

Oxidative Stress and Vitamin D as Predictors in Multiple Sclerosis

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ABSTRACT: Multiple Sclerosis (MS) is a multifactorial demyelinating diseases that affect mostly the young and active people. Here, is crucial to identify new strategies in order to slow down the diseases progression and maintain a good functional outcome. Our hypothesis was that the interconnection between anti-oxidant molecules and anti-inflammatory or neuroprotective molecules can act as predictors of diseases progression. In the study were included 36 patients with MS. Inclusion criteria were the following: patients over 18 years old were divided in three groups, 16 relapsing-remitting MS (RRMS) group, 10 secondary progressive MS (SPMS) group and 10 healthy control group. We showed that the vitamin D sufficiency did not improve de EDSS score in the later stage of diseases. Also, we showed that in the early stage (RRMS) the vitamin D status can significantly improve the EDSS and IADL score and may slow down the diseases progression. started with the early stage of diseases (RRMS) we found that catalase activity, an enzyme that act as anti-oxidant, is significantly decreased compare with healthy people, and can be associated with a low level of vitamin D. we concluded that a pro-oxidative and anti-oxidative balance is an important player in the multifactorial mechanism of MS diseases progression and additional prospective studies are needed to determine optimal vitamin D levels that lead to clinical and immunological benefits for patients with MS. Long-term follow-up studies using high-dose vitamin D supplementation are needed to confirm the preliminary results of the studies.

KEYWORDS: Multiple Sclerosis, Vitamin D, anti-oxidative molecules, neuroprotection.

Introduction

Multiple sclerosis (MS) is a demyelinating inflammatory disease of the central nervous system (CNS) of autoimmune nature [1].

MS is a heterogeneous [2] disease and in young adults it is the most common cause of non-traumatic primary neurological disability [3].

A complex and intricate interaction between variate genetic and environmental factors has been considered as the aetiology of MS [4-6].

There are several forms of MS: PPMS (primary progressive MS), SPMS and relapsing-remitting MS (RRMS). A neurodegenerative process is more strongly expressed in SPMS and PPMS than in RRMS where predominates an inflammatory process. MS is connected with demyelination, neuroinflammation, and axonal loss [7].

The attention in last years has been also paid to oxidative stress (OS) which is one of the main factors in MS responsible for demyelination [1,8,9].

In the pathogenesis of MS, oxidative stress has a crucial role, the presence of oxidative damage both in the nervous system and in the blood of patients of MS [10,11].

In post-mortem brains of MS patient, increased protein carbonyls [12], in addition to elevated contents of OS markers in plasma and cerebrospinal fluid (CSF) were found [9,12,13].

According to existing evidence, oxidative stress contributes to oligodendrocyte loss, neuronal damage and myelin degeneration as pathological landmarks of MS [14-17].

There is evidence that patients with MS display a low levels of antioxidant enzymes such as catalase, glutathione peroxidase (GPx) and manganese superoxide dismutase (MnSOD) [1,18-21].

Oxidative stress results as an imbalance between the production of reactive oxygen species (ROS) and the limited capacity of the cellular metabolism to catabolise these radicals and to repair the resulting functional and structural alterations [22].

Reactive nitrogen species (RNS) and ROS results from the activity of tissue macrophages and microglia, are most probably also involved in damaging the tissue in MS [19].

Patients with RRMS exhibit higher levels of oxidative stress compared to healthy controls, and it is a proven fact that lipid and protein oxidation markers do correlate with the overall disability, as evaluated by the Expanded Disability Status Scale (EDSS) [18].

The brain is particularly vulnerable to oxidative damage due to low antioxidant levels, elevated use of oxygen and high phospholipid levels [23].

OS biomarkers may be used for the assessment of the treatment response or the prognosis of exacerbation [24].

Vitamin D shows important immunomodulatory and anti-inflammatory effects [25,26] and some studies have reported possible association between oxidative stress markers and vitamin D level [27,28].

Published data indicate that supplementation with vitamin D3 leads to a lower risk for developing MS [29,30].

In the same line, the antioxidant effect of vitamin D3 seems to be exhibiting a protective role on neurons [31], and this fact has been proposed to be utilised for MS patients to alleviate the progression of the disease [32].

