The role of Monocyte Chemoattractant Protein-1 (MCP-1) as an immunological marker for patients with leprosy: a systematic literature review (#93807)

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The role of Monocyte Chemoattractant Protein-1 (MCP-1) as an immunological marker for patients with leprosy: a systematic literature review

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Introduction: Leprosy contributes to a significant number of cases worldwide and impacts predominantly the peripheral nerves and integumentary system. Its pathology, disease progression and reaction occurrence depend on the host cells' immune system. MCP-1 is involved in leprosy' immunological process therefore it has potential ability in diagnosing leprosy. This systematic review aims to investigate the involvement of MCP-1 in leprosy as a diagnostic tool and predicting reactions occurrence. **Methods:** Literature search was conducted with specified keywords from Medical Subject Headings (MeSH) using several databases (PubMed, Scopus, ScienceDirect, and Wiley Online Library). We only included literature that was conducted in humans and published in English up until September 30th, 2023. Each study's quality was assessed using the Newcastle-Ottawa Scale (NOS), and the risk of bias was then investigated using the Risk of Bias Assessment tool for Non-randomized Studies (RoBANS). After that, a narrative synthesis was carried out to compile all findings. **Results:** Thirteen distinct studies were included, each characterized by variations in study design, sample size, population demographics, inclusion and exclusion criteria, and outcome measures. Significant findings suggest that MCP-1 could be utilized for diagnosing leprosy, distinguishing it from control groups, and discerning between different types of leprosy. Additionally, MCP-1 shows promise in predicting the occurrence of leprosy reversal reactions. **Conclusion:** In summary, MCP-1 offers clinical benefits in diagnosing leprosy, particularly for early diagnosis and differentiation between distinct types of leprosy. Nevertheless, further studies with larger sample sizes and standardized

methodologies covering various parameters are still necessary to confirm the diagnostic properties of MCP-1.

The Role of Monocyte Chemoattractant Protein-1 (MCP-1) as an Immunological Marker for Patients with Leprosy: A Systematic Literature Review

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Abstract

Introduction: Leprosy contributes to a significant number of cases worldwide and impacts predominantly the peripheral nerves and integumentary system. Its pathology, disease progression and reaction occurrence depend on the host cells' immune system. MCP-1 is involved in leprosy' immunological process therefore it has potential ability in diagnosing leprosy. This systematic review aims to investigate the involvement of MCP-1 in leprosy as a diagnostic tool and predicting reactions occurrence.

Methods: Literature search was conducted with specified keywords from Medical Subject Headings (MeSH) using several databases (PubMed, Scopus, ScienceDirect, and Wiley Online Library). We only included literature that was conducted in humans and published in English up until September 30th, 2023. Each study's quality was assessed using the Newcastle-Ottawa Scale (NOS), and the risk of bias was then investigated using the Risk of Bias Assessment tool for Non-randomized Studies (RoBANS). After that, a narrative synthesis was carried out to compile all findings.

Results: Thirteen distinct studies were included, each characterized by variations in study design, sample size, population demographics, inclusion and exclusion criteria, and outcome measures. Significant findings suggest that MCP-1 could be utilized for diagnosing leprosy, distinguishing it from control groups, and discerning between different types of leprosy. Additionally, MCP-1 shows promise in predicting the occurrence of leprosy reversal reactions.

Conclusion: In summary, MCP-1 offers clinical benefits in diagnosing leprosy, particularly for early diagnosis and differentiation between distinct types of leprosy. Nevertheless, further studies with larger sample sizes and standardized methodologies covering various parameters are still necessary to confirm the diagnostic properties of MCP-1.
Keywords: MCP-1, Leprosy, Immunological Marker

Introduction

Leprosy, which first appeared in an Egyptian skeleton from the second century BCE and for which the earliest documented accounts date to 600 BCE from India, is one of the earliest diseases to afflict mankind.[1,2] The infectious agent responsible for causing leprosy is *Mycobacterium leprae*, which results in a chronic granulomatous disease that impacts predominantly peripheral nerves and integumentary system.[3] By changing the mitochondrial glucose metabolism in Schwann cells (SC), *M. leprae* infects both macrophages and these cells.[4] WHO data from 2021 demonstrate that we have 133.781 cases and 140.546 new cases, with India, Brazil, Indonesia continue to contribute a significant number of new cases of leprosy worldwide (74%).[5,6] 17,439 new cases of leprosy were reported in Indonesia, 1,121 of which had grade-2 disabilities (G2D)[7,8]. The susceptibility of an individual to leprosy is established by multiple variables: idiosyncratic, immunological, and environmental factors of the host.[9] Symptoms might vary from person to person due to immunogenic differences that result in a particular clinical appearance.[10]

Clinical diagnosis of leprosy is confirmed if one out of three cardinal signs are present : Cutaneous lesions with hypopigmentation or erythema, such as macules or plaques, accompanied by the loss of sensation on the skin; Thickening or enlargement of peripheral nerves and signs of its damage, such as loss of sensory, paralysis or motoric dysfunction with or without nerve enlargement; Findings of acid-fast bacilli (AFB) on skin biopsy and/or lesion scraping.[11] *Mycobacterium sp*. are one of the acid-fast bacilli due to their capacity to withstand acid-induced color loss during staining processes.[12] *M. leprae* has a highly specific antigen which is phenolic glycolipid-I (PGL-I) and it has the ability to attach to the basal lamina of Schwann cell-axon units.[13] Toll-like receptors (TLR) identify PGL-I and present it to APC. APC introduces *M. leprae* to lymphoid naïve T-cells which then can transform into Th1, Th2, Treg, and Th17.[14] Leprosy develops because of an imbalanced immune response, marked by T-cell dysfunction, heightened cell death, and an imbalance between the Th1 and Th2 immune responses.[15] Th1 dominant immune responses are mediated by protective IFN-γ and IL 2 with microbicidal properties which more prevalent in PB type leprosy.[14] MCP-1 is associated with Th1 responses and has an antagonistic association with IFN-γ, which both cytokines play a crucial role in *M. leprae* elimination.[15,16] In addition, it is well recognized, that the family of transcription factors named nuclear factor kappa B (NFκB) plays a central role in modulation of innate and adaptive immunity.[17,18]

Chemotactic cytokines are classified into two main classes (CXC and CC) and manage how other cells response to a chemical stimulation (chemotaxis).[19] Monocyte chemotactic protein (MCP-1)/CC chemokine ligand-2 (CCL2), a member of the CC chemokine family, is involved in regulation of monocyte, microglia, and memory T cell passage and penetration to the site of injury and infection in a variety of diseases.[20,21] MCP-I has been identified as a potent inducer of macrophage infiltration, a reliable marker of inflammation, and a potential therapeutic target for a variety of inflammatory illnesses.[22] Since MCP-I facilitates the recruitment of macrophages to the leprosy nerves, it is possible that MCP-I is related to the severe nerve fibrosis.[3] MCP-I is

significantly higher in PB patients, however some literatures stated MCP-I is higher in MB patients.[13,23,24] MCP-I indicates a more vigorous reaction to *M. leprae*.[25] MCP-I is useful in understanding the pathogenesis of leprosy because of its involvement between *M. leprae* and host cells' immune system. [26] MCP-I can be used to determine the degree of inflammation in a variety of medical conditions.[21] Due to the difference in expression between PB/MB and TT/BT leprosy patients, MCP-I could be utilized to distinguish between different kinds of leprosy. [13,23,24] MCP-1 was found sensitive only to PB leprosy. MCP-I can also be used as an additional marker to enhance the accuracy of leprosy diagnosis because the current diagnostic testing for IgM antibodies against PGL-I is not able to represent household leprosy contacts.[27] With IFN-γ, MCP-I are potential indicators of subclinical infection of *M. leprae* in household contacts, also as a parameter of early infection monitoring.[16] MCP-I is currently under investigation as a potential immunotherapy as shown in previous study which immunotherapy with *Mycobacterium* vaccine has shown benefit to MB leprosy patients.[22,28] Therefore, the goal of this systematic review is to completely synthesize all findings on MCP-I's potential as a biomarker to diagnose and distinguish different types of leprosy, as well as its potential as a therapeutic intervention.

