ISSN: 2709-5215 Volume 6, Issue 1, 2025
DOI: 10.48173/jwh.v2i2.102 page 001-007
Journal Wetenskap Health

Assessment of Serum Interleukin 40 and 41 Levels in Patients with Multiple Sclerosis

Aya Ahmed Ghani[1], Zahra'a Abdul-Raheem Ahmed[1]

[1]Assistant Lecturer, Medical Laboratory Technologies, Dijlah University, Baghdad, Iraq

[2]Prof. Dr Department of Biology, College of Science for Women, University of Baghdad, Baghdad, Iraq

E-mail: aya.ahmmed@duc.edu.iq

Abstract

Multiple sclerosis (MS) is a chronic immune-mediated disease characterized by inflammation, demyelination, gliosis, and neurodegeneration in the central nervous system (CNS). Interleukin 40 and 41 are novel immune-modulatory cytokines associated with inflammatory and autoimmune diseases. The study aims to evaluate novel cytokines in MS patients and their role as a potential biomarker for JCV reactivation in MS patients. Five millilitres of blood were taken from 165 individuals (age range 18–53 years), divided into 109 samples from MS patients (37 males and 72 females) who enrolled at Dr. Saad AlWitry Hospital for Neurosciences in Baghdad and 56 healthy volunteers (18 males and 38 females). The patients were distributed into three groups: MS patients, JCV-associated MS patients, and MS patients without therapy. ELISA has been used to measure the cytokine levels in the blood of MS patients and healthy volunteers. The serum level mean of IL-40 decreased significantly ($p \leq 0.001$) in the three groups of patients: MS patients (20.005±1.346 ng/ml), JCV-associated MS patients (24.520±1.454 ng/ml), and MS patients without therapy (24.686±3.008 ng/ml) compared to controls (42.287±4.742 ng/ml). For IL-41, its mean level also decreased significantly ($p \leq 0.001$) in MS patients (1.397±0.224 ng/ml), JCV-associated MS patients (1.545±0.175 ng/ml), and MS patients without therapy (1.161±0.276 ng/ml) compared to controls (3.044±0.321 ng/ml). Newly discovered cytokines (IL-40 and IL-41) were negatively associated with the severity of the disease and may have a role as a potential biomarker for MS.

Keywords: Autoimmune disease, Interleukin 40, Interleukin 41, Multiple sclerosis, JC virus

Introduction

MS is a chronic autoimmune disease resulting from immune disorders in the CNS and is characterized by inflammation, demyelination, and axonal transection, causing a range of debilitating symptoms that can significantly impact a person's quality of life (Nociti & Romozzi, 2022). The pathophysiology of MS, a malfunctioning immune system that targets components of the CNS. Autoreactive peripheral T cells that are out of whack enter the brain across the blood-brain barrier and launch an assault on myelin and oligodendrocytes, two components of the CNS (Bsteh et al., 2016). On the other hand, primary degeneration of

Article Info:
Received: January 2, 2025
Revised: February 11, 2025
Accepted: March 3, 2025

oligodendrocytes and myelin is the first occurrence of MS, with oligodendrocyte mortality and mild myelinopathy, thus generating a secondary autoimmune response. Demyelination and other symptoms of MS are caused by a subsequent autoimmune onslaught (Titus et al., 2020). Among the debilitating symptoms of MS are difficulties with movement, stiffness, incontinence, discomfort, impaired vision, distorted speech, and cognitive decline (Dennison et al., 2018).

Cytokines are implicated in the pathogenesis of MS through CNS demyelination, which occurs when immune system cells (β and T) invade the CNS and produce antibodies and cytokines that target myelin antigens (Lassmann, 2005). The novel cytokine IL-40 regulates immune processes and the maintenance of B cells in a stable state and thus may play a role in the development of autoimmune diseases (Abed et al., 2023). Likewise, the new cytokine IL-41 regulates the immune system. Macrophages and barrier tissues have high levels of IL-41 expression, which is stimulated by several cytokines such as IL-17A, IL-12, and IL-4, while interferon-γ and transforming growth factor-β suppress its production (Gong et al., 2023).

