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Original Article

Immune Status in Patients of Leprosy before and after Treatment

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Abstract:

Immunological disturbances are frequently observed in leprosy patients, particularly in the lepromatous (LL) form compared to the tuberculoid (TT) form. This study investigated immunological changes in active lepromatous leprosy patients, revealing significant lymphopenia and a proportional reduction in OKT3-positive (pan T), OKT4-positive (helper/inducer), and OKT8-positive (suppressor/cytotoxic) cells. However, no alterations in percentages or helper-to-suppressor ratios were noted.

The research was conducted at the Microbiology, Clinical Pathology and Medicine Departments of Sylhet MAG Osmani Medical College and Hospital, along with the Leprosy Hospital in Seikhghat, Sylhet, from January to December 2012. A quasi-experimental design was employed, involving 30 leprosy patients. Immunological parameters, including total blood count, serum total protein, serum albumin, serum globulin (and their ratios), CD4+, and CD8+ T-cell counts, were measured before and three months after multidrug therapy (MDT).

Following MDT, lymphocyte counts showed a significant increase ($p < 0.001$), whereas other parameters such as total white blood cell count ($p = 0.226$), polymorph neutrophils ($p = 0.110$), monocytes ($p = 0.213$), eosinophils ($p = 0.104$), basophils ($p = 0.388$), red blood cells ($p = 0.893$), and hemoglobin levels ($p = 0.982$) remained unchanged. Serum total protein ($p = 0.358$), serum albumin ($p = 0.299$), serum globulin ($p = 0.179$), and the albumin/globulin ratio ($p = 0.148$) also showed no significant changes. Significant increases were observed in CD4+ ($p < 0.001$) and CD8+ ($p = 0.001$) T-cell counts after MDT in lepromatous leprosy patients. However, the CD4+/CD8+ ratio remained unchanged.

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Introduction:

Leprosy, also known as Hansen's disease, is a disease of chronic, multisystem infection caused by a rod-shaped, acid-fast bacillus named *Mycobacterium leprae*.[1-4]It predominantly affects the skin, peripheral nerves, and upper respiratory tract mucosa, eyes, and other structures.

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The disease involves complex immunological processes, with inflammatory cells infiltrating the skin and various organs. Clinical manifestations of leprosy are largely determined by the host's cellular immune response, which can vary widely, resulting in different forms of the disease.[5]Tuberculoid leprosy is characterized by a strong cell mediated immune response,

leading to localized, lymphocyte-rich granulomas with limited bacterial presence. The predominant immune cells in tuberculoid lesions are CD4+ T cells, which are responsible for effectively controlling the growth of *M. leprae*. Patients with this form of leprosy demonstrate strong in vitro responses to *M. leprae* antigens and exhibit positive skin test reactions to lepromin. In some cases, the infection can become self-limiting.[6] In contrast, lepromatous leprosy occurs when the host's cellular immune response fails to control the infection. Lesions in this form consist of loose macrophage infiltrates that permit the intracellular multiplication of *M. leprae*.[7] CD8+ T cells are predominant, while CD4+ T cells are largely absent. These patients show minimal response to *M. leprae* antigens in vitro, and the disease often progresses to a disseminated cutaneous infection.[8] The Th1/Th2 paradigm explains how lepromatous and tuberculoid responses differ from one another. Th1 cells promote cellular immunity, as seen in tuberculoid leprosy, while Th2 cells promote humoral immunity, which is dominant in lepromatous leprosy.[9] Leprosy reactions are categorized into two main types. Type 1 (reversal) reactions, which occur in approximately 30% of borderline cases (BT, BB, or BL), involve delayed hypersensitivity. Type 2 reactions, known as erythema nodosum leprosum (ENL), are associated with immune complex deposition and occur in patients with high antibody production and antigen load, primarily in BL and LL forms.[8] Treatment with multiple drug therapy (MDT), recommended by the World Health Organization (WHO), has proven effective in managing leprosy. However, monitoring treatment progress remains challenging, as current methods, such as clinical observation and bacterial index (BI), are subjective. Some studies have explored changes in antibody levels to *M. leprae* antigens, including phenolic glycolipid (PGL-I), to assess response to treatment. Evidence suggests that enhancing immune function could play a crucial role in improving treatment outcomes, particularly for lepromatous leprosy patients.[10]

Although a few international studies have examined the immune status of leprosy patients before and after MDT[11], no such research has been conducted in Bangladesh. This study aims to evaluate the immunological status of leprosy patients, with a focus on cell-mediated immunity, before and three months after initiating MDT. The findings will provide a comprehensive understanding of immune changes associated with treatment in this population

