

ORIGINAL ARTICLE

Human Interleukin-12 p40 Induced Immune Responses in Heterogenic Group of Healthy Leprosy Contacts

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ABSTRACT

Leprosy, caused by Mycobacterium leprae, remains poorly understood despite reduced global attention and the misconception that it has been eradicated. Key aspects of its transmission and pathogenesis are still unclear. Remarkably, M. leprae can spread among hospital staff despite stringent hygiene, yet resists experimental transmission in humans. Prolonged, close contact with untreated patients, especially among malnourished individuals, appears to increase susceptibility. This study aimed to explore immunological mechanisms behind natural resistance to M. leprae, with a focus on the cytokine IL-12p40. Three groups were analysed: healthy individuals with long-term exposure to leprosy patients in caregiving roles, clinically diagnosed leprosy patients, and healthy, unexposed volunteers. Biochemical parameters, including albumin, globulin, and C-reactive protein (CRP), were assessed to evaluate immune and inflammatory status. Special emphasis was placed on regulatory IL-12p40, a cytokine that supports protective immunity against intracellular pathogens. Notably, exposed but healthy individuals, including both household contacts and healthcare workers, showed elevated IL-12p40 levels, indicating a possible adaptive immune mechanism conferring resistance to infection. Additionally, a link between nutritional status and disease susceptibility was observed, reinforcing the impact of malnutrition on leprosy progression. These findings highlight the role of IL-12p40 in mediating immune protection in naturally exposed yet uninfected individuals. The results enhance our understanding of host-pathogen interactions and suggest that immune modulation via IL-12p40 may offer potential pathways for future prevention and control strategies in endemic regions.

KEYWORDS: *Mycobacterium leprae, Cytokines, Interleukin-12p40, Autoimmune, Inflammatory.*

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INTRODUCTION

Leprosy, also known as Hansen's disease, was first described in 1873 when G.A. Hansen discovered the causative bacillus. The disease is primarily transmitted through droplets from the nose, mouth, and skin during close and frequent contact with untreated infected individuals. *Mycobacterium leprae* (*M. leprae*) has not been successfully cultivated in vitro. It is believed that one of the significant routes of transmission to medical personnel is via airborne droplets. This view is supported by the presence of a large number of viable bacilli in the nasal discharge of lepromatous patients, as reported (1,2). Leucocytosis has been observed in both febrile cases and early aesthetic leprosy. The persistence of *M. leprae* infection is largely influenced by the host's immune response (3). Lymphocytes (both T and B cells), along with macrophages, migrate to the affected area, sensitizing or immunizing the host against the pathogen. Neutrophils can be activated by cytokines such as interferon-gamma (IFN- γ) and tumour necrosis factor-alpha (TNF- α), and play a crucial role in defending the body against infection (4). TNF- α , produced by monocytes, is essential for host immunity, although its overproduction may lead to harmful pathological consequences. Monocytes and monocyte-derived macrophages serve as vital antigen-presenting cells and are key players in the development of adaptive immune responses. Tissue

macrophages, monocytes, and granulocytes possess some intrinsic anti-parasitic activity, which is significantly enhanced by antibodies and cytokines secreted by sensitized lymphocytes (5). Approximately 80% of circulating lymphocytes are T cells, 15% are B cells, and the remaining 5% are 'null' cells (cells that are neither T nor B cells). Natural killer (NK) cells, although classified differently, are functionally similar to T cells. An extended haematological study is to be discussed in another paper with complete information. The albumin-to-globulin (A/G) ratio is often used to assess disease status, although it is not specific to any particular disease, as it does not indicate which proteins are altered. C-reactive protein (CRP) is an acute-phase protein found in normal serum, which increases significantly in response to tissue injury, infections, inflammation, or malignant conditions (6). Interleukins (ILs) are a group of cytokines, secreted proteins and signalling molecules, produced by leukocytes that mediate immune communication. The name "interleukin" reflects their function as communicators ("inter-") and their origin in leukocytes ("-leukin"). The human genome encodes more than 50 interleukins and related proteins, which are grouped based on structural features. IL-12 is a lymphokine that stimulates the proliferation of responsive T cells and can also act on certain B cells by promoting growth and antibody production (7,8). Medical personnel, including leprosy workers, often come into direct contact with leprosy patients through touch, particularly via their hands. Despite this, healthy leprosy contacts have rarely been examined in detail to assess their immune status. This study is part of a broader research project examining different groups of healthy leprosy contacts, aiming to underscore the continued need for research into the immunological aspects of leprosy.

