Immune Response Dynamics and Chemokine Profile from MCP-1 and MIP-1α in Toxoplasma gondii Infection

Nawras Alwan Hussain Ali [1], Saleem Khteer Al-Hadraawy [1*]

Abstract

Background: The delicate interplay between host immune responses and the evasion strategies of parasites is pivotal in determining disease outcomes. Toxoplasma gondii, a widespread protozoan parasite, triggers complex immune reactions involving various cell types and cytokines. Here, we investigated the levels of two key chemokines, monocyte chemoattractant protein-1 (MCP-1) and macrophage inflammatory protein-1 alpha (MIP-1α), in male patients with Toxoplasma infection compared to healthy controls. Method: We recruited 78 male subjects suspected of Toxoplasmosis, alongside 30 healthy males as controls, and collected blood samples from August 2023 to February 2024. MCP-1 and MIP-1α concentrations were measured using ELISA kits. Results: Our findings revealed a significant increase in serum MCP-1 levels among Toxoplasma-infected patients compared to healthy controls ($p < 0.001$). Additionally, a positive correlation between MCP-1 and MIP-1 was observed. Specifically, serum levels of MIP-1 positively and significantly correlated with MCP-1 levels in patients with chronic Toxoplasmosis (R2 = 0.0147). The mean concentration of MCP-1 was markedly higher in patients with Toxoplasmosis (41.08 ± 6.410 pg/ml) compared to the control group (27.73 ± 1.124 pg/ml) ($p < 0.001$). Similarly, the mean concentration of MIP-1α was elevated in Toxoplasma-infected individuals (12.06 ± 2.122 pg/ml) compared to healthy controls (6.971 ± 0.4809 pg/ml). Conclusion: These findings highlight the role of MCP-1 and MIP-1α in the immune response to Toxoplasma infection and suggest their potential as biomarkers for disease monitoring. Understanding the dynamics of these chemokines may aid in the development of targeted interventions and therapeutic strategies for Toxoplasmosis.

Keywords: Toxoplasmosis, MCP-1, MIP-1α, Immune response, Chemokines

Significance | This study determined the MCP-1 and MIP-1α Dynamics in Toxoplasma Infection as an implications for Immune Response and Biomarkers

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Introduction

Maintaining a delicate balance between the host's anti-parasitic immunity and the adaptive strategies employed by parasites is crucial, as any disruption can lead to severe consequences (Miller et al., 2009). Central to this equilibrium are immune cells, which play a pivotal role in initiating the immune response and fostering the development of adaptive immunity (Borges et al., 2019; Al-Hadraawy et al., 2022).

During the early stages of infection with Toxoplasma gondii (T. gondii), dendritic cells (DCs), macrophages, and monocytes are among the first responders of the host's immune system. Upon encountering pathogens, the host cells utilize pattern recognition

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receptors (PRRs), such as Toll-like receptors (TLRs), to identify "non-self" components known as microbial or pathogen-associated molecular patterns (MAMPs/PAMPs). This recognition triggers the production of key cytokines like interleukin-12 (IL-12), which plays a pivotal role in mounting resistance against bacterial and parasitic infections (Gee et al., 2009).

The ensuing immune response involves the secretion of pro-inflammatory chemokines and cytokines, which act on receptors present on Th1 cells and other T-cells, thereby stimulating and attracting these cells to the site of infection (Miller et al., 2009). This orchestrated immune cascade is essential for containing and eliminating the invading pathogens, highlighting the critical role of innate immunity in combating parasitic infections.

Dendritic cells, neutrophils, and other immune cells play crucial roles in stimulating the synthesis of interleukin-12 (IL-12), while natural killer cells and T lymphocytes induce interferon-gamma (INF-ɤ) production, which is vital for combating infections (Halonen & Weiss, 2013). Maturation and activation of dendritic cells are essential for effective infection control (Mihret, 2011), with macrophages and neutrophils being among the first immune cells recruited to the site of infection (Borges et al., 2019). Neutrophils eliminate parasites through IL-17 signaling, a key cytokine involved in their recruitment and development (Mills, 2022).

Monocyte chemoattractant protein-1 (MCP-1), also known as CCL2, acts as a biomarker and chemokine that recruits immune cells to sites of inflammation (Jedrysik et al., 2024). MCP-1 regulates the biological activities of monocytes/macrophages and facilitates the recruitment of various inflammatory cells to damaged tissues. Although CCR2 is the main receptor for MCP-1, other receptors may also be involved (He et al., 2023).

MCP-1 has been implicated in inflammatory diseases and neurodegenerative conditions such as atherosclerosis, rheumatoid arthritis, and certain neurodegenerative disorders (Kadomoto et al., 2021). Macrophages play critical roles in innate immune responses through phagocytosis and serve as a link between innate and acquired immunity (Do-Thi et al., 2023). Additionally, chemokines from the macrophage inflammatory protein-1 (MIP-1) family, including CCL3 (MIP-1-alpha), CCL4 (MIP-1-beta), and CCL9 (MIP-1-gamma), exhibit potent pronociceptive properties (Ciechanowska et al., 2023).