The lipid peroxidation levels and the catalase (CAT) activities were assessed as indices of oxidative status into the lesion site [33].

Vitamin D insufficiency is linked to increased MS activity to the patients with established disease and is a risk factor for developing MS or accelerate the disease progression [34,35].

Vitamin D supplementation positively affects many other metabolic markers and reduces markers of oxidative stress [36,37].

Some publications showed that supplementation with vitamin D might reduce the levels of oxidative stress markers [27,28], while other publications did not reach at the same conclusion [38,39].

Several studies have demonstrated in different conditions the effect of vitamin D deficiency on oxidative stress [40-43], and only a few studies have been performed in MS patients.

The aim of our study was to find potential correlation between level of vitamin D in peripheral blood samples collected from RRMS and SPMS patients. Also, our objective was to identify new potential predictive biomarkers, such as inflammatory and/or anti-oxidant molecules, in RRMS and SPMS.

Material and Methods

This research protocol was approved by the Ethics Committee of the University of Medicine and Pharmacy of Craiova (Registration No. 96/2019), and all the patients signed a written informed consent of acceptance in order to be included in the study. A number of 36 patients with MS were included in this study. The participants were selected based on the

following inclusion criteria: patients over 18 years old were divided in three groups, 16 RRMS group, 10 SPMS group and 10 healthy control group. MS groups were included according to McDonald 2010 criteria for MS diagnostics. As exclusion criteria we used: drug abuse, pregnancy, comorbidities that might increase the systemic inflammation (e.g., metabolic syndrome, diabetes). All patients included in our study were non-smokers. The duration and the severity of the disease were recorded for this study. The severity of MS was quantified using the Instrumental Activities of Daily Living Scale (IADL) and Expanded Disability Status Scale (EDSS).

25-OH vitamin D level was measured in serum MS patients using automated chemiluminescent immunoassay technology (CLIA, Abbott U.S.). Vitamin D sufficiency was considered above or equal to 20ng/dl of 25-OH-vitamin D serum level and vitamin D deficiency less than 20ng/dl. Monocyte and Lymphocyte ratio (MLR) were calculated by dividing monocyte number and lymphocyte number counted using automated flow cytometry analyser.

Catalase activity was analysed in red blood cells lysate obtained from EDTA blood sample. The catalase activity was measured using spectrophotometric method [44], by reading the decreasing of absorbance at 240nm after hydrogen peroxide addition in the sample (UV spectrophotometer, Beckman, U.S.A.). The catalase activity was expressed as unit per mg of haemoglobin (U/mgHb).

Statistical analysis was performed with SPSS version 20 (SPSS Inc. SPSS for Windows, Version 20.0. Chicago, SPSS Inc) and GraphPad Prism version 5.0 (GraphPad for Windows, San Diego, California USA). Descriptive statistics was utilised to assess the characteristic features of the patients, using percentages for categorical variables and mean±standard deviation of the mean (SD) or standard error of mean (SEM) for continuous variables. Group comparisons were performed using Student's t test and chi square test; p-values<0.05 were considered statistically significant.

Results

We found that in our MS study group is a significant difference in the period of onset between patients with relapsing-remitting form and those with secondary progressive form, those with progressive secondary form tending to have the onset more time ago (Figure 1).

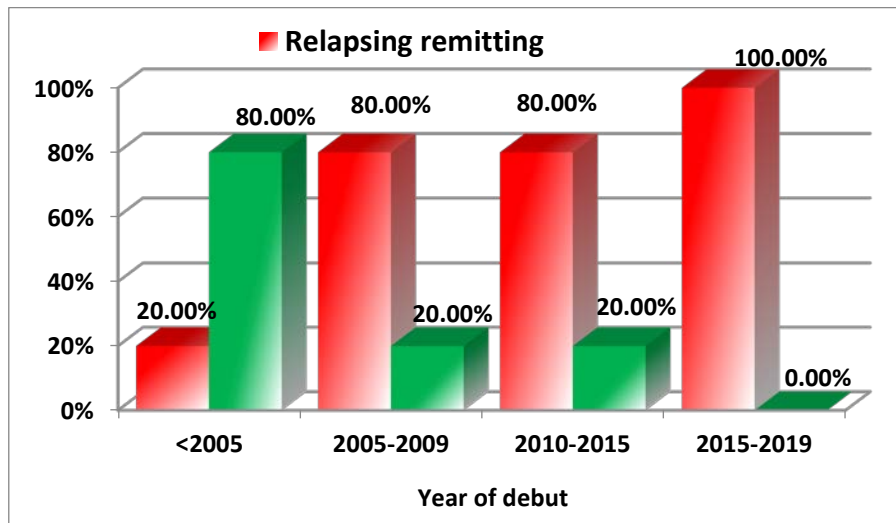


Figure 1. MS Pattern in our study group.

