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Correlation Analysis of Immunological Status and Clinical Parameters with Their Histological Subtype during the Treatment of Leprosy Patients

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Abstract: Leprosy is a skin disease affecting also the nerves was caused by *Mycobacterium leprae*. Multidrug therapy (MDT) cures the infection, but immunological reactions may occur and neuropathy may lead to disability and deformity. The current study aimed to understand the immune status of the patient before and after treatment by correlating histological subtype and peripheral blood lymphocyte count. Total number of 35 cases was studied. Blood samples were taken from the patients and complete blood picture was done before instituting the MDT including TLC and DLC. After 6 months of treatment, patients were followed up with complete blood picture. In lepromatous leprosy group (BL+LL) total mean WBC count was increased with 11,414 (range 7,000- 22,600 mm^3) above the range of normal limits before treatment. It was in the mean normal range after treatment with 8514 (range 5,900- 11,000mm^3). Lymphocyte count has increased after treatment. After the treatment, there is considerable increase in lymphocyte count. It was in the range of 34% to 42% with a mean value of 38.1%.

Keywords: leprosy, immunology, reactions, treatment.

INTRODUCTION

Leprosy is a chronic granulomatous infection principally affecting the skin and peripheral nerves caused by the obligate intracellular organism *Mycobacterium leprae* [1]. The overall prevalence of leprosy in India has declined from 5.27/10000 in the year 2000 to 0.66/10000 in the year 2016, but still it continues to be a sizable public health problem [2].

India represents approximately 60% of the global burden [3]. Ridley and Jopling were the first to suggest a subclassification of leprosy based on immunological aspects, as five types; Tuberculoid (TT), Borderline Tuberculoid (BT), Mid borderline (BB), Borderline Lepromatous (BL) and Lepromatous Leprosy (LL) [4].

Multiple drug therapy (MDT) has been recommended by the World Health Organization (WHO) during the last decades for the treatment of leprosy and has been very effective. One of the difficulties in treatment is to find a satisfactory quantitative measurement of a patient's progress toward a successful outcome.

Currently used methods of assessing response to drug therapy are still subjective: clinical observation and bacterial index (BI). Many studies have reported the changes in antibody levels of patients to *Mycobacterium leprae* sonicated antigens and specific antigens, including the phenolic glycolipid (PGL-I) and the specific disaccharide (ND-O-BSA), during MDT, but long-term studies on the changes in cell-mediated responses have not been reported [5-8].

In this study, we attempt to document the immunological status before and after MDT for up to 6 months, and to find out whether a change in clinical presentation due to MDT was associated with immunological changes with respect to) total WBC count.

MATERIALS AND METHODS

This observational study was conducted at department of pathology, narayana medical college, Nellore. Total number of 35 cases was studied. All clinical and histopathological aspects will be anlaysed. H and E stain performed.

Blood collection:

5 ml of blood was collected before treatment (To) and after 6 months for complete blood picture. At the same time, the patients were examined clinically and bacteriologically and skin tests were done. The two regimens recommended by the WHO Study Group were used for treat ment of paucibacillary (PB) and multibacillary (MB) leprosy by department of dermatology. Regimen I for PB leprosy: Rifampin 600 mg once monthly for 6 months (supervised) plus dapsone 100 mg (1-2 mg/kg body weight) daily (self-

administered) for 6 months. Regimen II for MB leprosy: Rifampin 600 mg once monthly (supervised); 50 mg of clofazimine daily and 300 mg once monthly (supervised) and 50 mg of dapsone daily (self-administered).

Blood samples were taken from the patients and complete blood picture was done before instituting the MDT including TLC and DLC. After 6 months of treatment, patients were followed up with complete blood picture.

RESULTS

Information about retrospective cases was collected from histopathology records of department of pathology, Narayana Medical College and Hospitals Nellore. During the total study period of 2 years, 35 cases of leprosy were studied. According to clinical types of cases; 1 (2.85%) case was diagnosed as TT, 19 (54.28%) cases of BT, 5 (14.28%) cases of BB, 5 (14.28%) cases of BL, 5 (14.28%) cases of LL& no cases of IL type.

