© Aktualn Neurol 2022, 22 (2), p. 75–79
DOI: 10.15557/AN.2022.0008

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Received: 29.03.2022
Accepted: 28.05.2022
Published: 07.12.2022

Do increased serum IL-12 and IL-23 levels affect cognitive function in patients with multiple sclerosis? A preliminary study

Czy podwyższone stężenie IL-12 i IL-23 w surowicy krwi pacjentów ze stwardnieniem rozsianym wpływa na ich funkcjonowanie poznawcze? Badanie pilotażowe

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Abstract

Aim of the study: To compare the serum levels of IL-12 and IL-23 between healthy volunteers and patients with multiple sclerosis with regard to their cognitive function. **Materials and methods:** A total of 21 patients with multiple sclerosis and 21 healthy individuals were enrolled into the study. The individuals were age- and sex-matched. Each participant was evaluated using the Montreal Cognitive Assessment (MoCA), the Beck Depression Inventory (BDI), and the Pittsburgh Sleep Quality Index (PSQI). The enzyme-linked immunosorbent assay was performed to assess the serum levels of IL-12 and IL-23. **Results:** The concentration of IL-12 was 1.61 ± 4.61 pg/mL in the group of patients with multiple sclerosis and 1.78 ± 3.54 pg/mL in the control group, $p = 0.5009$. The concentration of IL-23 was 19.04 ± 75.50 pg/mL in the study group and 5.50 ± 14.4 pg/mL in the control group, $p = 0.5170$. A significant difference was found between the control and study groups in the MoCA cognitive test (28 vs. 24 points, respectively, $p < 0.0001$). There was no significant difference in the Beck Depression Inventory and PSQI between the control and study groups. No significant correlations were found between the IL-12/IL-23 serum levels and psychological evaluations. **Conclusions and clinical implications:** The results obtained indicate that IL-12 and IL-23 may not play a role in the development of cognitive impairment. The assessment of cognitive impairment in patients with multiple sclerosis may have a screening value in preventing their cognitive deterioration.

Keywords: multiple sclerosis, IL-12, IL-23

Streszczenie

Cel badania: Porównanie stężeń IL-12 i IL-23 w surowicy krwi zdrowych ochotników oraz pacjentów ze stwardnieniem rozsianym w odniesieniu do ich funkcji poznawczych. **Materiał i metody:** Do badania włączono 21 pacjentów ze stwardnieniem rozsianym i 21 zdrowych osób, dopasowanych pod względem wieku i płci. U wszystkich badanych funkcje poznawcze zostały ocenione przy pomocy Montreal Cognitive Assessment (MoCA), testu Becka i Pittsburgh Sleep Quality Index (PSQI). U wszystkich uczestników oznaczono stężenie IL-12 i IL-23 we krwi metodą immunoenzymatyczną. **Wyniki:** Stężenie IL-12 wyniosło w grupach badanej i kontrolnej odpowiednio 1,61 ± 4,61 pg/ml i 1,78 ± 3,54 pg/ml, $p = 0,5009$, natomiast stężenie IL-23 – odpowiednio 19,04 ± 75,50 pg/ml i 5,50 ± 14,4 pg/ml, $p = 0,5170$. Wykazano istotną różnicę między grupami kontrolną i badaną w teście funkcji poznawczych MoCA (odpowiednio 28 i 24 pkt, $p < 0,0001$). Nie wykazano istotnej różnicy w teście Becka i PSQI między grupą badaną i kontrolną, podobnie nie stwierdzono istotnej korelacji między stężeniami IL-12/IL-23 a psychologiczną oceną. **Wnioski i implikacje kliniczne:** Otrzymane wyniki

wskazują, że IL-12 i IL-23 mogą nie odgrywać roli w rozwoju zaburzeń poznawczych. Obecność zaburzeń funkcji poznawczych u pacjentów ze stwardnieniem rozsianym może mieć wartość przesiewową w zapobieganiu ich poznawczej deterioryzacji.

