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Original Article

CD4+ T cell and CD8+ T Cell Count with their Ratio in Patients of Leprosy Before and After Treatment

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ABSTRACT

Immunological disturbances have often been described in leprosy patients-more so in the lepromatous (LL) than in the tuberculoid (TT) end of the clinicopathological spectrum of the disease. Studies suggested that active lepromatous patients showed a significant lymphopenia and a significant proportionate reduction in the number of OKT3-positive (Pan T), OKT4-positive (Helper/inducer/CD4), and OKT8-positive (Suppressor/cytotoxic/CD8) cells, but no alteration in distribution as judged by percentage and no abnormality in the helper-suppressor ratio. This research work was performed at the department of microbiology and clinical pathology, department of medicine, Sylhet MAG Osmani Medical College and Hospital, Sylhet; and leprosy hospital, Sheikhghat, Sylhet, during the period of January 2012 to December 2012. The objective of the study was to determine cell-mediated immunity by measuring $CD4^+$ and $CD8^+$ cells, and their ratio in patients with leprosy before and after treatment. In this quasi-experimental study, 30 patients with leprosy were enrolled to measure the status of CD4 and CD8 cell counts with their ratios before intervention, and the values were compared to the second sample from the same patients who had multi-drug treatment for leprosy. Lymphocyte count ($p<0.001$) significantly increased after treatment of leprosy. $CD4^+$ T cell count ($p<0.001$) and $CD8^+$ T cell count ($p=0.001$) were significantly increased after treatment of all 30 leprosy cases, with no significant changes in the $CD4^+/CD8^+$ ratio ($p=0.072$). $CD4^+$ T cell count ($p=0.001$) and $CD8^+$ T cell count ($p=0.018$) were significantly increased after treatment of the 17 specific lepromatous leprosy cases, and there was no significant change in the $CD4^+/CD8^+$ ratio ($p=0.070$) in the same cases. The immune parameter showed a significantly upward change in the form of a raised lymphocyte count, and $CD4^+$ and $CD8^+$ T cell counts, while $CD4^+/CD8^+$ ratios are insignificantly raised after treatment of leprosy and also of lepromatous leprosy.

Keywords: *$CD4^+$ T cell, $CD8^+$ T cell, Leprosy, Immune parameter.*

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INTRODUCTION

Leprosy is a multisystem and immunological disease in which *Mycobacterium leprae* (*M. leprae*) and inflammatory cells infiltrate the skin and various

