Tropical Journal of Pharmaceutical Research October 2024; 23 (10): 1647-1652
ISSN: 1596-5996 (print); 1596-9827 (electronic)

Available online at http://www.tjpr.org
http://dx.doi.org/10.4314/tjpr.v23i10.8

Original Research Article

Expression patterns of inflammatory cytokines in multiple sclerosis patients

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Sent for review: 10 April 2024 *Revised accepted: 3 October 2024*

Abstract

Purpose: *To determine the serum levels of cytokines, interleukins (IL) and tumor necrosis factor (TNF) in multiple sclerosis (MS) patients and compare with those in healthy individuals.*
Methods: *Twenty-two (22) adult patients with MS and 44 healthy controls, matched for age and gender, and visiting the King Abdulaziz University Hospital, Jeddah, Saudi Arabia were divided into groups I and II, respectively. Serum levels of interferon-gamma (IFN-γ), tumor necrosis factor and interleukins were determined for both groups. Cytokine levels were assessed using Luminex® xMAP® technology in 22 Saudi MS patients and 22 matched healthy controls.*
Results: *There were no significant differences in mean age between Group I (30.14 ± 10.51) and Group II (30.22 ± 5.22) participants (p = 0.982). Furthermore, the levels of IFN-γ, IL-6, IL-8, IL-1β and TNF-α in the serum were significantly higher in Group I patients than in controls (p < 0.0001 for all). Furthermore, a strong positive correlation was found between IFN-γ and IL-8 (r = 0.467, p = 0.028) in MS patients.*
Conclusion: *Patients with MS release significant amounts of inflammatory cytokines, which play a crucial role in understanding the clinical course of MS. These findings enhance the understanding of MS pathophysiology and guide future studies toward developing targeted treatments to mitigate disease progression and improve patients' quality of life.*

Keywords: *Multiple sclerosis, Neurodegenerative diseases, Inflammatory cytokines, Interleukins, Interferons, Tumor necrotic factors*

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INTRODUCTION

Multiple sclerosis (MS) is a long-term condition that results in substantial disability. It primarily affects the central nervous system (CNS), triggering inflammatory responses that lead to demyelination, neurodegeneration and axonal loss [1]. The disease is mainly caused by activated T cells, with contributions from B cells and cells of the innate immune system. Its symptoms include paraesthesia, ataxia, cognitive decline, vision loss, and immobility [2].

Multiple sclerosis is classified into four primary types based on severity and progression viz primary progressive (PPMS), secondary progressive (SPMS), relapsing-remitting (RRMS) and progressive-relapsing (PRMS) MS [3]. Two new classifications were introduced in 2013 which include radiologically isolated syndrome (RIS) and clinically isolated syndrome (CIS). The latter refers to the first clinical manifestation of MS, characterized by a neurological event lasting at least 24 hours and caused by inflammation or demyelination [4]. Given the recurring nature of MS, patients often face a poor prognosis, which significantly impacts their families and society [2]. It is an autoimmune disease caused by the activation of Th1 and Th17 cells. This process triggers the release of proinflammatory cytokines, leading to the upregulation of Th cells and formation of metalloproteinases. This leads to the breakdown of the blood-brain barrier, allowing Th cells to attack the CNS. B cells also play a complex role in the pathogenesis of MS, as evidenced by the effectiveness of B cell-targeting drugs [4]. Infectious pathogens trigger the development of pathogenic T cells that react to myelin. There are several possible explanations for MS development, including cross-reactivity with CNS myelin antigens, activation of pre-existing autoreactive immune cells, or a self-limiting brain infection that releases myelin antigens. However, persistent viral infection or a transmissible agent has not been established as a cause of MS [5]. The inference that MS primarily affects CD4 T cells is supported by the fact that the major histocompatibility complex (MHC) class II locus is the most significant genetic risk factor for MS development [6].

This study aimed to determine serum levels of interferon-gamma (IFN-γ), interleukins (IL-8 and IL-6, IL-1β) and tumor necrosis factor-alpha (TNF-α) in MS patients compared with healthy individuals at the King Abdulaziz University Hospital (KAUH) in Jeddah, Saudi Arabia and to explore correlations between the cytokine levels in MS patients.

