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ORIGINAL ARTICLE

# Oxidative stress and neuroinflammation should be both considered in the occurrence of fatigue and depression in multiple sclerosis

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## Abstract

Oxidative stress and neuroinflammation have a role in the pathogenesis of multiple sclerosis (MS) and in depression. Fatigue is the most disabling symptom in patients with MS and could also be a part of depressive symptomatology. In this study, we measured the serum levels of uric acid (UA) as a marker of oxidative stress and C-reactive protein (CRP) as an inflammatory marker, in 98 patients with MS in relapse and remitting phase of illness and 35 healthy subjects. Degree of depressive symptomatology and fatigue were assessed with Beck's Depression Inventory (BDI) and Fatigue Severity Scale (FSS). Further, we examined the possible correlation of these biomarkers with symptoms of depression and fatigue. Relapse and remitting MS had a lower serum UA levels than controls (236.97 ± 9.25 µmol/L vs. 268.27 ± 0.09 µmol/L vs. 314.82 ± 11.02 µmol/L; $p = 0.000$), while sera levels of CRP were higher in relapse than remitting patients (4.46 ± 0.40 mg/L vs. 1.01 ± 0.38 mg/L; $p = 0.000$). Patients in relapse had higher BDI scores (15.68 ± 16.62 vs. 8.36 ± 7.10; $p = 0.045$). Decreased UA levels showed weak negative correlation with the presence of sadness and disturbed daily activities, higher CRP levels positively correlated with severe depression and the correlation between depression and fatigue was also observed ($p < 0.05$). It is possible that decreased UA levels lead to sadness, disturbed daily activities and severe disability. Every attack of CRP elevation in relapse could additionally precipitate the depression onset. The clinicians must pay special attention to early detection of fatigue because it could precede depression and improve further treatment.

**Keywords** Multiple sclerosis · Uric acid · C-reactive protein · Fatigue · Depression

## Introduction

Multiple sclerosis (MS) is a neurological disease of still unresolved etiology. Neuroinflammation and oxidative stress are very important elements in the pathogenesis of MS. These processes cause demyelination, axonal damage, loss of neurons over years and consequently lead to neurodegeneration [1].

The relationship between neuroinflammation and oxidative stress is still poorly understood. Chronic oxidative stress and reactive oxidative species are involved in autoimmune-mediated tissue damage in MS by inducing proliferation, inflammation and activation of astrocytes, microglia and T cells [2, 3]. Elevated oxidative stress markers are strongly associated with higher sera C-reactive protein (CRP) levels [4]. Important fact is that CRP levels in peripheral blood mirror inflammation in the central nervous system (CNS) [5]. CRP is used as a marker in assessing inflammation and disease progression in MS patients [6, 7].

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Previous data pointed out that peroxynitrite, as a marker of oxidative stress, has also a role in the MS evolution [8]. Two decades ago, the endogenous antioxidant uric acid (UA) was marked as a strong peroxynitrite scavenger [9]. Patients with MS have significantly lower levels of serum UA than control subjects [10, 11] and it was observed that MS and gout (hyperuricemia) are almost mutually exclusive diseases and thereby further raising the possibility that hyperuricemia may have protective anti-inflammatory properties in MS [9].

Fatigue is considered to be a consequence of activated nitro-oxidative stress pathways, thereby it is more frequently observed in neuroinflammatory diseases, including MS [12]. Fatigue is one of the three most frequently occurring disabling symptoms in diverse clinical presentations of MS and it has been identified as a significant problem in 84% of patients [13]. The MS Council for Clinical Practice Guidelines defines fatigue as a sense of exhaustion, lack of energy, or tiredness, and "a subjective lack of physical and/or mental energy that is perceived by the individual or caregiver to interfere with usual or desired activity" [14]. Fatigue in MS can be a direct consequence of MS pathology or secondary caused by MS unrelated factors, which can be clinically presented at the same time or even potentiate each other [15]. Fatigue can be directly worsened by depressive symptomatology or be a consequence of physiological changes [16, 17]. Lifetime prevalence of depression in MS is 50% and depression itself can be manifested and misinterpreted as fatigue, loss of motivation and anhedonia, making this condition difficult to distinguish from MS-associated isolated fatigue [18–20]. Previous studies indicated that oxidative stress and neuroinflammation play an important role in the pathophysiology of major depressive disorder [21]. Also, MS-related depression was associated with increased serum levels of nitro-oxidative and immune-inflammatory biomarkers [22, 23].