Table-1: Distribution of Lesions in Clinical Spectrum

CLINICAL TYPE	NUMBER OF CASES	PERCENTAGE
IL	0	0%
TT	1	2.85%
BT	19	54.28%
BB	5	14.28%
BL	5	14.28%
LL	5	14.28%
TOTAL	35	100%

Table-2: Immunological Status in Tuberculoid Leprosy Patients before and After Treatment

	Before treatment	After treatment
Sample Size **Mean Age In Years & Range**	35 39 Years (22-66 Years)	
Total Wbc Count Range	4600-11000cells/mm^3	4,400-7900cells/mm^3
Mean	7200 cell/mm^3	6350 cells/mm^3
Absolute Lymphocyte Count		
Range	1240-2880 cells/mm^3	1232-3160 cells/mm^3
Mean	2034cells/mm^3	2276cells/mm^3

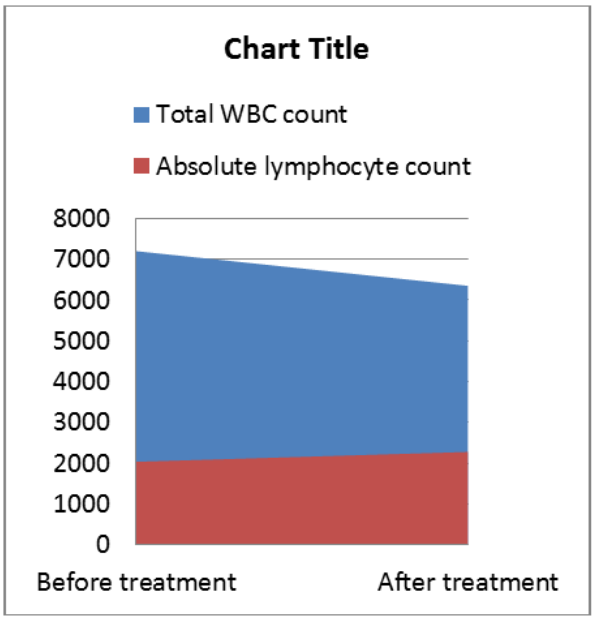

Fig-1: Immunological Status in Tuberculoid Leprosy Patients before and After Treatment

In tuberculoid leprosy group (TT+BT) total WBC count & Lymphocyte count are within normal range and did not show much variability before and after treatment

Table-3: Immunological Status in Lepromatous Leprosy Patients before Nd After Treatment

Before treatment		After treatment
Sample size	07	
Mean age in years & range	38.5 Years (15-50 Years)	
Total wbc count Range	7000-22600 cells/mm^3	5900-11000 cells/mm^3
Mean	11414 cells/mm^3	8514 cells/mm^3
Absolute lymphocyte count		
Range	1288-3792cells/mm^3	2360-3880 cells/mm^3
Mean	1970 cells/mm^3	3204cells/mm^3

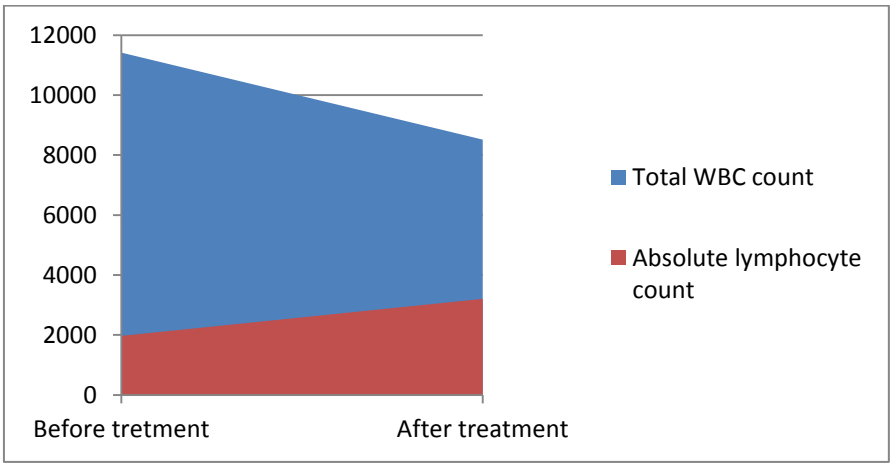

Fig-2: Immunological Status in Lepromatous Leprosy Patients before And After Treatment

In lepromatous leprosy group (BL+LL) total WBC count was increased above the range of normal limits before treatment. It was in the normal range after treatment. Lymphocyte count has increased after treatment.