New disease-modifying treatments (DMTs) have emerged in the last few years and can help MS patients live better lives by reducing relapse rates and slowing the disease's development (Stamatellos & Papazisis, 2023). Natalizumab, interferon beta, and fingolimod are the most common of the DMTs available on the market. Although these drugs have demonstrated encouraging outcomes in clinical trials, they come with some unwanted side effects (Rommer et al., 2014). Interferon beta is a popular treatment for MS because of its ability to affect the activity of immune cells, particularly T and B lymphocytes, which play a crucial role in the body's defense against infections (Severa et al., 2015). Patients taking immunosuppressant drugs are at higher risk of developing a John Cunningham virus (JCV) infection. The use of immunosuppressive medication and immune system deficiency are the main causes of JCV reactivation (Hussein et al., 2023). The JC virus can enter a permanent latent state in the kidneys, the central nervous system, and hematopoietic progenitor cells (Arthur & Shah, 1989; Schneider & Dörries, 1993). Immunosuppressive medications such as natalizumab, efalizumab, and rituximab reduce the mobilization of CD4+ and CD8+ T cells. As a result, the JCV can infect and kill oligodendrocytes in the brain (Wijburg et al., 2018). The study aims to highlight the evaluation of IL-40 and 41 levels, which may have a significant impact on host immune responses.

Methods

Blood samples were collected from 109 patients (age range: 18 to 53 years) with relapsing-remitting multiple sclerosis (RRMS) at Dr. Saad AlWitry Hospital for Neurosciences, Baghdad, Iraq, from July 2023 to the end of December 2023. RRMS was determined by McDonald's criteria revision in 2017, and the diagnosis was done by the consultant medical staff at the hospital. According to the clinical examination, magnetic resonance imaging (MRI) examination, hematology indicators, biochemical indicators, and immunological indicators, the study samples were divided into four groups: MS patients (N = 52), JCV infection in MS patients (N = 45), MS patients without therapy (first diagnosis) (N = 12), and a control group (N = 56) consisting of healthy volunteers who were matched for gender and age with MS patients.

Assessment of Interleukins Serum Levels

The levels of IL-40 and IL-41 were measured in the sera of MS patients and healthy controls using the ELISA technique. The assessment was performed at the College of Science for Women, University of Baghdad, using a sandwich ELISA kit for each interleukin that was

produced by the Bioassay Technology Laboratory in China. These kits were designed for quantitative measurement of human cytokines based on the manufacturer's protocol.

Ethical approval

Informed consent and Ethics Committee approval were obtained from the Iraqi Ministry of Health and the Department of Biology, College of Science for Women, University of Baghdad, under number 22/4000 on 16-7-2023.

Statistical Analysis

Frequencies and percentages are used to present data. A significant threshold of $P < 0.05$ was established. Version 14 of SPSS was used for statistical analysis. The study employed one-way analysis of variance (ANOVA) to assess group differences and a t-test to compare cytokine levels between groups. The correlation between the levels of interleukin was ascertained using Pearson's correlation coefficient.

Results and Discussion

We included 109 patients with MS: 52 (47.7%) were relapsing-remitting MS (RRMS) patients, 12 (11.0%) were RRMS patients without therapy (first diagnosis), and 45 (41.3%) were JCV infection in RRMS patients. The mean age was 32.477± 8.868 years in the patient group and 34.107±10.188 years in the control group. The age and gender distribution of patients with different groups of MS and the control group are shown in Table (1) and show that the prevalence of the disease is greater in females.

