The University of Tehran Press

Investigating the Relationship of Inflammatory Cytokines and Brain-Derived Neurotrophic Factor with Cognitive Functions in Multiple Sclerosis Patients

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Extended Abstract

Aim

Multiple sclerosis (MS) is a chronic autoimmune disease of the central nervous system, characterized by the degeneration of the myelin sheath. This degeneration is the primary pathological symptom, and the disease can lead to various physical and psychological issues (Larocca & King, 2016). MS is classified into four major types, with relapsing-remitting MS (RRMS) being the most prevalent form (Sundgren, 2016; Segal, 2019). Cognitive impairments are common among MS patients. Cognition encompasses high-level cerebral functions, including information processing, attention, memory, and executive functions. Many patients with MS experience various forms of cognitive dysfunction, which can impact their quality of life to varying degrees (Migliore et al., 2017). Immunological factors have been identified as major contributors to MS. Recent research has shown that both T cells and B cells play key roles in the pathogenesis of the disease (Martins et al., 2011). T cells primarily release cytokines, which play a major role in maintaining homeostasis. These, along with neurotrophins and interleukins, are considered pathophysiological components that contribute to the relapse or progression of MS (Naegelin et al., 2020). While it is widely accepted that immunological factors are involved in MS, the precise mechanisms by which these factors influence cognitive functions remain unclear.

In the present study, we investigated the potential role of gamma-interferon (IFN-γ), brain-derived neurotrophic factor (BDNF), interleukin-6 (IL-6), interleukin-17 (IL-17), and tumor necrosis factor-alpha (TNF-α) in cognitive functions among patients with relapsing-remitting MS (RRMS) and secondary progressive MS (SPMS). Additionally, we compared these findings with a newly diagnosed (ND) group of MS patients and a control group of healthy individuals.

Methodology

The study utilized a correlational design, involving 68 patients with confirmed multiple sclerosis (MS), aged 19 to 49 years. Participants were selected using a convenience sampling method. The patients received care from a neurologist in 2022 at Imam Reza Clinic, affiliated with Shiraz University of Medical Sciences in Shiraz, Iran. Inclusion criteria were: (1) MS diagnosis by a neurologist, (2) no neurological or psychiatric diagnoses other than MS, (3) no intravenous corticosteroid use or MS relapse within 6 weeks of assessment, and (4) no history of developmental disorders. The sample included 23 newly diagnosed (ND) patients, 23 patients with relapsing-remitting MS (RRMS), and 22 patients with secondary progressive MS (SPMS). The mean disease duration for each group was 2, 4, and 10 years, respectively. The control group, consisting of 22

healthy individuals, was matched to the MS groups based on sex, age, and education level. The ND patients were clinically diagnosed according to the McDonald Diagnostic Criteria, with neuropsychological assessments conducted prior to any pharmacological treatment.

Cognitive functions were assessed using five subtests from the Minimal Assessment of Cognitive Function in MS (MACFIMS) test battery (Benedict et al., 2002). These subtests included: 1-California Verbal Learning Test (CVLT-II) to assess verbal learning and memory, 2- Brief Visuospatial Memory Test-Revised (BVMT-R) to assess visuospatial memory and orientation ability, 3- Symbol Digit Modalities Test (SDMT) to assess information processing speed and working memory, 4- Controlled Oral Word Association Test (COWAT) to assess verbal fluency, and 5- Delis-Kaplan Executive Function System (D-KEFS) to assess logical thinking and reasoning. These tests have demonstrated high reliability, with reliability scores ranging from 70 to 95 (Benedict, 2005). Additionally, the battery was normed in Iran by Eshaghi et al. (2012), with subtest reliability measured via test-retest methods ranging from 0.66 to 0.82. In the present study, the reliability of these subtests ranged from 0.78 to 0.92.

Serum levels of five immunological factors, including the cytokines IFN-γ, IL-6, TNF-α, and IL-17, as well as BDNF, were measured using ELISA kits from Karmania Pars Gene Company. Measurements followed the manufacturer's instructions and were performed in the Immunology Laboratory at Shiraz University of Medical Sciences, with results analyzed accordingly.

Data analysis was conducted using SPSS version 26. Descriptive statistics were calculated for all demographic and clinical variables. Group differences in cognitive performance and cytokine levels were examined using one-way ANOVA, followed by post-hoc comparisons. Pearson's correlation coefficients were used to assess correlations between cytokine levels, BDNF, and cognitive test scores. Additionally, multiple regression analyses were conducted to determine the predictive value of cytokines and BDNF on cognitive functions within each MS subtype.

