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Why Do Multiple Sclerosis and Migraine Coexist? Running Title: Multiple Sclerosis and Migraine

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Highlights

- Lower vitamin D levels were detected in the coexistence of MS and migraine.
- Lower antioxidant enzyme levels were detected in the coexistence of MS and migraine.
- Higher oxidative stress was detected in the coexistence of MS and migraine.
- Higher hs-CRP levels were detected in the coexistence of MS and migraine.
- There is a need to better understand the coexistence of MS and migraine.

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Why Do Multiple Sclerosis and Migraine Coexist?

Running Title: Multiple Sclerosis and Migraine

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Background: Migraine coexistence, which is high in multiple sclerosis (MS), is reported. To better understand the etiology of the coexistence of MS and migraine and the outcomes of this relationship, the vitamin D, vitamin D-binding protein (VITDBP), vitamin D receptor (VITDR), high-sensitivity C-reactive protein (hs-CRP), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), total antioxidant status (TAS), total oxidant status (TOS), and Oxidative Stress Index (OSI) values were examined in patients with the coexistence of relapsing-remitting multiple sclerosis (RRMS) and migraine.

Methods: This study was conducted between January 1, 2019, and July 25, 2019, at the neurology and biochemistry clinics of two different tertiary hospitals simultaneously. Overall, 50 RRMS patients with migraine, 50 RRMS patients without migraine, and 50 healthy volunteers were included in the study. The participants' vitamin D, VITDBP, VITDR, hs-CRP, SOD, CAT, GSH-Px, TAS, TOS, and OSI values were measured.

Results: The vitamin D and VITDR values of the RRMS patients with migraine were lower than those of the RRMS patients without migraine (respectively, $p = 0.014$, $p < 0.001$). There was no significant difference between the RRMS patients with and without migraine in terms of their VITDBP values ($p = 0.570$). The SOD, CAT, GSH-Px, and TAS values of the RRMS patients with migraine were lower than those without migraine (all $p < 0.001$). The hs-CRP and TOS values of the RRMS patients with migraine were higher than those without migraine (all $p < 0.001$).

Conclusion: To the best of our knowledge, this is the first study on this topic to date. Based on the results, our study may shed light on the etiopathogenesis of the coexistence of MS and migraine and new treatments. However, more studies are needed to better understand the etiology of this relationship and its negative effects.

Keywords; Comorbidity; Vitamin D; High-sensitivity C-reactive protein; Oxidative stress; Relationship

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1. Introduction

Multiple sclerosis (MS) is the most common immune-mediated disease of the central nervous system and affects more than 2 million people worldwide (Díaz et al., 2019). It has well-known characteristic early symptoms such as loss of strength, loss of sensation, visual disorders, spasticity, and fatigue (Patejdl and Zettl, 2017). Several recent studies have shown high rates of migraine in MS (Beckmann and Türe, 2019; Gebhardt et al., 2019). This has a particular significance, because headaches were accepted as an exclusion criterion for MS in the past (Gebhardt et al., 2019). It was shown that MS patients at young ages and with the relapsing-remitting form are especially more likely to experience headaches at the early stages of the disease compared to MS patients at advanced ages and in other forms (Möhrke et al., 2013).

While migraine affects approximately 11.7% (Lipton et al., 2007) of the general population, studies have found migraine in 19.8-82% of MS patients, and migraine is also the most commonly observed type of headache in cases of MS (Nicoletti et al., 2008; Vacca et al., 2007). This coexistence is important because the rate of relapse was reported to increase in MS patients with migraine (Kowalec et al., 2017), and this relationship may also have negative effects on patients (Marrie, 2017). A literature review found that studies that have been conducted in the last decade were usually focused on the prevalence of migraine in MS patients (Beckmann and Türe, 2019). A literature examination indicated that this relationship has been demonstrated by prevalence studies, and new studies are necessary to explain the causes and effects of this coexistence.

While vitamin D deficiency has been reported as a possible risk factor for MS, sufficient vitamin D levels have also been reported as a possible protective factor (Pierrot-Deseilligny and Souberbielle, 2017). One study found a significant relative correlation between vitamin D levels and disability in RRMS patients (Ibrahim et al., 2019). Another study showed that vitamin D supplementation reduced the number of attacks in RRMS patients treated with natalizumab (Laursen et al., 2016). High rates of vitamin D deficiency/insufficiency have been shown in migraine patients (Ghorbani et al., 2019). Another study also found an association between migraine headache frequency and vitamin D levels (Song et al., 2018).

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While there are many questions that should be asked on the coexistence of MS and migraine, the following two questions may be important.

1) Is there a common mechanism underlying this coexistence?

2) What kind of changes does this relationship cause to the human body?

To answer these questions, we planned a study that we did not find in the literature. Thus, we examined the vitamin D, vitamin D-binding protein (VITDBP), vitamin D receptor (VITDR), high-sensitivity C-reactive protein (hs-CRP), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), total antioxidant status (TAS), total oxidant status (TOS), and Oxidative Stress Index (OSI) values in relapsing-remitting multiple sclerosis (RRMS) patients with migraine and compared their results to those of MS patients without migraine and a healthy control group.

2. Materials and methods

2.1. Topic

This study was conducted between January 1 2019, and July 25, 2019, simultaneously at the neurology and biochemistry clinics of two different tertiary hospitals. The study was approved by the Ethics Committee of the Faculty of Medicine at the University (protocol number: 2017-KAEK-189_2019.03.13_01). The principles of the Declaration of Helsinki were used as a reference throughout the study, and written informed consent was obtained from all of the participants.

