

The Role of Interleukin-6 as an Indicator of Multiple Sclerosis Progression From Relapse Remitting to Secondary Progressive Status



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ABSTRACT

Background: Multiple Sclerosis (MS) is the most common chronic inflammatory disease of the white matter of the Central Nervous System (CNS). Proinflammatory cytokine network such as interleukin-6 (IL-6) has a role in the initiation of a destructive immune response in CNS and the progression of the disease. This study aimed to evaluate the comparative levels of IL-6 and IL-8 in MS patients and healthy controls and also to assess any fluctuation in IL-6 and IL-8 levels in MS progression.

Materials and Methods: This case-control study recruited a total of 183 subjects, including 103 MS patients and 80 sex- and age-matched healthy controls. MS patients included 51 Relapsing Remitting MS (RRMS), 25 secondary progressive MS (SPMS), 27 Primary Progressive MS (PPMS), and Progressive Relapsing MS (PRMS). Clinical findings were collected, and serum levels of IL-6, IL-8, and 25-hydroxyl vitamin D3 (25(OH)D3) were determined with ELISA.

Results: The mean serum level of IL-6 was significantly higher in MS patients than healthy controls (23.8±2.1 vs. 15.6±2.7 pg/mL, P=0.043). MS patients with SPMS had more prominent IL-6 levels than RRMS or PPMS (P=0.008). However, IL-8 levels did not show a significant change either in the patients compared with the controls or in the different forms of MS. MS was more prone to progressive form in male patients. Mean 25(OH)D3 level was significantly lower in MS patients than the controls. Meanwhile, the mean 25(OH)D3 level was surprisingly higher in MS patients with SPMS.

Conclusion: The increased serum level of IL-6 in more advanced MS status, SPMS, suggests that IL-6 but not IL-8 might be a prognostic marker for the disease deterioration. Besides, the tendency for MS to progress to worse stages in affected men is an important finding that needs further clarification.

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Introduction

Multiple Sclerosis (MS) is an immune-mediated disease that attacks axons surrounded by myelin in the Central Nervous System (CNS), destroys the myelinated axon in variable degrees, which leads to physical disabilities [1-3]. MS is associated with a wide spectrum of sensory, motor, and psychological disorders. Clinically, the disease is characterized by abnormal sensation, weakness and fatigue, lack of coordination in body movements, imbalance, paralysis, and ocular symptoms [4]. MS patients are classified into four major groups based on the severity of disease: Relapsing Remitting MS (RRMS), Secondary Progressive MS (SPMS), Primary Progressive MS (PPMS), and Progressive Relapsing MS (PRMS) [5]. Most patients (85%) experience RRMS form and only 15% have PPMS. Approximately 50% of RRMS patients convert to SPMS after 15 years and 75% after 25 years [6]. Clinical symptoms such as fatigue, pain, depression, are more common in SPMS than in the RRMS patients, because of the disease progression and the deteriorating condition of MS patients with SPMS [7].

The etiology and pathogenesis of MS are not well understood, but susceptibility to MS is influenced by genes and environmental factors such as 25-hydroxyl vitamin D3 (25(OH)D3) deficiency [8]. Mounting evidence suggests that 25(OH)D3 can be protective against MS development [9]. Epidemiological studies have shown that the prevalence of MS increases with increasing northern latitudes, where ultraviolet B irradiation decreases significantly, especially during autumn and winter. Also, an association has been reported between 25(OH)D3 deficiency and the rate of MS attacks. This prohormone plays an immunomodulatory role in the CNS, which inhibits proinflammatory responses and promotes anti-inflammatory cytokines [10, 11].

It also has an immunomodulatory effect, which alters the balance between T helper 1 (Th1) and T helper 2 (Th2) in favor of Th2 cells. 25(OH)D3 deficiency is associated with the increased production of inflammatory cytokines such as interleukin-2 (IL-2), interferon-gamma (IFN- γ), tumor necrosis factor-alpha (TNF- α), IL-17A, IL-1, IL-6, and IL-8, as well as decreased production of anti-inflammatory cytokines such as Transforming growth factor beta 1 (TGF- β_1) and IL-10 [8, 11].

IL-6 is a proinflammatory cytokine released by myeloid cells and other cell types such as fibroblasts and endothelial cells. IL-8 is another proinflammatory cytokine

produced by a variety of cell types, including endothelial cells, fibroblasts, hepatocytes, and keratinocytes [12]. The amount of these cytokines increases in the serum and lesions of MS patients [13-15].