The result showed that IL-40 concentrations were significantly greater ($p = 0.001$) in healthy controls (42.287±4.742 ng/ml) compared to MS patient groups (22.296± 0.960 ng/ml). IL-41 concentrations were also significantly greater ($p = 0.001$) in healthy controls (3.044±0.321 ng/ml) compared to MS patient groups (1.406± 0.131 ng/ml), while the differences for IL-40 and IL-41 were not significant when comparing a patient with MS to a patient with JCV and first diagnosis of MS, Table (2).

Healthy females had a slight increase in IL-40 concentration compared to males (43.444± 6.032 vs. 39.845± 7.654 ng/ml, respectively), while male MS patients showed a slightly increased mean compared to females. However, no significant gender-related variation was observed between females and males in patients or controls. IL-41 concentration was lower in males than in females in all study groups, with a significant difference in JCV infection in MS patients (1.687± 0.196 ng/ml in females vs. 0.885± 0.308 ng/ml in males) and MS patients (1.723± 0.312 ng/ml in females vs. 0.987± 0.306 ng/ml in males). While no significant difference ($P > 0.05$) was observed between females and males of MS patients without therapy and healthy controls Table (3).

Ninety-seven MS patients were on medication and were undergoing two lines of therapy: first-line therapy (Interferon-beta) and second-line therapy (Gilenya or Natalizumab). Therefore, when serum levels of IL-40 were evaluated, no significant differences were found in MS patients treated with Betaferon (23.536±1.803 ng/ml) compared to MS patients treated with Fingolimod (22.785±2.401 ng/ml) or Tysabri (20.309±1.662 ng/ml). While the serum level of IL-41 demonstrated increased levels in MS patients treated with Tysabri (1.839±0.273 ng/ml) compared to MS patients treated with Betaferon and Fingolimod (1.296±0.201 ng/ml, 1.190±0.216 ng/ml, respectively).

The correlation coefficient (rs) and two-tailed probability (p) are shown in Table (4). IL-40 showed a significant positive correlation with IL-41 ($p = 0.001$) in all study groups

except JCV infection in MS patients, which showed a negative correlation but no significant differences.

Circulatory system infections account for the majority of CNV infections. White blood cells are crucial for the onset and course of inflammatory diseases like multiple sclerosis. MS is an autoimmune neurodegenerative disease that affects the central nervous system (CNS) white matter. Women are twice as likely as males to get MS. Young adults typically experience relapsing-remitting episodes of the disease, which cause a variety of neurological impairments with each episode. Between episodes, symptoms may totally vanish, although they frequently linger as the illness worsens (Najafi et al., 2022). The presence of cytokines and activated lymphocytes is a characteristic of these illnesses. After adhering to cerebral endothelial cells, lymphocytes go through blood vessel walls and into the central nervous system. A key factor in the demyelination process seen by MS patients is the extravasation of T cells over the blood-brain barrier. Because lymphocytes emit lytic agents including antibodies and inflammatory cytokines extracellularly, they are frequently linked to tissue destruction (Gloudina et al., 2012).

Pro-inflammatory cytokines (such as IL-1, IL-6, and TNF-alpha) may contribute to the development of MS by promoting inflammation and tissue damage in the CNS. Conversely, other cytokines (such as IL-10 and TGF-beta) have anti-inflammatory cytokines and help regulate the immune response in MS, which may limit tissue damage (Navrátilová et al., 2023). Therefore, cytokines play diverse roles, sometimes providing signals for inflammation and other times providing signals for protection (Catalan-Dibene et al., 2018). Therefore, cytokines and their receptors are used as therapeutic targets. Medication that inhibits specific pro-inflammatory cytokines or enhances the activity of anti-inflammatory cytokines may help reduce disease activity and slow the progression of MS (Al Rubaye et al., 2023). In addition, the immune system plays a crucial role in controlling JCV infection and preventing the development of PML. Cytokines produced by immune cells help regulate the immune response to JCV and other pathogens. In individuals with MS, immunosuppressive therapies cause dysregulation of cytokine production, which may contribute to impaired immune surveillance and an increased risk of JCV reactivation. Common MS therapies, such as natalizumab and rituximab, lead to a decrease in mature B cells in the periphery and subsequent mobilization of immature B cells from the bone marrow, which may lead to the disseminating of latent viruses to the brain (Branco et al., 2018).