organs[1-4]. It is a chronic infectious disease affecting the skin and peripheral nerves, caused by the intracellular bacillus *M. leprae*[5]. The incidence of new cases of leprosy remains constant at 286,000 per year, and Brazil is one of the worst-affected countries, accounting for the majority of new cases reported in the Americas[6]. Much of the morbidity of leprosy results from episodic inflammatory exacerbations of leprosy lesions in the skin and nerves, called 'lepra' reactions, thought to be caused by spontaneous shifts in host immunity[6]. Leprosy is caused by *M. leprae*, an obligate intracellular acid-fast bacillus. With the exception of armadillos and certain primates, humans are the only reservoir and the only source of infection for *M. leprae*[7]. The route of transmission of the disease is uncertain but is probably primarily through nasal droplet infection. Other modes of transmission have been documented and include contact with infected soil and, rarely, direct dermal implantation, such as occurs during tattooing. Skin-to-skin contact is thought not to be an important route of infection. There is some controversy about the precise route the organism uses to enter the nerve. A postulated route has been the direct binding of these organisms to Schwann cells that are found in dermal nerves of superficial skin in the cooler parts of the body; in one study, the bacillus appeared to enter the nerve directly through the perineurium[8]. Tuberculoid leprosy develops a cell-mediated immune response, while lepromatous leprosy develops an initially infectious metabolic disease with secondary reactions related to humoral immunity. This mechanism depends on accessory immune system reactivity, which is responsible for the infectious metabolic disease of leprosy and for T or B lymphocyte stimulation[8]. Although great advances have been made in basic research, the knowledge of the pathogenesis of leprosy continues to be a formidable challenge, and the disease has not yet been eradicated. To unmask anergy and cell-mediated and humoral immune phenomena, electron microscopy findings should be re-examined and supplemented with histochemical, immunohistochemical, and immunologic data[7]. The clinical manifestations of leprosy are determined by the cellular immune response of the host. There is a wide spectrum in the host response to *Mycobacterium leprae*, which is evident in both clinical and histologic findings. When the cellular immune response is not induced, the patient manifests the lepromatous form of leprosy. The lesions of lepromatous leprosy are typically loose infiltrates of macrophages permissive for the intracellular multiplication of *M. leprae*. The uncontrolled growth of the bacilli and the spread of infection from cell to cell result in a disseminated cutaneous infection. The low numbers of T cells in the lesions of lepromatous leprosy are almost exclusively $CD8^+$ T cells, with few if any $CD4^+$ T cells present. In vitro, cells from these patients fail to respond t*o M. leprae* in the lymphocyte proliferation assay[8]. 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 finding a satisfactory quantitative measurement of a patient's progress towards a successful outcome. The 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)[9]. Because of the importance of immunological mechanisms in the pathogenesis of leprosy and because of the varieties of granulomatous responses and reactional states that constitute the broad clinical canvas of leprosy, the study of peripheral blood T cell subsets in patients with leprosy as well as immunoglobulin level assessment is logical. So the objective of the study was to determine the cell mediated immunity by measuring $CD4^+$ and $CD8^+$ cells and their ratio in patients with leprosy before and after treatment.

MATERIALS AND METHODS

This quasi-experimental study (Before and after treatment) was designed to determine the cell mediated immunity by measuring $CD4^+$ and $CD8^+$ cells, and their ratio in patients with leprosy before and three months after treatment. This experiment was carried out in the department of microbiology and clinical pathology, and department of medicine, Sylhet MAG Osmani Medical College and Hospital, Sylhet; and Leprosy Hospital, Seikhghat, Sylhet, during the period of January 2012 to December 2012. A total of 30 newly diagnosed cases of leprosy with an age between 18 and 55 years of either sex attending Sylhet leprosy hospital, Seikhghat, Sylhet, and the department of medicine, Sylhet MAG Osmani Medical College Hospital, Sylhet, were included in the study by the consecutive sampling method. Ethical issues were maintained properly. Informed written consent was obtained from each of the participants. Patients with suspected leprosy were interviewed for a detailed history, and a clinical examination was performed in all cases, followed by investigations,

including slit skin smear in all patients. After confirmation of leprosy, before starting treatment, 3 ml of venous blood was collected for $CD4^+$, $CD8^+$ counts and $CD4^+/CD8^+$ ratio. Then multi-drug treatment (MDT) was started. Three months after treatment, 3 ml of venous blood was again collected from the same patient for detection of $CD4^+$, $CD8^+$ count, and $CD4^+/CD8^+$ ratio. The flow cytometric counting procedure for $CD4^+$ and $CD8^+$ cells was utilised fulfilling all the methodical conditions. Data regarding age, sex, socioeconomic status, and type of leprosy were collected and recorded in a data collection sheet. Data were processed and analysed with the help of the computer programme Statistical Package for Social Sciences (SPSS) version 14. Quantitative data were presented as mean and standard deviation, and comparison between groups was done by a paired "t" test. Qualitative data were presented as frequency and percentage.