Materials and Method

This quasi-experimental study (before-and-after treatment) was conducted in the Microbiology, Clinical Pathology and Medicine department of Sylhet MAG Osmani Medical College and Hospital, Sylhet, as well as the Leprosy Hospital, Seikhghat, Sylhet, from January to December 2012. The study involved patients with suspected leprosy who attended the Sylhet Leprosy Hospital and the Department of Medicine at Sylhet MAG Osmani Medical College Hospital. All participants were interviewed, and a thorough medical history was obtained, followed by a clinical examination. Among the investigations, slit-skin smears were done for all participants. After confirming the diagnosis of leprosy, patients were enrolled based on inclusion and exclusion criteria. A total of 30 patients aged between 18 and 60 years, of both sexes, were selected for the study. Written informed consent was obtained from each participant after a full explanation of the study objectives. Prior to initiating treatment, 3 mL of venous blood was collected from each patient to measure CD4+, CD8+ counts, and the CD4+/CD8+ ratio. Multi-drug therapy (MDT) was then started for the diagnosed cases of leprosy, classified either as tuberculoid (paucibacillary) or lepromatous (multibacillary). Three months after starting treatment, 5 mL of venous blood was collected again from the same patients to assess full blood count, serum total protein, serum albumin and globulin levels, albumin/globulin ratio, and CD4+, CD8+ counts, as well as the CD4+/CD8+ ratio.

Flow cytometric analysis was used for counting CD4+ and CD8+ cells, while a hematology analyzer employing light scatter techniques was used to differentiate white blood cell (WBC) counts into neutrophils, lymphocytes, eosinophils, monocytes and basophils. All

procedures were conducted following standard methodological protocols. The collected data were processed and analyzed using the Statistical Package for Social Sciences (SPSS) software version 16 for Windows. Quantitative data were presented as mean and standard deviation, and comparisons between groups were performed using a paired t-test. Qualitative data were presented as frequencies and percentages. Ethical approval for this study was obtained from the Ethical Committee of Sylhet MAG Osmani Medical College prior to its commencement.

Results

The age of the patients in this study ranged from 18 to 55 years, with a mean age of 41.5 years (SD ± 10.2). Among the participants, 12 patients (40.0%) were in the 36-45 years age group, 11 patients (36.7%) in the 46-55 years group, 4 patients (13.3%) in the 26-35 years group, and 3 patients (10.0%) in the 18–25 years group **(Figure 1)**. There were 24 male patients (80.0%) and 6 female patients (20.0%), resulting in a male-to-female ratio of 4:1. Among the 30 patients, lepromatous leprosy was observed in 17 patients (56.7%), tuberculoid leprosy in 7 patients (23.3%), and leprosy with reaction in 6 patients (20.0%) **(Figure 2).**

Following MDT, lymphocyte counts showed a significant increase ($p < 0.001$), whereas other parameters such as total WBC count ($p = 0.226$), polymorph neutrophils ($p = 0.110$), monocytes ($p = 0.213$), eosinophils ($p = 0.104$), basophils ($p = 0.388$), red blood cells ($p = 0.893$), and hemoglobin levels ($p = 0.982$) remained unchanged **(Table 1)**. Serum total protein ($p = 0.358$), serum albumin ($p = 0.299$), serum globulin ($p = 0.179$), and the albumin/globulin ratio ($p = 0.148$) also showed no significant changes **(Table 2)**. Significant increases were observed in CD4+ ($p < 0.001$) and CD8+ ($p = 0.001$) T-cell counts after MDT in lepromatous leprosy patients. However, the CD4+/CD8+ ratio remained unchanged **(Table 3)**.