In the present study, we measured the serum levels of UA as a marker of oxidative stress and CRP as an inflammatory marker in different phases of MS and compared it with those in healthy control subjects. Considering contradictory results from previous studies, we also assessed a level of fatigue, disability and depression symptoms in relapse and remitting patients and examined the possible correlation of these biomarkers with assessed clinical features of this disease. This is the first study, to our knowledge, that at the same time explores the possible relationship of UA and CRP levels with fatigue and depression in relapse and remitting MS patients.

## Subjects and methods

### Subjects

The study was approved by the Ethic Committee of the Clinical Centre Kragujevac and conducted in compliance with the ethical principles of the Declaration of Helsinki. Written informed consent was obtained from all patients. The study was conducted at the Clinic of Neurology, Clinical Centre Kragujevac and in the Central Laboratory Clinical Centre Kragujevac, from February 2016 to February 2017.

Patients with a diagnosis of MS were included into the study according to the revised Mc Donald's criteria [24]. Patients were classified into two groups: MS relapse group and MS remitting group. A relapse was defined as the presence of new symptoms existing longer than 24 h and in absence of fever or subacute worsening of existing symptoms after 30 days of a stable phase of the disease. Patients in MS relapse group were not on immunomodulatory therapy, corticosteroid therapy or some other MS treatment options in previous 3 months. All patients in remitting group have relapse–remitting form of disease and were treated with immunomodulatory therapy (interferon-beta-1a, administered subcutaneously three times weekly). The control group consisted of healthy subjects, matched by gender and age with MS patients, recruited during blood donation at Service supply of blood and blood products, Clinical Center Kragujevac.

The exclusion criteria were comorbidity of stroke, myocardial diseases, renal or hepatic dysfunction, other neurodegenerative diseases, acute infections, recent surgical interventions, alcohol or drug abuse, all disorders which are immune- and redox-mediated illnesses, such as fibromyalgia, generalized anxiety disorder, psychosis, bipolar disorder (bipolar depression) and treatment with medications that can influence on uric acid levels (vitamin C, diuretics, epinephrine, levodopa, methyldopa, acetylsalicylic acid and azathioprine).

### Somatic examination and laboratory measurements

Somatic examination was done and vital signs were measured at admission. Patients were fasting and the blood samples for routine laboratory analysis were taken the next day, between 9am and 12 pm, before the application of corticosteroid therapy. Levels of serum UA and CRP were determined on Beckman Coulter AU 680 analyzer using reagents from the same manufacturer (Beckman Coulter Inc., USA). Referent values for UA were in the following range: 154–428 μmol/L. The CRP values greater than or equal to 5 mg/L were considered elevated.

### Clinical assessment

Data concerning sociodemographic characteristics, duration of illness and clinical course of the disease were collected. In the relapse patients group, the scales were conducted immediately after admission and before the use of pulsed

corticosteroid therapy. At patients in remission the scales were carried out on scheduled 3-month visits.

Neurological status in all patients was assessed using the Expanded Disability Status Scale (EDSS) score [25]. EDSS consists of eight functional domains: visual (optic), brainstem, pyramidal, cerebellar, sensory, bowel and bladder, cerebral function and ambulation. The EDSS is an ordinal clinical rating scale, ranging from 0 (normal neurologic examination) to 10 (death due to MS), in half-point increments to indicate mild, moderate or severe disability.

The occurrence of fatigue was determined with Fatigue Severity Scale (FSS). It is a self-reported questionnaire designed by Krupp for fatigue identification [26]. The scale consists of nine items on a seven-point scale. The presence of mild fatigue is marked with an average score over 3 points, and as disabling fatigue with FSS average score higher than 4.