In this study, patients with MS showed decreased serum IL-40 and IL-41 levels, and we think that the decline in the IL-40 and IL-41 concentrations is under the strict influence of immunosuppressant drugs. IL-40 is a recently identified cytokine associated with B cells. This cytokine is involved in several biological processes, such as the production of IgA and the development of B cells in bone marrow. In addition, IL-40 has a role in a wide variety of inflammatory and autoimmune diseases, including rheumatoid arthritis, Sjogren's syndrome, ankylosing spondylitis, type 2 diabetes, and Graves' disease (Al Rubaye et al., 2023). Extracellular IL-40 stimulates neutrophils through pro-inflammatory effects, which helps to increase the immune response in multiple sclerosis. IL-40 release necessitates a particular pro-inflammatory milieu (Bani-Wais & Ad'hiah, 2023). When studying the contribution of IL-40 to the pathogenesis of MS, we observed that IL-40 is decreased in the serum of patients with MS and may be affected by anti-suppressive drugs. Therefore, the decrease of IL-40 in MS after therapy indicates its role in inflammation and tissue destruction in MS and may also have a role in acute disease flare-ups. A recent study showed that the expression of IL-40 was downregulated in a human cell model of pneumonia after treatment with anti-inflammatory

drugs, confirming that the concentration of IL-40 may be altered and impacted in conditions of inflammation and therapy (Dabbagh-Gorjani, 2024).

The immunomodulatory IL-41 has anti-inflammatory effects and is expressed in many tissues and immune cells, especially activated macrophages. IL-41 has been proven to inhibit inflammation, improve metabolism, and regulate adipose function (Shi et al., 2024). IL-41 is also involved in Th1, Th2, and Th17 immune responses. The anti-inflammatory IL-41 plays a role in many diseases, including colitis and arthritis. However, serum IL-41 levels are decreased in other diseases, especially in patients with inflammation-related diseases (Bridgewood et al., 2019). If we assume that IL-41 is involved in MS development through its association with macrophage polarization, IL-41 would be produced by alternatively activated macrophages (M2), which inhibit inflammation. Therefore, the decrease in serum IL-41 in MS patients may be related to an imbalance of M1/M2. In addition, IL-41 is also stimulated by a variety of cytokines (such as TNF-α, IL-4, IL-12, and IL-17A) and inhibited by IFN-γ and TFG-β. Therefore, MS Patients who took immunosuppressive drugs showed decreased IL-41 concentration in serum because IL-41 production in bone marrow is stimulated by IL-4, IL-12, IL-17A, and TNF-α.

Therefore, B cells are the cause of the drop in cytokines from the usual range. Eliminating B cells can lessen the onset or severity of autoimmune or inflammatory disorders, which are diseases that are heavily influenced by them. However, activated B cells, not plasma cells, are the target of the majority of therapy. The generation of cytokines is one significant effector of activated B lymphocytes. According to this perspective, B cell elimination decreased the production of IL-40 and IL-41, indicating that treatments aimed at B cells may offer relief from autoimmune diseases where IL-40 and IL-41 are involved.

Conclusion

Serum levels of IL-40 and IL-41 were significantly decreased in MS patients and were likewise suppressed in patients with JCV-associated MS and therapeutic patients. Therefore, IL-40 and IL-41 are negatively associated with disease severity and may have a role as a potential biomarker for MS.

Acknowledgments:

The authors express their gratitude and deepest thanks to the consultant medical staff and all the patients and volunteers for their cooperation during sample collection.