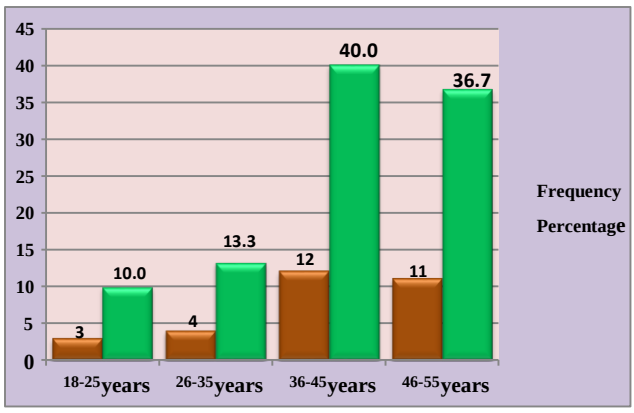

Figure 1: Age distribution among participants

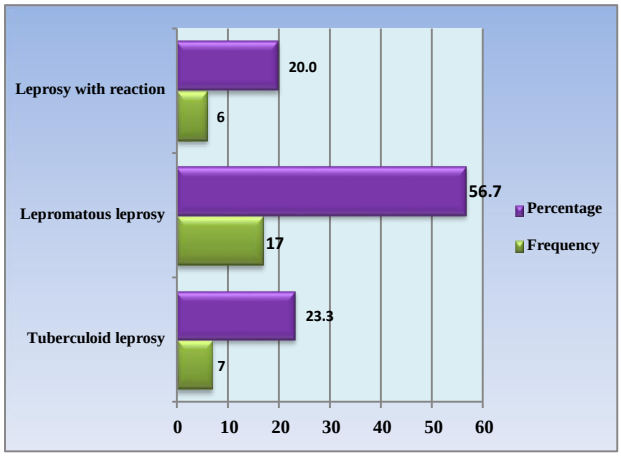

Figure 2: Types of leprosy among participants

Table 1: Hematological profile of participants before and after treatment

	Parameter	Before treatment	After treatment	T test	P value
1	TC	10.3 ± 4.6	11.4 ± 4.6	t = -1.237	p=0.226
2	Polymorph	52.8 ± 10.4	48.3 ± 10.2	t = 1.651	p=0.110
3	Monocyte	8.1 ± 3.8	7.2 ± 2.7	t = 1.273	p<0.001
4	Eosinophil	7.9 ± 6.0	5.7 ± 4.0	t = 1.678	p=0.213
5	Basophil	0.76 ± 0.45	0.68 ± 0.42	t = 0.876	p=0.104
6	RBC	3.99 ± 0.96	4.03 ± 1.33	t = -0.136	p=0.388
7	Hemoglobin (gm/dl)	10.20 ± 2.71	10.19 ± 2.99	t = 0.023	P=0.893
8	Lymphocyte	30.4 ± 5.2	37.4 ± 6.5	t = -4.624	p < 0.001

Table 2: Serum protein profile of participants before and after treatment

	Parameters	Before treatment	After treatment	p-value
1	Serum total protein (gm/dl)	7.04 ± 0.39	7.15 ± 0.24	p=0.358
2	Serum albumin (gm/dl)	3.75 ± 0.36	3.90 ± 0.26	p=0.299
3	Serum globulin (gm/dl)	3.36 ± 0.31	3.22 ± 0.22	p=0.179
4	Serum A/G ratio	1.13 ± 0.15	1.21 ± 0.14	p=0.148

Table 3: CD4+ and CD8+ counts before and after treatment

			Before treatment	After treatment	P value
1	**CD4+ T cells /µL**	Mean	637.1	1111.2	p<0.001
		SD	± 260.0	± 255.7	
2	**CD8+ T cells /µL**	Mean	416.1	639.9	p=0.001
		SD	± 218.8	± 236.5	
3	**CD4+/ CD8+**	Mean	1.63	1.88	p=0.072
		SD	± 0.42	± 0.64	

Discussion:

Leprosy is a disease caused by *Mycobacterium leprae* (M. leprae) which is an obligate intracellular acidfast bacillus. Apart from armadillos and certain primates, humans are the only known reservoir and source of infection for *M. leprae*.[12] In order to study the T helper-1/T helper-2 (Th1/Th2) paradigm, leprosy has been suggested as a human infection model. That allows researchers to examine polarized T helper responses to a single pathogen. A significant portion of leprosy-related morbidity results from "lepra reactions," which are episodic inflammatory flare-ups of leprosy lesions in the skin and nerves. These reactions are believed to result from spontaneous shifts in host immunity.[13]

Generalized, reversible depression of T-lymphocytes has been previously demonstrated in tuberculosis[14] as well as in other infectious diseases, such as lepromatous leprosy[15]. In all these studies, lymphocyte count returned to normal levels following therapy. However, no similar study has been conducted in the Bangladeshi population. Therefore, this study was conducted mainly in the Department of Microbiology at Sylhet MAG Osmani Medical College in Bangladesh, within the timeframe of January 2012 to December 2012, with the purpose of evaluating the immune status of leprosy patients before and after treatment. For this purpose, 30 patients with leprosy were selected. The age of the patients in this study ranged from 18 to 55 years, with a mean age of 41.5 years (SD ± 10.2). Some of the studies have reported a lower mean age of detection[16,17], while others have documented a higher mean age of detection[18,19,20].