Depressive symptomatology was assessed with Beck's Depression Inventory (BDI), suggested for depressive symptoms screening in MS patients by American Academy of Neurology [27]. The 21-item BDI consists of four statements, describing increasing intensities of depression symptoms. It includes somatic and cognitive-affective symptoms and every item is graded into a scale from 0 to 4, reflecting the patient's feelings in the previous 2 weeks. The degree of severity of depression symptoms was quantified as follows: normal (range 0–10), mild mood disturbance (range 11–16), borderline clinical depression (17–20), moderate depression (21–30), severe depression (31–40) and extreme depression (over 40).

### Statistical analyses

The statistical analyses were performed using SPSS 20.0 software. Normal distribution of data was tested with Kolmogorov–Smirnov test. Numerical values are presented as mean ± standard deviation and as percentages. We used Student's *t* test for parametric and Mann–Whitney *U* test for non-parametric variables to evaluate mean differences between two groups of patients. We used General Linear Model (GLM) analyses to determine levels of UA and CRP as dependent variables between groups while controlling gender and body mass index (BMI) and to show effect of these parameters. We examined the correlations between serum UA and CRP levels and scores on clinical assessment scales using Pearson's or Spearman's correlation. To delineate the best prediction on presence of illness onset, depression and fatigue in MS patients, we performed a binary logistic regression analysis. The presence of depressive symptomatology was assessed using BDI. In patients with MS, a cut-off score > 13 on BDI scale can be used as criterion for depression with a sensitivity of 71% and specificity of 79% [27]. Fatigue was assessed using FSS scale and suggested cut-off score for MS patients is > 4, with acceptable internal consistency, stability over time and sensitivity to clinical change [26]. For binary logistic regression analysis we used these cut-off values (BDI > 13, FSS > 4) to discriminate patients with and without depression, and also those with and without fatigue. A multiple regression analysis was used to evaluate the significant sociodemographic and clinical predictors for FSS and BDI scores. A *p* value of 0.05 was considered to be statistically significant.

## Results

### Sociodemographic and clinical data

Sociodemographic data are presented in Table 1. The study included $n = 98$ MS patients, $n = 48$ MS relapse patients (48.9%) and $n = 50$ MS remitting patients (51.1%). No differences were found in relation to gender and age between groups. The mean duration of disease was significantly longer in MS remitting patient's group ($6.19 \pm 7.11$ vs. $8.64 \pm 4.46$; $p = 0.01$), while relapse rate was higher in MS relapse patient's group ($2.42 \pm 1.22$ vs. $1.24 \pm 1.39$; $p = 0.05$). The patients included in MS relapse group were those with Clinically Isolated Syndrome (CIS), Relapse Remitting MS (RRMS) and Secondary Progressive MS (SPMS), with the following distribution, respectively: 20 patients (41.7%), 17 patients (35.4%) and 11 patients (22.9%). The mean duration of treatment in remitting group was 4.42 years ± 3.64 (in the range of 1–12).

### Lower serum levels of UA in all MS patients and higher CRP in MS relapse patients

We measured significantly lower mean serum UA levels in relapse MS patients than in healthy control subjects ($236.97 \pm 9.25$ vs. $314.82 \pm 11.02$ µmol/L, $F = 14.339$, $df = 2/128$, $p = 0.000$). In relapse group, we did not find significantly different serum UA levels between CIS, RRMS and SPMS ($223.60 \pm 14.77$ µmol/L vs. $225.69 \pm 15.43$ µmol/L vs. $264.80 \pm 20.36$ µmol/L, $F = 1.497$, $df = 2/86$, $p = 0.230$). The patients in MS remitting group also had the lower mean serum UA level than healthy control subjects ($268.27 \pm 0.09$ µmol/L vs. $314.82 \pm 11.02$ µmol/L, $F = 14.339$, $df = 2/128$, $p = 0.000$). We did not find significantly different serum UA levels between patients in relapse and remission ($238.03 \pm 9.67$ vs. $258.88 \pm 9.08$, $F = 3.307$, $df = 1/86$, $p = 0.07$). We found that patients in remitting phase had higher UA levels in sera than patients with CIS and RRMS, but it was not significant ($223.60 \pm 14.77$ µmol/L vs. $225.69 \pm 15.43$ µmol/L vs. $258.88 \pm 9.08$; $F = 1.497$, $df = 2/86$, $p = 0.230$). Tests between subject effects showed that sex ($F = 14.360$, *df* 1/86,

