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High Serum Levels of Transforming Growth Factor β1 (TGF-β1) a Risk Factor for Neuropathic Pain in Patients with Multibacillary Leprosy

Dewa Ayu Narha Suari, I Putu Eka Widyadharma*, Desak Ketut Indrasari Utami

Neurology Department, Faculty of Medicine, Universitas Udayana,
Prof. Dr. IGNG Ngoerah General Hospital, Denpasar, Indonesia

*Corresponding author details: I Putu Eka Widyadharma; eka.widyadharma@unud.ac.id

ABSTRACT

Leprosy caused by Mycobacterium leprae is a chronic infectious disease, affecting the peripheral nerves, skin, and other organs. Neuropathic pain is a common complication, occurring in approximately 15% of leprosy patients, even after treatment completion. Proinflammatory cytokines that play a role in the immune response and nerve injury in leprosy include Transforming Growth Factor β1 (TGF-β1). This review article aims to analyze the relationship between elevated serum TGF-β1 levels and the risk of neuropathic pain in leprosy patients. Literature findings indicate that increased TGF-β1 expression is associated with nerve damage, Schwann cell apoptosis, and sensitization of pain pathways, contributing to chronic neuropathic pain development. The article highlights the importance of understanding the pathophysiology of TGF-β1 in leprosy-related neuropathic pain for early detection and targeted therapy. Evaluating serum TGF-β1 levels may serve as a biomarker for assessing neuropathic pain risk and developing immunomodulatory treatments to improve pain management in leprosy patients. Identifying TGF-β1 as a therapeutic target could lead to novel strategies for reducing neuropathic pain and enhancing the quality of life for affected individuals.

Keywords: leprosy; neuropathic pain; TGF-β1; Schwann cell apoptosis.

INTRODUCTION

Leprosy (Hansen Disease) remains one of the oldest infectious diseases and continues to be a global health concern. This infection leads to peripheral neuropathy, which, in some patients, can progress to chronic neuropathic pain.[1] According to a World Health Organization (WHO) report from 184 countries, there were 182,815 new cases of leprosy in 2023. The global detection of new cases increased by 5% compared to 2022. Of these new cases, 125,752 (68.8%) were classified as multibacillary (MB) leprosy. New cases were reported from all regions worldwide, with the highest incidence (71.9%) occurring in the Southeast Asia region. Indonesia is among the countries that reported an increase in new cases, with a 15.6% rise in 2023.[2] The prevalence rate of leprosy in Indonesia in 2023 was 0.63 cases per 10,000 population, with a new case detection rate of 5.2 cases per 100,000 population.[3]

Neuropathic pain is a clinical manifestation of sensory impairment due to peripheral nerve damage, occurring in approximately 15% of leprosy patients.[4] It can develop during or after the resolution of leprosy. Neuropathic pain in leprosy is considered a long-term consequence of the disease, as it frequently persists even after the completion of leprosy treatment, affecting patients who have been previously declared cured and discharged from care. This condition can cause mood changes, disrupt daily activities, and cause a burden on the patient's quality of life so that it can have an impact on productivity and the economy.[5]

Transforming Growth Factor β1 (TGF-β1) is a proinflammatory cytokine that plays a crucial role in the immune response to leprosy and is suspected to be associated with the development of neuropathic pain. Neuropathic pain in leprosy patients is often underdiagnosed due to the variability of symptoms and the limited availability of diagnostic tools in endemic areas. [6,7] Therefore, further research is needed to elucidate the pathophysiological mechanisms of neuropathic pain and identify potential biomarkers for early detection and management of neuropathic pain in leprosy.

Leprosy and Neuropathic Pain

Mycobacterium leprae infection primarily affects the skin and peripheral nerves but can also involve other tissues, including the eyes, respiratory mucosa, muscles, bones, and testes. When leprosy affects the peripheral nerves, it leads to muscle weakness and sensory loss in the hands, feet, and eyes, predisposing patients to ulceration and deformities.[8]

Leprosy is classified into paucibacillary (PB) and multibacillary (MB) types based on the number of lesions and skin smear results. In smear-based classification, patients with negative smears at all tested sites are categorized as PB leprosy, while those with at least one positive smear are classified as MB leprosy. However, due to the limited availability of skin smear tests in many leprosy programs, an alternative classification system based on the number of skin lesions and nerve involvement is commonly used, with PB leprosy defined as fewer than five skin lesions and MB leprosy as five or more skin lesions.

The clinical variants of leprosy are influenced by the mycobacterial tropism for skin and peripheral nerves, as well as the host's genetically determined susceptibility to *M. leprae*.[1,9] MB leprosy is more frequently associated with neuropathic complications due to its high bacillary load. Mycobacteria differ from other bacteria in their unique lipid composition, particularly mycolic acids, which form their cell membrane and confer distinctive characteristics. This large hydrophobic cell membrane prevents polar molecules and most drugs from entering the bacterial cell. [1,10]

TABLE 1: Ridley Jopling and WHO Classification of Leprosy[9].