The primary purpose of our study was to investigate the 25(OH) D3, IL-6, and IL-8 serum levels in MS patients and compared them with healthy controls. Evaluation of the role of these proinflammatory cytokines in MS exacerbation was also investigated.

Participants and methods

Study subjects

In this case-control study, serum samples were obtained from 103 patients with clinically definite MS (31 men and 72 women; Mean \pm SD age: 34.7 \pm 8.4 years) referred to Bu Ali Sina Hospital, Mazandaran University of Medical Sciences, Sari City, Iran, from April 2017 to September 2019. Only patients meeting the McDonald Criteria for clinically definite MS were recruited in this study [16]. An experienced MS neurologist examined all patients and classified them into one of the MS types using the Expanded Disability Status Scale (EDSS). The control group comprised 80 people (17 men and 63 women; Mean \pm SD age: 34.2 \pm 7.5 years), apparently healthy asymptomatic volunteers who referred for a health checkup. The control group matched with MS patients with respect to age, sex, and ethnicity. The exclusion criteria were a history of autoimmune or inflammatory diseases, cancer, and other relevant disorders.

Serum collection

Whole blood samples were collected in the anticoagulant-free sterile tubes. Sera were isolated from coagulated blood using centrifugation at 2500 rpm for 10 min at room temperature and stored at -80 $^{\circ}$ C until used.

IL-6 and IL-8 measurements

Serum levels of IL-6 and IL-8 were measured using the target-specific solid-phase sandwich Enzyme-Linked Immunosorbent Assay (ELISA) kits according to the manufacture's instruction (Thermo Fisher Scientific, MA). These kits were designed to measure the amount of IL-6 or IL-8 bound between a matched specific antibody pair. At the end of the reaction, the developed-color signals were detected at 450 nm using BioTek Synergy H1 hybrid multi-mode plate reader (BioTek, VT). The sensitivity of the kit for measuring IL-6 and IL-8 are 1.6 and 2 pg/mL, respectively.

Vitamin D (25(OH)D3) assay

The level of 25(OH)D3 was assessed by a commercially available ELISA kit (bioactiva diagnostica GmbH, Homburg, Germany) according to the manufacturer's instruction. The sensitivity of the assay is 1.98 ng/mL.

Statistical analyses

Statistical analysis was performed in SPSS V. 17. The Kolmogorov-Smirnov test was used to examine the normal distribution of quantitative variables. To compare binominal data between groups, we used the Chi-square test. Also, continuous variables were analyzed using the Student's t test or 1-way Analysis of Variance (ANOVA) for normally distributed data and Mann-Whitney U test or Kruskal-Wallis test for non-normally distributed variables.

Results

Demographic and clinical characteristics of MS patients

The Mean±SD ages of MS patients and the healthy controls were 34.7±8.4 and 34.2±7.5 years, respectively (P=0.706). Among 103 MS patients and 80 controls, 72 (69.9%) and 63 (78.7%) were females, respectively (P=0.119). **Table 1** presents the demographic and clinical characteristics of the study population. There is no significant difference between MS patients and controls with regard to age, sex, and tobacco smoking. The clinical exacerbations and physical disability of MS patients

were determined with EDSS. The overall Mean±SD EDSS score was 2.8±2.3 and the MS patients have experienced recurrence rate of 2.5±2.4 times. The Mean±SD age of MS diagnosis in the patients was 28.07±8.3 years.

Increased serum levels of IL-6 in MS patients

Figure 1 shows the serum levels of 25(OH)D3, IL-6, and IL-8 in MS patients and controls. Mean serum level of 25(OH)D3 was significantly lower in MS patients than that in the controls (23.2±12.2 vs. 30.8±14.8 ng/mL, P<0.0001). The levels of IL-6 significantly differed in two study groups. On the other hand, MS patients produced higher levels of IL-6 than the controls (23.8±2.1 vs. 15.6±2.7 pg/mL, P=0.043). The levels of IL-8 were not significantly different between the two study groups (26.2±2.5 vs. 24.8±5.2, P=0.798).