**Table 1** Sociodemographic data in relapse and remitting MS patients

| Variables | MS relapse group $n=48$ | MS remitting group $n=50$ | *P* value |
|---|---|---|---|
| Age (years) | 41.7 ± 9.13 | 38.6 ± 7.88 | $p=0.18$ |
| Sex (female/male) | 30/18 | 35/15 | $p=0.08$ |
| BMI (kg/m$^2$) | 22.98 ± 3.67 | 22.502 ± 1.78 | $p=0.40$ |
| Mean duration of illness (years) | 6.19 (1–34) | 8.64 (2–18) | $p=0.01$ |
| Relapse rate | 2.42 (0–5) | 1.24 (0–6) | $p=0.05$ |
| MS clinical types | | | |
| CIS | 20 (41.7%) | / | |
| Relapsing–remitting MS | 17 (35.4%) | 50 | |
| Secondary progressive MS | 11 (22.9%) | / | |

*MS* multiple sclerosis, *CIS* clinical isolated syndrome, *BMI* body mass index
$p < 0.05$

$p = 0.000$, partial eta squared = 0.142) and BMI ($F = 4.515$, $df = 1/86$, $p = 0.036$, partial eta squared = 0.050) had significant effect on sera levels of UA. Mean value of CRP was significantly higher in the relapse patients than in the remitting MS patients (4.46 ± 0.40 mg/L vs. 1.01. ± 0.38 mg/L; $F = 15.906$, $df = 1/86$, $p = 0.000$). The effects of sex ($F = 2.044$, $df = 1/86$, $p = 0.156$, partial eta squared = 0.023) and BMI ($F = 1.908$, $df = 1/86$, $p = 0.171$, partial eta squared = 0.025) on CRP were not significant (Fig. 1).

## More severe disability and depressive symptomatology in MS relapse group

Clinical characteristics of MS relapse and MS remitting patients are shown in Table 2. Relapse MS group patients had significantly higher EDSS scores (4.02 ± 1.92 vs. 2.07 ± 1.17; $p = 0.000$) and BDI scores (15.68 ± 16.62 vs. 8.36 ± 7.10; $p = 0.045$). We did not find difference in FSS scores between these patient groups (data not presented).

Fatigue was present in 32 patients with relapse (66.7%) and depressive symptomatology was present in 21 patients (43.7%). In MS remitting group, we observed the fatigue occurrence in 28 patients (56%) and depression symptoms in 12 patients (24%).

## Negative correlation of UA sera levels with the presence of sadness and disturbed daily activities and positive correlation of CRP levels with EDSS, BDI and FSS scores

We found significant, but weak positive correlation with CRP levels and physical and daily activities (overall $p < 0.05$,