This study assessed volunteers between the ages of 18 and 60. Before starting the study, equal numbers of participants were included in the groups to provide more statistically significant results. The patients were included from among those who were admitted to the clinics until 50 MS patients with migraine and 50 MS patients without migraine were reached.

The participants were divided into 3 groups as follows:

Group 1: 50 RRMS patients between the ages of 18-60 with the coexistence of migraine.

Group 2: 50 RRMS patients between the ages of 18-60 without the coexistence of migraine.

Group 3: 50 healthy individuals who were age- and sex-matched and had no neurological pathology.

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MS patients who satisfied the 2017 revised McDonald criteria and received a definite MS diagnosis were included (Thompson et al., 2018). All patients, regardless of existing migraine diagnoses, were re-evaluated according to the third edition of the International Classification of Headache Disorders (ICHD-3) (Arnold, 2018). According to these criteria, patients who met the diagnostic criteria of migraine were included in the coexistence of migraine and multiple sclerosis. Patients with possible migraine were not included in the groups. Furthermore, patients with possible migraine without a definite diagnosis were not included in the calculation of rate of MS and migraine coexistence.

The demographic and clinical characteristics of the cases were recorded including age, sex, disease duration, Body Mass Index (BMI), and Expanded Disability Status Scale (EDSS) values. A complete neurological examination was performed, and the disability rates were determined by using the EDSS for each MS patient. The EDSS is scored between 0 and 10, and higher scores indicate higher degrees of disability (Kurtzke, 1983).

Multiple sclerosis patients who received steroid treatment one month before blood collection or with a history of attacks were excluded from the study. Blood samples of patients with migraine were collected on a headache-free day. Patients with Parkinson's disease, dementia, vascular diseases, metabolic disease (diabetes mellitus or hypertension), epilepsy, or psychiatric diseases were excluded from the study. Other exclusion criteria were congenital anomalies of the brain, history of head trauma in the last year, acute chronic infection, peripheral neuropathy, diseases of the heart, thyroid, and lungs, collagen tissue diseases, liver diseases, kidney failure, alcohol consumption, and pregnancy.

A total of 143 RRMS patients were screened to reach 50 MS patients with migraine who satisfied the inclusion criteria. The coexistence of migraine was found in 54 of the 143 RRMS patients. Four of these patients were excluded based on the exclusion criteria.

2.2. Blood collection and preparation

Venous blood samples were collected from an antecubital vein of each patient after 12 hours of overnight fasting, and a 10 ml sample of venous blood was placed into a biochemistry tube. Blood

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samples were withdrawn, and their sera were separated with centrifugation at 3000 rpm for 10 min. All of the materials were stored at -80°C until analysis.

2.3. Analysis of vitamin D, vitamin D-binding protein (VITDBP), vitamin D receptor (VITDR), and high-sensitivity C-reactive protein (hs-CRP)

Commercial ELISA kits were used to measure the serum levels of vitamin D, VITDBP, and VITDR (Elabsciences, Beijing, People's Republic of China) and hs-CRP (DRG International, Inc., Springfield Township, NJ, USA). The ELISA measurements were conducted with a microplate reader branded Multiscan GO (Thermo Fisher Scientific, Waltham, MA, USA).

2.4. Determination of superoxide dismutase (SOD) activity

Total SOD activity was determined according to the manufacturer's instructions using an SOD Activity Assay kit (Rel Assay Diagnostics kit; Mega Tıp, Gaziantep, Turkey).

2.5. Measurement of glutathione peroxidase (GSH-Px)

GSH-Px activities were measured using the modified method of Paglia and Valentine in which GSH-Px activity was coupled to the oxidation of NADPH by glutathione reductase (Paglia and Valentine, 1967). Oxidation of NADPH was followed spectrophotometrically at 340 nm and 37°C. The reaction mixture consisted of 50 mM of potassium phosphate buffer (pH = 7), 1 mM of EDTA, 1 mM of NaN_3, 0.2 mM of NADPH, 1 mM of glutathione, and 1 U ml-1 of glutathione reductase. The absorbance at 340 nm was recorded for 5 min. The activity was the slope of the lines expressed as μmol of NADPH oxidized per min. The results are expressed as U/ml.

2.6. Measurement of catalase (CAT) activity

Catalase (CAT, EC, 1.11.1.6) activities were determined using the modified Aebi method (Aebi, 1974). The assay principle was based on the determination of the rate constant k (dimension: s-1) of hydrogen peroxide decomposition. By measuring the absorbance changes per minute, the rate constant of the enzyme was determined. The activities are expressed as U/ml.

2.7. Determination of total antioxidant status (TAS) and total oxidant status (TOS)

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Serum TAS and TOS levels were determined with commercial kits (Rel Assay Diagnostics kit; Mega Tıp, Gaziantep, Turkey) developed by Erel, and the Oxidative Stress Index (OSI) values were calculated.

The total antioxidant status (TAS) was measured in the serum by the generation of 2,2′-azino-di-(3-ethylbenzthiazoline sulfonate) (ATBS) radical cations using a commercial TAS kit according to the manufacturer's instructions.