Survey methodology

Study Design

The review protocol for this investigation was registered with the International Prospective Register of Systematic Reviews (PROSPERO; ID: CRD42023460380), and the study was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA). The search was conducted in October 2023 in four databases (PubMed, Scopus, Wiley Online Library, ScienceDirect). Medical subject headings (MeSH)-based keywords were utilized in the search approach. Keywords used were: (Leprosy OR "Hansen disease" OR "Hansen's disease" OR "Morbus Hansen" OR Leprae) AND (MCP-1 OR CCL2 OR CCL#2 OR "Chemokine (C-C Motif) Ligand#2" OR "C-C chemokine ligand#2" OR MCP1 OR MCP#1 OR "Monocyte Chemotactic and Activating Factor" OR "Monocyte Chemoattractant Protein#1" OR "Monocyte Chemotactic Protein#1") AND ("Immunological Marker" OR "Immunologic Marker" OR "Immunological Marker$" OR Marker$ OR Biomarker$). We used these keywords for PubMed, Scopus, and Wiley Online Library. For ScienceDirect, we use: (Leprosy OR "Hansen's disease" OR "Leprae") AND (MCP-1 OR CCL2 OR CCL#2) AND ("Immunological Marker$" OR Biomarker$). To ensure that no pertinent papers were overlooked, reference lists of the included studies were reviewed.

Inclusion and Exclusion Criteria

Studies giving data regarding leprosy, MCP-I, and immunological markers up until September 30th 2023 were evaluated. Only studies in humans were included. However, we only included publications that were written in English. All types of reviews are excluded in this study. No limitations on time were placed on this study.

Study Selection

The screening process began by importing all search results upon titles and abstracts into rayyan.ai, and duplicate articles were subsequently excluded. F.R.S.P. and E.J.H, two reviewers, independently examined the obtained articles' titles, abstracts, and full texts in accordance with

inclusion and exclusion criteria. A third reviewer (E.D.R) arbitrated any disagreements between the two reviewers.

Quality Assessment

To assess each quality of the study, we used Newcastle-Ottawa Scale (NOS) that consisted of three major items: selection of study groups (0-4 points), comparability of cases and control studies (0-2 points) or cohorts, and ascertainment of exposure/outcome (0-3 points). This scale applies to cohort and case control study, however for cross-sectional study, the NOS items were selection of study group (0-5 points), comparability of cases and control studies (0-1 points) and ascertainment of exposure/outcome (0-3 points). Studies were considered high-quality if they received six points or higher. This assessment of study quality was conducted by two reviewers, F.R.S.P and E.J.H, with any disparities resolved through the intervention of a third reviewer, E.D.R.

Risk of Bias Assessment

The Risk of Bias Assessment tool for non-randomized research (RoBANS) is utilized to evaluate the potential for bias in the research that are incorporated. This tool consists of six items: participant selection, confounding variables, exposure measurement, blinding of outcome assessments, incomplete outcome data, and selective outcome reporting. Two independent reviewers, namely F.R.S.P and E.J.H, carried out the risk of bias assessment using RoBANS. In the event of any discrepancies or disagreements, a third reviewer (E.D.R) was consulted to reach a consensus.

Data Analysis

Information such as the date and location of testing, aim of study, population description and setting, inclusion and exclusion criteria, diagnostic methods and findings, tools for measurement, leprosy classification, treatment, and outcomes were all gathered from previous studies and reviewed by E.J.H, N.H. and M.F.I. A qualitative analysis was then conducted to cross-examine all the findings.

Results

Study Selection

This systematic review was carried out using the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guideline. We retrieved a total of 93 studies from the following databases: Scopus (n=14), PubMed (n=14), Wiley Online Library (n=19), and ScienceDirect (n=46). We eliminated 16 duplicate studies before commencing the screening process. Following a review of titles and abstracts, we excluded 48 studies. Unfortunately, one article could not be retrieved. The remaining twenty-eight articles were assessed for eligibility; eight studies were eliminated due to an inaccurate study design, and seven studies were removed due to insufficient outcome data. Ultimately, thirteen studies were included in the review. All review process are described in Figure 1.

Quality and Risk of Bias

Our studies' eligibility was assessed further for its quality using Newcastle-Ottawa scale (NOS) instrument and risk of bias using Risk of Bias Assessment tool for Non-randomized Studies

(RoBANS) tool. The results of the quality assessment were presented in Table 1. All the included studies scored more than six points in the quality assessment. Following, risk of bias assessment results using RoBANS tool was presented in Figure 2 in which most of the items have 'low' score, however some confounding variables item and incomplete outcome data were 'unclear'.

Study Characteristics

Majority of study designs were cross-sectional (n=7), others were case controlled studies (n=2) and cohort studies (n=4). Samples were varied from 8 to 188, with a total sample size of 737 patients. Studies varied from multiple countries. Comprehensive explanation of study characteristics can be observed in Table 1.

Study Results

MCP-1' diagnostic, genetic and neuropathic properties

From our systematic review, we found that MCP-1 has potential diagnostic abilities[3,29,30]. A cross-sectional study by Medeiros *et al.,* (2015) in 23 Pure Neural Leprosy (PNL) patients found MCP-1' immunoreactivity in PNL Schwann cells' biopsy samples from either Acid-Fast Bacilli $(AFB)^+$ or AFB^-. MCP-1 was detected in 13 out of 23 PNL patients (66.7% in PNL AFB^+ & 81.8% in PNL AFB^-). MCP-1 expression showed a correlation with fibrosis that was not influenced by HLA-DR, CD3, CD4, CD8, CD45RA, CD68, or any other immunologic markers ($p = 0.026$)[3]. A cohort study conducted on 160 patients by Geluk *et al*. (2012) discovered that MCP-1 (or CCL2) was considerably increased in TT/BT patients following stimulation with *M. leprae* in contrast to endemic controls (ECs) ($p = 0.0021$). In Bangladesh, there is good to excellent differentiation between the TT/BT and EC groups, as indicated by the MCP-1 area under the curve (AUC) of 0.94[29]. Study conducted by Meneses *et al*., (2014) on 44 patients found that leprosy patients had higher urinary MCP-1 (101.0 ± 79.8 vs. 34.5 ± 14.9 mg/g-Cr, $p = 0.006$) and urinary MDA levels (1.77 ± 1.31 vs. 1.27 ± 0.66 mmol/g-Cr, $p = 0.0372$) than healthy controls[30].