MATERIAL AND METHODS

This study was conducted at the Central Leprosy Teaching and Research Institute (CLT&RI), Thirumani, Chengalpattu, Tamil Nadu, India, during the months of January to March 2018. Participants included in the study were those who provided written informed consent by signing the 'Human Informed Consent Form'. The study population comprised healthy individuals with varying durations and types of exposure to leprosy patients, including those with less than 10 years and more than 25 years of professional contact in leprosy hospitals, as well as male and female hospital workers who also had household exposure, amounting to a combined exposure of over 40 years. The control group consisted of 30 healthy young adults (15 males and 15 females), aged between 25 and 35 years, with no known history of contact with leprosy patients. Leprosy is known as a 'Great Imitator' (9). Understanding the cellular immunologic phenomena of leprosy has been challenging due to the lack of specific antigens. The Diaclone IL-12p40 Enzyme-Linked Immunosorbent Assay (ELISA) kit was purchased from DIACLONE SAS, France. This ELISA kit is a solid-phase sandwich ELISA for the in-vitro qualitative and quantitative determination of IL-12p40 (p40 subunit) in supernatants, and buffered serum or plasma samples. The assay can detect both natural and recombinant human IL-12p40 monomers. The kit is configured for research use only.

Albumin and Globulin Ratio: Photometric methods were employed using the EM DESTINY 180 ERBA MANNHEIM-TRANSASIA instrument to estimate albumin and globulin levels. An inversion of the A/G ratio is considered significant in the immunological context of leprosy. The normal A/G ratio ranges from 0.8 to 2.0. A decreased A/G ratio may result from low albumin levels or elevated globulin concentrations. Total globulins can be elevated in several chronic inflammatory conditions, including tuberculosis, syphilis, multiple myeloma, and leprosy. A reversed A/G ratio may serve as a diagnostic tool and a useful indicator for managing individuals affected by leprosy (10).

C-reactive protein (CRP): The CRP-Turbilatex is a quantitative turbidimetric test used for the measurement of C-reactive protein (CRP) levels in human serum or plasma. In this method, latex particles coated with anti-human CRP antibodies agglutinate when mixed with CRP-containing samples. The test kit, supplied by BEACON, was used to measure CRP levels in all samples. To quantify the degree of agglutination, the PHOTOMETER 5010 V5+ (ROBERT BLELE KG, Berlin) was used. Based on this method, the normal serum CRP level was estimated to be up to 6 mg/L, which was adopted as the reference range for this study.

Human Interleukin (IL) - 12p40: IL-12p40 has been identified as a potential target in the treatment of autoimmune and systemic inflammatory diseases. The DIACLON Human IL-12p40 ELISA kit is a solid-phase sandwich ELISA designed for the quantitative determination of IL-12p40 (p40 subunit) in serum, plasma, and supernatant samples from tissue cultures (11). This kit is intended solely for research use. The assay procedure, including the preparation of the standard curve and controls, was carried out according to the manufacturer's specifications. Pre-coated ELISA plates were washed at the required intervals using the automated LISAWASH 4000 TULIP Microplate ELISA Washer. The absorbance of the samples was measured at 450 nm using the LISAQUANT-TS TULIP Microplate ELISA reader. Results were

calculated from the standard curve generated using the reagents and buffers supplied with the research kit.

RESULTS AND DISCUSSION

Participants who signed the consent forms were categorized into four equal groups, each comprising 15 males and 15 females within the same age range. Group I included healthy professionals with less than 10 years of contact exposure to leprosy. Group II consisted of healthy professionals with more than 30 years of contact exposure. Group III comprised healthy professionals who also had household contact with leprosy patients for more than 40 years. Group IV, the control group, included healthy individuals with no history of contact with leprosy patients.