The total oxidant status (TOS) was measured as described by the manufacturer's protocol. In this method, the oxidants present in the sample oxidized the ferrous ion-o-dianisidine complex to ferric ions. Ferric ions produce a colored complex with xylenol orange in an acidic medium. The color intensity, which can be measured spectrophotometrically, is related to the total amount of the oxidant molecules present in the sample. The assay was calibrated with hydrogen peroxide, and the results are expressed in terms of μmol H_2O_2 equivalent/L of serum.

2.8. Calculation of Oxidative Stress Index (OSI)

The TOS:TAS ratio was used as the Oxidative Stress Index (OSI) and calculated as follows: OSI (arbitrary units) = [(TOS, μmol H2O2/L)/(TAS, mmol Trolox equiv./L)].

2.9. Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences (version 22.0, IBM Inc., Chicago, IL, USA) software. The descriptive statistics of the data were calculated, and Kolmogorov-Smirnov tests were applied to test the normal distribution of the data. The chi-squared test was used for comparison of groups regarding the categorical variables. ANOVA and post hoc Tukey's tests were used for comparisons among the three groups in terms of data with normal distribution. The Mann Whitney-U Test was performed for pairwise comparisons in the presence of significant differences. Pearson's correlation test was used for the normally distributed data, and Spearman's correlation test was used for the data without a normal distribution. A P-value less than 0.05 was considered statistically significant.

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3. Results

In this study, migraine was found in 54 of the 143 RRMS patients, and the coexistence rate of RRMS and migraine was 37.8%. No significant difference was found among the groups in terms of age, sex. and BMI. Table 1 shows the sociodemographic and clinical characteristics of the groups.

Table 1. Sociodemographic and clinical characteristics of the groups

	Group 1 (***n*** **= 50)**	**Group 2** (***n*** **= 50)**	**Group 3** (***n*** **= 50)**	***p***
Age, (mean ± standard deviation)	35.26 ± 7.89	37.58 ± 8.84	35.82 ± 9.54	$p = 0.389$ F = 0.949
Female	40 (80%)	36 (72%)	34 (68%)	$p = 0.385$
Male	10 (20%)	14 (28%)	16 (32%)	$X^2 = 1.909$
BMI, (median)	27.29 (min: 17.19, max: 36.73)	24.94 (min: 17.47, max: 40.57)	25.15 (min: 18.37, max: 37.04)	$p = 0.092$ $X^2 = 4.777$
EDSS	3 (min: 0, max: 6)	3 (min: 0, max: 6.5)		$p = 0.368$ U = 1120.50
MS Disease-Modifying Therapies	14 (28%) Interferon beta-1a 11 (22%) Interferon beta-1b 10 (20%) Glatiramer acetate 6 (12%) Teriflunomide 4 (8%) Dimethyl fumarate 3 (6%) Fingolimod 2 (4%) No prophylactic treatment	13 (26%) Interferon beta-1a 11 (22%) Glatiramer acetate 10 (20%) Interferon beta-1b 7 (14%) Teriflunomide 4 (8%) Dimethyl fumarate 2 (4%) Fingolimod 1 (2%) Natalizumab 1 (2%) Ocrelizumab 1 (2%) No prophylactic treatment		
Patients with existing migraine diagnosis before MS diagnosis, n (%)	31 (62%)			
Chronic migraine	3 (6%)			
Migraine with aura	9 (18%)			
Attack frequency (attack/month), (median)	4 (min: 1, max: 18)			
Attack duration (hours) (median)	8 (min: 4, max: 72)			

Group 1: relapsing-remitting multiple sclerosis patients with migraine, Group 2: relapsing-remitting multiple sclerosis patients without migraine, Group 3: healthy volunteers. BMI: Body Mass Index, EDSS: Expanded Disability Status Scale, MS: Multiple sclerosis, F: ANOVA, X^2: Pearson's chi-squared test.

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Table 2 presents the vitamin D, VITDBP, VITDR, hs-CRP, SOD, CAT, GSH-Px, TAS, TOS, and OSI values of the groups and their comparison.

Table 2. Group values and comparisons of Vitamin D, VITDBP, VITDR, hs-CRP, SOD, CAT, GSH-Px, TAS, TOS and OSI

	Group 1 (*n*=50)	**Group 2 (*n*=50)**	**Group 3 (*n*=50)**	***p***
VITAMIN D	31.99±7.06[a,b]	36.61±6.77[a]	53.76±10.11	***p* = <0.001** F = 99.714
VITDBP	42.15±13.52[a]	45.30±14.93[a]	73.40±17.89	***p* = <0.001** F = 61.151
VİTDR	8.49±2.53[a,b]	12.59±2.59[a]	19.92±2.84	***p* = <0.001** F = 236.985
hs-CRP	9.94±1.23[a,b]	6.59±0.36[a]	2.65±0.10	***p* = <0.001** F = 1214.018
SOD	14.21±1.19[a,b]	15.82±0.72[a]	19.07±0.76	***p* = <0.001** F = 365.057
CAT	24.93±1.97[a,b]	27.24±1.29[a]	32.73±1.62	***p* = <0.001** F = 293.559
GSH-Px	112.79±15.19[a,b]	134.41±8.48[a]	176.83±7.14	***p* = <0.001** F = 450.118
TAS	0.88±0.18[a,b]	1.01±0.11[a]	1.65±0.11	***p* = <0.001** F = 460.140
TOS	15.44±1.91[a,b]	12.18±0.92[a]	7.64±0.42	***p* = <0.001** F = 493.985
OSI index	1.86±0.56[a,b]	1.22±0.20[a]	0.47±0.05	***p* = <0.001** F = 204.779

Group 1: relapsing-remitting multiple sclerosis patients with migraine, Group 2: relapsing-remitting multiple sclerosis patients without migraine, Group 3: healthy volunteers. [a]Significant difference with Group 3. [b]Significant difference with Group 2.