Clinical characteristics of various courses of MS

MS patients were categorized into three groups: RRMS (n=51), PPMS (n=27), and SPMS (n=25). All three MS subgroups were comparable with respect to the age of MS diagnosis (**Table 2**). However, female MS patients were more affected by RRMS form than the other forms (P=0.037). Disease duration was significantly longer in MS patients with SPMS compared to RRMS and PPMS (11.2±4 vs. 5.1±4.8 and 5.2±6.9 years, respectively, P<0.0001). Vision problems, numbness or tingling, fatigue, and cognitive dysfunction were the most common

Table 1. Demographic and clinical characteristics of MS patients and healthy controls

Variables	Controls n=80	MS Patients n=103	P
Age (y)	34.2±7.5	34.7±8.4	0.706
Sex – No. (%)	-	-	-
Female	63 (78.7)	72 (69.9)	0.119
Male	17 (21.3)	31 (30.1)	-
Positive Family history of MS	-	6 (5.6)	-
Smoking – No. (%)	7 (8.7)	6 (5.8)	0.124
Duration of illness (y)	-	6.26±5.7	-
Age at MS diagnosis (y)	-	28.07±8.3	-
EDSS score	-	2.8±2.3	-
Number of relapses	-	2.5±2.4	-

Abbreviations: MS, multiple sclerosis; EDSS, expanded disability status scale.

All quantitative variables are presented as mean ± SD or No. (%).

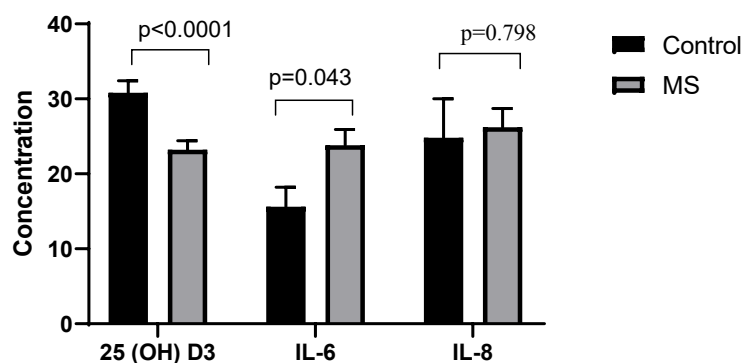


Figure 1. Serum levels of IL-6, IL-8, and Vitamin D in MS patients and control subjects (Error bars represented as mean \pm SE)

Table 2. Clinical characteristics in different types of MS patients.

Variables	RRMS n=51	PPMS n=27	SPMS n=25	P
Age (y)	33.7 \pm 7.5	33.8 \pm 8.3	37.6 \pm 9.8	0.139
Female- No. (%)	41 (56.9)	18 (25)	13 (18.1)	0.037
Age of the onset (y)	28.7 \pm 8.1	27.3 \pm 6.3	27.7 \pm 10.4	0.769
Disease duration (y)	5.1 \pm 4.8	5.2 \pm 6.9	11.2 \pm 4	<0.0001

RRMS: Relapsing Remitting MS; SPMS: Secondary Progressive MS; PPMS: Primary Progressive MS.

symptoms and depression was the less common problem in MS patients with RRMS (Figure 2).

Fatigue, vision problem, muscle spasms, numbness or tingling, sexual dysfunction were found in more than half of the MS patients with SPMS (Figure 2). To evaluate disease progression, we compared the disability status of MS subgroups. As Figure 3 shows, the EDSS score was significantly higher in MS patients with SPMS than those with RRMS (5.7 \pm 1.7 vs. 1.6 \pm 1.2, P=0.006). Surprisingly, the MS patients with SPMS had higher levels of 25(OH)D3 compared to RRMS and PPMS groups (28.8 \pm 14.2 vs. 21.4 \pm 12.4 and 21.5 \pm 8.1 ng/mL,

P=0.031). The high level of 25(OH)D3 might be because of 25(OH)D3 supplement therapy in the MS patients with SPMS.