Author Contributions:

	Contributor 1	Contributor 2	Contributor 3	Contributor 4
Concepts	*	*		
Design	*			
Definition of intellectual content	*	*		
Literature search	*	*		
Clinical studies	*			
Experimental studies	*			
Data acquisition	*	*		
Data analysis		*		
Statistical analysis	*			
Manuscript preparation	*	*		
Manuscript editing	*	*		
Manuscript review	*	*		
Guarantor	*	*		

References

Abed, R. M., Abdulmalek, H. W., Yaaqoob, L. A., Altaee, M. F., & Kamona, Z. K. (2023). Serum level and genetic polymorphism of IL-38 and IL-40 in autoimmune thyroid disease. *Iraqi Journal of Science*, 2786-2797. https://doi.org/10.24996/ijs.2023.64.6.12

Al Rubaye, A. M., Sharquie, I. K., & Gorial, F. I. (2023). Serum interleukin 40: An innovative diagnostic biomarker for patients with systemic lupus erythematosus. *The Medical Journal of Malaysia, 78*(5), 609-615.

Arthur, R. R., & Shah, K. V. (1989). Occurrence and significance of papovaviruses BK and JC in the urine. *Progress in Medical Virology, 36*, 42-61.

Bani-Wais, D. F., & Ad'hiah, A. H. (2023). A novel intergenic variant, rs2004339 A/G, of the gene encoding interleukin-40, C17orf99, is associated with risk of rheumatoid arthritis in Iraqi women. *Molecular Immunology, 164*, 39-46. https://doi.org/10.1016/j.molimm.2023.11.002

Branco, L. P., Adoni, T., Apostolos-Pereira, S. L., Brooks, J. B. B., Correa, E. C., Damasceno, C. A., Eboni, A. C. B., Fezer, L., Gama, P. D. D., Goncalves, M. V. M., & Gomes, S. (2018). Serological profile of John Cunningham virus (JCV) in patients with multiple sclerosis. *Arquivos de Neuro-Psiquiatria, 76*, 588-591. https://doi.org/10.1590/0004-282X20180083

Bridgewood, C., Russell, T., Weedon, H., Baboolal, T., Watad, A., Sharif, K., Cuthbert, R., Wittmann, M., Wechalekar, M., & McGonagle, D. (2019). The novel cytokine Metrnl/IL-41 is elevated in psoriatic arthritis synovium and inducible from both entheseal and synovial fibroblasts. *Clinical Immunology, 208*, 108253. https://doi.org/10.1016/j.clim.2019.108253

Bsteh, G., Ehling, R., Lutterotti, A., Hegen, H., Di Pauli, F., Auer, M., Deisenhammer, F., Reindl, M., & Berger, T. (2016). Long-term clinical prognostic factors in relapsing-remitting multiple sclerosis: Insights from a 10-year observational study. *PLoS ONE, 11*(7), e0158978. https://doi.org/10.1371/journal.pone.0158978

Catalan-Dibene, J., McIntyre, L. L., & Zlotnik, A. (2018). Interleukin 30 to interleukin 40. *Journal of Interferon & Cytokine Research, 38*(10), 423-439. https://doi.org/10.1089/jir.2018.0089

Dabbagh-Gorjani, F. (2024). A comprehensive review on the role of interleukin-40 as a biomarker for diagnosing inflammatory diseases. *Autoimmune Diseases, 2024*(1), 3968767. https://doi.org/10.1155/2024/3968767

Dennison, L., Brown, M., Kirby, S., & Galea, I. (2018). Do people with multiple sclerosis want to know their prognosis? A UK nationwide study. *PLoS ONE, 13*(2), e0193407. https://doi.org/10.1371/journal.pone.0193407

Gloudina, M. H., Mogamat, S. H., Rajiv, T. E., & Tandi, M. (2012). The haematological profile of patients with multiple sclerosis. *Open Journal of Modern Neurosurgery, 2012*.