Vitamin D-binding protein (VITDBP), vitamin D receptor (VITDR), high-sensitivity C-reactive protein (hs-CRP), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), total antioxidant status (TAS), total oxidant status (TOS), and Oxidative Stress Index (OSI). F: ANOVA. Bold values represent significant findings at $p < 0.05$.

The results of the correlation analyses of Groups 1 and 2 between their EDSS values and values of vitamin D, VITDBP, VITDR, hs-CRP, SOD, CAT, GSH-Px, TAS, TOS, and OSI are shown in Table 3.

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Table 3. Correlation analysis of the EDSS values of Group 1 and Group 2 and their vitamin D, VITDBP, VITDR, hs-CRP, SOD, CAT, GSH-Px, TAS, TOS, and OSI values

	Group 1 (***n*** **= 50)**	**Group 2** (***n*** **= 50)**
Vitamin D	*p* = 0.053 r = - 0.276	*p* = 0.264 r = - 0.161
VITDBP	*p* = 0.874 r = 0.023	*p* = 0.637 r = 0.068
VITVDR	*p* = 0.871 r = 0.024	*p* = 0.222 r = 0.176
hs-CRP	***p* = 0.004** r = 0.399	***p* = < 0.001** r = 0.797
SOD	***p* = 0.038** r = -0.294	***p* = < 0.001** r = -0.600
CAT	***p* = 0.034** r = -0.300	***p* = < 0.001** r = -0.549
GSH-Px	*p* = 0.119 r = -0.223	***p* = < 0.001** r = -0.655
TAS	*p* = 0.236 r = -0.236	***p* = 0.027** r = -0.313
TOS	*p* = 0.210 r = 0.180	***p* = < 0.001** r = 0.684
OSI	*p* = 0.220 r = 0.177	***p* = < 0.001** r = 0.511

Group 1: relapsing-remitting multiple sclerosis patients with migraine, Group 2: relapsing-remitting multiple sclerosis patients without migraine, EDSS: Expanded Disability Status Scale, vitamin D-binding protein (VITDBP), vitamin D receptor (VITDR), high-sensitivity C-reactive protein (hs-CRP), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), total antioxidant status (TAS), total oxidant status (TOS), and Oxidative Stress Index (OSI).

Bold values represent significant findings at $p < 0.05$. All of the parameters were determined as significant (Spearman's correlation analysis).

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4. Discussion

When patients who met migraine criteria who were excluded from the study plan were included in the analysis, migraine coexistence in RRMS was found as 37.8%. It is known that the prevalence of migraine in MS patients (19.8-82%) is higher than in the general population (Nicoletti et al., 2008; Vacca et al.,

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2007). This difference in prevalence rates of migraine in MS patients may be explained by the racial characteristics of the relevant patient populations and the methodological differences among such studies. A recent study of 754 MS patients reported 202 (39%) cases of migraine (Beckmann and Türe, 2019). The prevalence of migraine in MS patients in our study seems to be in agreement with other studies.

The vitamin D, VITDBP, and VITDR values of the RRMS patients without migraine were lower than those of the healthy control group. Additionally, the vitamin D and VITDR values of the RRMS patients with migraine were significantly lower than those of the RRMS patients without migraine. Especially studies on animal models showed that vitamin D contributes to neural plasticity, neuroprotective effects, dopaminergic system physiology, and neural connections (Eyles et al., 2007; Pertile et al., 2016). There is increasing evidence that vitamin D plays a positive role in the regulation of inflammation and oxidative stress by reducing tumor necrosis factor α, interleukine-1, nitric acid synthase, and oxidative stress markers. Vitamin D also decreased neuronal loss by increasing neurotrophic and neuroprotective factors (Mpandzou et al., 2016). In addition, low vitamin D serum levels were found in patients who were affected by neurodegenerative, neuroinflammatory, and neuropsychological diseases (Hiller et al., 2018; Pierrot-Deseilligny and Souberbielle, 2017). It was shown that both vitamin D deficiency and migraine risks vary based on latitudinal differences (Prakash et al., 2010). Lower vitamin D levels were found in patients with migraine, and a relationship was demonstrated between the pain severity of migraine and vitamin D (Song et al., 2018). In addition, one study found that vitamin D combined with a statin decreased the number of attacks in migraine patients (Buettner et al., 2015). Hypovitaminosis D is common in countries with temperate climates due to insufficient sunlight and variable lifestyles. Some epidemiological studies have found high prevalence rates of MS in countries at temperate regions and theorized a relationship between latitude differences and MS risk, assuming that insufficient sunlight and vitamin D deficiency may be the cause of the increased risk of MS in these countries; however, this theory is still under debate (Bezzini and Battaglia, 2017; Pierrot-Deseilligny and Souberbielle, 2017; Zheng et al., 2018). Low vitamin D levels were found in MS patients (Mazdeh et al., 2013), and it was shown that vitamin D intake may reduce the risk of MS (Cortese et al., 2015). It was also determined that the risk of

development of MS in children of mothers with low vitamin D levels in early pregnancy increased by 90% (Munger et al., 2016). When the results of our study are examined in the light of the information in the literature, it may be important in terms of indicating the effect of low vitamin D in the association of MS and migraine. Nevertheless, it remains unclear whether vitamin D deficiency may lead to the coexistence of multiple sclerosis and migraine or if this relationship may cause vitamin D deficiency.