One cross-sectional study (Dias *et al.,* (2021)) discovered MCP-1 response in live and killed *M. leprae* in A59 alveolar epithelial cells. 24 hours of incubation resulted in higher MCP-1 levels ($p<0.05$) in the treated cells' supernatants compared to control cells. At a later stage of incubation (48 hours), only bacteria that had been destroyed could cause MCP-1 to be produced ($p<0.05$). The impact of the pharmacological inhibitor wedelolactone on MCP-1 was also investigated in this research, but no effect was found; hence, its regulation is controlled by a different mechanism that is independent of NF-kB[31]. Several studies were investigating MCP-1 genetic properties[32,33]. Carriers of the TT genotype (TC and TT) in TLR1_rs5743551 produced decreased serum levels of MCP-1, according to Santana et al. (2017)[32]. Based on TLR4 rs1927914 alleles/genotype, Cunha et al. (2023) found that the AA genotype (CXCL8, MCP-1, TNF, and IL-2) was linked to a more prominent secretion in vitro culture of HHC (PB) and HHC (MB)[33]. Another study compared the levels of MCP-1 between leprosy neuropathy and diabetic neuropathy. This was performed by a cross-sectional study in Brazil by Morales Angst *et al*., (2020) that found MCP-1 value in diabetic neuropathy group was statistically significant compared to leprosy neuropathy group ($p = 0{,}001$ and $p = 0{,}01$)[4].

MCP-1 to classify leprosy' types and leprosy reaction

MCP-1 can also be used in discriminating types of leprosy[33,34]. A cohort study by Yuan *et al*., (2021) on 82 patients found that MCP-1 showed an excellent performance in diagnosing types of leprosy. Between leprosy patients vs endemic controls (ECs) with AUC of 0,87 (95% CI 0,75-0,98), sensitivity of 50,00% and specificity of 95,45%. In MB leprosy patients vs ECs with AUC of 0,91 (95% CI 0,81-1,00), sensitivity of 66,67% and specificity of 95,45%. However, sensitivity was 90,00% in comparison between PB leprosy vs ECs and specificity was 81,82%. MCP-1 is more sensitive in PB leprosy diagnosis (sensitivity 100,00% and specificity 66,67%) compared to MB leprosy diagnosis. Both sensitivity (72,22%) and specificity (82,35%) were lower in comparison between leprosy vs household controls (HHCs). Overall, MCP-1 is more specific rather than sensitive in diagnosing leprosy, however this study found that MCP-1 are sensitive only to PB leprosy[34]. Similar findings of higher MCP-1 in household controls (HHC) paucibacillary (PB) as compared to HHC multibacillary (MB) were found in a cross-sectional study conducted by Cunha *et al*., (2023)[33] .

However, MCP-1 was found higher in MB patients in two studies conducted by Meneses *et al*., (2014) and Queiroz *et al*., (2020)[16,33]. Urinary MCP-1 was shown to be greater in multibacillary patients (122.1 ± 91.9 vs. 72.0 ± 46.1 mg/g-Cr, p = 0.023) than in paucibacillary patients. Additionally, a significant association was found between urine MCP-1 and the bacteriological index in skin smears (r = 0.322, p = 0.035). Urinary MCP-1 levels and the duration of symptoms were not significantly correlated (r = 0.014, p = 0.938)[30]. Queiroz *et al*., (2020) found that during the initial visit MB patients had higher levels of MCP-1 than PB patients. However, MCP-1 expression was found higher after 1 year of treatment in PB patients. A significant association (R2 = 0.05/p = 0.02), as well as negative correlation (r = -0,25/p=0.00) between MCP-1 and IFN-γ was found only in HHC group[16].

MCP-1 can also be used as a predictive value for the occurrence of a reversal reaction (or type 1 reaction). This was stated by a prospective cohort study in 2019 (Tio-Coma *et al*.,) on 10 patients that MCP-1 is useful in comparing the development of reversal reaction (RR) (patients who developed RR (n=30) vs did not developed RR (n=184)) because MCP-1 was significantly increased reversal reaction (RR) patients (p < 0,05)[35].

Even though most studies we reviewed had shown that MCP-1 was beneficial in diagnostic and predictive outcome, some studies stated that MCP-1 did not have significant diagnostic properties[36–38]. A cross-sectional study by Geluk *et al*., (2010) found that production of MCP-1 in response to ML2531 p1-15 and IL-12 tended to be increased by IL-12, although this was not statistically significant (P = 0.2 and 0.4)[36]. Stefani *et al*., (2009) discovered that MCP-1 levels for non-reactional type 1 reaction-controls (T1R-controls) and type-2 reaction-controls (T2R-controls) groups were not statistically significant[37]. Mendonca *et al.* (2009) conducted a cross-sectional investigation and found that there were no significant variations in plasma concentrations between infected and non-infected persons among 33 leprosy patients before and during multi-drug therapy (MDT)[38].

Discussion

Current systematic review investigated MCP-1' potential in relation to leprosy diagnosis. Thirteen studies formed the qualitative analysis. Regarding its diagnostic skills, there was a significant degree of variation among the included studies. Some studies were investigating its ability to diagnose leprosy and differentiate between controls; some were investigating the tendency of leprosy' reaction occurrence; some were measuring levels of MCP-1 in different

types of leprosy; some were discovering its genetic properties, and some were assessing different levels of MCP-1 between each leprosy classification. Most studies used ELISA to measure MCP-1 levels, some used PCR, and others assessed histopathological staining under the microscope.

MCP-1' diagnostic and genetic properties

According to Medeiros *et al*. (2014), MCP-1 is involved in PNL. In macrophages or Schwann cells present in the majority of nerves with leukocytic inflammatory infiltrate, MCP-1 levels were shown to be greater. This occurred because of Schwann cells' capacity to coordinate a response to peripheral nerve injury, including leprosy nerve damage. Leukemia inhibitory factor release, and IL-6 were released prior to MCP-1 secretion. Following the release of the MCP-1 signal, macrophages begin to infiltrate the endoneurial compartment. MCP-1 expression was linked to nerve fibrosis and was detected in PNL Schwann cell biopsy samples.. Because macrophages are essential to the inflammatory healing process, they are implicated in the generation of angiogenic and fibrogenic cytokines. MCP-1 increases the production of the pro-α1 chain and TGFβ1 in type I collagen. Therefore, MCP-1 is associated with nerve fibrosis[3].
Previous research has revealed that *M. leprae* may enter the lungs, infiltrate pulmonary epithelial cells, and thrive within them. In cells infected with *M. leprae*, MCP-1 was found to be upregulated. Additionally, exposure to *M. leprae* increased the production of IL-8 in human primary nasal epithelial cells, supporting the possibility that this reaction occurs when the bacteria enter the respiratory system. MCP-1 functions as a chemoattractant for CD4+ T cells and monocytes, whereas IL-8 primarily attracts neutrophils—the initial inflammatory cells that arrive at the infection site to limit the spread of germs[31].
MCP-1 effectively distinguishes leprosy patients from healthy controls. It has been demonstrated that leprosy patients have significantly higher levels of MCP-1 compared to healthy controls. However, there is no specific test to determine whether exposure to HHC will result in leprosy development[33]. In asymptomatic people with latent infection, MCP-1 may contribute to the integrity of the granuloma by attracting monocytes, memory T cells, and dendritic cells to areas of tissue damage and infection[29,30]. Therefore, there was a considerable increase in MCP-1 in TT/BT leprosy patients compared to healthy controls[29]. The MB and LL polar forms of leprosy were reported to have higher urine MCP-1 levels in an investigation by Meneses *et al*. (2014), despite the absence of clinical renal damage in these leprosy patients. Leprosy patients often experience renal problems due to inflammation caused by *M. leprae*. Renal inflammation in leprosy patients is believed to be associated with the T helper 2 (TH2) response, which is more pronounced in the lepromatous type of the disease. Although chronic kidney disease may not manifest for a long time, urinary MCP-1 has the potential to be a useful early biomarker for identifying individuals at risk[30].
Due to the general chemokine and cytokine profile of the AA genotype, TLR4 rs1927914, and other genetic features are connected to the HHC immune response. Reduced exposure to *M. leprae* (HHC coexisting with PB patients) was associated with higher MCP-1 levels. Similar claims about single nucleotide polymorphisms in TLR genes increasing leprosy susceptibility by raising the likelihood of developing clinical illness or leprosy reactions were found in earlier investigations. Because of its relevance in subclinical infection, MCP-1, which is related to IFN-γ, is important to be utilized as a metric for early infection monitoring[33]. MCP-1 is only expressed on the surface of monocytes; it is not expressed on neutrophils or eosinophils, according to studies by Yuan *et al*. (2021). Increased MCP-1 levels suggest that it plays a role in