METHODS

Study design and groups

This observational case-control study involved 22 adult MS patients and 44 healthy controls matched for age and gender. Before the study, all participants were provided with detailed information about its purpose and methodology, and their consent was obtained. This study was approved by the Institutional Review Board (IRB) Committee (approval no. 202403-076-018743-042550), and protocols followed the guidelines of Helsinki Declaration was followed to conduct the protocol [7]. Consent was duly obtained from the participants. Group I (study group) consisted of 22 participants, with approximately 45.5 % males and 54.5 % females, and an age range of 15 - 49 years. Group II (control) was composed of 44 healthy individuals, with an equal distribution of males and females and ages ranging from 23 to 40 years. Among the patients, the duration of disease ranged from a few weeks to 17 years, with 22.7 % having a family history of MS.

Inclusion criteria

Group I were Saudi MS patients aged between 15 - 50 years in which Multiple sclerosis was confirmed based on McDonald's criteria [8]. Group II were healthy Saudi individuals also aged 15 - 50 years and with no history of chronic diseases.

Exclusion criteria

Those excluded were pregnant women, patients below 15 years and above 50 years, patients with other major co-morbidities such as hypertension and diabetes and those on other therapies such as anti-retroviral, antibiotics and anti-inflammatory agents that may interfere with the outcome of this investigation.

Data collection

Data collected from participants consisted of two parts: the first part pertained to participants' demographic information, whereas the second part involved clinical characteristics of patients, including the duration of MS (in years), age at MS diagnosis, family history of MS, and any treatment drugs used.

Inflammatory biomarker assessment

Serum samples were obtained from all participants to determine the levels of various cytokines. The human neurodegenerative disease panel tool kit from MILLIPLEX (catalogue no. HCYTOMAG - 60K) was used to assay for the levels of IFN-γ, IL-8, TNF-α, IL-6, and IL-1β. The analysis was performed in duplicate using a single plate, following the manufacturer's assay protocols. In summary, 25 µg of serum (diluted at a 1:2 ratio) was mixed with antibody-conjugated magnetic beads and incubated overnight at 4 °C. After rinsing, the bead complexes were combined with 50 µL of biotinylated detection antibody and agitated on a plate shaker at room temperature for 30 minutes. Subsequently, they were incubated with 50 µL of streptavidin phycoerythrin on a plate shaker at 20

– 25 °C for another 30 minutes. Following three washes, 100 µL of sheath fluid was added to all wells. MAGPIX® then analyzed the bead complexes using the xPONENT® software on a run plate utilizing Luminex® 200TM. Data analysis was performed using the Luminex 200 machine and MILLIPLEX Analyst software. The Multidimensional Fatigue Inventory (MFI) measurements were analyzed for sensitivity, consistency and reproducibility.

Statistical analysis

Statistical analyses were conducted using the Statistical Package for Social Science (SPSS) software for Windows, version 8.0. Data are presented as mean ± standard error of means (SEM) or frequency (%) as appropriate. Statistical comparisons between groups were performed using one-way analysis of variance (ANOVA), followed by post hoc Tukey test for between-group comparisons. Correlation between the levels of serum cytokines in the participants was assessed using Spearman correlation. Statistical significance was set at $p < 0.05$.

RESULTS

Demographic characteristics

Table 1 details the demographic characteristics of participants in Group I and Group II. The average ages for Group I and Group II were 30.14 ± 10.51 and 30.22 ± 5.22 years, respectively and there were no significant differences in age between the groups ($p = 0.982$). Average disease duration in Group I was 7.03 ± 4.30 years (range: 0.06 - 17 years). A positive family history of the disease was found in five (22.7 %) patients viz: two patients (40.0 %) had a brother with MS, one patient (20.0 %) had an uncle, one patient (20.0 %) had a niece, and one patient (20.0 %) had a mother with the disease. Conversely, up to 77.3 % of patients had no family history of MS. Only one participant in Group I had another condition, asthma, in addition to MS. Six patients (27.3 %) received medications, of these, five (83.3 %) took Gilenya and one (16.7 %) took Avonex.

Inflammatory biomarkers

Serum levels of IFN-γ, IL-8, TNF-α, IL-6 and IL-1β were significantly higher in Group I than in Group II ($p < 0.0001$; Table 2). There were no significant differences ($p > 0.05$) in cytokines levels between male and female patients (Table 3). A correlation coefficient test revealed a moderately significant correlation between IFN-γ and IL-8 ($r = 0.467$, $p = 0.028$; Table 4 and Figure 1).