The hs-CRP values in the RRMS patients without migraine were higher than those in the healthy control group. Additionally, the hs-CRP values of the RRMS patients with migraine were significantly higher than those of the RRMS patients without migraine comorbidity. The histopathological analyses of the brain biopsies of post-mortem MS patients demonstrated the presence of lymphoid follicle-like structures in the meninges (Howell et al., 2011; Magliozzi et al., 2010). Findings on meningeal inflammation that contains B-cell and T-cell activation seen in MS patients may explain why the prevalence of headaches is so high at the onset of the disease (Howell et al., 2011; Moreno et al., 2018). Cortical spreading depression is currently one of the most widely accepted notions in migraine formation (Ferrari MD et al., 2015). However, there are still questions that haven't been completely answered. On the other hand, one study showed that cortical spreading depression opens pannexin-1 megachannels. The same study demonstrated that pannexin-1 stimulates parenchymal inflammation through the high mobility group B1 pathway (Karatas H et al., 2013). C-reactive protein was also reported to increase in migraine patients (Avci et al., 2015; Güzel et al., 2013). The relapse rate was stated to increase in MS patients with migraine (Kowalec et al., 2017). Elevated hs-CRP levels could be attributed to the cumulative effects of migraine and MS coexistence, or it could be suggested that migraine could aggravate MS, and several other potential causes could be proposed. Therefore, hs-CRP elevation associated with MS and migraine coexistence cannot be linked to a cause and effect relationship.

RRMS patients without migraine had lower SOD, CAT, and GSH-Px values than the healthy controls. Additionally, the SOD, CAT, and GSH-Px values of the RRMS patients with migraine were significantly lower than those of the RRMS patients without migraine. The body contains a complex antioxidant defense based on endogenous enzymatic and non-enzymatic antioxidants. There are first-, second-, third-,

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and even fourth-line defense mechanisms against free radicals. In humans, SOD, CAT, and GSH-Px are the first-line defense mechanisms (Ighodaro and Akinloye, 2018). A study showed higher morbidity and increased gadolinium involvement in MS patients with the glutathione S-transferase supergene family polymorphism (Mann et al., 2000). Additionally, individuals with migraine had more SOD and CAT polymorphisms that provide less antioxidant protection (Saygi et al., 2015). The results of this study may indicate that disruption in the body's antioxidant system may be an underlying factor in the coexistence of MS and migraine.

The RRMS patients without migraine had worse oxidant and antioxidant values than the healthy controls. Additionally, the oxidant and antioxidant values of the RRMS patients with migraine were significantly worse than those of the RRMS patients without migraine. Oxidative stress is caused by the increased production of reactive oxygen species and failure to sufficiently eliminate these by antioxidants (Yigit et al., 2018). Increased oxidative stress in MS was shown in animal experiments and pathological samples of those with MS (Haider et al., 2011; Juybari et al., 2018; Waslo et al., 2019; Witte et al., 2009). Studies have also shown that reactive oxygen species and oxidative stress contribute to the formation and permanence of MS lesions (Schreibelt et al., 2007; Tasset et al., 2012). Despite considerable data on the mechanisms of the pathogenesis of MS, it was assumed that oxidative stress is a turning point that plays a key role at the center of all mechanisms that lead to neuronal injury and neurodegeneration in MS (Seven et al., 2013). It was reported that acute inflammatory responses caused by oxidative stress may play an important role in the pathogenesis of pain in migraine attacks (Eren et al., 2015; Geyik et al., 2016). Moreover, a significant relationship was found between oxidative changes and migraine (Borkum, 2016; Yigit et al., 2018). Additionally, it was argued that frequent and prolonged migraine attacks will increase oxidative stress even further, possibly resulting in chronic migraine (Ferroni et al., 2018).

In this study, while the EDSS values of the RRMS patients without migraine were strongly correlated with their hs-CRP, GSH-Px, and TOS values, they were correlated on a medium level with SOD and CAT and a weak level with TAS. The EDSS values of the RRMS patients with migraine were only weakly correlated with hs-CRP, SOD, and CAT. This situation could have been caused by the presence of

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migraine, which was independent of the EDDS scores. Therefore, this finding indicates that correlation analysis between EDSS and blood parameters cannot be performed without excluding migraine in MS patients. This finding also indicates that migraine comorbidity causes a higher burden of disease in MS. Comorbid conditions such as hypertension and diabetes are common in MS and have been reported to be associated with increased rates of hospitalization (Marrie et al., 2015), greater disability progression (Marrie et al., 2010), and mortality risk (Marrie et al., 2015) in MS. Comorbid conditions in MS are also associated with poor quality of life (Berrigan et al., 2016). However, migraine comorbidity in MS reportedly leads to an increased number of attacks (Kowalec et al., 2017) and contrast-enhancing lesions (Graziano et al., 2015). When this finding in our study is evaluated in context with the literature, it may raise many questions including whether or not MS patients with migraine comorbidity should be treated differently or more aggressively.