leprosy etiology. In addition to TLR4, this study also discovered decreased MCP-1 levels in carriers of the TT genotype (TC and TT)[32] Leprosy in household contacts is not solely associated with immunological characteristics; other contributing factors include the physical environment of the home, access to latrines, clean water sources, facilities for waste disposal, personal cleanliness, and nutritional condition. Improved hygiene lowers the risk of leprosy among household contacts[39].

MCP-1 to classify leprosy' types and leprosy reaction

MCP-1 is a chemokine ligand that is surface-expressed on monocytes and is implicated in inflammatory reactions and immunological regulation[40]. Variations in MCP-1 levels throughout leprosy subtypes suggest that this marker can be used to categorize the illness. MCP-1 is typically more specific than sensitive for leprosy diagnosis, especially in PB leprosy[34]. Lower exposure to *M. leprae* in HHC (PB) was linked to a modulatory axis (marked by greater MCP-1 and IL-10 levels); whereas higher exposure to *M. leprae* in HHC (MB) did not exhibit any modulatory axis. Therefore, it can be utilized as a measurement tool for monitoring early infection in PB patients[33]. In lepromatous form patient cell cultures, TNF-induced MCP-1 expression was found to be lower, which may have contributed to the dissemination of the bacillus and the development of a more robust inflammatory process in MB patients[16].
This result, however, is incompatible to some other research that discovered elevated MCP-1 levels in MB patients[16,30]. One possible explanation is that MCP-1 was initially higher in MB patients at the time of diagnosis and later became higher in PB patients one year after treatment. Increased MCP-1 in PB indicated a strong cellular immunological response, which may operate as a leprosy protective factor[16]. This statement was supported by a study conducted by Prakoeswa *et al*. (2022), which found that PB patients had higher Th17 cell counts, resulting in better clinical symptoms and a stronger immune response, thereby corroborating this claim[41].
Reversal reactions (RRs) may occur during, prior to, or following MDT. Although previous research suggested that genetic predisposition plays a role in the immunological shift from Th2 to Th1 in RRs, the precise mechanism of RRs remains unclear[35]. The leprosy reaction is linked to Th1 cells[14]. Clinical results for RR could be significantly improved by early diagnosis, particularly in terms of minimizing nerve damage, yet there is currently no established biomarker for RR[35]. But according to our reviews, future RR patients had higher levels of MCP-1 because of its correlation to excessive extracellular matrix deposition and macrophage recruitment, which triggers pro-inflammatory cytokines and draws CD4+ T cells. This could be because the immune system is exposed to more *M. leprae* antigens following MDT, as indicated by future RR patients' similarly elevated expression of IL-2[35].
While MCP-1 may hold potential for predicting reversal reactions, no statistically significant difference in its levels was observed between type 1 and type 2 reactions. Stefani *et al*. (2009) reported a lack of correlations between the duration of response symptoms and the levels of cytokines or chemokines, possibly due to an inadequate sample size. In contrast, Mendonca *et al*. (2014) noted elevated MCP-1 plasma levels in patients with PB; however, it is important to note that all patients in the current investigation were MB, which suggests that the specific MB type may have masked the elevated MCP-1 levels[38].

Clinical Implications

These findings imply the possibility that MCP-1 may serve as a diagnostic biomarker for leprosy. Most of our included studies used humans as the study population; however, there were

still too few studies for each diagnostic parameter. Future research with larger populations, lower risk of bias, assessments of confounding variables, and systematic procedures for sample retrieval is needed. Several potential areas for future research include studies focusing on MCP-1's diagnostic properties for differentiating between leprosy patients and healthy controls, assessing MCP-1's predictive value in predicting leprosy reactions, distinguishing between different types of leprosy, and identifying genetic properties to predict leprosy's prognostic values. Along with the earlier statement, the variety of the included studies in terms of diagnostic characteristics, different parameters of studies' variables and varying results, it is tricky to reach firm conclusions. To ensure MCP-1' ability to diagnose leprosy and its clinical staging, additional research is needed.

Limitations

This study was limited to a systematic review and did not proceed to a meta-analysis due to the heterogeneity of the included studies and because each of these studies assessed different parameters, making meta-analysis impossible to conduct. During the 'Quality and Risk of Bias Assessment' process, we found that most of our included studies did not explain the investigation of potential confounders. None of the case-control and cohort studies stated the ascertainment of exposure. Two out of four cohort studies did not mention the adequacy of follow-up for their cohorts. Additionally, each study acknowledged its limitations. Several patients were excluded because there was insufficient data, and the sample size was lowered due to the non-availability of blood samples. Another potential bias arose from data collection performed by different examiners. The results may also be affected by using multiple comparisons without correcting for confounding variables and different sample retrieval environments. More included studies involving a larger population of leprosy patients and healthy controls are needed to determine which biomarker profiles are best for discriminating *M. leprae*-infected individuals from controls.

Conclusions

In conclusion, our systematic review suggests that MCP-1 possesses diagnostic potential for leprosy. The cross-sectional, case-control, and cohort studies included in this review have consistently shown significant associations of MCP-1 levels with the leprosy group, despite the findings of three out of thirteen included studies indicating otherwise. Moreover, MCP-1 has the potential to be beneficial in predicting the occurrence of reversal reactions in leprosy. Therefore, further studies with larger sample sizes and standardized methodologies covering various parameters are necessary to confirm MCP-1's diagnostic properties.