IL-6 and IL-8 serum levels

Figure 4 shows the serum levels of IL-6 and IL-8 in patients with different forms of MS. IL-6 level increased in line with the severity progression of the patients. On the other hand, the IL-6 level was significantly prominent in MS patients with SPMS than the RRMS (30.2 \pm 3.2 vs. 15.7 \pm 2.4 pg/mL, P=0.008). There was no significant difference between MS patients with SPMS and

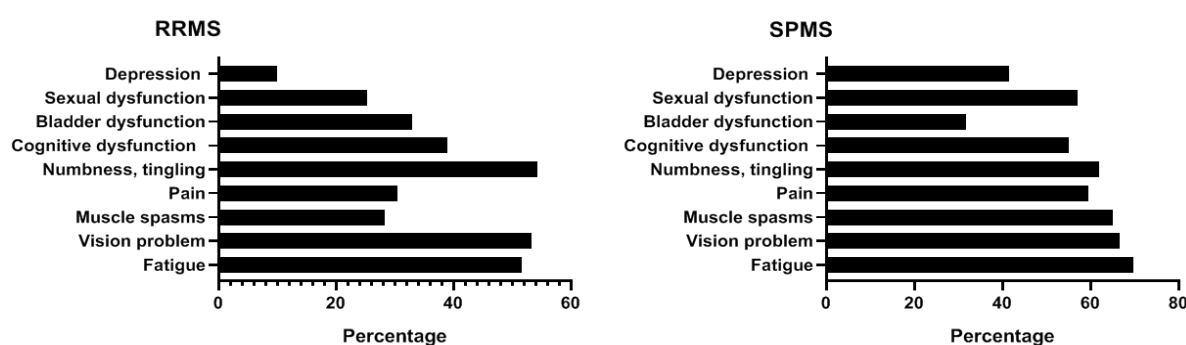


Figure 2. Clinical symptoms of MS patients with relapsing remitting MS and secondary progressive MS

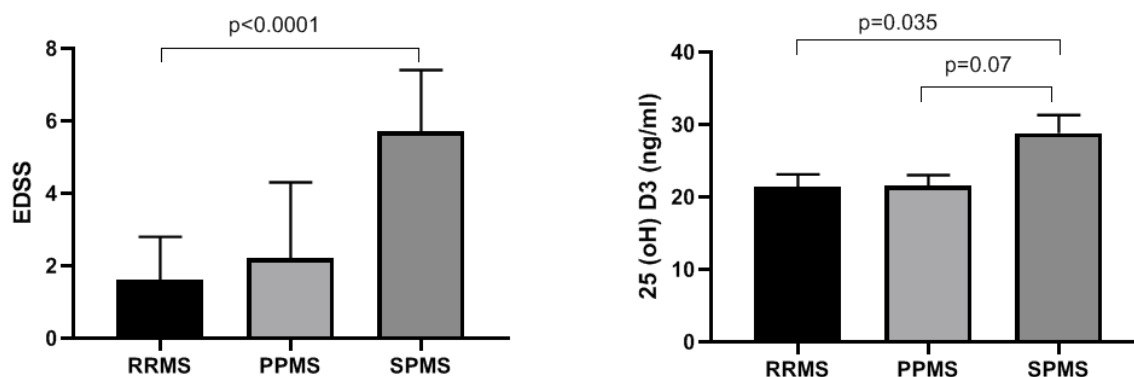


Figure 3. Disability scale and 25(OH)D3 levels in various types of MS (Error bars represented standard deviation)

RRMS: Relapsing Remitting MS; SPMS: Secondary Progressive MS; PPMS: Primary Progressive MS

PPMS ($P=0.0197$). The levels of IL-8 were not significantly altered during MS progression ($P>0.05$). On the other hand, the Mean±SD levels of IL-8 were 27.1 ± 2.7 , 24.3 ± 5.7 , and 26.7 ± 6.8 pg/mL, in the MS patients with SPMS, RRMS, and PPMS, respectively ($P=0.89$).

To explore whether disease characteristics could affect IL-6 and IL-8 production rate, we evaluated the correlation between the cytokine levels and the clinical findings of MS, in all and MS subgroups. No significant association was seen between IL-6 or IL-8 and EDSS score, disease duration, and the first symptom. However, a positive correlation was observed between the EDSS score and disease duration ($r=0.534$, $P<0.0001$).

Discussion

The main finding of this study was an increased serum level of IL-6 in MS patients than the controls. It was also higher in the more advanced stage of MS, i.e., SPMS. This finding is consistent with other reports which show the higher levels of IL-6 in Cerebrospinal Fluid

(CSF) and serum of MS patients [17]. IL-6 is a critical pro-inflammatory cytokine that has a dual effect in a chronic inflammatory condition. IL-6 in combination with TGF- β , has a drastic role in the differentiation of naive CD4+ T cells to Th17 cells. Nowadays, the effect of Th17 cells is evident in the pathogenesis and progression of MS disease.