Using the Student's t test, we demonstrated that there is a significant difference between the mean age values measured in patients with recurrent remissive form and those with progressive secondary form, those with progressive secondary form having a higher average than others ($p=0.005<0.05$) (Figure 2).

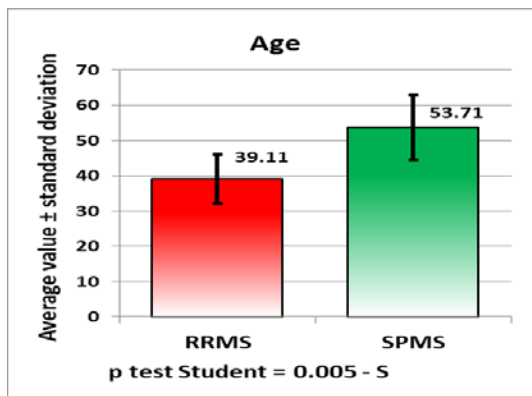


Figure 2. Age mean of RRMS vs. SPMS groups.

Following the Student's t test, it was shown that there was a significant difference between the IADL- $p=0.021$ (Figure 3) measured in patients with recurrent remissive form and those with secondary progressive form and those with recurrent remissive form with a higher mean than the others.

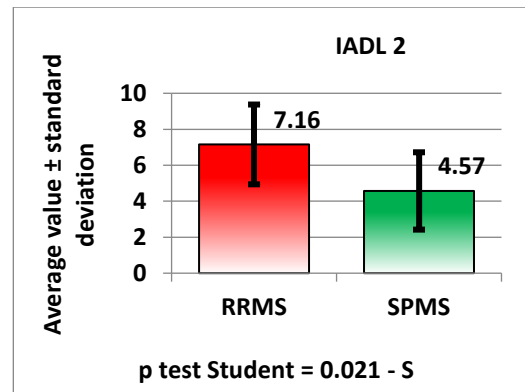


Figure 3. IADL in RRMS vs. SPMS ($p<0.05^*$).

In order to appreciate the immune status in MS subgroups we quantify the monocyte and lymphocyte ratio in MS groups. There is no significant difference between the values of the monocytes/lymphocytes ratio measured in patients with recurrent remissive form and those with progressive secondary form, the two groups having close mean values ($p=0.285>0.05$) (Figure 4).

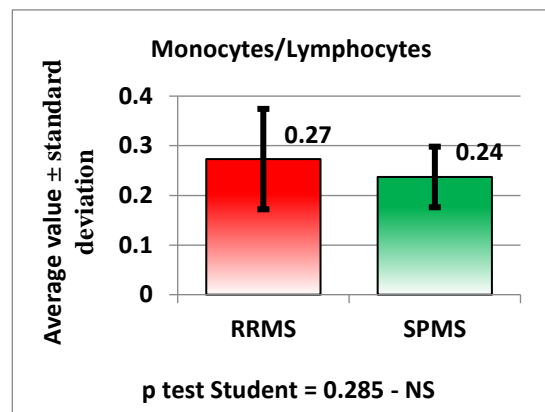


Figure 4. MLR in RRMS vs. SPMS groups ($p>0.05$).

In order to evaluate the impact of vitamin D status on functional outcome of MS groups we divided the RRMS and SPMS groups in vitamin D sufficiency and vitamin D deficiency subgroups, according with 25-OH vitamin D serum level.

In our study groups we found no significant correlation between IADL and vitamin D status ($p=0,29$) but, a significant difference ($p=0.04$) of EDSS between SPMS subgroups (Figure 5).

