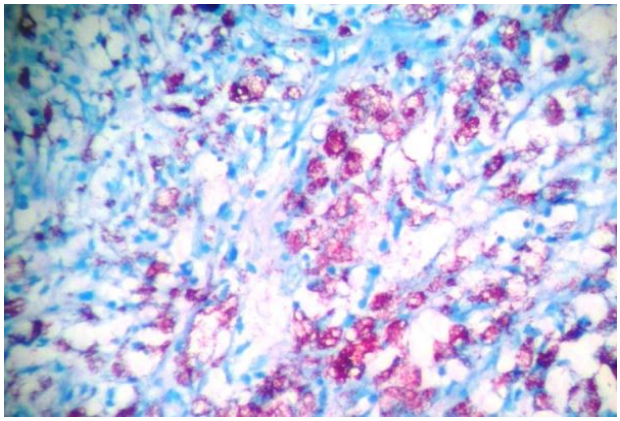

Figure 2: Foamy macrophages containing numerous AFB (globi) in an extrapulmonary lesion of non responder (ZN stain x 3200).

Table I: Patterns of Histomorphological lesions seen in non responders (n=29)

Histomorphological lesion	Number of cases (%age)
Size of granuloma	
Small	8 (27.6)
large	21 (72.4)
Type of granuloma	
Caseous	9 (31.0)
Solid	3 (10.3)
Suppurative	2 (6.9)
mixed	15 (51.7)
Distribution pattern of granuloma	
Focal	3 (10.3)
Multifocal	9 (31.0)
Coalescing	17 (58.6)
Cellular composition of granuloma	
Lymphocytic cuff	23 (79.3)
Multinucleated giant cell	16 (55.2)
Foamy macrophages	3 (10.3)
Fibrosis	10 (34.5)

Table -2: Showing location & load of bacilli withstanding drug therapy. Persisting AFB (Acid Fast Bacilli) in non responders (n=29)

AFB in tissue sections	n (%)
Bacterial load	
None	6 (20.7)
Scanty	14 (48.3)
Moderate	3(10.3)
Numerous	6 (20.7)
Bacterial location	
Necrotic centre	11 (37.9)
Outer rim	9 (31.0)
Foamy macrophages	3 (10.3)
Area of Fibrosis	-

DISCUSSION

The tissue morphology in infection with resistant strains of *Mycobacterium tuberculosis* is not yet very clear. Moreover, less data is available regarding the presence of bacteria in patients in which they have acquired drug resistance during therapy[16].

In this paper we present data supporting the idea that in the lungs and extrapulmonary tissue of patients during or after antituberculosis treatment, few bacilli acquire

drug resistance independently in discrete physical locales. It was found that there was diversity of histopathological lesions at these sites. By studying the histology and characterizing the bacterial populations present, we sought to ascertain whether significant correlation exists between the type of pathological lesion and presence of existing AFB in non responders, during or after long-term anti tuberculosis therapy. This could help in early diagnosis of MDR-TB and to find out new drugs targeting the residing bacteria in specific locations.

Majority of non responders included in this study were males with pulmonary pathology. Higher drug resistance amongst males is in accordance with previous studies reporting male gender[17,18] as a risk factor for resistant TB. Pulmonary cases are also reported to show highest records of non responders[17].

Histologic examination of lung and extrapulmomary tissue examined in the study revealed heterogeneous morphology and distribution of acid fast bacilli.

Majority of the cases exhibited multifocal lesions in 9 cases (31%) and contained necrotic material in the centre (31% of the cases). Abundant lymphocytic infiltrate (23 cases) and multinucleated giant cells were observed in 16 (55.2%) of the study cases. These results match with the findings seen in mice model by Srivastava et al[19]. They found that lung parenchyma infected with MDR-TB bacteria contains dense inflammatory infiltrate and extensive caseation and same was our observation. Accumulation of monocytes / macrophages, lymphocytes, and polymorphonuclear leukocytes in tuberculous lesions is cellular immune response to the tubercle bacilli[19].

Tuberculous granuloma is a primary lesion of the disease. A granuloma can have different morphological forms, including solid granuloma comprising of macrophages without necrosis or a granuloma with caseous necrosis in the center surrounded by lymphocytes and macrophage[12]. Histological analysis of the present study did not show any solid granulomas. These findings support the data[15] that solid granulomas are seen in the initial stages of infection. A large percentage of cases exhibited large coalescing granulomas with central necrosis. Subsequent necrosis indicates continued proliferation of bacteria. Presence of extensive necrosis and abundant inflammatory infiltrate in a granuloma may reflect a localized response in which a high degree of intracellular killing with resulting necrosis occurs.

Foamy macrophages, seen as globi constitute an important reservoir used by the tubercle bacillus for long-term persistence within its human host[20]. Only three cases exhibited foamy macrophages mixed with necrotic material. Although a small percentage of case comprised of these cells but they contained a heavy load of persisting bacilli. Surrounding fibrous tissue did not contain any bacilli. Same observations were found in a study where granulomatous fibrotic layer with abundant macrophages and giant cells, was essentially devoid of visible AFB[16]. The same study demonstrated small to moderate number of AFB in macrophages infiltrating the necrotic areas.

Regarding other locales of AFB withstanding drug therapy, perinecrotic rim and caseous centres of granulomas were found to contain persisting bacteria in 9 cases (31.0%) and 11 cases (37.9%) respectively. These results are in accordance with a previous data described in the guinea-pig model[21]. In that model, both the rim and the necrotic region appear to be the main locations of bacilli persisting after standard drug treatment. In contrast, however, Kaplan et al.[16] show that area of acellular necrotic material had few, if any, visible AFB. In the perinecrotic zone of the granulomas, bacillary numbers were substantially lower.

Understanding these host-pathogen interactions is critical to our understanding of MDR-TB as well as for the development of drugs treatment and diagnostic approaches to eradicate this disorder. Continued study of lung and extrapulmonary tissues from patients with MDR-TB will provide important benchmarks for validation of disease and may suggest

alternative therapeutic strategies for the treatment of chronic and MDR-TB. A major limitation of this study was small sample size due to incomplete clinical/ therapeutic data required to differentiate primary and treated cases. Due to rising number of non responders in our population, there is an urgent need for a drug resistance survey for early detection and treatment of previous TB cases and to prevent the load of additional non responders.

CONCLUSIONS

The TB cases resistant to conventional antimycobacterial drugs show distinct tissue pathology. The findings highlight mild to moderate chronic inflammatory changes in the form of large coalescing granulomas with few persisting mycobacterium mainly within the necrotic foci of granulomatous tissue.

By determining the bacterial load and studying the tissue histopathology and by finding the location of persisting bacilli after drug therapy, identification of non responder, probably MDR-TB cases can be identified and new drugs may be tried that can penetrate and hit exactly the persistent bacteria and hence reduce the burden of MDR-TB.

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