Ridley Jopling Classification	WHO Classification
Tuberculoid leprosy (TT) Borderline tuberculoid (BT) Borderline – borderline (BB)	Paucibacillary (PB): 1–5 skin lesions, negative skin-slit smears.
Borderline lepramatous (BL) Lepramatous leprosy (LL)	Multibacillary (MB): >5 skin lesions, positive skin-slit smears at any site

Neuropathic pain in leprosy patients is generally chronic and involves several peripheral mechanisms. Abnormal neuronal activity in the dorsal root ganglia or afferent nerves leads to an increased expression of sodium, calcium, and potassium channels, resulting in a lowered action potential threshold. This process triggers abnormal electrical discharges that do not originate from nociceptors, known as ectopic discharges. The inflammatory response and the upregulation of these ion channels contribute to the development of neuropathic pain. Pathological interactions occur between afferent nerve fibers, leading to increased nociceptor sensitization even in the absence of a noxious stimulus. Consequently, non-painful stimuli can activate nociceptors and induce pain. Beyond local responses, intact surrounding nerves also participate in this process. Spontaneous electrical discharges spread through adjacent intact nerve fibers. When ectopic discharges occur, neurotransmitters accumulate in the extracellular space and diffuse to surrounding, unconnected neurons, causing spontaneous excitation thus causing chronic neuropathic pain. [11, 12]

Pathophysiology of Neuropathic Pain in Leprosy

M. leprae predominantly invades macrophages and Schwann cells, leading to nerve damage, loss of axonal conductance, and demyelination. The bacterium invades Schwann cells via laminin-binding receptors and the conjugated protein PGL1. Additionally, research suggests that *M. leprae* interacts with Schwann cell receptors like dystroglycan, which may facilitate bacterial adhesion. The direct ligation of *M. leprae* to the neuregulin receptor activates ErbB2 and Erk1/2, triggering MAP kinase signaling and cell proliferation, ultimately causing demyelination. Involvement of the skin and mucosa results from the infection of histiocytes and keratinocytes.[1]

Recent findings indicate that *M. leprae* enters through endoneurial laminin-2 isoforms and alpha-dystroglycan receptors, elucidating the mechanisms of peripheral nerve damage in leprosy. Although alpha-dystroglycan is primarily linked to early development and muscular dystrophy, it also functions as a receptor for Schwann cells during *M. leprae* infection. [10] The precise mode of *M. leprae* transmission remains unclear; however, it is thought to spread through direct contact with infected individuals via inhalation or skin contact. Studies suggest that multibacillary (MB) leprosy cases are more infectious than paucibacillary (PB) cases. [13] Additionally, transplacental transmission and blood transfusion have been proposed as possible transmission routes, with the skin and upper respiratory tract considered the main portals of entry. Many studies support the upper respiratory tract as the primary transmission route.[14]

The clinical signs of leprosy are often difficult to detect, particularly in PB cases. Although early detection is challenging, certain clinical features should raise suspicion, including pale or reddish skin patches, loss or reduction of sensation in affected skin areas, numbness or tingling in the hands or feet, weakness in the hands, feet, or eyelids, neuropathic pain, swelling or nodules on the face or earlobes, and painless wounds or burns on the hands or feet.[2] Peripheral neuropathy in leprosy affects motor, sensory, and autonomic nerves. If nerve damage exceeds 30%, clinical manifestations become evident.[15] Neuropathic pain occurs in 11% to 21.8% of leprosy patients, significantly higher than the prevalence in the general population, which is approximately 7%. Neuropathic pain in leprosy patients may arise before, during, or after antibiotic therapy. Studies have shown that 85% of leprosy patients who have completed treatment continue to experience chronic neuropathic pain.

This pain may persist for years after bacterial elimination, significantly impacting patients' quality of life.[5]

The pathogenesis of neuropathic pain in leprosy involves a complex interaction between the immune and nervous systems. *Mycobacterium leprae* infects Schwann cells and alters their glucose metabolism, leading to reduced axonal metabolism, axonal loss, and demyelination. Schwann cells play a crucial role in pain modulation, as they can proliferate and secrete mediators that contribute to Wallerian degeneration - one such mediator proinflammatory cytokines, which act as chemoattractants and also sensitize nociceptors.[16] Schwann cells and macrophages trigger an inflammatory response that produces various cytokines, including TNF-α, IL-1β, and TGF-β1. These cytokines play a key role in nociceptor activation, demyelination, and apoptosis. Additionally, Schwann cell damage contributes to both peripheral and central sensitization, leading to neuropathic pain and loss of nerve function essential for pain perception, thus resulting in complex neuropathic pain symptoms. Neuropathic pain is characterized as nerve pain associated with dysfunction of the peripheral or central nervous system, which may arise as a consequence of neuritis.[17] Genetic factors and the patient's immunological condition also influence the severity of neuropathic pain. Several studies have identified that nerve thickening, nerve tenderness, trigeminal nerve dysfunction, and painful skin lesions are significant risk factors for neuropathic pain in leprosy patients.[18]