Słowa kluczowe: stwardnienie rozsiane, IL-12, IL-23

INTRODUCTION

Multiple sclerosis (MS) is a chronic, incurable, organ-specific, inflammatory demyelinating disease (Berer and Krishnamoorthy, 2014). The onset of the condition usually occurs in young adults, leading to permanent disability. Cognitive impairment affects approximately 43–65% of adults with MS (Charvet et al., 2015). The inflammatory process taking place in the nervous system stimulates the proliferation of pro-inflammatory cytokines, including IL-12 and IL-23 (Oppmann et al., 2000). IL-23 and IL-12 are heterodimeric interleukins having the same p40 subunit (Vojdani and Lambert, 2011). IL-23 additionally has a role in the induction of pro-inflammatory IL-17, which has been proven to be involved in the pathogenesis of MS (Cua et al., 2003). Although several roles of IL-12 in MS pathogenesis have been described, studies investigating the serum levels of IL-12 and IL-23 in MS patients are controversial (Jahanbani-Ardakani et al., 2019; International Multiple Sclerosis Genetics Consortium et al., 2007). In the present study, we determined the serum levels of IL-12 and IL-23, and evaluated MS patients and healthy volunteers using the Montreal Cognitive Assessment (MoCA), the Beck Depression Inventory (BDI), and the Pittsburgh Sleep Quality Index (PSQI).

MATERIALS AND METHODS

Study design

This was a prospective study in which the serum levels of IL-12 and IL-23 were evaluated, and the cognitive function by MoCA, susceptibility of depressive disorders by Beck test, and sleep quality by PSQI were compared between MS patients and healthy volunteers. The study was approved by the local Research Ethics Committee (RNN/75/18/KE), and all participants were provided with written consent according to the guidelines established by the Declaration of Helsinki.

Participants

The study included a total of 21 patients with relapsing-remitting MS (RRMS) in remission (average age: 42.95 ± 10.24) and 21 healthy people (average age: 42.00 ± 6.11). All patients were undergoing MS therapy with drugs including Tecfidera, Copaxone, Betaferon, Avonex, Extavia, Aubagio, Gilenya. The groups were matched in terms of age and sex. The study involved 11 men and 10 women in each group.

The criteria for inclusion in the study were the ability to obtain medical history and the history of use of drugs, alcohol and other psychoactive agents (within 24 hours prior to testing). The criteria for excluding participants from the study included a history of past or active diseases with a proven association with elevated levels of IL-12 or IL-23 including colorectal cancer (Hu et al., 2017), rheumatoid arthritis (Zaky and El-Nahrery, 2016), schizophrenia (O'Connell et al., 2015), and psoriasis (Teng et al., 2015). Another exclusion criterion was illiteracy.

Material

Prior to the study, all subjects provided written consent to participate in the study. Each participant was assessed for cognitive function using MoCA. Depressive disorders were

	Multiple sclerosis ($n = 21$)	Control group ($n = 21$)	p-value
Sex	M = 11, F = 10	M = 11, F = 10	-
Age	42.95 ± 10.24	42.00 ± 6.12	0.8206
Years since diagnosis	6.76 ± 6.43	-	-
Number of relapses	2.33 ± 2.24	-	-
Beck Depression Inventory	6.86 ± 6.58	4.90 ± 4.38	0.4707
PSQI	5.33 ± 4.20	5.00 ± 3.62	0.9697
MoCA	24.05 ± 2.06	28.0 ± 1.35	<0.0001
IL-12 [pg/mL]	1.61 ± 4.61	1.78 ± 3.54	0.3439
IL-23 [pg/mL]	19.04 ± 75.50	5.50 ± 14.4	0.4684
PSQI – Pittsburgh Sleep Quality Index; **MoCA** – Montreal Cognitive Assessment.			

Tab. 1. Comparison between demographic parameters, psychological assessments and IL-12/IL-23 serum level in the control and study groups

DOI: 10.15557/AN.2022.0008

Correlation		Multiple sclerosis		Control group	
		R	*p*-value	*R*	*p*-value
IL-12 vs.:	BDI	−0.2603	0.2545	−0.0444	0.8486
	PSQI	−0.3568	0.1123	0.1239	0.5925
	MOCA	0.1022	0.6593	−0.1946	0.3980
IL-23 vs.:	BDI	0.0449	0.8469	−0.1148	0.6203
	PSQI	−0.0455	0.8448	0.0600	0.7962
	MOCA	0.2734	0.2304	−0.0565	0.8078

BDI – Beck Depression Inventory; **PSQI** – Pittsburgh Sleep Quality Index; **MoCA** – Montreal Cognitive Assessment.