5. Conclusion

The results of our study indicate that migraine comorbidity in MS leads to lower vitamin D levels, increased oxidative stress, and increased hs-CRP levels. Reverse causality cannot be excluded, as these results may have caused the coexistence of MS and migraine, or these values may have led to MS and migraine coexistence. It should be kept in mind that this study included few sets of biochemical analyses that may lead to multiple sclerosis and migraine coexistence, but there may be many more mechanisms that may cause this association. Raising awareness about the coexistence of MS and migraine will lead to new studies. In addition, long-term prospective studies are needed to understand the pathophysiology underlying the coexistence of MS and migraine. If the etiopathogenesis underlying coexistence of MS and migraine can be better understood, new perspectives for the treatment of diseases can be achieved. Finally, few pre-clinical and clinical studies showed the benefits of antioxidant treatments for MS (Waslo et al., 2019). Considering this information and the results of our study, antioxidant treatment may be considered to be more effective in MS patients with migraine, and it may be concluded that further studies on this topic are necessary.

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References

Aebi, H., 1974. Catalase, Methods of Enzymatic Analysis (Second Edition). Academic Press, New York pp. 673–684. doi: 10.1016/B978-0-12-091302-2.50032-3.

Arnold, M., 2018. Headache classification committee of the international headache society (IHS) the international classification of headache disorders. Cephalalgia 38(1), 1-211.

Avci, A.Y., Lakadamyali, H., Arikan, S., Benli, U.S., Kilinc, M., 2015. High sensitivity C-reactive protein and cerebral white matter hyperintensities on magnetic resonance imaging in migraine patients. The journal of headache and pain 16(1), 9. doi: 10.1186/1129-2377-16-9.

Beckmann, Y., Türe, S., 2019. Headache characteristics in multiple sclerosis. Multiple sclerosis and related disorders 27, 112-116. doi: 10.1016/j.msard.2018.09.022.

Berrigan, L.I., Fisk, J.D., Patten, S.B., Tremlett, H., Wolfson, C., Warren, S., Fiest, K.M., McKay, K.A., Marrie, R.A., 2016. Health-related quality of life in multiple sclerosis: direct and indirect effects of comorbidity. Neurology 86(15), 1417-1424.

Bezzini, D., Battaglia, M.A., 2017. Multiple sclerosis epidemiology in Europe, Multiple Sclerosis: Bench to Bedside. Springer, pp. 141-159.

Borkum, J.M., 2016. Migraine triggers and oxidative stress: a narrative review and synthesis. Headache: The Journal of Head and Face Pain 56(1), 12-35.

Buettner, C., Nir, R.R., Bertisch, S.M., Bernstein, C., Schain, A., Mittleman, M.A., Burstein, R., 2015. Simvastatin and vitamin D for migraine prevention: a randomized, controlled trial. Annals of neurology 78(6), 970-981.

Cortese, M., Riise, T., Bjørnevik, K., Holmøy, T., Kampman, M.T., Magalhaes, S., Pugliatti, M., Wolfson, C., Myhr, K.-M., 2015. Timing of use of cod liver oil, a vitamin D source, and multiple sclerosis risk: The EnvIMS study. Multiple Sclerosis Journal 21(14), 1856-1864.

Díaz, C., Zarco, L., Rivera, D.M., 2019. Highly active multiple sclerosis: An update. Multiple sclerosis and related disorders 30, 215-24. doi: 10.1016/j.msard.2019.01.039.

Journal Pre-proof

Eren, Y., Dirik, E., Neşelioğlu, S., Erel, Ö., 2015. Oxidative stress and decreased thiol level in patients with migraine: cross-sectional study. Acta Neurologica Belgica 115(4), 643-649.

Eyles, D., Almeras, L., Benech, P., Patatian, A., Mackay-Sim, A., McGrath, J., Feron, F., 2007. Developmental vitamin D deficiency alters the expression of genes encoding mitochondrial, cytoskeletal and synaptic proteins in the adult rat brain. The Journal of steroid biochemistry and molecular biology 103(3-5), 538-545.

Ferrari, M.D., Klever, R.R., Terwindt, G.M., Ayata, C., van den Maagdenberg, A.M., 2015. Migraine pathophysiology: lessons from mouse models and human genetics. The Lancet Neurology 14(1), 65-80.

Ferroni, P., Barbanti, P., Della-Morte, D., Palmirotta, R., Jirillo, E., Guadagni, F., 2018. Redox mechanisms in migraine: novel therapeutics and dietary interventions. Antioxid. Redox Signal. 28(12):1144-1183.

Gebhardt, M., Kropp, P., Hoffmann, F., Zettl, U.K., 2019. Headache in the course of multiple sclerosis: a prospective study. Journal of Neural Transmission 126(2), 131-139.

Geyik, S., Altunısık, E., Neyal, A.M., Taysi, S., 2016. Oxidative stress and DNA damage in patients with migraine. The journal of headache and pain 17(1), 10.

Ghorbani, Z., Togha, M., Rafiee, P., Ahmadi, Z.S., Magham, R.R., Haghighi, S., Jahromi, S.R., Mahmoudi, M., 2019. Vitamin D in migraine headache: a comprehensive review on literature. Neurological Sciences, 1-19.