SERUM ALBUMIN AND GLOBULIN RATIO

Albumin accounts for more than 50% of total serum proteins. Total globulin levels tend to increase in certain chronic inflammatory diseases. A reversed Albumin-to-Globulin (A/G) ratio may serve as a useful indicator of such conditions (10). In our study, no significant differences in the A/G ratio were observed across the four groups, indicating the absence of any active inflammatory conditions during the sampling period.

Table 1: Comparative Analysis of Serum Albumin/Globulin (A/G) Ratio in Leprosy Contact Groups and Healthy Controls (g/dL)

Groups	Female Mean $\pm$ SD	Male Mean $\pm$ SD
Group I	2.97 $\pm$ 0.12	3.01 $\pm$ 0.10
Group II	3.20 $\pm$ 0.19	3.32 $\pm$ 0.19
Group III	3.22 $\pm$ 0.21	3.28 $\pm$ 0.24
Group IV (Healthy Controls)	2.84 $\pm$ 1.33	2.81 $\pm$ 0.09
Two-Way ANOVA	Group: $F = 6.78$, $P = 0.0012$	Sex: $F = 0.94$, $P = 0.336$ Interaction: $F = 1.28$, $P = 0.291$

Statistical analysis using a two-way analysis of variance (ANOVA) revealed a significant effect of exposure group on the serum albumin-to-globulin (A/G) ratio ($F(3,112) = 6.78$, $P = 0.0012$). Participants in Group II and Group III, both consisting of individuals with prolonged or household exposure to *Mycobacterium leprae*, showed significantly higher A/G ratios compared to the control group (Group IV). There was **no** significant main effect of sex ($F(1,112) = 0.94$, $P = 0.336$), nor was there a significant interaction between group and sex ($F(3,112) = 1.28$, $P = 0.291$), indicating that the observed differences in A/G ratio were independent of sex (Table 1).

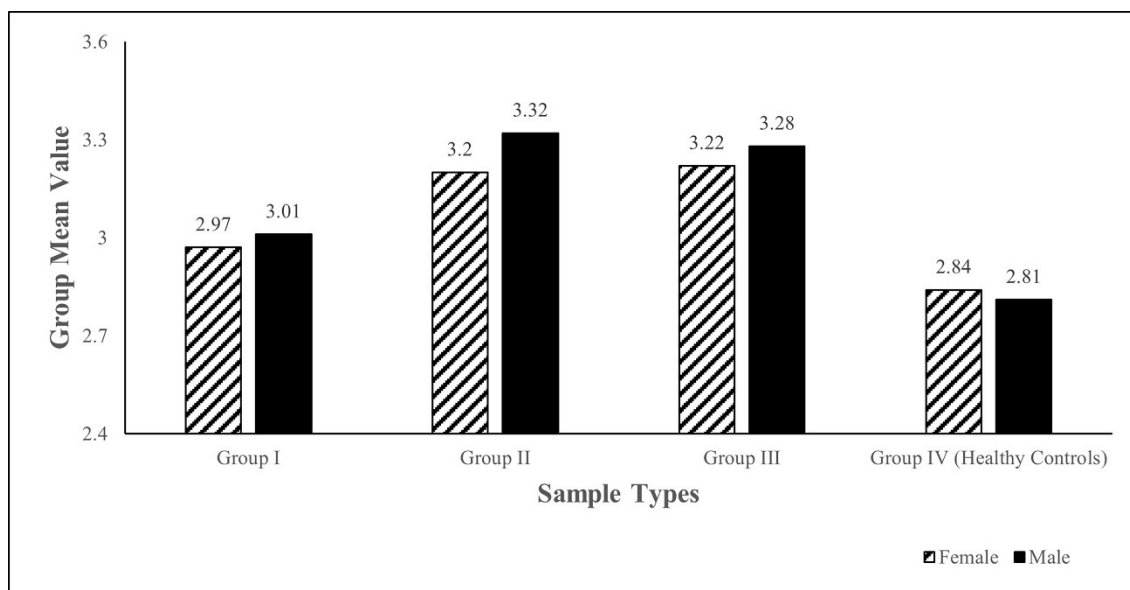


Figure 1: Graphical representation of Serum Albumin and Globulin Ratio in Leprosy-Exposed Individuals and Healthy Controls.