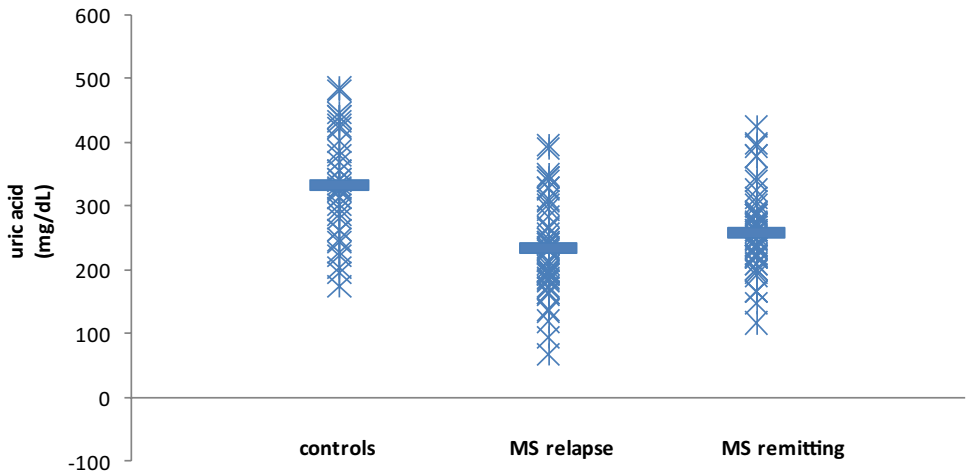


**Fig. 1** Serum levels of uric acid. Serum UA levels in healthy control subjects ($n=35$), MS relapse patients ($n=48$) and MS remitting patients ($n=50$) were determined by Beckman–Coulter autoanalyzer. Data are presented as mean ± SE. Statistical significance was tested by General Linear Model (GLM). Serum levels of UA were significantly lower in MS relapse patients than controls [236.97 ± 9.25 µmol/L vs. 314.82 ± 11.02 µmol/L; $p=0.000$]. Serum levels of UA were significantly lower in MS remitting patients than controls [268.27 ± 0.09 µmol/L vs. 314.82 ± 11.02 µmol/L; $p=0.000$]

**Table 2** Clinical features at relapse and remitting phases of multiple sclerosis patients

| Variables | MS relapse group | MS remitting group | *P* values |
|---|---|---|---|
| EDSS score | 4.02 ± 1.92 | 2.07 ± 1.17 | $p = 0.000$ |
| FSS score | 4.91 ± 1.18 | 4.80 ± 1.06 | $p = 0.711$ |
| BDI score | 15.68 ± 16.62 | 8.36 ± 7.10 | $p = 0.045$ |

*MS* multiple sclerosis, *EDSS* Expanded Disability Status Scale, *FSS* Fatigue Severity Scale, *BDI* Beck's Depression Inventory

We performed Student's *t* test ($p < 0.05$). Data are expressed as mean and standard deviation

$r = 0.40$). Further, we observed significant positive correlation between CRP and BDI scores ($p = 0.000$, $r = 0.40$) and positive correlations with EDSS scores ($p = 0.000$, $r = 0.57$) and FSS scores ($p = 0.03$, $r = 0.40$). A statistically significant positive correlation was found between FSS and BDI scores ($p = 0.00$, $r = 0.45$), FSS and EDSS scores ($p = 0.00$, $r = 0.40$) and BDI and EDSS score ($p = 0.00$, $r = 0.45$).

We did not establish statistically significant correlation between serum UA levels with an overall score on the BDI scale ($p = 0.259$), FSS scale ($p = 0.080$) and EDSS scale ($p = 0.117$), but we found a statistically significant negative correlation with the BDI items that represent the symptoms of sadness ($p = 0.008$, $r = -0.266$) and with the FSS item which refers to the effect of fatigue on daily physical activity ($p = 0.032$, $r = -0.21$).

Table 3 shows the results of three different binary logistic regression analyses: the first one used serum UA level data, while adjusting sex and BMI, and showed that UA levels were significantly associated with illness onset ($\chi^2 = 28.933$, $df = 1$, $p = 0.000$; Nagelkerke = 0.289; 77.4% of all cases were correctly classified with 92.9% sensitivity and 34.3% specificity). The second analysis was performed using sex, age, level of education, duration of illness, EDSS score data and showed that age and EDSS score affect the occurrence of fatigue ($\chi^2 = 20.059$, $df = 5$, $p = 0.001$; Nagelkerke = 0.247; 71.4% of all cases were correctly classified with 63.0% sensitivity and 78.8% specificity). The third binary logistic regression analysis was performed using sex, age, level of education, duration of illness, EDSS scores and showed that EDSS score affects the occurrence of depression ($\chi^2 = 15.214$, $df = 5$, $p = 0.009$; Nagelkerke = 0.199; 75.5% of all cases were correctly classified with 62.4% sensitivity and 92.3% specificity). Multiple regression analysis indicated that age, clinical type of MS ($F$ (1/95) = 4.339, $p = 0.040$, $R^2 = 0.164$, adjusted $R = 0.146$) and EDSS score ($F$ (1/96) = 16.243, $p = 0.000$, $R^2 = 0.145$, adjusted $R = 0.136$) are significant predictors for FSS score. Also, clinical type of MS, immunomodulatory therapy ($F$ (1/95) = 13.236, $p = 0.000$, $R^2 = 0.198$, adjusted $R = 0.182$), CRP and EDSS score ($F$ (1/95) = 4.128, $p = 0.040$, $R^2 = 0.203$, adjusted $R = 0.187$) are significant predictors of BDI score (results are presented in Table 4).