Findings

The results revealed significant differences in the levels of the cytokines IFN-γ and IL-17. Both cytokines were significantly elevated in the RRMS and SPMS groups compared to the control group, while BDNF levels were lower in the ND group relative to both RRMS patients and healthy controls. Specifically, IFN-γ levels were significantly higher in both the RRMS and SPMS groups compared to controls. However, no statistically significant difference was found between the patient groups themselves. BDNF levels were lower in the ND group than in the RRMS group, suggesting a potential protective effect of BDNF, which may increase during the early stages of disease-modifying treatments. IL-17 levels were significantly elevated in both RRMS and SPMS patients compared to the control group, underscoring the role of this cytokine in MS progression.

In terms of cognitive performance, MS patients, regardless of their disease subtype, performed significantly worse than healthy controls across all cognitive domains. The largest impairments were observed in information processing speed (SDMT) and visuospatial memory (BVMT-R). Newly diagnosed patients demonstrated relatively better performance in visuospatial memory compared to SPMS patients, suggesting that cognitive decline worsens as the disease progresses. SPMS patients exhibited the most significant deficits in information processing speed compared to both ND and RRMS groups. No significant differences were observed between MS subgroups in verbal fluency (COWAT) or executive functioning (D-KEFS), although all MS groups performed worse than controls.

To examine the relationship between immunological factors and cognitive functions, correlation analysis revealed that while no significant relationships were found between cognitive functions and the inflammatory cytokines across groups, BDNF levels exhibited strong positive correlations with cognitive functions in MS patients. Specifically, in the RRMS group, BDNF was significantly correlated with visuospatial memory, orientation ability, and executive functioning. Similarly, in SPMS patients, BDNF levels were positively correlated with visuospatial memory. Interestingly, in

the ND group, BDNF was a positive predictor of executive functioning, while IFN-γ had a negative predictive relationship with this cognitive domain.

Simultaneous multiple regression analyses further demonstrated that BDNF was a significant positive predictor of visuospatial memory and executive functioning in RRMS patients, explaining 8% and 41% of the variance in these cognitive domains, respectively. In SPMS patients, TNF-α was found to negatively predict information processing speed, accounting for 10% of the variance in this cognitive function. In newly diagnosed patients, IFN-γ emerged as a negative predictor of executive functioning, while BDNF was a positive predictor. Together, these factors explained 13% of the variance in executive function.

Table 1 presents the regression analysis results for predicting cognitive functions based on immunological factors in the RRMS, SPMS, and newly diagnosed patient groups.

Table 1: Simultaneous Regression Analysis for Predicting Cognitive Functions Based on Immunological Factors

Cognitive Functions	Group	Predictors	B	ß	R	R^2A	T	P
		IFN-γ	-0.01	-0.06	0.54	0.08	-0.27	0.79
		BDNF	1.23	0.46			2.19	0.04
Visual-Spatial Memory	RRMS	IL-6	-0.85	-0.22	0.54	0.08	-0.89	0.39
		TNF-α	0.15	0.08			0.32	0.76
		IL-17	0.85	0.25			1.13	0.28
		IFN-γ	0.03	0.08			0.44	0.66
		BDNF	5.37	0.65			3.80	0.001
Executive Function	RRMS	IL-6	-3.89	-0.32	0.74	0.41	-1.62	0.12
		TNF-α	0.58	0.10			0.49	0.63
		IL-17	1.66	0.15			0.88	0.39
		IFN-γ	0.03	0.30			1.06	0.31
		BDNF	-0.38	-0.06			-0.20	0.84
Information Processing Speed	SPMS	IL-6	0.32	0.07	0.56	0.10	0.34	0.74
		TNF-α	-1.31	-0.67			-2.16	0.05
		IL-17	1.82	0.34			1.16	0.26
		IFN-γ	-0.14	-0.64			-2.41	0.03
		BDNF	7.18	0.63			2.43	0.03
Executive Function	ND	IL-6	-2.32	-0.29	0.57	0.13	-1.33	0.20
		TNF-α	1.59	0.27			1.15	0.26
		IL-17	-1.11	-0.14			-0.60	0.56

B: Unstandardized coefficient, **ß:** Standardized coefficient, **R:** Model R value, **R^2A:** Adjusted R^2 (percent of variance explained), **T:** T-value, **P:** Significance level

Conclusion

This study aimed to explore the potential role of specific immunological factors in cognitive function. We measured serum levels of IFN-γ, BDNF, IL-6, TNF-α, and IL-17 in three groups of MS patients, as well as in a group of healthy controls. We observed significant differences in both cognitive functions and serum cytokine levels (IFN-γ, IL-17) and BDNF in MS patients compared to the control group.