Graziano, E., Hagemeier, J., Weinstock-Guttman, B., Ramasamy, D.P., Zivadinov, R., 2015. Increased contrast enhancing lesion activity in relapsing–remitting multiple sclerosis migraine patients. NeuroImage: Clinical 9, 110-116.

Güzel, I., Taşdemir, N., Çelik, Y., 2013. Evaluation of serum transforming growth factor β1 and C-reactive protein levels in migraine patients. Neurol Neurochir Pol 2013;47(4):357-362. doi: 10.5114/ninp.2013.36760.

Journal Pre-proof

Haider, L., Fischer, M.T., Frischer, J.M., Bauer, J., Höftberger, R., Botond, G., Esterbauer, H., Binder, C.J., Witztum, J.L., Lassmann, H., 2011. Oxidative damage in multiple sclerosis lesions. Brain 134(7), 1914-1924.

Hiller, A.L., Murchison, C.F., Lobb, B.M., O'Connor, S., O'Connor, M., Quinn, J.F., 2018. A randomized, controlled pilot study of the effects of vitamin D supplementation on balance in Parkinson's disease: Does age matter? PloS one 13(9), e0203637.

Howell, O.W., Reeves, C.A., Nicholas, R., Carassiti, D., Radotra, B., Gentleman, S.M., Serafini, B., Aloisi, F., Roncaroli, F., Magliozzi, R., 2011. Meningeal inflammation is widespread and linked to cortical pathology in multiple sclerosis. Brain 134(9), 2755-2771.

Ibrahim, I., El Wassefy, M., Metwally, S.S., Elmenshawi, I., 2019. Matrix metalloproteases 9 rs3918242 gene polymorphism and serum vit D in MS Egyptian patients. Multiple sclerosis and related disorders 32, 103-106. doi: 10.1016/j.msard.2019.04.029.

Ighodaro, O., Akinloye, O., 2018. First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid. Alexandria Journal of Medicine 54(4), 287-293.

Juybari K.B., Ebrahimi G., Momeni Moghaddam M.A., Asadikaram G., Torkzadeh-Mahani M., Akbari M., Mirzamohammadi S., Karimi A., Nematollahi M.H., 2018. Evaluation of serum arsenic and its effects on antioxidant alterations in relapsing-remitting multiple sclerosis patients. Multiple sclerosis and related disorders 19, 79-84. doi: 10.1016/j.msard.2017.11.010.

Karatas, H., Erdener, S.E., Gursoy-Ozdemir, Y., Lule, S., Eren-Koçak, E., Sen, Z.D., Dalkara, T., 2013. Spreading depression triggers headache by activating neuronal Panx1 channels. Science 339(6123), 1092-1095.

Kowalec, K., McKay, K., Patten, S., Fisk, J., Evans, C., Tremlett, H., Marrie, R., 2017. CIHR Team in Epidemiology and Impact of Comorbidity on Multiple Sclerosis (ECoMS) Comorbidity increases the risk of relapse in multiple sclerosis. Neurology 89(24), 2455-2461.

Journal Pre-proof

Kurtzke, J.F., 1983. Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). Neurology 33(11), 1444-1444.

Laursen, J.H., Søndergaard, H.B., Sørensen, P.S., Sellebjerg, F., Oturai, A.B., 2016. Vitamin D supplementation reduces relapse rate in relapsing-remitting multiple sclerosis patients treated with natalizumab. Multiple sclerosis and related disorders 10, 169-173. doi: 10.1016/j.msard.2016.10.005.

Lipton, R.B., Bigal, M.E., Diamond, M., Freitag, F., Reed, M., Stewart, W.F., 2007. Migraine prevalence, disease burden, and the need for preventive therapy. Neurology 68(5), 343-349.

Magliozzi, R., Howell, O.W., Reeves, C., Roncaroli, F., Nicholas, R., Serafini, B., Aloisi, F., Reynolds, R., 2010. A gradient of neuronal loss and meningeal inflammation in multiple sclerosis. Annals of neurology 68(4), 477-493.

Mann, C., Davies, M., Boggild, M., Alldersea, J., Fryer, A., Jones, P., Ko, C.K., Young, C., Strange, R., Hawkins, C., 2000. Glutathione S-transferase polymorphisms in MS: their relationship to disability. Neurology 54(3), 552-552.

Marrie, R., Rudick, R., Horwitz, R., Cutter, G., Tyry, T., Campagnolo, D., Vollmer, T., 2010. Vascular comorbidity is associated with more rapid disability progression in multiple sclerosis. Neurology 74(13), 1041-1047.

Marrie, R.A., 2017. Comorbidity in multiple sclerosis: implications for patient care. Nature Reviews Neurology 13(6), 375--382.

Marrie, R.A., Elliott, L., Marriott, J., Cossoy, M., Blanchard, J., Leung, S., Yu, N., 2015. Effect of comorbidity on mortality in multiple sclerosis. Neurology 85(3), 240-247.

Mazdeh, M., Seifirad, S., Kazemi, N., Seifrabie, M.A., Dehghan, A., Abbasi, H., 2013. Comparison of vitamin D3 serum levels in new diagnosed patients with multiple sclerosis versus their healthy relatives. Acta Medica Iranica 51(5), 289-292.

Moreno, M.A., Or-Geva, N., Aftab, B.T., Khanna, R., Croze, E., Steinman, L., Han, M.H., 2018. Molecular signature of Epstein-Barr virus infection in MS brain lesions. Neurology-Neuroimmunology Neuroinflammation 5(4), e466. doi: 10.1212/NXI.0000000000000466.