## Discussion

In the present study, we observed lower serum UA levels in MS patients than in healthy control subjects in all phases of MS and higher sera CRP levels at relapse patients. The serum levels of UA affect the occurrence of the disease. More severe disability and depressive symptomatology were presented in MS relapse patients. Sera levels of UA negatively correlate with the presence of sadness and disturbed daily activities and CRP levels correlate with EDSS, BDI and FSS scores. CRP was a significant predictor of BDI scores, while EDSS score had influence on FSS and BDI scores.

In the past few years, various studies have presented conflicting results regarding serum UA levels in MS patients. To this day, it is still not clear whether UA should be considered in disease onset or only as a consequence of a disease progression in MS. Our results are consistent with the results of the recent meta-analysis [11], indicating that MS patients have a significantly lower serum UA levels compared with those in healthy control subjects. UA levels were evaluated in different stages of MS and according to clinical

**Table 3** Results of binary logistic regression analysis with disease onset, depression and fatigue as dependent variables and demographic, clinical and biological data as explanatory variables

| Categories | Explanatory variables | Wald | *df* | *P* | Odds ratio | Lower and upper 95% CI |
|---|---|---|---|---|---|---|
| Illness onset | UA | 21.902 | 1 | 0.000 | 0.986 | 0.97–0.99 |
| Depression | EDSS | 10.970 | 1 | 0.001 | 0.636 | 0.48–0.83 |
| Fatigue | Age | 4.114 | 1 | 0.043 | 0.935 | 0.87–0.99 |
| | EDSS | 5.472 | 1 | 0.019 | 0.727 | 0.55–0.95 |

MS patients were divided into two groups: with and without depression, using cut-off score > 13 on the Beck's Depression Inventory

MS patients were divided into two groups: with and without fatigue, using cut-off score > 4 on the Fatigue Severity Scale

*UA* uric acid, *EDSS* Expanded Disability Status Scale

**Table 4** Results of multiple regression analysis with FSS and BDI scores as dependent variables and demographic and clinical data as explanatory variables

| Dependent variables | Explanatory variables | *B* | SE | *β* | *T* | *P* |
|---|---|---|---|---|---|---|
| FSS scores | Age | 0.060 | 0.020 | 0.291 | 2.627 | 0.004 |
| | MS clinical types | 1.141 | 0.307 | 0.355 | 3.715 | 0.000 |
| | EDSS scores | 0.364 | 0.091 | 0.380 | 4.030 | 0.000 |
| BDI scores | MS clinical types | 8.293 | 2.197 | 0.352 | 3.775 | 0.000 |
| | Immunomodulatory therapy | −8.289 | 2.439 | −0.339 | −3.411 | 0.001 |
| | CRP | 0.850 | 0.419 | 0.200 | 2.032 | 0.040 |
| | EDSS scores | 2.383 | 0.696 | 0.337 | 3.424 | 0.001 |

*MS* multiple sclerosis, *FSS* Fatigue Severity Scale, *EDSS* Expanded Disability Status Scale, *BDI* Beck's Depression Inventory, *CRP* C-reactive protein

$p < 0.05$

phenotypes. Our data suggested that the patients with CIS and RRMS in relapse have lower UA levels than patients with SPMS, but the difference was not statistically significant. The results of previous studies indicated that immunomodulatory drugs increase serum UA levels [28], which is in accordance with our findings of higher levels of UA in MS remitting patients on therapy in comparison to patients in early, inflammatory phase of illness and without therapy.

Previous studies indicated sex differences in UA serum levels, with significantly lower values UA in female patient [29]. A possible explanation for this phenomenon is reduced antioxidant activity and higher incidence of MS in females. Also, BMI is a major determinant of lower UA sera levels [30]. Our results are in accordance with these findings and emphasize that possible sex and BMI differences should be especially considered.

Also, we measured significantly higher CRP levels in relapse MS patients. Hanninen et al. showed that CRP values are higher during relapses than in remission [7]. All these findings support the fact that in the early, inflammatory disease stages, neuroinflammation and oxidative stress are predominant, while the neurodegeneration is prominent in progressive form of MS, in line with the results of Zoccolella et al. [31]. Our results point out that UA could be considered as a trait marker in MS and that decreased level of UA enhances the risk for disease.