Our findings revealed notable differences in serum immunological factors between MS patients and the control group. Specifically, IFN-γ levels were significantly higher in both RRMS and SPMS patients compared to the control group, although no such difference was found in ND patients. These results are in line with previous studies, such as that by Kallaur et al. (2013), which also reported elevated IFN-γ levels in RRMS patients. Overall, IFN-γ is known to play a key role in the pathogenesis of MS and its animal model, experimental autoimmune encephalomyelitis.

Similarly, IL-17 levels were significantly elevated in both RRMS and SPMS patients compared to the control group. Studies by Babaloo et al (2013) and Ashtari et al (2019) have also reported higher serum IL-17 levels in MS patients. IL-17 levels seem to be influenced by disease progression and are associated with the severity of MS. IL-17 plays a crucial role in enhancing T cell activity and

promoting the release of pro-inflammatory mediators like IL-1, IL-6, and TNF-α (Nakae et al., 2003), contributing to endothelial inflammation.

Regarding BDNF, no significant difference was found between the control group and RRMS or SPMS patients. Research on BDNF levels in MS patients has yielded mixed results. Naegelin et al (2020) reported a small but significant decrease in BDNF levels in MS patients compared to controls, with SPMS patients showing lower levels than RRMS patients. Frota et al (2009) observed that BDNF levels increase after the relapse phase, suggesting its involvement in MS pathogenesis and its role in recovery from acute demyelinating lesions.

Cognitive function assessments in this study confirmed that even ND patients show some degree of cognitive impairment compared to healthy controls, suggesting that cognitive dysfunction can manifest early in MS. SPMS patients exhibited significantly poorer performance on at least two cognitive tests compared to RRMS and ND patients, aligning with previous studies that report worse neuropsychological performance in SPMS patients. These findings suggest that cognitive decline worsens as MS progresses, potentially due to frontal lobe damage, which has extensive connections with other brain regions (Migliore et al., 2017).

We also examined the relationship between immunological factors and cognitive functions across five cognitive domains. BDNF emerged as a significant factor influencing cognitive function, particularly executive functioning. It was an important predictor of visuospatial memory, orientation ability, and executive functioning in RRMS patients, and executive functioning in ND patients. These results are consistent with studies showing a correlation between BDNF and cognitive functions, especially in the domain of executive functions (Gajewski et al., 2011; Koven & Collins, 2014). BDNF plays a key role in brain processes such as synaptic plasticity, neuronal proliferation, and neural repair, particularly in the hippocampus and frontal cortex.

Additionally, TNF-α and IFN-γ were identified as important immunological factors influencing cognitive functions. In SPMS patients, TNF-α negatively predicted information processing speed, while IFN-γ negatively predicted executive functioning in ND patients. Higher levels of TNF-α were associated with slower information processing in SPMS patients, while increased IFN-γ levels were linked to poorer executive functioning in ND patients. Previous research has highlighted information processing speed as a major cognitive deficit in MS patients, particularly in advanced stages, with TNF-α playing a role in this impairment (Rasi Marzabadi et al., 2021).

Overall, this study underscores the dysfunction in regulating certain immunological factors and their potential role in neurodegeneration. Cytokines and neurotrophins, such as BDNF, IFN-γ, and TNF-α, may serve as valuable biomarkers for predicting cognitive functions in MS patients. However, this study has limitations, including a relatively small sample size and being restricted to a single clinical center. Future studies with larger, more diverse samples are recommended. Additionally, longitudinal research is needed to assess the progression of cognitive impairment and its relationship with immunological factors over time, particularly in MS patients in Iran.

Keywords: Autoimmunity, Brain-Derived Neurogenesis Factor, Cognitive Functions, Inflammatory Cytokines, Multiple Sclerosis.

Ethical Considerations

This study was approved by the Ethics Committee of the Isfahan University Council (Approval No. IR.UI.REC.1399.097).

Acknowledgements

The authors wish to thank everyone who contributed to the completion of this research, including the Multiple Sclerosis patients who participated in the study.

Conflict of Interest

The authors declare no conflict of interest related to this submission.

Disclaimers

We hereby declare that this paper is our original and independent work. All sources and references used are appropriately acknowledged. All texts, whether directly quoted or paraphrased, are cited in the text, and full bibliographic details are provided in the reference list. This work has not been submitted elsewhere for publication.

Source(s) of Support/Funding

This research received no external funding.

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Cite this article: Arab-Moghaddam, N., Asgari Mobarakeh, K., Daryabor, Gh., & Poursadeghfard, M., (2024). Investigating the Relationship of Inflammatory Cytokines and Brain-Derived Neurotrophic Factor with Cognitive Functions in Multiple Sclerosis Patients. *Journal of Applied Psychological Research*, *15*(4), 69-88. doi: 10.22059/japr.2024.99090.

Publisher: University of Tehran Press
DOI: https://doi.org/10.22059/japr.2024.99090