Materials and Methods

Subjects

The study involved 78 male individuals suspected of Toxoplasmosis, aged between 16 and 56 years old. These participants were recruited from AL-Hakeem Hospital and underwent examination by measuring Toxo IgG serum levels. Additionally, a control group comprising 30 healthy males was randomly selected from AL-Najaf province. Sample collection took place between August 2023 and February 2024. It's important to note that none of the participants were using medication or undergoing treatment for any other diseases during the study period (Muslim & Al-Hadraawy, 2023). The study protocol was approved by the ethical committee of [Institution Name]. Informed consent was obtained from all participants before their inclusion in the study. Confidentiality of participant information was strictly maintained throughout the study, and all procedures were conducted in accordance with ethical standards and guidelines for human research.

Blood sample collection

Out of the total 78 suspected patients and 30 healthy individuals from AL-Hakeem Hospital and AL-Najaf province, respectively, only 48 samples tested positive. The samples were collected between August 2023 and February 2024. Blood samples were obtained from patients via vein puncture, with one tube containing EDTA for gene expression analysis according to (Shlash & Aldujaili, 2023), and another tube with serum for estimating MIP-1 and MCP-1 levels. The serum tubes were allowed to stand at room temperature for 30 minutes before centrifugation at 3000 rpm for 5 minutes using a Backman counter (Germany) to separate the serum. The serum was then transferred to sterile tubes, divided into two parts, and stored at -20°C until further use for MIP-1 and MCP-1 estimation. The biomarkers were quantified using Eliza Kits (Abd & Al-Hadraawy, 2023).

Statistical analysis

Graph pad prism for Windows (5.04, Graph pad software Inc. USA) was used to analyze the data, and the results are reported as the mean, standard error (SE). A student t-test was used to examine the differences between the patient and control groups (Al-Hadraawy *et al.,* 2022)

Results

Concentration of Serum MCP-1

The present study indicates a significant elevation in serum MCP-1 and MIP-1α levels in patients with Toxoplasmosis compared to healthy controls. Specifically, MCP-1 concentrations were notably higher in Toxoplasmosis patients (41.08 ± 6.410 pg/ml) compared to controls (27.73 ± 1.124 pg/ml) (P=<0.001), while MIP-1α levels also exhibited a positive correlation with MCP-1 levels in patients with chronic Toxoplasmosis (R2 = 0.0147), as illustrated in Figure 1, 2, and 3.

Discussion

The present study unveiled a significant elevation in serum MCP-1 levels among patients infected with Toxoplasmosis compared to their healthy counterparts. In response to T. gondii infection, the host's immune system orchestrates a protective response, wherein immune cells, notably macrophages, collaborate to mount an innate

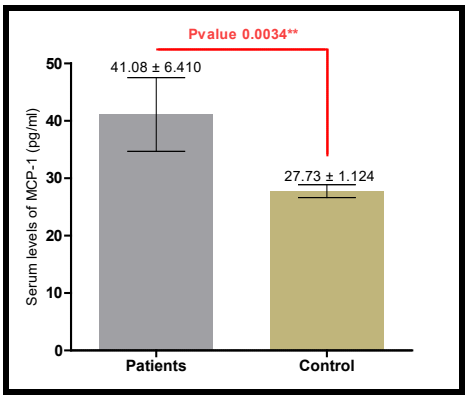

Figure 1. Mean serum level of MCP-1in patients and healthy controls.

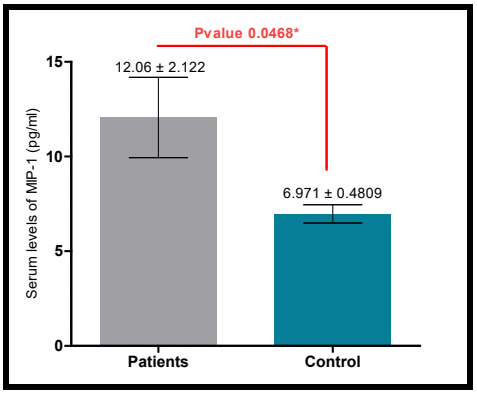

Figure 2. Mean serum level of MIP-1α in patients and healthy controls.

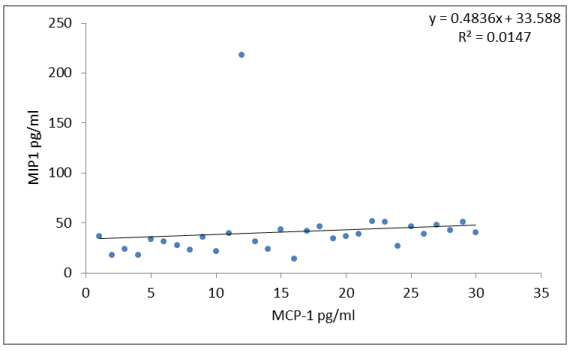

Figure 3. Correlation between MIP-1 (pg/ml) Levels and MCP-1 (pg/ml) in patients infected with Toxoplasmosis.

immune defense against the parasite. Macrophages play a pivotal role in this response, swiftly migrating to infection sites and employing various mechanisms to combat T. gondii, including direct phagocytosis of the parasite and secretion of cytokines and chemokines to foster an adaptive immune response. Among these chemokines, monocyte chemoattractant protein-1 (MCP-1), also recognized as C-C motif chemokine ligand 2, along with its principal receptor C–C chemokine receptor type 2 (CCR2), plays a crucial role.