In this research, we examined the frequency and degree of disability, depression and fatigue in patients in relapse and remission. Our findings suggest that the more severe disability and depressive symptomatology occur in MS relapse group of patients, but we did not find a statistically significant difference in fatigue scores between groups.

Further, we examined a possible relationship of UA and CRP levels with disability, symptoms of depression and fatigue. Association between UA and disability has been shown in some studies, while other studies did not confirm these data [32]. We also did not establish the correlation between UA levels and EDSS scores. Moccia et al. indicated that patients with impaired ambulation (EDSS ≥ 5) presented lower UA levels than fully ambulatory patients (EDSS ≤ 4.5) [33]. We suppose that this correlation was not observed because most of our patients had EDSS ≤ 4.5. On the other side, degree of disability in our study significantly correlated with higher CRP levels. This fact is in line with the results of Guzel et al. which indicate that patients with higher CRP levels have higher level of disability on EDSS [34].

As already presented in depression, lower UA levels in MS can be a consequence of minimized adenosine turnover and an increased adenosine activity in CNS, causing depression-like behavior [35]. Bartoli et al. in their meta-analysis indicate that patients with major depression have lower serum UA levels than healthy controls and that antidepressant treatment increases UA levels [36]. Similarly, in our study, UA levels negatively correlated with the presence of sadness. Oliveira et al. have investigated sera UA levels in MS patients with and without depression and did not find differences in UA levels with and without comorbid depression and MS [32].

Maes et al. [37] indicated that general anxiety disorder is characterized by increased nitro-oxidative stress with lipid peroxidation, lower protein oxidation and lipid-associated antioxidant defenses. Increased UA levels may be regarded as a beneficial compensatory mechanism in response to oxidative stress, because the formation of peroxynitrite is inhibited by increased UA levels and consequently protein oxidation is less sever than in depression. It could be said that higher UA levels may be associated with lower risk of depression [38].

Additionally, major depressive disorders are characterized by low-grade inflammation and it was shown that depression is associated with increased CRP sera levels [39]. Our data suggest the positive correlation of serum CRP levels with BDI scores and subscores in MS patients. Also, we pointed out that CRP is a significant predictor of BDI scores. All these findings suggest that decrease of UA levels could be an underlying mechanism in depression onset, potentiated with CRP elevation.

In this study, more disabled MS patients (with higher EDSS score) had more severe fatigue and depression symptoms and we observed the association between fatigue and depression. Our data indicated that EDSS score affected the occurrence of fatigue and depression and also was a significant predictor of BDI and FSS scores. Previous studies showed that disability status is an independent, but moderate determinant of depression in MS patients and that fatigue may be due to disability level [40, 41]. We did not find a statistically significant correlation between UA sera levels with fatigue, but we observed significant correlation with lower daily activity on FSS scale. Kawase et al. show in their study the relationship between lower serum UA levels and poor activities of daily living in patients with ischemic stroke, and suggest that UA could be a predictive marker of lower daily activity in these patients [42]. It is possible that the same approach could be applied in MS indication field. We demonstrated significant correlation between CRP levels with fatigue, and the result is in line with Cho et al. who reported that plasma CRP was prospectively associated with new-onset fatigue [43]. The established correlations were statistically significant but weak correlations, so the larger sample could reveal much stronger relations of these factors.

## Conclusion

Our results indicate that in patients with MS, both low-grade inflammation and oxidative stress have a role in fatigue and depression occurrence and must be simultaneously considered. It is possible that UA depots could be emptied in MS evolution, leading to sadness, disturbed daily activities and severe disability. Every attack of CRP elevation in relapse could additionally precipitate the depression onset.

The clinicians must pay special attention to early detection of fatigue because of its potential to precede depression. This also implies that effective treatment of depression in MS could markedly improve recovery.

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### Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethical approval** The study was approved by the Ethics Committee of the Clinical Centre of Kragujevac and conducted in compliance with the ethical principles of the Declaration of Helsinki.

**Informed consent** Informed consent was obtained from all individual participants included in the study.

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