Chronic pain mechanisms involve not only peripheral mechanisms but also central mechanisms, where a reduction in central inhibitory processes occurs. The descending inhibitory pathways from the brainstem and nuclei in the mesencephalon modulate signal transmission at the dorsal horn, involving the monoaminergic and adrenergic systems. Central sensitization is enhanced through the activation of N-methyl-D-aspartate (NMDA) receptors, leading to spontaneous impulses that cause hyperexcitability of central nociceptive neurons. This hyperexcitability may result from the loss of γ-aminobutyric acid (GABA) interneurons.[12] In the context of neuropathic pain in leprosy, hyperexcitability of the dorsal horn is attributed to the increased levels of interferon-gamma (IFN-γ) observed in animal studies. Additionally, neuropathic pain in leprosy is associated with elevation of several pro-inflammatory cytokines, including interleukin-1 beta (IL-1β), tumor necrosis factor-alpha (TNF-α), transforming growth factor-beta (TGF-β), and interleukin-17 (IL-17). TNF-α and IL-1β stimulate and sensitize Aδ and C nerve fibers. Aδ fibers are responsible for sharp pain sensations, while C fibers mediate burning pain.[16]

Peripheral nerve damage due to *Mycobacterium leprae* infection is categorized into three stages: the stage of involvement, characterized by nerve thickening; the stage of damage, where incomplete nerve damage occurs; and the stage of destruction, where permanent nerve damage is observed. Disability is defined as an impairment of bodily function (biological), activity, personality (psychological aspects), environment, or social participation experienced by an individual due to a specific disease. The World Health Organization (WHO) classifies leprosy-related disability into three grades (0–2). Grade 0 indicates normal sensation without visible deformity. Grade 1 signifies sensory impairment without visible deformity, whereas Grade 2 denotes the presence of visible deformity or disability resulting from leprosy.[18,19]

Transforming Growth Factor β1 (TGF-β1) in the Pathophysiology of Neuropathic Pain in Leprosy

TGF-β1 is a versatile cytokine that modulates nociceptive transmission and plays a fundamental role in immune system regulation. Initially identified as an inhibitor of cell proliferation, TGF-β1 is widely expressed across the hematopoietic system. Within the immune system, TGF-β1 signaling is essential for the function of T cells, B cells, and phagocytes.[20] Moreover, TGF-β1 acts as a crucial inducer of integrins, particularly CD103, in CD8+ resident memory T cells, Th1 cells, and regulatory T cells (Tregs), thereby influencing cell migration during and after inflammatory responses. The diverse effects of TGF-β1 signaling are mediated through the integration of multiple cytokine signals (e.g., IL-6, IL-1, and IL-21, in conjunction with TGF-β1, promote Th17 differentiation), which are dependent on the surrounding cytokine milieu.[21] TGF-β1 plays different roles in various pain conditions but has been demonstrated to mitigate neuropathic pain associated with peripheral nerve injury. Its pain-relieving mechanism involves the suppression of activated astrocytes and microglia.[16,21]

Proinflammatory cytokines (e.g. TNF-α and IL-1β) can stimulate and sensitize Aδ and C nerve fibers, which are involved in neuropathic pain mechanisms in leprosy patients. TNF-α and TGF-β1 contribute to Schwann cell apoptosis and peripheral nerve damage. Moreover, TGF-β1, as a proinflammatory cytokine, stimulates the Th2 immune response. TGF-β1 levels have been found to be significantly elevated in leprosy patients with neuropathic pain compared to those without neuropathic pain.[16] The lowest TGF-β1 expression in Tregs has been observed in patients with the BB subtype of leprosy, potentially due to the persistent presence of high proinflammatory cytokine levels, which remain active in eliminating *Mycobacterium leprae*.[8] During the early immune response following *M. leprae* entry into macrophages, the bacteria induce the production of TNF-α and TGF-β1 by infected macrophages. TNF-α activates macrophages, facilitating intracellular pathogen destruction and potentiating the Th1 response. Conversely, TGF-β1 deactivates macrophages, promotes bacillary proliferation, and counteracts TNF-α by shifting the immune response toward Th2 dominance. In the humoral response, bacterial clearance is inefficient, as evidenced by the presence of antibodies (anti-PGL-1) specific to the phenolic glycolipid of the *M. leprae* cell wall.