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Figure 1

PRISMA 2020 Flow Diagram

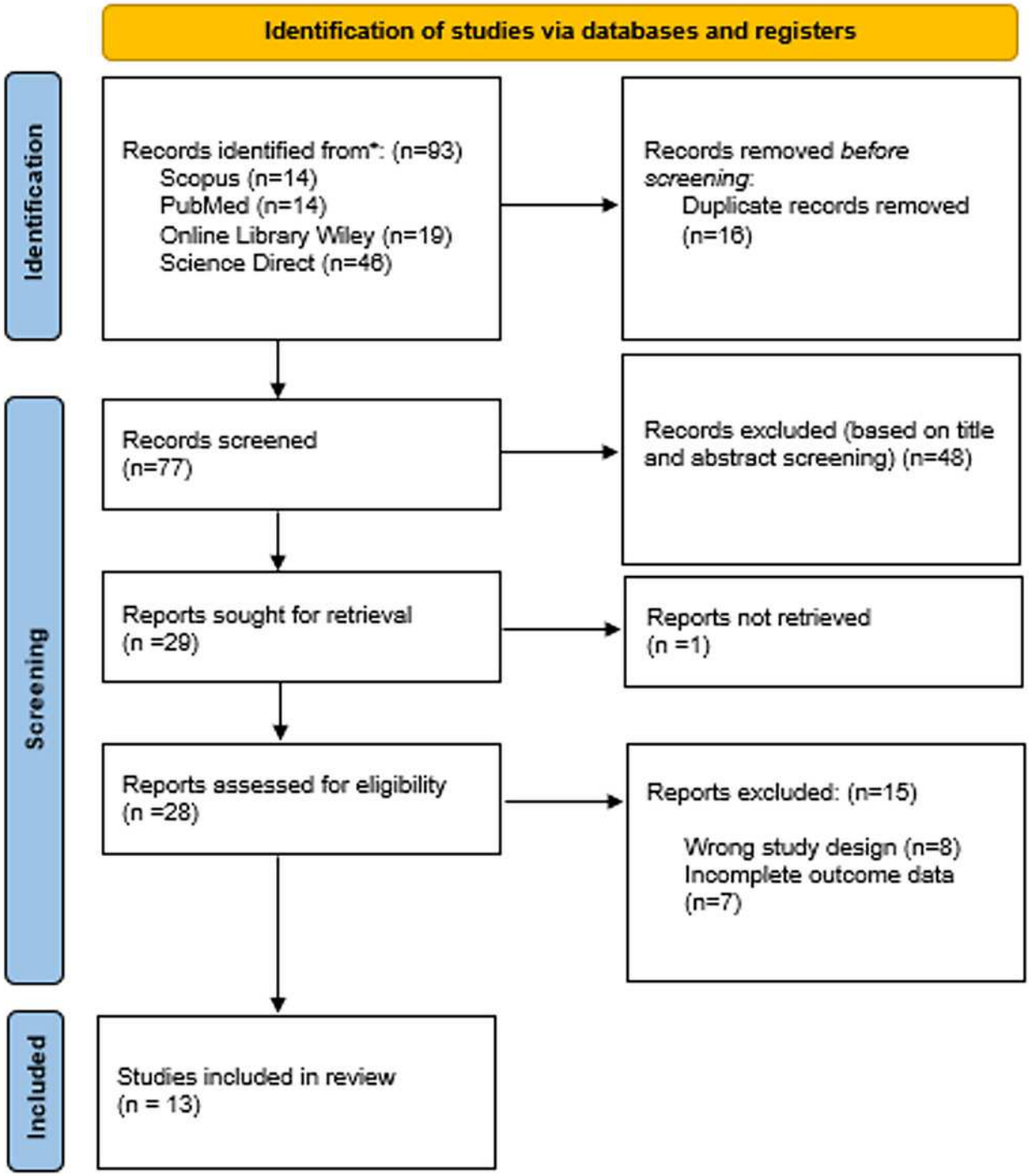

Figure 2

Risk of Bias Assessment tool for Non-randomized Studies (RoBANS) Graph

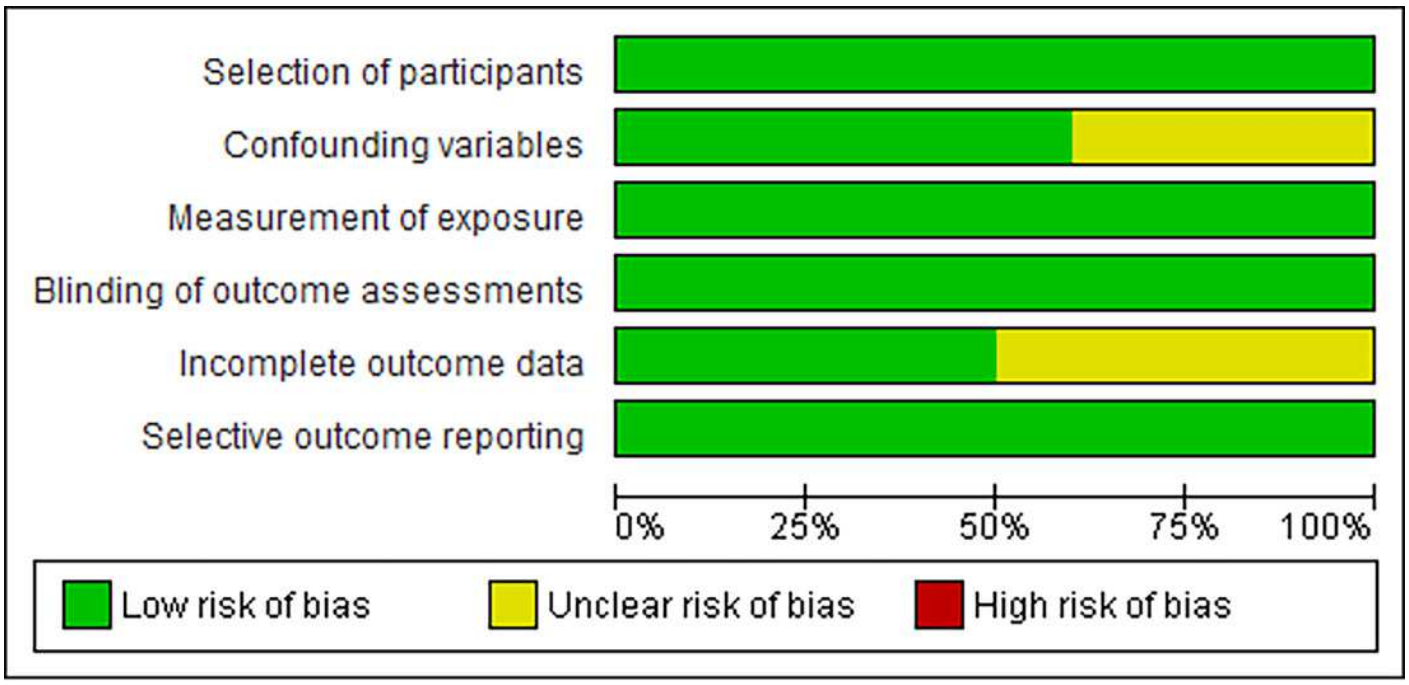

Figure 3

Summary of Risk of Bias Assessment tool for Non-randomized Studies (RoBANS)