Tab. 2. Correlation coefficients between interleukin level and psychological parameters

assessed with the Beck test. Sleep quality was assessed using the PSQI. After testing, 5 mL blood samples were collected from the ulnar vein into vials with anticoagulant (K_2EDTA dipotassium ethylenediaminetetraacetic acetate).

Procedure

The collected blood samples were centrifuged (3000 × *g*, 20 min, 20°C). Next, the plasma was harvested, aliquoted, and stored frozen (−80°C) for further analysis. The levels of studied proteins (IL-23, IL-12) in the test material were determined with the enzyme-linked immunosorbent assay (ELISA) according to an optimised protocol provided by the test manufacturer (Thermo Fisher Scientific, Waltham, Massachusetts, US). In brief, wells of half-area polystyrene plates (Greiner) were coated with specific polyclonal antibodies (overnight incubation 4°C), and standard and tested samples were applied in duplicate. Subsequent incubation steps with specific biotinylated antibodies, horseradish peroxidase and substrate for the enzymatic reaction led to the formation of a coloured, soluble product. Then the quantity of product was determined colourimetrically, which allowed estimating the amount of the studied protein in the sample.

Statistical analysis

Distribution of variables was evaluated using the Shapiro–Wilk test. Due to non-Gaussian distribution of variables, the Mann–Whitney *U* test was used to compare the differences between the control and study groups. Correlations between each variable were examined using the Spearman's rank correlation. A *p* value below 0.05 was considered statistically significant. All calculations were made using Statistica 13 software.

RESULTS

Detailed results are shown in Tab. 1. A significant difference between the control and study groups was found in MoCA (28.00 ± 1.35 vs. 24.05 ± 2.06, $p < 0.0001$, respectively).
We found no significant difference between the control and study groups with regard to the Beck Depression Inventory (4.90 ± 4.38 vs. 6.86 ± 6.58, $p = 0.4707$, respectively), and PSQI (5.00 ± 3.62 vs. 5.33 ± 4.20, $p = 0.9697$). The serum levels of IL-12 and IL-23 were not significantly different between the control and study groups (IL-12: 1.78 ± 3.54 pg/mL vs. 1.61 ± 4.61 pg/mL, $p = 0.3439$, respectively, IL-23: 5.50 ± 14.4 vs. 19 04 ± 75.50, $p = 0.4684$, respectively). We did not find any significant correlation between the serum levels of IL-12/IL-23 and psychological assessments (Tab. 2).

DISCUSSION

A better understanding of the causes of MS will allow the introduction of prophylaxis, screening, and earlier initiation of treatment.
It is postulated that IL-12 and IL-23 may play an important role in the aetiopathogenesis of MS. Administration of antibodies against the p40 subunit significantly inhibits the action of IL-12 and IL-23, which facilitates the renewal of neurological damage (Cua et al., 2003). However, current study findings do not give a definite answer on the effects of IL-12 and IL-23 in the pathogenesis process, offering a field for future research.
Nicoletti et al. (1996) described elevated serum IL-12 levels in patients with chronic progressive MS. They also studied healthy volunteers and people suffering from another neurological diseases. Significantly higher levels of IL-12 occurred only in patients with MS, regardless of their age and sex (Nicoletti et al., 1996).
In our study, there was no statistically significant difference in the measurement of serum IL-12 level in MS patients and healthy volunteers. Drulović et al. (1998), in their study, divided the subjects into three groups: with an active form of MS, with inactive MS, and without inflammatory neurological diseases. The authors observed an increased mean serum IL-12 level in patients with MS compared to the control group, but the difference did not reach the level of statistical significance. However, detectable serum IL-12 levels were only noted in MS patients who were in the clinically active phase of disease, and in none of those with inactive MS (Drulović et al., 1998). Bahner et al. (2002) examined the levels of IL-12 p40 and IL-12 p70 separately. Subunit levels were tested before and after interferon beta-1b treatment in patients with primary progressive MS.

The authors observed a significantly elevated IL-12 p40 subunit level after treatment, while the IL-12 p70 subunit level did not increase significantly (Bahner et al., 2002). Orbach et al. (2014) measured the level of the IL-12 p40 subunit in cerebrospinal fluid in patients with clinically isolated syndrome (CIS), RRMS, and other neurologic disorders (OND). They observed significantly higher levels of IL-12 p40 in patients with CIS and RRMS compared to patients with OND (Orbach et al., 2014). These studies indicate a possible involvement in the MS pathogenesis of the IL-12 p40 subunit, which is also common for IL-23 (Oppmann et al., 2000).