High concentrations of these antibodies in peripheral blood correlate with the bacterial load observed in BL and LL patients, whereas TT patients exhibit antibody titers similar to those of healthy controls.[22]

Mycobacterium destruction or proliferation within macrophages is determined by immune mechanisms involving major histocompatibility complex (MHC) antigen presentation and human leukocyte antigen (HLA), both of which are genetically regulated. TT-type leprosy is predominantly associated with the HLA-DR2 and HLA-DR4 phenotypes, whereas LL and borderline types are characterized by the dominance of the HLA-DQ1 phenotype, which is linked to individual susceptibility. [22,23] The expression of TGF-β1 in regulatory T cells (Tregs) from leprosy lesions has been examined using immunohistochemistry. This analysis utilized monoclonal anti-CD25 (Santa Cruz Inc.) and monoclonal anti-TGF-β (Santa Cruz Inc.), with immunodoublestaining employed to observe TGF-β1 expression in CD25+ cells. Serum-based assays are more commonly used due to the ease of sample collection.[8] *Mycobacterium leprae*-infected macrophages induce TGF-β1 production by Tregs during the immune response in leprosy. As a suppressor cytokine, TGF-β1 inactivates macrophages and inhibits TNF-α activity, leading to increased bacterial proliferation. Recent studies on TGF-β1 expression in Tregs have indicated that TGF-β1 secretion by Tregs is elevated in lepromatous leprosy lesions. Tregs are hypothesized to be anergic T cells that fail to mount an effective immune response against *M. leprae*, thereby contributing to disease progression toward the lepromatous form.[8]

Erythema nodosum leprosum (ENL) is characterized by increased serum levels of TGF-β1, IFN-γ, IL-10, IL-6, IL-8, and IL-1β, while IL-4 and IL-5 remain unchanged. A study by Hamzah et al. measured serum TGF-β1 levels in patients with ENL, reporting a mean TGF-β1 concentration of 62.6 ± 30.4 pg/mL in leprosy patients compared to 47 ± 21.6 pg/mL in controls. The study suggested that higher TGF-β1 levels correlate with an increased risk of recurrent ENL episodes.[24] In multibacillary (MB) leprosy, TGF-β1 levels were found to be higher than in paucibacillary (PB) leprosy, which has been associated with the severity of nerve damage. TGF-β1 contributes to Schwann cell apoptosis and enhances the expression of other proinflammatory cytokines, exacerbating neuropathy. Several studies have demonstrated a link between increased TGF-β1 levels and heightened neuropathic pain in leprosy patients. This is supported by findings showing higher TGF-β1 levels in patients with neuropathic pain compared to those without. Additionally, TGF-β1 interacts with neuroinflammatory mechanisms that lead to peripheral and central nerve hyperexcitability. Therefore, targeting the TGF-β1 signaling pathway may offer a novel approach for managing neuropathic pain in leprosy patients.[16,25]

Clinical Implication and Prognosis Factor

The determination of TGF-β1 levels can be a potential biomarker parameter to detect the risk of neuropathic pain in MB leprosy patients. This biomarker is important for the management of MB leprosy so that it can reduce the risk of neuropathic pain. The development of immunomodulator-based therapies that target cytokines such as TGF-β1 can be an innovative solution for the management of neuropathic pain in leprosy patients in the future. If symptoms of neuropathic pain have appeared, an examination can be carried out using the Douleur Neuropathique en 4 Questions (DN4) questionnaire, Leeds Assessment of Neuropathic Symptoms and Signs (LANSS), and painDETECT as well as nerve conduction examination with electromyography (EMG) or nerve conduction studies (NCS).[1,17] Immunological assessment of TGF-β1 levels may also provide an additional tool for evaluating the progression of neuropathy in leprosy patients. The management of neuropathic pain in leprosy involves a multimodal approach, including the use of anticonvulsants (e.g., gabapentin, pregabalin), antidepressants (e.g., amitriptyline, duloxetine), and corticosteroids to reduce inflammation. Rehabilitation therapies, such as physiotherapy and nerve stimulation, may also help mitigate the functional impact of neuropathic pain.[17,26] The development of immunomodulatory therapies targeting cytokines such as TGF-β1 could offer an innovative solution for neuropathic pain management in leprosy patients in the future.

CONCLUSION

High levels of TGF-β1 are associated with an increased risk of neuropathic pain in multibacillary (MB) leprosy patients. The elevation of this cytokine contributes to inflammatory processes, Schwann cell apoptosis, and nervous system sensitization, all of which play key roles in the pathogenesis of neuropathic pain. Assessing TGF-β1 levels may serve as a novel approach for predicting neuropathic pain risk in leprosy patients and could represent a potential therapeutic target.

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