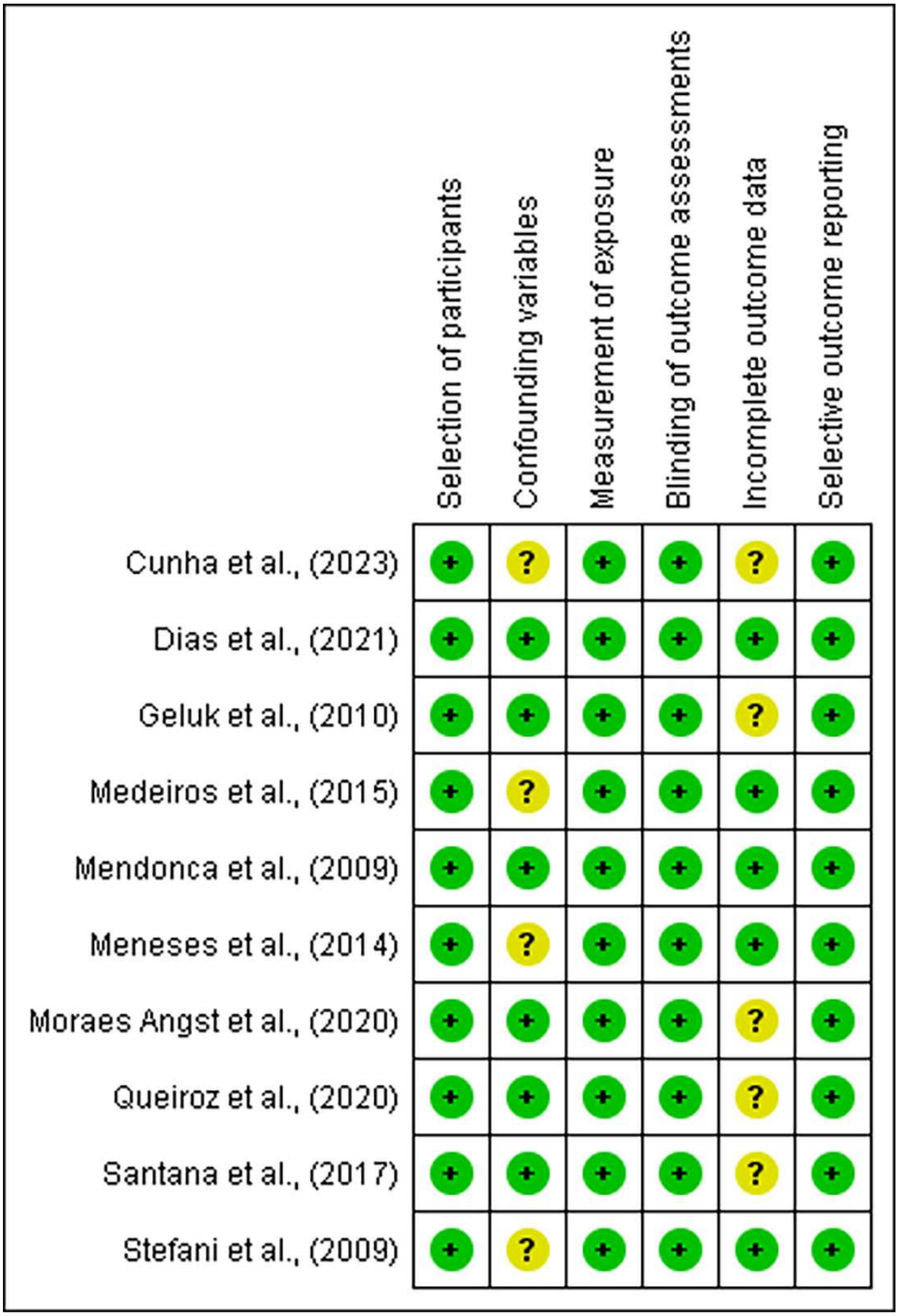
Selection of participants
Confounding variables
Measurement of exposure
Blinding of outcome assessments
Incomplete outcome data
Selective outcome reporting
Cunha et al., (2023)
Dias et al., (2021)
Geluk et al., (2010)
Medeiros et al., (2015)
Mendonca et al., (2009)
Meneses et al., (2014)
Moraes Angst et al., (2020)
Queiroz et al., (2020)
Santana et al., (2017)
Stefani et al., (2009)

Table 1(on next page)

Characteristic of Study

Sample Size	Study Duration	Population Description and Setting	Inclusion Criteria	Exclusion Criteria
n=28		Adult patients with Pure Neural Leprosy (PNL) attending single institution.	The patient, who ranged in age from 17 to 75, had a prior diagnosis of PNL based on laboratory, histopathological, neuroelectrophysiological, and clinical findings.	
n=56	January 1998 – December 2017.	Leprosy patients collected from Souza Araujo Out-Patient Unit (ASA), Brazil and Diabetes Outpatient Clinic of Pedro Ernesto University Hospital, Brazil. Data on histology of nerve biopsy were gathered from medical records.	Data on the histology of nerve biopsy taken from leprosy patients with and without pain to identify the cause of neuropathy. Furthermore, individuals who suffer from both neuropathic pain and diabetic neuropathy.	Individuals on corticosteroid therapy in reaction, patients without a blood sample stored in a laboratory, individuals with medical conditions that are known to cause peripheral neuropathy, and patients whose medical records are incomplete.
		Human alveolar epithelial cell line A549 and infected with M. leprae cultures.		
n=44	August 2012 – August 2013	With no prior anti-mycobacterium treatment and diagnosed with leprosy.	(1) No previous anti-mycobacterium treatment. (2) Patients signed informed consent.	Individuals having a history of systemic lupus erythematosus, diabetes mellitus, erythema nodosum leprosum response episode, and arterial hypertension.

Exclusion Criteria	No	Author (Year)	Country	Study Design	Aim of Study
	1.	Medeiros *et al*., (2015)	Brazil	Cross-sectional	(1) To expand on the investigation of the nerves impacted by leprosy. (2) To determine the relationship between the local MCP-1 expression and the histopathologic changes reported in neural leprosy.
Staff members working in leprosy centres or TB clinics were excluded as Endemic Controls.	2.	Moraes Angst *et al.*, (2020)	Brazil	Cross-sectional	To investigate the role of cytokine profiles in understanding the pathophysiology of leprosy-related pain and to assess patients with leprosy who primarily experience nociceptive or neuropathic pain.
	3.	Dias *et al.*, (2021)	Brazil	Cross-sectional	(1) To determine if M. leprae may elicit an immunological response in alveolar epithelial cells. (2) To investigate the role of TLR-9 in respiratory epithelial cells' expression and mycobacterial detection. (3) To discover the potential function of DNA-Hlp complexes exposed on the bacterial surface.
	4.	Meneses *et al.,* (2014)	Brazil	Cross-sectional	(1) To assess oxidative stress and urine MCP-1 in leprosy patients as opposed to healthy control group. (2) To compare patients based on the clinical picture of polar leprosy and those that tested positive for Bacilli smear.

Aim of Study	Sample Size	Study Duration	Population Description and Setting	Inclusion Criteria
To examine alternative cytokines/chemokines as putative WBA readouts and investigate potential augmentation of IFN-gamma production in response to *M. leprae* peptides with the inclusion of several cytokines and antibodies.	n=8		Individuals with leprosy attending a single institution.	
(1) To measure the levels of several other cytokines in addition to IFN-gamma in different cohorts from leprosy-endemic regions in Ethiopia, Brazil, and Bangladesh following a 24-hour whole blood stimulation with 17 M. leprae Ags. (2) To report on the discovery of novel cellular host indicators in one endemic location that distinguish leprosy patients from EC.	Bangladesh (n=50), Brazil (n=40), Ethiopia (n=70)	August 2008 - February 2011	Based on clinical, bacteriological, and histological data, an adult was diagnosed with leprosy and had a skin biopsy performed to classify the condition using the Ridley-Jopling classification system.	(1) Leprosy patients received chemotherapy for less than three months without exhibiting any leprosy reactions. (2) HHC: Adults who had spent at least the previous six months living in the same home as a BL/LL index patient. (3) Patients with tuberculosis (TB) had chemotherapy for minimum three months. Diagnosis of TB was based on a positive culture of M. tuberculosis in sputum. (4) Endemic controls are patients with absence of signs and symptoms of TB and leprosy.
To compare MCP-1 levels in leprosy patients' plasma levels to those of non-infected people at various phases of multidrug therapy (MDT).	n=33		Adults who were newly diagnosed with leprosy and were not receiving treatment.	Positive response to therapy (dapsone 100 mg and clofazimine 50 mg daily for a year). Additionally received 300 mg of clofazimine and 600 mg of rifampicin per month under supervision.
To assess how leprosy susceptibility is influenced by polymorphisms in the TLR1, TLR2, and TLR4 genes; to confirm the relationship between these markers' genotypes and leprosy patients' serum immune profiles.	n=52		Adult leprosy patient from two institutions (diagnosed by ELISA).	(1) Leprosy patients with diagnostic confirmation (2) Subjects with reactions were free of immunosuppressive drugs