Miteva et al. (2019) measured the level of IL-12 p40 and IL-23 in serum in patients with RRMS treated with interferon beta (IFN-β), and observed a significantly higher level of these interleukins in relation to the control group. Interestingly, when it comes to sex, women in the study group had significantly higher levels of both interleukins. Higher levels of IL-12 p40 and IL-23 did not correlate with the duration of the disease or the degree of disability (Miteva et al., 2019). This may be because women more often than men suffer from autoimmune diseases, including MS (Duquette, 1998). The relationship between IL-23 concentration and MS patients is not confirmed by our study. Shajarian et al. (2015) observed a significantly higher serum IL-23 concentration in patients with RRMS compared to the control group.

In our study, we used the MOCA test, which was employed to assess the cognitive functions of the subjects. Test results showed a significant statistical difference in the level of cognitive functions between the patients with MS and the control group. In several other studies, worse cognitive function was also observed in patients with MS compared to the control group. These authors also used the MoCA test. Charvet et al. (2015) point out that cognitive impairment occurs in approximately 43–65% of adults with MS. However, it is relatively often overlooked in the patients' clinical assessment. 259 patients with diagnosed MS took part in the study of these authors. They achieved an average MoCA score of 25.86 ± 2.92 points. From the whole sample, 40% (n = 103) of the respondents had a result lower than the cut-off point – 26 points (Charvet et al., 2015).

Similar results were obtained in another study involving only subjects with diagnosed MS. The study group had a total of 41 patients. The subjects were divided into two groups: cognitively intact (n = 27) and cognitively impaired (n = 14). The average MoCA test result was 26.02 ± 2.30, with lower results in the group with previously diagnosed cognitive impairment (Dagenais et al., 2013). In the study conducted by Freitas et al. (2018), similarly to our study, patients with MS were compared with healthy volunteers. Both the study and the control groups consisted of 59 subjects, 25.03 ± 2.51 vs. 26.76 ± 2.44, respectively (Freitas et al., 2018). Researchers also point to the relationship between the duration of the disease, the effectiveness of treatment, and the gradual decline in cognitive function.

Depression is one of the most important factors that can impair cognitive function not only in patients affected by neurological diseases. Its effect on memory and concentration as well as the effectiveness of implemented therapies has also been pointed out (Glanz and Houtchens, 2012).

We found no difference between the groups with regards to BDI. However, they differ from the data presented by other authors. Brola et al. (2007) examined 94 patients with MS in their study evaluating the impact of depression on the quality of life of patients with MS. Mild depressive disorders were found in 62% (n = 58) participants. The Hamilton Depression Rating Scale was used to assess depression (Brola et al., 2007). Lobentanz et al. (2004) found depressive disorders in 46.2% (n = 233) of patients with MS. The Self-Rating Depression Scale was used to assess depression (Lobentanz et al., 2004).

Already in the 5th century B.C., Hippocrates mentioned sleep disturbance among factors contributing to melancholy (Musiał, 2007). Also modern research, including ours, confirms the relationship between depressive tendencies and sleep disorders.

In our study, no statistically significant difference was found between the groups in terms of the quality of sleep measured by PSQI. These results do not coincide with those obtained by other authors. Lobentanz et al. (2004) in their work observed an almost two-fold deterioration of sleep quality in patients with MS compared to the control group (61.9% vs. 32.1%). Higher results in the PSQI test in MS patients were also observed in a Norwegian hospital study (Bøe Lunde et al., 2012).

The relationship between depressed mood and sleep disorders has long been reported in the literature.

Our study was limited to a small number of participants. Medications taken by the patients are another limiting factor impacting the study findings.

IMPLICATIONS/FUTURE DIRECTIONS

1. The results obtained indicate that IL-12 and IL-23 may not play a role in the development of cognitive impairment.
2. Susceptibility to depression may be associated with poor sleep quality both in healthy individuals and MS patients.
3. The assessment of cognitive impairment in patients with MS may have a screening value in preventing their cognitive deterioration.

Conflict of interest

The authors declare that they have no conflict of interest related to the publication of this article.

Funding/Support and role of the sponsor

The study was financed from the funds for statutory activity of the USK-WAM Department of Neurology and Stroke (503/5-062-01/503-51-002).

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