A significant positive correlation was reported between IL-8 and INF-γ in MS patients ($r = 0.467$, $p = 0.028$; Table 4, Figure 1).

Table 1: Demographic data of MS patients and control

Characteristic	Control	MS patients	*P*–value
Age (years)	30.22±5.22 (23.00-40.00)	30.14±10.51 (15.00-49.00)	0.982
Gender			
Male	11 (50 %)	10 (45.5%)	
Female	11 (50 %)	12 (54.5%)	
Disease duration (years)	-	7.03±4.30 (0.06-17.00)	
Family history of MS			
No		*17 (77.3%)*	
Yes		*5 (22.7%)*	
Brother		2 (40.0%)	
Uncle		1 (20.0%)	
Niece		1 (20.0%)	
Mother		1 (20.0%)	
Treatment			
No		16 (72.7%)	
Yes		6 (27.3%)	
Gilenya		5 (83.3%)	
Avonex		1 (16.7%)	
Comorbidities			
No		21 (95.5%)	
Yes		1 (4.5%)	

Data presented as mean ± SEM (minimum-maximum) and frequency (%)

Table 2 Error! Reference source not found.**:** Comparison of cytokines serum levels between MS patients and controls

Cytokines	Control	MS patients	*P*-value
INF - γ (pg/mL)	35.78±10.26 (17.69-47.41)	72.77±13.72 (50.12-91.57)	0.0001
IL - 8 (pg/mL)	102.23±11.14 (88.18-119.45)	324.52±53.65 (224.30-406.32)	0.0001
TNF - α (pg/mL)	57.02±7.92 (48.86-75.85)	85.78±16.18 (50.71-109.30)	0.0001
IL - 6 (pg/mL)	14.79±3.20 (10.13-19.62)	167.20±19.21 (144.90-198.48)	0.0001
IL - 1β (pg/mL)	11.01±1.74 (8.26-13.16)	44.47±3.87 (38.00-48.51)	0.0001

Data presented as mean ± SEM (minimum-maximum)

Table 3: Comparisons of cytokines serum levels between male and female MS patients

Cytokines	Male	Female	*P*-value
INF - γ (pg/mL)	75.63±14.29 (50.39-88.85)	70.38±13.36 (50.12-91.57)	0.384
IL - 8 (pg/mL)	333.29±47.76 (258.63-397.02)	317.21±59.16 (224.30-406.32)	0.456
TNF - α (pg/mL)	87.81±13.38 (68.97-108.00)	84.09±18.61 (50.71-109.30)	0.141
IL - 6 (pg/mL)	173.87±21.03 (152.77-198.48)	161.64±16.39 (144.90-198.03)	0.080
IL - 1β (pg/mL)	45.20±3.59 (38.00-48.51)	43.87±4.15 (38.00-48.51)	0.381

Data presented as mean ± SEM (minimum-maximum)

Table 4: Correlations between measured cytokines serum levels in patients

Cytokines	IFN - γ	IL8	TNF - α	IL - 6
IL - 8 (pg/mL)	0.467 (p = 0.028*)			
TNF - α (pg/mL)	0.145 (p = 0.520)	0.198 (p = 0.378)		
IL - 6 (pg/mL)	0.091 (p = 0.688)	0.244 (p = 0.274)	0.278 (p = 0.210)	
IL - 1β (pg/mL)	0.319 (p = 0.148)	- 0.229 (p = 0.304)	0.349 (p = 0.111)	0.147 (p = 0.514)

Data expressed as r (significance)

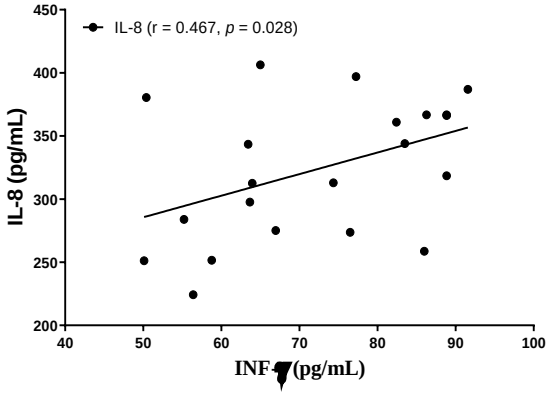

Figure 1: Positive significant correlation between IL - 8 (pg/mL) and INF - γ (pg/mL) serum levels in MS patients

DISCUSSION

The exact causes of MS remain unknown, but cytokines have been found to play an essential role in triggering immune responses and the formation of MS lesions. This study aimed to evaluate various cytokines involved in immune processes in MS patients. White matter lesions in MS patients exhibit elevated levels of B cells, B cell-related cytokines (e.g. BAFF and CXCL13) and myelin-specific antibodies [3]. B cell depletion therapy, particularly with ocrelizumab, has shown favorable outcomes [9].