RESULTS

Among 30 newly diagnosed leprosy patients, 24 (80%)

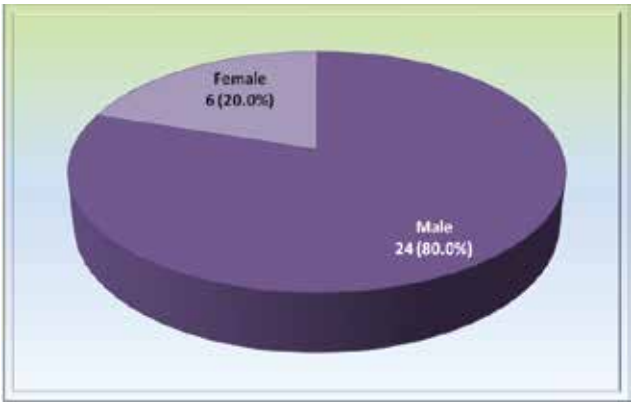

Figure-1: *Distribution of the patients according to sex, N=30*

Table-I: *Distribution of the patients according to age, N=30*

Age (Years)	Frequency	Percentage
18-25	3	10
26-35	4	13.3
36-45	12	40
46-55	11	36.7
Mean±SD (Years)	41.5±10.2	

Table-II: *Disribution of the patients according to type of leprosy, N=30*

Type of Leprosy	Frequency	Percentage
Lepromatous Leprosy	17	56.7
Tuberculoid Leprosy	7	23.3
Leprosy with reaction	6	20

Table-III: *Comparison of $CD4^+$ T cell, $CD8^+$ T cell count and $CD4^+/CD8^+$ ratio before and after treatment of leprosy, N=30*

T cell count	T cells /µL, Mean±SD		*p -value
	Before treatment	After treatment	
$CD4^+$ T cell count	637.1±260	1111.2±255.7	<0.001
$CD8^+$ T cell count	416.1±218.8	639.9±236.5	0.001
$CD4^+/CD8^+$ ratio	1.63±0.42	1.88±0.64	0.072

*Pair-t test was applied to analyze the data.

were males and 6 (20%) were females, with a ratio of males to females of 4:1 (Figure-1). There were 3 (10%) patients in the age group 18-25 years, 4 (13.3%) patients in the age group 26-35 years, 12 (40%) patients in the age group 36-45 years, 11 (36.7%) patients in the age group 46-55 years (Table-I).

Regarding the type of leprosy, 17 (56.7%) were lepromatous leprosy, 7 (23.3%) were tuberculoid leprosy and 6 (20%) were leprosy with reaction (Table-II). Table-III shows the comparison of $CD4^+$ T cell count before and after treatment of leprosy. $CD4^+$ T cell count before treatment was 637.1±260 /uL and after treatment was 1111.2±255.7 /uL. $CD4^+$ T cell count was significantly increased after treatment (t=7.299; p<0.001). $CD8^+$ T cell count before treatment was 416.1±218.8 /uL and after treatment was 639.9±236.5 /uL. $CD8^+$ T cell count was significantly increased after treatment (t=-3.821; p=0.001). $CD4^+/CD8^+$ ratio before treatment was 1.63±0.42 and after treatment was 1.88±0.64. $CD4^+/CD8^+$ ratio was not significantly increased after treatment (t=1.869; p=0.072).

Table-IV shows the comparison of T cell count before and after treatment of lepromatous leprosy. $CD4^+$ T cell count [648.5±242.4 vs 1099.8±278.1; t=-4.239; p=0.001] and $CD8^+$ T cell count [425.6±195.5 vs 593.9±200.5; t=-2.624; p=0.018] were significantly increased after treatment of lepromatous leprosy but $CD4^+/CD8^+$ ratio [1.63±0.37 vs 2±0.77; t=-1.940; p=0.070] was not significantly increased after treatment of lepromatous leprosy.

Table-IV: *Comparison of $CD4^+$ T cell, $CD8^+$ T cell count and $CD4^+/CD8^+$ ratio before and after treatment of lepromatous leprosy, N=17*

T cell count	T cells /µL, mean±SD		p-value
	Before treatment	After treatment	
$CD4^+$ T cell count	648.5±242.4	1099.8±278.1	0.001
$CD8^+$ T cell count	425.6±195.5	593.9±200.5	0.018
$CD4^+/CD8^+$ ratio	1.63±0.37	2±0.77	0.070

*Pair-t test was applied to analyze the data.