Therefore, the increased production of IL-6 in MS patients well justifies its pathogenic role in the initiation of neuronal destruction and the progress of demyelination disorders. On the other hand, the presence of local or systemic higher production of IL-6 significantly inhibits the development of regulatory T cells (Tregs). It has been demonstrated that increased Th17/Treg balance is closely correlated with the destruction of immunological tolerance and provides conditions for the development of pathological effects of autoimmune and inflammatory responses. This inference suggests that the increase in IL-6 levels is in line with the clinical trend of manifestations from RRMS to SPMS status.

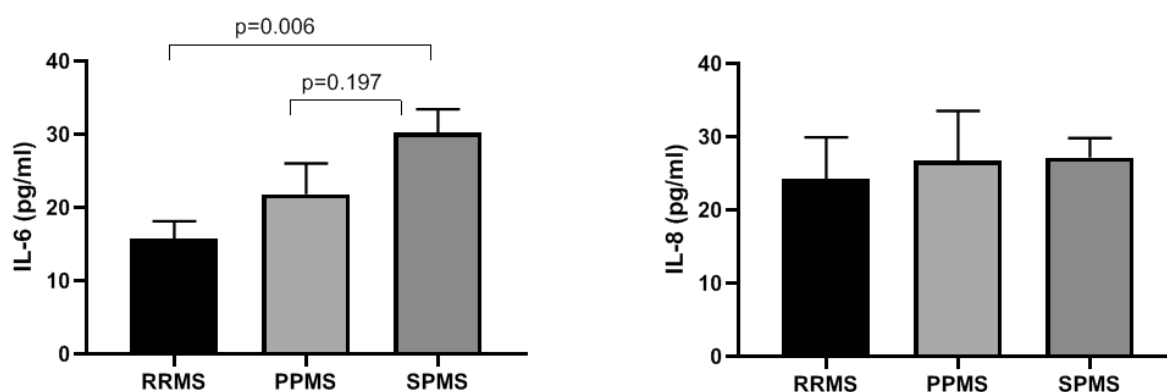


Figure 4. Serum levels of IL-6 and IL-8 in MS patients with RRMS, PPMS, and SPMS

IL-8, another inflammatory cytokine which is a potent neutrophil chemoattractant chemokine, did not show a correlation to disease activity. Besides, its serum concentration was not different between MS patients and controls. IL-8 levels were paradoxes in previous reports. Lower IL-8 has been reported in serum levels of MS patients compared to healthy controls [18, 19]. However, other reports show elevated levels of IL-8 in serum or CSF in the early diagnosed MS patients [15, 20, 21]. These discrepancies may be due to different study population and sampling, severity and disease stage, ethnicity, treatment protocol, and the time of sampling.

On the other hand, in the acute phase of MS or relapse occurrence phase, the inflammatory condition is activated by a surge release of proinflammatory cytokine/chemokine. In the acute stage, this phenomenon leads to the infiltration of neutrophils and other leukocytes through the blood-brain barrier and their accumulation around the target neurons. However, it is demonstrated that a therapeutic regimen, including IFN- β for 6 to 12 months, leads to a significant reduction in IL-6 levels.

25(OH)D3 was significantly lower in MS patients than that in the controls. This finding is in line with many reports shown lower 25(OH)D3 is associated with higher MS risk and more exacerbation of MS symptoms [9]. However, our MS patients with SPMS surprisingly have higher levels of 25(OH)D3 than those with RRMS. This discrepancy might be due to receiving 25(OH)D3 supplements in SPMS to prevent disease activity and progression.

Ethical Considerations

Compliance with ethical guidelines

The study was performed according to the Declaration of Helsinki and approved (IR.MAZUMS.REC.1398.1281) by the Research Ethics Committee of Mazandaran University of Medical Sciences.

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Authors contribution's

Alireza Rafiei conceived and designed the study. Mohammad Baghbanian helped to select the patients. Mina Eslami and Araz Mohammad Mirabi performed the experiments. Alireza Rafiei and Mina Eslami wrote the

manuscript. All authors read the paper and approved the final manuscript.

Conflict of interest

The authors declared no conflict of interest.

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