Gong, L., Zhou, Y., Shi, S., Ying, L., Li, Y., & Li, M. (2023). Increased serum IL-41 is associated with disease activity in rheumatoid arthritis. *Clinica Chimica Acta, 538*, 169-174. https://doi.org/10.1016/j.cca.2022.11.021

Hussein, R. K., Al-Obaidi, A. B., & Hussein, M. R. (2023). Prevalence of Anti JC Polyomavirus IgG in a sample of Iraqi kidney transplant recipients and healthy blood donors. *Iraqi Journal of Medical Sciences, 21*(2).

Lassmann, H. (2005). Mechanisms of multiple sclerosis. *Drug Delivery Today: Disease Mechanisms/Nervous System, 2*(4), 448-452. https://doi.org/10.1016/j.ddmec.2005.11.007

Najafi, P., Hadizadeh, M., Cheong, J. P. G., Mohafez, H., & Abdullah, S. (2022). Cytokine profile in patients with multiple sclerosis following exercise: A systematic review of randomized clinical trials. *International Journal of Environmental Research and Public Health, 19*(13), 8151. https://doi.org/10.3390/ijerph19138151

Navrátilová, A., Bečvář, V., Hulejová, H., Tomčík, M., Štolová, L., Mann, H., Růžičková, O., Šléglová, O., Závada, J., Pavelka, K., & Vencovský, J. (2023). New pro-inflammatory cytokine IL-40 is produced by activated neutrophils and plays a role in the early stages of seropositive rheumatoid arthritis. *RMD Open, 9*(2), e002894. https://doi.org/10.1136/rmdopen-2022-002894

Nociti, V., & Romozzi, M. (2022). Multiple sclerosis and autoimmune comorbidities. *Journal of Personalized Medicine, 12*(11), 1828.

Rommer, P., Zettl, U., Kieseier, B., Hartung, H., Menge, T., & Frohman, E. (2014). Requirement for safety monitoring for approved multiple sclerosis therapies: An overview. *Clinical & Experimental Immunology, 175*(3), 397-407. https://doi.org/10.1111/cei.12206

Schneider, E. M., & Dörries, K. (1993). High frequency of polyomavirus infection in lymphoid cell preparations after allogeneic bone marrow transplantation. *Transplantation Proceedings, 25*(1 Pt 2), 1271-1273.

Severa, M., Rizzo, F., Giacomini, E., Salvetti, M., & Coccia, E. M. (2015). IFN-β and multiple sclerosis: Cross-talking of immune cells and integration of immunoregulatory networks. *Cytokine & Growth Factor Reviews, 26*(2), 229-239. https://doi.org/10.1016/j.cytogfr.2014.11.005

Shi, R., He, M., Peng, Y., & Xia, X. (2024). Homotherapy for heteropathy: Interleukin-41 and its biological functions. *Immunology*. https://doi.org/10.1111/imm.13791

Stamatellos, V. P., & Papazisis, G. (2023). Safety and monitoring of the treatment with disease-modifying therapies (DMTs) for multiple sclerosis (MS). *Current Reviews in Clinical and Experimental Pharmacology, 18*(1), 39-50. https://doi.org/10.2174/2772432817666220412110720

Titus, H. E., Chen, Y., Podojil, J. R., Robinson, A. P., Balabanov, R., Popko, B., & Miller, S. D. (2020). Pre-clinical and clinical implications of "inside-out" vs. "outside-in" paradigms in multiple sclerosis etiopathogenesis. *Frontiers in Cellular Neuroscience, 14*, 599717. https://doi.org/10.3389/fncel.2020.599717

Wijburg, M. T., Kleerekooper, I., Lissenberg-Witte, B. I., de Vos, M., Warnke, C., Uitdehaag, B. M. J., Barkhof, F., Killestein, J., & Wattjes, M. P. (2018). Association of progressive multifocal leukoencephalopathy lesion volume with JC virus polymerase chain reaction results in cerebrospinal fluid of natalizumab-treated patients with multiple sclerosis. *JAMA Neurology, 75*(7), 827-833. https://doi.org/10.1001/jamaneurol.2018.0094