The present study revealed that 12 patients (40.0%) were in the age group of 36–45 years, 11 patients (36.7%) in the age group of 46–55 years, 4 patients (13.3%) in the age group of 26–35 years, and 3 patients (10.0%) in the age group of 18–25 years. In this regard, de Sousa et al.[21] reported that 91.5% of their patients were aged 15 years or older, while 8.5% were under 15 years. The frequency of cases increased with age, with the highest incidence observed in the 20–59 years age group. Similarly, Mathuret al.[22] found that the majority of leprosy patients (23.57%) were in the 21–30 years age group, with children under 10 years being the least affected (0.007%). Madanet al.[16] reported that the majority of cases (52.45%) were in the 20–40 years age group. Poojabylaiahet al.[18] observed that 121 patients (74.3%) were above 30 years of age, while 42 patients (25.7%) were below 30 years.

In the present study, there were 24 male patients (80.0%) and 6 female patients (20.0%), resulting in a male-to-female ratio of 4:1. Similar findings were reported by Poojabylaiahet al.[17] and Shen et al.[18]. Poojabylaiahet al.[18] observed 126 male patients and 37 female patients, with a male-to-female ratio of 3.41:1. Shenet al.[17] found a male-to-female ratio of 3.05:1 among leprosy patients. Madanet al.[16] reported a male-to-female ratio of 3:1. Other studies have also documented a male predominance among leprosy patients.[19,21,22]In this study, the total count of WBC in peripheral blood [10.3 ± 4.6 vs. 11.4 ± 4.6; p = 0.226] did not change significantly after the treatment of leprosy. This finding is in line with the study by Sheret al.[23], which also reported no significant changes in WBC count following leprosy treatment.

However, the lymphocyte count in our study [30.4 ± 5.2 vs. 37.4 ± 6.5; p < 0.001] showed a significant increase after treatment. This result aligns with the findings of Sheret al.[23], who also observed a significant increase in lymphocyte count post-treatment. In this study, serum total protein [7.04 ± 0.39 vs. 7.15 ± 0.24; p = 0.358], serum albumin [3.75 ± 0.36 vs. 3.90 ± 0.26; p = 0.299], serum globulin [3.36 ± 0.31 vs. 3.22 ± 0.22; p = 0.179], and the serum albumin-to-globulin ratio [1.13 ± 0.15 vs. 1.21 ± 0.14; p = 0.148] did not change significantly after treatment. In the current study, the CD4+ T cell count before treatment was 637.1/µL (SD ± 260.0), and after treatment, it increased to 1111.2/µL (SD ± 255.7). This increase was statistically significant (p < 0.001). Similarly, Brown et al.[8] reported an increase in CD4+ T cell count after treating leprosy with cimetidine, although they did not specify whether the change was statistically significant. Cimetidine is thought to act as a nonspecific stimulant of cell-mediated immunity (CMI), with its effects on T cell functions and suppressor cell activation potentially mediated in part via H-2 receptors.

In the current study, the CD8+ T cell count before treatment was 416.1/µL (SD ± 218.8), which increased to 639.9/µL (SD ± 236.5) after treatment. This increase was statistically significant (p = 0.001). Similarly, Brown et al.[8] reported an increase in CD8+ T cell count following the treatment of leprosy with cimetidine, although they did not specify whether this change was statistically significant. The CD4+/CD8+ ratio in this study was 1.63 (SD ± 0.42) before treatment and 1.88 (SD ± 0.64) after treatment, reflecting a non-significant increase (p = 0.072). Brown et al.[8] also reported an increase in the CD4+/CD8+ ratio after treatment with cimetidine but did not indicate whether the change was significant. It is essential to note that cimetidine was not used in the current study population. Some variations were observed in our findings. For instance, serum total protein, serum albumin, serum globulin, and the serum albumin-to-globulin ratio did not change significantly after three months of leprosy treatment. This lack of significant change may be attributed to the short treatment duration in the present study, as other studies have reported changes with long-term treatment. Additionally, the inclusion of patients with lepromatous leprosy, tuberculoid leprosy, and leprosy with reaction (erythema nodosum leprosum) may have contributed to the variations in results.

Conclusion:

Leprosy remains endemic in a lot of developing countries, where it continues to be a significant health problem. The immune response plays a key role in controlling mycobacterial infections, and monitoring immune responses during leprosy could provide valuable tools for managing patients during and after treatment. This study demonstrated that the immune status of leprosy patients remains suppressed until effective treatment is administered. In conclusion, immune parameters showed a significant improvement, reflected by an increase in lymphocyte count, as well as CD4+ and CD8+ T cell counts. Therefore, measuring immune parameters could aid as a valuable and cost-effective guideline for the follow-up and management of leprosy patients.

Conflict of interest: This was a self-funded research and the authors declare no conflict of interest.

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