A recent study suggests that TNF-α signaling involves two receptors – TNFR1 and TNFR2. These receptors mediate demyelination/ apoptosis and remyelination/ neuroprotection, respectively, which may explain the lack of efficacy of non-selective TNF-α blockers in MS treatment [10]. This study demonstrated a significant elevation in TNF-α serum levels of MS patients compared with the control group, which contrasts with the findings of previous studies [11,12]. Miller *et al* found significantly elevated serum IFN-γ levels in MS patients compared with healthy controls [13]. However, conflicting findings exist regarding IFN-γ levels in CSF

samples [12-14]. This present study reveals a moderate correlation between IFN-γ and IL-8 in MS patients, warranting further research to confirm and elucidate the relationship between these cytokines.

Furthermore, results of this study show a significant increase in serum IL-8 levels in MS patients, which contradicts some previous findings [15,16]. For instance, Malekzadeh *et al*. reported a substantial decrease in IL-8 serum levels in both treated and untreated MS subgroups compared with healthy controls [1**7**]. Also, a significant increase in serum IL-6 levels in MS patients compared with the control group was revealed in this study which is consistent with the findings of other studies [17,18]. Interleukin 1β (IL-1β) is a proinflammatory cytokine associated with increased immune responses and the severity of various CNS conditions, including MS. This study also showed a significant increase in IL-1β serum levels in the MS group compared with the control group, as reported in other studies [17,19], whereas one report noted insignificant changes [20].

Limitations of this study

This study had some limitations. First, the sample size of patients was small. Second, only Saudi patients from a single tertiary hospital were included and the disease stage was in remission. To generalize the results, a new investigation involving a larger cohort of patients across different disease stages, nationalities and tertiary hospitals would be required.

CONCLUSION

This study reveals a significant increase in various inflammatory cytokines in MS patients, providing crucial insights into the disease's clinical progression. The evident rise in cytokine levels highlights their potential as biomarkers for monitoring MS activity and establishes a basis for investigating therapeutic strategies to manage inflammatory responses in these patients. These findings enhance the understanding of MS pathophysiology and guide future studies toward developing targeted treatments to mitigate disease progression and improve patients' quality of life.

DECLARATIONS

Acknowledgment

The authors acknowledge the technical and financial support of the Deanship of Scientific Research (DSR) at King Abdulaziz University, Jeddah, Saudi Arabia.

Funding

This project was funded by the Deanship of Scientific Research (DSR)، Institutional Fund Projects under grant no (IFPIP:2085-290-1443) at King Abdulaziz University, Jeddah, Saudi Arabia.

Ethical approval

The investigation was approved by the National Committee for Bioethics at King Abdulaziz City for Science and Technology (no. 202403 - 076 - 018743 - 042550).

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. All authors contributed significantly to the conception, design, acquisition, analysis and interpretation of data. They were involved in drafting the article, critically revising it for important intellectual content and agreeing to submit it to the current journal.

REFERENCES

1. *Yamout BI, Alroughani R. Multiple Sclerosis. Semin Neurol 2018; 38(2): 212–25.*