This bar graph (Figure 1) illustrates the mean serum albumin to globulin (A/G) ratios across four groups of individuals. Leprosy-exposed subjects (Groups I, II & III) and healthy controls (Group IV) were identified by gender variation.

C - REACTIVE PROTEIN

C-reactive protein (CRP) is a non-specific indicator of inflammation (6). Male healthy contacts in Group III exhibited a marked increase in CRP levels compared to the male controls and other test groups. Interestingly, a significant decrease in CRP levels was observed among the male controls.

Table 2: Comparative Analysis of C-Reactive Protein (CRP) Levels Among Leprosy Contact Groups and Healthy Controls (mg/L)

Groups	Female Mean $\pm$ SD	Male Mean $\pm$ SD
Group I	2.62 $\pm$ 1.171	2.99 $\pm$ 0.882
Group II	2.84 $\pm$ 0.681	3.26 $\pm$ 0.714
Group III	3.98 $\pm$ 0.539	4.75 $\pm$ 0.286
Group IV (Healthy Controls)	1.26 $\pm$ 0.557	0.04 $\pm$ 0.124
Two-Way ANOVA	Group: $F = 45.32$, $p < 0.0001$	Sex: $F = 12.48$, $p = 0.0007$ Interaction: $F = 2.81$, $p = 0.043$

A two-way ANOVA revealed a highly significant effect of exposure group on C-reactive protein (CRP) levels ($F (3,112) = 45.32$, $P < 0.0001$). Participants in Group III (female: 3.98 ± 0.539 mg/L; male: 4.75 ± 0.286 mg/L) showed the highest CRP concentrations, significantly elevated compared to all other groups ($p < 0.05$). The control group (Group IV) exhibited the lowest CRP levels, with males having markedly reduced values (0.04 ± 0.124 mg/L). A significant main effect of sex was observed ($F (1,112) = 12.48$, $P = 0.0007$), indicating that in the test group, males generally exhibited higher CRP levels than females. Additionally, a significant interaction between group and sex ($F (3,112) = 2.81$, $P = 0.043$) suggests that the pattern of group differences varied by sex (Table 2).

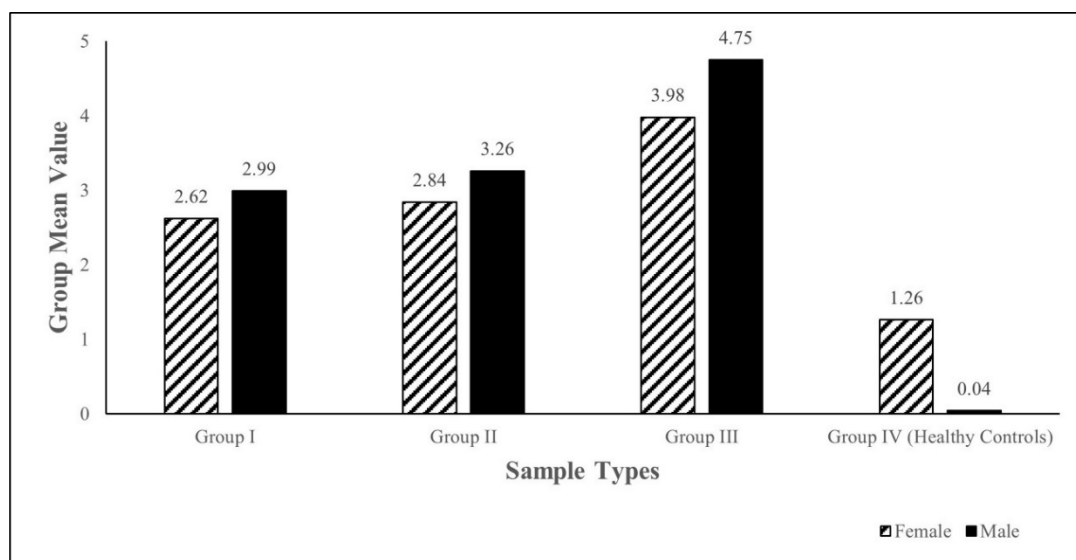


Figure 2: Graphical representation of C-RP in Leprosy-Exposed Individuals and Healthy Controls

This bar graph (Figure 2) illustrates the mean C-reactive protein (C-RP) levels in leprosy leprosy-exposed group (Groups I, II & III) with healthy controls (Group IV), by gender variation.