Journal Pre-proof

Möhrke, J., Kropp, P., Zettl, U.K., 2013. Headaches in multiple sclerosis patients might imply an inflammatorial process. PLoS One 8(8), e69570.

Mpandzou, G., Haddou, E.A.B., Regragui, W., Benomar, A., Yahyaoui, M., 2016. Vitamin D deficiency and its role in neurological conditions: A review. Revue neurologique 172(2), 109-122.

Munger, K.L., Åivo, J., Hongell, K., Soilu-Hänninen, M., Surcel, H.-M., Ascherio, A., 2016. Vitamin D status during pregnancy and risk of multiple sclerosis in offspring of women in the Finnish maternity cohort. JAMA Neurol. 73(5), 515-519.

Nicoletti, A., Patti, F., Fermo, S.L., Liberto, A., Castiglione, A., Laisa, P., Garifoli, A., Naia, F.L., Maimone, D., Sorbello, V., 2008. Headache and multiple sclerosis: a population-based case-control study in Catania, Sicily. Cephalalgia 28(11), 1163-1169.

Paglia, D.E., Valentine, W.N., 1967. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. The Journal of laboratory and clinical medicine. 70(1), 158-169.

Patejdl, R., Zettl, U.K., 2017. Spasticity in multiple sclerosis: Contribution of inflammation, autoimmune mediated neuronal damage and therapeutic interventions. Autoimmunity reviews 16(9), 925-936.

Pertile, R.A., Cui, X., Eyles, D.W., 2016. Vitamin D signaling and the differentiation of developing dopamine systems. Neuroscience 333, 193-203.

Pierrot-Deseilligny, C., Souberbielle, J.-C., 2017. Vitamin D and multiple sclerosis: an update. Multiple sclerosis and related disorders 14, 35-45. doi: 10.1016/j.msard.2017.03.014.

Prakash, S., Mehta, N.C., Dabhi, A.S., Lakhani, O., Khilari, M., Shah, N.D., 2010. The prevalence of headache may be related with the latitude: a possible role of Vitamin D insufficiency? The journal of headache and pain 11(4), 301-7.

Saygi, S., Erol, İ., Alehan, F., Yalçın, Y.Y., Kubat, G., Ataç, F.B., 2015. Superoxide dismutase and catalase genotypes in pediatric migraine patients. Journal of child neurology 30(12), 1586-1590.

Journal Pre-proof

Schreibelt, G., Van Horssen, J., Van Rossum, S., Dijkstra, C.D., Drukarch, B., de Vries, H.E., 2007. Therapeutic potential and biological role of endogenous antioxidant enzymes in multiple sclerosis pathology. Brain research reviews 56(2), 322-330.

Seven, A., Aslan, M., İncir, S., Altıntaş, A., 2013. Evaluation of oxidative and nitrosative stress in relapsing remitting multiple sclerosis: effect of corticosteroid therapy. Folia neuropathologica 51(1), 58-64.

Song, T.-J., Chu, M.-K., Sohn, J.-H., Ahn, H.-Y., Lee, S.H., Cho, S.-J., 2018. Effect of Vitamin D Deficiency on the Frequency of Headaches in Migraine. Journal of Clinical Neurology 14(3), 366-373.

Tasset, I., Agüera, E., Sánchez-López, F., Feijóo, M., Giraldo, A.I., Cruz, A.H., Gascón, F., Túnez, I., 2012. Peripheral oxidative stress in relapsing–remitting multiple sclerosis. Clinical biochemistry 45(6), 440-444.

Thompson, A.J., Banwell, B.L., Barkhof, F., Carroll, W.M., Coetzee, T., Comi, G., Correale, J., Fazekas, F., Filippi, M., Freedman, M.S., 2018. Diagnosis of multiple sclerosis: 2017 revisions of the McDonald criteria. The Lancet Neurology 17(2), 162-173.

Vacca, G., Marano, E., Morra, V.B., Lanzillo, R., De Vito, M., Parente, E., Orefice, G., 2007. Multiple sclerosis and headache co-morbidity. A case-control study. Neurological Sciences 28(3), 133-135.

Waslo, C., Bourdette, D., Gray, N., Wright, K., Spain, R., 2019. Lipoic Acid and Other Antioxidants as Therapies for Multiple Sclerosis. Current treatment options in neurology 21(6), 26.

Witte, M.E., Bø, L., Rodenburg, R.J., Belien, J.A., Musters, R., Hazes, T., Wintjes, L.T., Smeitink, J.A., Geurts, J.J., De Vries, H.E., 2009. Enhanced number and activity of mitochondria in multiple sclerosis lesions. J. Pathol. 219(2), 193-204.

Yigit, M., Sogut, O., Tataroglu, Ö., Yamanoglu, A., Yigit, E., Güler, E.M., Ozer, O.F., Kocyigit, A., 2018. Oxidative/antioxidative status, lymphocyte DNA damage, and urotensin-2 receptor level in patients with migraine attacks. Neuropsychiatric disease and treatment 14, 367-374.

Zheng, C., He, L., Liu, L., Zhu, J., Jin, T., 2018. The efficacy of vitamin D in multiple sclerosis: A meta-analysis. Multiple Sclerosis and Related Disorders 23, 56-61. doi: 10.1016/j.msard.2018.05.008.

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