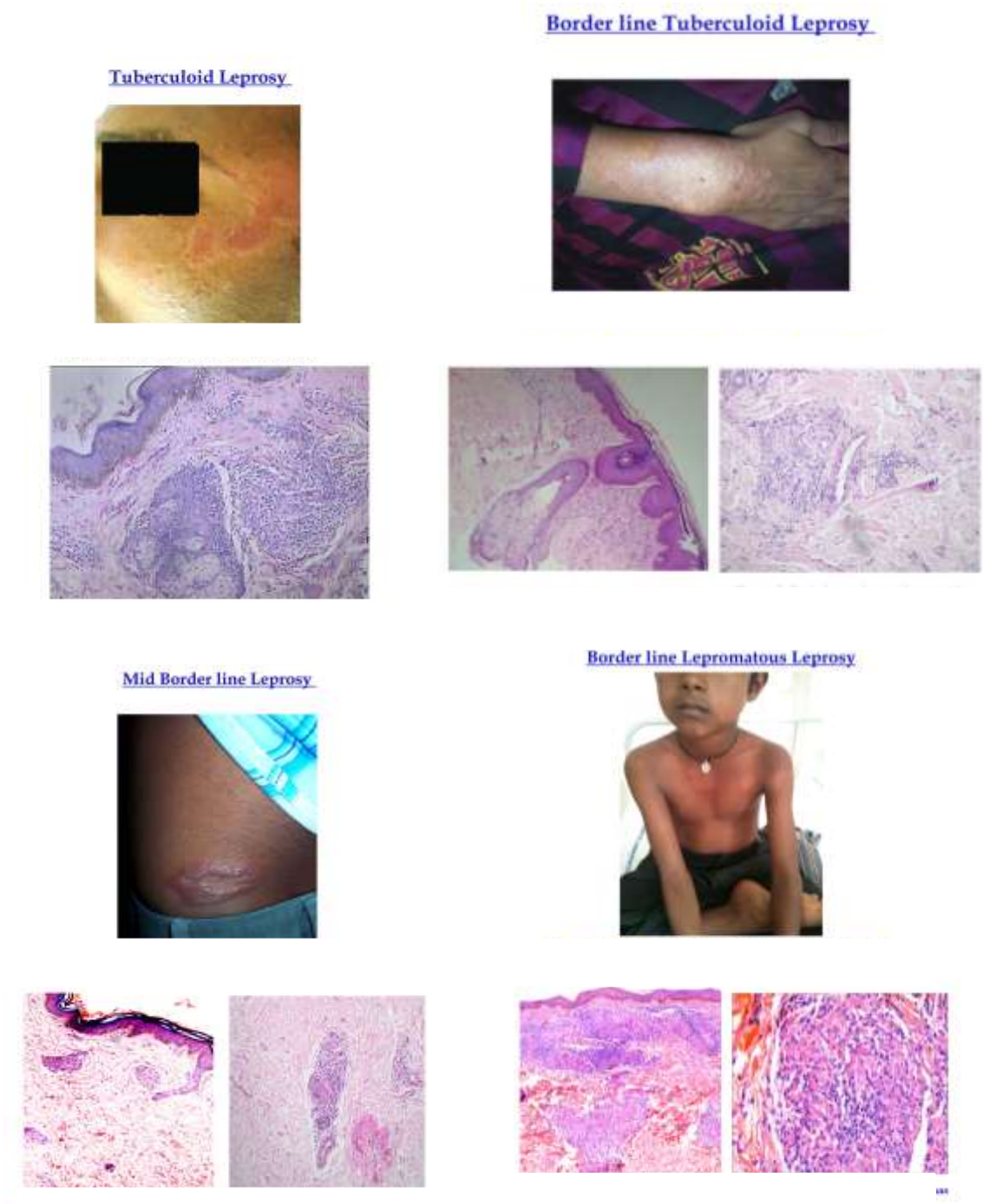

Fig-3: Types of leprosy (Hematoxyline & Eosin stain at 10X x 10X) .