HUMAN INTERLEUKIN-12p40/70

IL-12 p40 (p40 subunit) has been described as a target in the treatment of autoimmune and systemic inflammatory diseases (12). The potential influence of various drugs on the assay results has not been evaluated by the test kit manufacturer. IL-12p40 levels were estimated using freshly prepared standards. In comparison to their male counterparts, the female groups, both among the contact groups and the control group, showed elevated IL-12p40 levels.

Table 3: Comparative Analysis of Serum IL-12p40/70 in Leprosy Contact Groups and Healthy Controls (pg/mL)

Groups	Female Mean $\pm$ SD	Male Mean $\pm$ SD
Group I	230.62 $\pm$ 39.34	187.20 $\pm$ 37.81
Group II	195.46 $\pm$ 40.01	174.95 $\pm$ 28.08
Group III	181.7 $\pm$ 31.61	175.00 $\pm$ 29.31
Group IV (Healthy Controls)	179.37 $\pm$ 22.48	121.00 $\pm$ 23.35
Two-Way ANOVA	Group: $F = 9.64$, $p < 0.0001$	Sex: $F = 16.82$, $p = 0.0001$ Interaction: $F = 2.26$, $p = 0.086$

A two-way ANOVA revealed a statistically significant effect of group on serum IL-12p40/70 levels (F (3,112) = 9.64, $P < 0.0001$). Individuals in Group I (healthcare professionals with <10 years of contact) exhibited the highest cytokine levels (female: 230.62 $\pm$ 39.34 pg/mL; male: 187.20 $\pm$ 37.81 pg/mL), while the control group (Group IV) showed the lowest levels, particularly in males (121.00 $\pm$ 23.35 pg/mL). A significant effect of sex was also observed (F (1,112) = 16.82, $P = 0.0001$), with females demonstrating overall higher IL-12p40/70 concentrations than males. However, the interaction between group and sex was not statistically significant (F (3,112) = 2.26, $P = 0.086$), indicating that the pattern of group differences was consistent across sexes (Table 3).

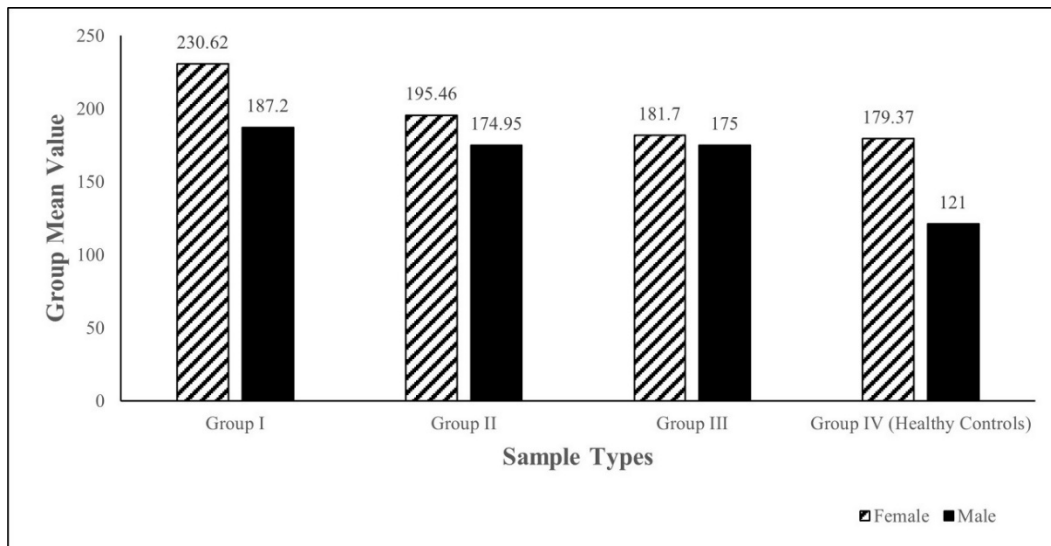


Figure 3: Graphical representation of Human IL-12p40 in Leprosy-Exposed Individuals and Healthy Controls.