2. Zheng C, He L, Liu L, Zhu J, Jin T. The efficacy of vitamin D in multiple sclerosis: A meta-analysis. Mult Scler Relat Disord 2018; 2356-61.
3. Lo Sasso B, Agnello L, Bivona G, Bellia C, Ciaccio M. Cerebrospinal fluid analysis in multiple sclerosis diagnosis: an update. Medicina 2019; 55(6): 245.
4. Klineova S, Lublin FD. Clinical course of multiple sclerosis. Cold Spring Harb Perspect Med 2018; 8(9): a028928.
5. Veroni C, Aloisi F. The CD8 T cell-Epstein-Barr virus-B cell trialogue: a central issue in multiple sclerosis pathogenesis. Front immunol 2021; 12665718.
6. Kunkl M, Frascolla S, Amormino C, Volpe E, Tuosto L. T helper cells: the modulators of inflammation in multiple sclerosis. Cells 2020; 9(2): 482.
7. World Medical Association. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. JAMA 2013; 310(20): 2191-2194.
8. Wexler M. Guidelines for MS diagnosis: McDonald criteria. Updated April 13, 2023. https://multiplesclerosisnewstoday.com/ms-diagnosis-mcdonald-criteria/
9. Ho S, Oswald E, Wong HK, Vural A, Yilmaz V, Tüzün E, Türkoğlu R, Straub T, Meinl I, Thaler F. Ocrelizumab treatment modulates B-cell regulating factors in multiple sclerosis. Neurol Neuroimmunol Neuroinflamm 2023; 10(2): e200083.
10. Zahid M, Busmail A, Penumetcha SS, Ahluwalia S, Irfan R, Khan SA, Reddy SR, Lopez MEV, Mohammed L. Tumor necrosis factor alpha blockade and multiple sclerosis: exploring new avenues. Cureus 2021; 13(10): e18847.
11. Magliozzi R, Pezzini F, Pucci M, Rossi S, Facchiano F, Marastoni D, Montagnana M, Lippi G, Reynolds R, Calabrese M. Changes in cerebrospinal fluid balance of TNF and TNF receptors in naïve multiple sclerosis patients: early involvement in compartmentalized intrathecal inflammation. Cells 2021; 10(7): 1712.
12. Talbot J, Chow HH, Mahler M, Buhelt S, Hansen RH, Lundell H, Vinther-Jensen T, Hellem MN, Nielsen JE, Siebner HR. Relationship between cerebrospinal fluid biomarkers of inflammation and tissue damage in primary progressive multiple sclerosis. Mult Scler Relat Disord 2022; 68104209.
13. Miller ED, Dziedzic A, Saluk-Bijak J, Bijak M. A review of various antioxidant compounds and their potential utility as a complementary therapy in multiple sclerosis. Nutrients 2019; 11(7): 1528.
14. Świderek-Matysiak M, Oset M, Domowicz M, Galazka G, Namiecińska M, Stasiołek M. Cerebrospinal fluid biomarkers in differential diagnosis of multiple sclerosis and systemic inflammatory diseases with central nervous system involvement. Biomedicines 2023; 11(2): 425.
15. Matejčíková Z, Mareš J, Sládková V, Svrčinová T, Vysloužilová J, Zapletalová J, Kaňovský P. Cerebrospinal fluid and serum levels of interleukin-8 in patients with multiple sclerosis and its correlation with Q-albumin. Mult Scler Relat Disord 2017; 1412-1415.
16. De Flon P, Söderström L, Laurell K, Dring A, Sundström P, Gunnarsson M, Svenningsson A. Immunological profile in cerebrospinal fluid of patients with multiple sclerosis after treatment switch to rituximab and compared with healthy controls. PLoS ONE 2018; 13(2): e0192516.
17. Malekzadeh A, Twaalfhoven H, Wijnstok NJ, Killestein J, Blankenstein MA, Teunissen CE. Comparison of multiplex platforms for cytokine assessments and their potential use for biomarker profiling in multiple sclerosis. Cytokine 2017; 91145-52.
18. Herrera MI, Kölliker-Frers RA, Otero-Losada M, Perez Lloret S, Filippo M, Tau J, Capani F, Villa AM. A pilot cross-sectional study to investigate the biomarker potential of phosphorylated neurofilament-H and immune mediators of disability in patients with 5-year relapsing-remitting multiple sclerosis. Front Neurol 2019; 101046.
19. D'Angelo C, Reale M, Costantini E, Di Nicola M, Porfilio I, de Andrés C, Fernández-Paredes L, Sánchez-Ramón S, Pasquali L. Profiling of canonical and non-traditional cytokine levels in interferon-β-Treated relapsing–remitting-Multiple sclerosis Patients. Front Immunol 2018; 91240.
20. Beheshti M, Salehi Z, Abolfazli R, Shirzad H, Izad M. Increased level of caspase-1 in the serum of relapsing-remitting multiple sclerosis (RRMS) patients. Iran J Allergy Asthma Immunol 2020; 19(5): 534-538.