DISCUSSION

Mycobacterium leprae probably enters the body via the nose and then spreads to the skin and nerves via the circulation.

The immunological response mounted by the host dictates the clinical phenotype that develops. People with leprosy show a spectrum of clinical types. Experimentally, the polar forms of the disease are said to conform to an immunological paradigm. Tuberculoid disease is the result of high cell-mediated immunity with a largely Th1 type immune response. Lepromatous leprosy however is characterized by low cell-mediated immunity with a humoral Th2 response. Intracellular pathogens are recognized by the innate immune system [9].

Total WBC count was in normal range (4,600-11,000) before treatment. It remained the same even after treatment. Lymphocyte count was in normal range (20-40) before treatment. It remained same after treatment. Total WBC count showed leucocytosis before treatment with a mean value of 11,414. Range is 7,000- 22,600.

After treatment range was 5,900- 11,000 with mean value of 8514/mm^3. Before treatment, lymphocyte count was below the normal range with mean value of 19% range is 82.26%.

Lepromatous leprosy patients (LL or BL forms) display a selective immunological unresponsiveness to Mycobacterium leprae antigen with the absence of delayed-typehypersensitivity, I T-cell proliferation, and deficiency in the production of growth factors such as IL-2 [10,11]. These patients also fail to produce interferon-gamma (IFN-y) in response to M. leprae.4 Active suppression by macrophages and/or T cells may explain their inability to respond to leprosy bacilli [12, 13]. Lepromatous patients carry a high load of bacilli which may play a role in vivo in the induction of immune tolerance [14]. Cellular anergy observed in lepromatous patients appears to be M. leprae specific since the immune response against other antigens is largely normal [15].

After treatment there is considerable increase in lymphocyte count. It was in the range of 34% to 42% with a mean value of 38.1%. In the study conducted by R. Sher et al 1976 [16].

In Tuberculoid Leprosy patients Immunological status has not changed significantly after treatment. In treated lepromatous leprosy group there was significant increase in the Immunological status when compared to the Immune status before treatment.

Before treatment there is lymphocytopaenia in the untreated LL group. After therapy total number of lymphocytes increased significantly in lepromatous patients.

Total lymphocytes were normal in untreated tuberculoid leprosy patients. A statistically significant decrease in T cells and total lymphocyte number was found in the untreated LL group.

The immune reactivity observed after chemotherapy suggests that the unresponsiveness in lepromatous patients might not be long lasting and unchangeable in all cases. The inability to kill and clear bacteria during the early phase of infection could result in a high antigenic load which may in turn induce a tolerant state. Some studies have demonstrated that immune tolerance may develop in the presence of a high concentration of antigens and this state may be reversed after decreasing the antigenic load [17, 18].

Above findings were correlating with the present study. One hypothesis for the depletion of T cells in untreated cases of LL is that there is a reduction in the proliferation of T cells in the paracortical areas of lymph nodes due to histiocytic accumulation in these regions [6, 1].

Increased destruction or trapping of T cells in the spleen may also play a role in depleting the T cell population. The effect of treatment on the recovery from the immunological anergy in lepromatous patients is a controversial subject. Findings from a number of studies suggest that an unresponsiveness to M. leprae seen in lepromatous patients is long-lasting and unrelated to the bacterial load [12]. However, some studies have revealed different immunological reactivity to mitogens and mycobacterial antigens when cellular immune responses of short-term treated patients were compared with untreated patients [13, 19-22].

CONCLUSION

Clinical examination or histopathological examination alone may not stand as ideal diagnostic tools in diagnosing and classifying leprosy. There are factors like interobserver variation, overlap between different types of leprosy. Continuous change of Immunological spectrum in borderline cases may be accounting for limitations of considering the single parameter alone as gold standard. A combined approach is always desired.

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