No	Author (Year)	Country	Study Design
7.	Geluk *et al.*, (2010)	Netherlands	Cross-sectional
8.	Geluk *et al.*, (2012)	Bangladesh, Brazil, Ethiopia, South Korea	Cohort
9.	Mendonca *et al.*, (2009)	Brazil	Cross-sectional
10.	Santana *et al.*, (2017)	Brazil	Case control

Population Description and Setting	Inclusion Criteria	Exclusion Criteria
Adult with leprosy (diagnosed using Ridley-Joppling criteria) from single institution.	(1) Type 1 reaction: Untreated leprosy patients with severely indurated and erythematous lesions at the site of previously indolent macules, according to medical history. (2) Type 2 reaction: Patients diagnosed with type 2 reaction at diagnosis or during follow up that characterized by the sudden appearance of tender erythematous skin nodules mainly accompanied by fever and other systemic symptoms. (3) Controls: Leprosy patients who did not exhibit a reaction at the time of initial diagnosis or during follow-up but were classified histopathologically similar to the reaction patients.	
Adult patients diagnosed with leprosy using Ridley-Jopling classification. Together with the adult leprosy patient, HHCs shared a home. ECs were among normal controls residing in the same neighbourhood as the leprosy patients.	(1) Age: 21-59 years old (2) From same ethnic group	
Newly diagnosed leprosy patients without clinical reactions according to Ridley-Jopling.	(1) Patients visits clinic monthly to monitor reactions. (2) Endemic controls: living in the same region without having known interaction with TB or leprosy patients and without developing any clinical manifestations of either disease. (3) Healthy household contacts: people who have lived with leprosy patients in the same home for the last six months or longer.	(1) Laboratories and personnel working in leprosy or tuberculosis clinics. (2) Those who experienced reactions throughout the first three months of treatment.

No	Author (Year)	Country	Study Design	Aim of Study	Sample Size	Study Duration	Follow up duration
11.	Stefani *et al*., (2009)	Brazil	Case control	To screen potential plasma markers in Type 1 and Type 2 leprosy reactions.	n=39	February 2004-October 2005	
12.	Yuan *et al*., (2021)	China	Cohort	(1) To produce a wide transcriptome profile that could be utilized as a biomarker to identify various disease stages. (2) To establish a practical blood test for early leprosy diagnosis.	n=82	February 2015 - May 2016	
13.	Tio-Coma *et al.,* (2019)	Bangladesh, Brazil, Ethiopia, Nepal, Netherlands	Prospective cohort	(1) Finding transcriptomic signatures which may be utilized in leprosy reaction monitoring. (2) To accommodate worldwide applicability. (3) To improve knowledge on longitudinal fluctuations of RNA expression associated with reactions.	n=10	February 2008 - March 2015	

Time of MCP-1 measurement	Tools for outcome measurement	Outcomes measured
Upon admission, following a 6-month multidrug regimen in accordance with WHO guidelines for PB leprosy and a 12-month regimen for MB leprosy.	MCP-1 was stained with mouse monoclonal antibodies anti-MCP1 (dilution 1:25; eBioscience) and then slides were assessed using microscope.	(1) Endoneurium, perineurium, and epineurium from PNL nerves were examined histopathologically. (2) Immunohistochemical analysis of cellular immunoactivation markers. (3) Immunohistochemical expression of CXCL10, CCL2 chemokines, MMP2 and MMP9. (4) Comparison of the Immunoreactivities in the AFB+ and AFB- samples. (5) Interaction between the immunolabeling markers and the histopathological changes.
At admission	A neurological examination was used to provide a clinical evaluation. The neurological examination included the following: pain location, pain severity (measured on a numerical pain rating scale of 0 to 10 or 11 points), and type of pain (stinging, burning, electric shock-like, cold, other). Neurophysiological evaluation was collected from a 4- channel Nihon-Koden-Neuropack S1 equipment. ELISA was used to assess serum cytokine levels in accordance with the manufacturer's instructions (eBioscience-San Diego, CA, United States).	Clinical characteristics of neural pain and serum cytokines in patients with pain.
At admission	ELISA kits (R&D Systems, Minneapolis, MN, USA).	(1) Measurement of IL-1 and MCP-1 in A549 cells. (2) NF-kB transcription factor in alveolar epithelial cells activation by M. leprae. (3) DNA sensing by TLR-9 has a role in alveolar epithelial cells' immunological detection of M. leprae. (4) Hlp ligands' function in enhancing mycobacterial immunostimulatory potential.
Approximately after an 8-hour fasting period.	ELISA from Boster Biological Technology, Fremont, CA, USA.	(1) Demographic data (age, time from symptom onset to leprosy diagnosis, types of leprosy according to skin-smear test, renal function). (2) Protein excretion and MCP-1 in the urine of leprosy patients and controls categorized based on clinical criteria. (3) Comparison between levels of urinary MDA of leprosy patients and healthy controls.

Outcomes measured	Follow up duration	No	Author (Year)	MCP-1/CCL2 Measurement
(1) Chemokine and cytokine signatures (2) Correlation between the chemokine and cytokine secretion of in vitro cultured PBMCs from leprosy patient' HHC and the TLR4 rs1927914 polymorphism. (3) PBMCs were stimulated to cluster subgroups of household contacts of leprosy patients by the release of chemokines and cytokines by *M. leprae.*		1.	Medeiros *et al*., (2015)	Sections were hydrated in phosphate-buffered saline (PBS) following the fixation and staining of histopathological materials. Anti-MCP1 mouse monoclonal antibodies (dilution 1:25; eBioscience) were used to stain MCP-1. Subsequently, the slides were counterstained with Mayer's hematoxylin and then mounted. Under a microscope, two impartial observers, who were blinded to the group, observed immunoreactivities.
Cytokine and chemokine serum level profile of household contact, paucibacillary type, and multibacillary type.		2.	Moraes Angst *et al.*, (2020)	Sensory nerve was biopsied, then serum cytokine levels and histopathological evaluation were done. Clinical and neurophysiological evaluation was also done to defined clinically or neurophysiologically detectable impairment of sensory and/or motor nerve.
(1) Impacts of cytokine or antibody addition on IFN-gamma release in 24-hour undiluted WBAs. (2) Impact of M. leprae peptides' mannosylation. (3) In-tube IFN-gamma assay. (4) Analysis of IFN-gamma responses to M. leprae antigens using flow cytometry. (5) Multiplex examination of whole-blood cultures containing M. leprae antigens.		3.	Dias *et al.*, (2021)	In culture supernatants from A549 cells, MCP-1 concentration was assessed by ELISA.
(1) IFN-gamma reactions to Ags from M. leprae (2) Multiplex investigation of chemokines and cytokines in Bangladesh, South Korea, and Ethiopia in response to M. leprae Ags in WBA. (3) IFN-gamma/IL-10 ratio measurements in WBA.		4.	Meneses *et al.,* (2014)	The sandwich enzyme-linked assay (ELISA) was used to measure urinary MCP-1.