This bar graph (Figure 3) illustrates mean levels of IL-12p40 in leprosy-exposed groups (Groups I, II & III) and healthy controls (Group IV), which were specifically identified by gender. This trend suggests a potential immunological response to *Mycobacterium leprae*. *Mycobacterium leprae* is a non-cultivable bacterium in artificial media and typically grows in the cooler regions of host tissues, particularly in humans (13). It is understood that the cell wall, cell membrane, and secreted proteins of *M. leprae* are the first components to interact with host immune cells, stimulating the host's immune system. In our view, identifying peptide antigens that interact with immune cells could provide potential targets for the development of diagnostic tools for leprosy. Notably, *M. leprae* has been shown to transmit even in hospital settings with high hygiene standards, suggesting that transmission risk is not limited to endemic areas. Defective Cell-Mediated Immunity (CMI) is a hallmark of *M. leprae* infection. A significant number of positive immune responses have been reported among health staff involved in treating leprosy and among household contacts of patients.

IL-12 plays a vital role in promoting cell-mediated immunity against microbial pathogens. To assess the stage and level of immune response in healthy leprosy contacts compared to healthy controls, groups were evaluated based on infection and immune parameters. Early tissue responses to infection involve the accumulation of mononuclear cells, primarily lymphocytes, crossing the perineurium. Schwann cells also respond to the presence of *M. leprae* or its antigens. This lymphocytic infiltration, with or without Schwann cell disorganization, represents an early inflammatory nerve response and is diagnostic of

indeterminate leprosy. Blood cells involved in CMI were analysed and compared across the four study groups. IL-12p40-dependent stimulation of T cell responses helps explain resistance to mycobacterial infections. Both CD4+ and CD8+ T cells contribute to protection against *M. leprae* (14). In response to bacterial products or upon interaction with activated T cells, monocytes, macrophages, and dendritic cells produce IL-12, a pleiotropic cytokine previously referred to as cytotoxic lymphocyte maturation factor (CLMF) or natural killer cell stimulatory factor (NKSF). IL-12 induces IFN- γ production and enhances T and NK cell proliferation and cytotoxic activity. Furthermore, IL-12 polarizes CD4+ cells toward the Th1 phenotype, which is crucial for immunity against intracellular pathogens (11). Healthy volunteers aged 25-35 years with active lifestyles were included as healthy controls, while the study's focus was on healthy leprosy contacts aged 40 - 50 years, who have been regularly involved in leprosy care for over 30 years.

CONCLUSION

We conclude that *Mycobacterium leprae* is frequently transmitted to contacts of individuals affected by leprosy, including medical personnel. However, the low prevalence of the disease among such contacts appears to be due to the development of effective immunity in the majority of those exposed. The significance of these findings must be discussed in relation to the pathogenesis of leprosy. It is particularly noteworthy that a large number of staff members exposed to *M. leprae* become immune without ever developing the disease. This could help to explain the strong Cell-Mediated Immunity (CMI) responses observed in tuberculoid leprosy patients. Until a specific diagnostic tool is developed and made available, assessing the infection or immune status of suspected individuals remains a challenging task. This project aimed to evaluate the immune status of healthy leprosy contacts, including nurses, laboratory technologists, lab assistants, hospital sanitary workers, paramedical staff, and others who have been involved in leprosy care. No previous study has focused specifically on this group of employees serving in leprosy healthcare. The control group comprised healthy young adults from non-leprosy-contact environments. The uniqueness of this study lies in the inclusion of Group III contacts, who have been exposed both professionally and domestically to leprosy for more than 40 years. IL-12p40 has been identified as a target for the treatment of autoimmune and systemic inflammatory diseases. In this study, we compared IL-12p40 levels, C-reactive protein (CRP), and Albumin and Globulin Ratio to assess the immune status of leprosy contacts. Although no conclusive evidence was found regarding the risk associated with prolonged contact with leprosy patients at home or in the workplace, several factors, such as awareness, infection probability, healthcare access, education, and personal hygiene, must be considered in the conclusion.

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