DISCUSSION

Leprosy is a chronic infectious disease affecting the skin and peripheral nerves, caused by the intracellular bacillus *Mycobacterium leprae*[5]. Much of the morbidity of leprosy results from episodic inflammatory exacerbations of leprosy lesions in the skin and nerves, called 'Lepra' reactions, thought to be caused by spontaneous shifts in host immunity[6]. The present study was conducted with a view to evaluate T-lymphocytes status in patients with leprosy before and after treatment. In this study, the age of the patients ranged from 18 to 55 years, with a mean age of 41.5±10.2 years. This result was supported by Sasiain et al[11]. They found the age of the patients ranged from 15 to 69 years, with a mean age of 40±14 years. However, a lower mean age were reported in some studies[12,13], while others reported a higher mean age[14-16]. The present study also showed 12 (40%) patients in the age group of 36-45 years, 11 (36.7%) patients in the age group of 46-55 years, 4 (13.3%) patients in the age group of 26-35 years, and 3 (10%) patients in the age group of 18-25 years. A study conducted by de Sousa et al.[17] found that 91.5% of the patients were 15 years of age or older, while 8.5% were under 15 years of age. The frequency of cases increased with age, with the highest incidence being in the 20-59 years age group. In another study, Mathur et al.[18] found that the majority of leprosy patients (23.6%) were in the age group of 21-30 years, and the least affected were children below 10 years (0.007%). A similar study conducted in India[19] reported that the majority of patients were in the age group of 20-29 years (20.7%) and the least affected were children below 9 years (6.5%). Madan et al.[12] reported that the majority of cases (52.5%) were in the age group of 20-40 years. Poojabylaiah et al.[14] found 121 (74.3%) leprosy patients were over 30 years old and 42 (25.7%) patients were below 30 years of age.

In the current study, $CD4^+$ T cell count before treatment was 648.5±242.4 /µL and after treatment was 1099.8±278.1 /µL. $CD4^+$ T cell count was significantly increased after treatment (p=0.001). In their study, Brown et al.[20] reported an increased $CD4^+$ T cell count

after treatment of leprosy with cemetidine, but they did not report whether this change was significant or not. Cimetidine may act as a nonspecific stimulant of cell-mediated immunity (CMI). Inhibition of various T cell functions and activation of suppressor cells appear to be mediated in part via H-2 receptors.

In the current study, $CD8^+$ T cell count before treatment was 425.6±195.5/µL and after treatment was 593.9±200.5 /µL. $CD8^+$ T cell count was significantly increased after treatment (p=0.001). In this regard, Brown et al.[20] reported an increased $CD8^+$ T cell count after treatment of leprosy with cemetidine, but they did not report whether this change was significant or not.

In their study, Brown et al.[20] reported an increased $CD4^+/CD8^+$ ratio after treatment of leprosy with cemetidine but they did not report whether this change was significant or not. Similar to this study, $CD4^+/CD8^+$ ratio was increased after treatment in our study, which was statistically significant (p=0.072).

CONCLUSION

This study revealed the fact that the immune status of a leprosy patient remains suppressed until effective treatment. This research proved that the pivotal factors of a patient's immune status, like the $CD4^+$ T cell count and $CD8^+$ T cell count were significantly increased after treatment with leprosy and also after treatment with lapromatous leprosy. Instead of such a scenario, $CD4^+/CD8^+$ ratio was not significantly increased after treatment with leprosy or after treatment with lepromatous leprosy. So immune parameters like measuring $CD4^+$ and $CD8^+$ T cell counts and their ratios before and after treatment of leprosy could establish a valuable, cost-effective yardstick for following up on leprosy patients for unburden the morbidity.

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