No	Author (Year)	MCP-1/CCL2 Measurement	Time of MCP-1 measurement	Tools for outcome measurement
5.	Cunha *et al.,* (2023)	Section Whole blood was extracted and utilized for TLR4 genotyping with PCR and chemokine and cytokine measurement using a cytometric beads array.	At admission	The Cytometric Bead Array (Human Chemokine Kit for CXCL8, CCL2, CXCL9, CCL5 and CXCL10; Human Cytokine Flex Set kit for IL-6, TNF, IFN-g, IL-17, IL-4, IL-10 and IL-2) was used to conduct a quantitative examination of chemokines and cytokines. It was purchased from BD Bioscience, Pharmingen (San Diego, CA, USA). Allelic discrimination based on validated Taqman® quantitative Polymerase Chain Reaction (qPCR) kits from Life Technologies® (Thermo Fisher, Inc.) was used to genotype SNP rs1927914 A/G in the TLR4 gene. The experiments used the Applied Biosystems® 7500 Fast Real-Time PCR instrument and were completed in accordance with the manufacturer's instructions.
6.	Queiroz *et al.,* (2020)	Serum levels of chemokines CXCL8 (IL-8), CCL2, CXCL9, and CXCL10, as well as cytokines TNF, IL-6, IFN-γ, IL-2, IL-17A, IL-4, and IL-10, were tested in all patients after blood was obtained.	2014 (Time 0-T0) and 2015 (Time 1-T1). On the initial visit, 15 out of the 79 patients were receiving typical multidrug therapy (Time 0 – T0) and the treatment was completed in it the second visit (Time 1 – T1). The remaining patients had already finished their therapy.	The CBA technique was utilized to quantify serum cytokines and chemokines (Becton Dickinson, BD-USA).
7.	Geluk *et al.,* (2010)	Whole blood was drawn and after incubated in 48-well plate with antigen, flow cytometry was performed. After determination of cytokines and chemokines, including CCL2 was performed.	At admission	Unstimulated, antigen-stimulated, or mitogen-stimulated samples were used to assess MCP-1 utilizing the Bio-Plex suspension array device, which runs by Luminex xMAP multiplex technology (Bio-Rad Laboratories, Veenendaal, Netherlands) and analysed with the Bio-Plex Manager software 4.0 (Bio-Rad Laboratories, Veenendaal, Netherlands).
8.	Geluk e*t al.,* (2012)	Whole blood was drawn and incubated with Ag solution. Supernatants were taken out of each well after a 24-hour period. WBA supernatants aliquots were used to measure the MCP-1 concentrations.	Approximately 24h after whole blood was drawn.	Using Bio-Plex suspension array system powered by Luminex xMap multiplex technology (Bio-Rad Laboratories, Veenendaal, The Netherlands).

Time of MCP-1 measurement	Tools for outcome measurement	Outcomes measured	Follow up duration
At admission	Serum cytokines and chemokines were quantified using the DuoSet R&D Systems, Minneapolis, MN, USA).	Chemokine levels in plasma from both uninfected and leprosy patients.	1 year
At admission	Commercial kits from R & D (R&D systems Inc. Minneapolis, MN, US) and BD OptEIA™ Set human (BD Biosciences, San Jose, CA, US).	(1) Study of the genes TLR 1, 2, and 4 using a population-based approach. (2) Leprosy patients' cytokine and chemokine trends in relation to their various TLR1, TLR2, and TLR4 SNP genotypes.	
At admission	Premixed human cytokine 27-plex panel of cytokines (Bio-Plex Cytokine reagent kit, BIO RAD Laboratories, Hercules, CA, USA).	(1) Patients' characteristics difference among T1R and T2R between cases and controls. (2) Cytokine analysis difference among T1R and T2R between cases and controls. (3) Pro-inflammatory cytokines difference among T1R and T2R between cases and controls. (4) Anti-inflammatory cytokines difference among T1R and T2R between cases and controls. (5) Growth factors difference among T1R and T2R between cases and controls.	
12h after blood was drawn	Using transcriptome sequencing with SYBR Premix Ex Taq™ II Kit (Perfect Real Time; Takara Bio, Cat. No. RR820A) and PrimeScript RT reagent Kit (Takara Bio, Cat. No. RR0377A). After that, three biological and three technical duplicates were used for RT-qPCR investigations on an Applied Biosystems 7500 Fast real-time PCR system (Applied Biosystems).	(1) Basic characteristics of participants (2) RNA-seq analysis of transcriptome variations in M. leprae Antigen-Stimulated PBMCs between leprosy patients and controls. (3) Among leprosy patients and non-leprosy controls, differentially expressed genes (DEGs) may serve as useful diagnostic markers. (4) The validation cohort's performance of particular DEGs in differentiating between leprosy patients and controls	
At admission (before the initiation of MDT).	dcRT-MLPA result' was analysed using Applied Biosystems 3730 capillary sequencer in GeneScan mode (Applied Biosystems, Foster City, CA).	(1) Leprosy-specific RNA-profiles s (increased: FCGR1A, IL6, IL15, LRKK2, MBP, MSR1, PACRGv1, TLR1, TLR4; decreased: CAMTA, CD3E, CTLA4, CXCL13, GATA3, LAG3, TFGB). (2) RNA-profiles for leprosy classification. (3) RNA-profles associated with exposure to M. leprae. (4) Risk factors for the transcriptome that lead to the development of RR. (5) Tracking the beginning and course of RR using longitudinal transcriptome alterations.	> 2 months for patients who developed reversal reaction.

No	Author (Year)	MCP-1/CCL2 Measurement
9.	Mendonca *et al*., (2009)	Chemokine concentrations were assessed in blood samples using sandwich ELISA kits that included CCL2, CCL3, CCL11, and CCL24 kits.
10.	Santana *et al*., (2017)	Whole blood was collected by venepuncture and centrifuged at 20,000g for 10 min for serum obtaining. Then ELISA chemokine assays were performed to measure levels of MCP-1.
11.	Stefani *et al*., (2009)	Prior to the assay, plasma aliquots were kept at -80°C after blood was taken in EDTA and centrifuged right away. After being frozen at -80°C, EDTA plasma samples were thawed and centrifuged at 1,000 × g for 10 minutes at 4°C. The supernatant was then filtered and used shortly thereafter.
12.	Yuan *et al*., (2021)	Peripheral blood was drawn and placed into EDTA tubes, then centrifuged to obtain a buffy coat. After, total RNA was extracted and measured with a spectrometer. Then, RNA sequencing was performed. The DESeq algorithm was used to analyze the differentially expressed genes (DEGs) between samples, and RT-qPCR was carried out to confirm the RNA-seq data.
13.	Tio-Coma *et al*., (2019)	Following the process of RNA separation, dual color reverse-transcription multiplex ligation-dependent probe amplification (dcRT-MLPA) tests were run. The result then analysed and transcriptomic risk factors was identified.