Yan et al. (2021) observed a notable increase in MCP-1 mRNA expression consequent to T. gondii infection, corroborating the heightened MCP-1 levels found in our study. Additionally, Rio et al. (2004) demonstrated that polymorphonuclear leukocytes (PMN) produce both IL-12p40 and MCP-1 in response to Toxoplasma, suggesting a link between PMN activity and MCP-1 secretion. Sukhumavasi et al. (2007) conducted a study on mice in Ithaca, NY, revealing significantly higher MCP-1 secretion 24 hours post-infection with live tachyzoites. Furthermore, Pierre et al. (2000) found that the inhibition of parasite multiplication by pyrimethamine did not alter MCP-1 secretion, aligning with the findings of our current investigation. These referenced studies consistently support the observations made in our study regarding the heightened MCP-1 levels in response to Toxoplasma infection.

The current study revealed a slight elevation in the level of MIP-1α in the blood serum of patients infected with toxoplasmosis compared to healthy controls. Chemokines play a crucial role in orchestrating the recruitment of various immune cells, including monocytes, macrophages, dendritic cells (DCs), natural killer (NK) cells, and various T cell subsets, thereby amplifying immune responses. Inflammatory monocytes are particularly vital in the immune defense against the parasite during both acute and chronic stages of infection. T. gondii infection may trigger an excessive release of pro-inflammatory cytokines, commonly referred to as a "cytokine storm," which is often associated with specific diseases, conditions, and treatments, including toxoplasmosis. Among these cytokines, Macrophage Inflammatory Protein-1α (MIP-1α) is noteworthy.

This finding is consistent with previous studies. Neutrophils, another significant source of chemokines, produce CCL2 (MCP-1), CCL3 (MIP-1α), CCL4 (MIP-1β), CCL17 (MIP-3β), and CCL20 (MIP-3α) upon activation. Neutrophils synthesize chemokines de novo and recruit immature DCs to the infection site via CCL3, CCL4, CCL5, and CCL20 during T. gondii infection (Shrestha & Hong, 2023). Similarly, a study conducted on pregnant women with toxoplasmosis by Ali (2016) yielded similar results. Furthermore, Yan et al. (2021) demonstrated a significant increase in MIP-1α mRNA expression in response to T. gondii infection, supporting our findings.

The present study demonstrates a positive and significant correlation between serum levels of MIP-1 and MCP-1 in patients with chronic toxoplasmosis. Chemokines, including MCP-1 and MIP-1, play crucial roles in recruiting and activating various white blood cells such as monocytes/macrophages, polymorphonuclear cells, and lymphocytes to sites of infection, leading to increased levels during inflammation. Released by various immune cells in response to infection, these chemokines facilitate cell migration to the infection site. MCP-1 and MIP-1α belong to the cysteine-cysteine (CC) chemokine family, acting as major chemoattractants for lymphocytes and monocytes, and are implicated in controlling T. gondii infection and its pathogenesis. Chemokines secreted by adipose tissue, including MIF, MCP-1, and MIP-1, are associated with elevated chemokine levels.

This finding is consistent with previous research. Jawad & Mushatt (2023) reported that the expressions of MIP-1α and MCP-1 gene transcripts are upregulated in T. gondii-stimulated macrophages and fibroblasts. Similarly, Yan et al. (2021) demonstrated a significant increase in MIP-1α and MCP-1 mRNA expression in response to T. gondii infection, aligning with the observations of our study. As the disease induces inflammation, the secretion of cytokines and chemokines is positively augmented.

Conclusion

In conclusion, our study highlights the intricate interplay between host immune responses and Toxoplasma gondii infection. Elevated serum levels of MCP-1 and MIP-1α in toxoplasmosis patients underscore the involvement of these chemokines in orchestrating immune cell recruitment and activation, crucial for combating the parasite. The positive correlation between MCP-1 and MIP-1α levels further emphasizes their coordinated roles in chronic toxoplasmosis. Consistent with previous research, our findings elucidate the importance of MCP-1 and MIP-1α in immune response modulation during T. gondii infection. Understanding these mechanisms can inform future therapeutic strategies targeting immune modulation in parasitic infections.

Author contributions

N.A.H. conducted study design, analyzed data, S.K.A.H. wrote and drafted the manuscript.

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Competing financial interests

The authors have no conflict of interest.

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