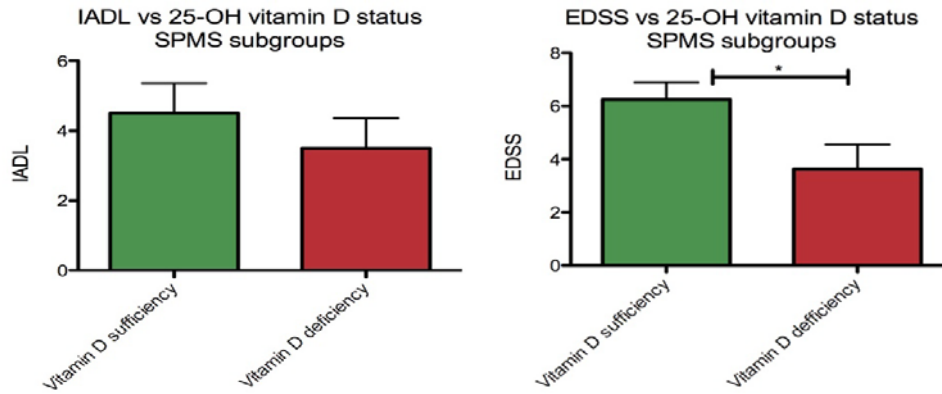


Figure 5. IADL ($p=0,29$) and EDSS ($p=0.04^*$) vs. 25-OH vitamin D level in SPMS subgroups.

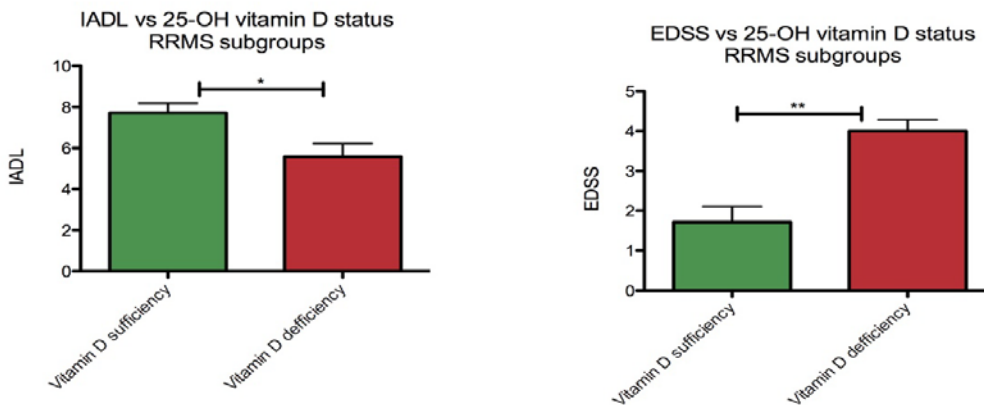


Figure 6. IADL ($p=0,04^*$) and EDSS ($p=0.06^{}$) vs. 25-OH vitamin D level in RRMS subgroups.**

In RRMS group, we showed a significant correlation of both IADL ($p=0.04^*$) and EDSS ($p=0.06^{**}$) in MS subgroups (Figure 6).

The anti-oxidant defense mechanism was quantified using catalase activity in blood sample of MS patients compared with healthy control group.

We found that the catalase activity is significant decreased in RRMS groups compare with control group ($p=0.04^*$) (Figure 7).

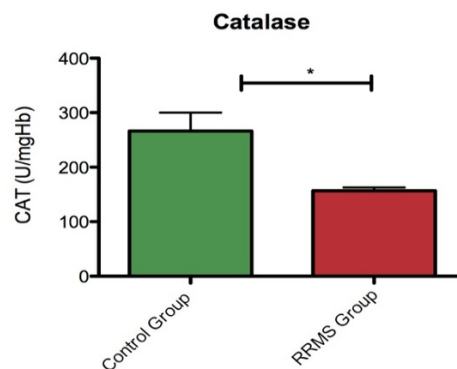


Figure 7. Catalase activity in RRMS group vs. Control Group ($p=0.04^*$).

Discussion

The monocyte-lymphocyte ratio (MLR) is easy to achieve, inexpensive and is related to psychological parameters and readily available components of the blood count. In patients with MS, neutrophils and monocytes have been shown to be prepared for pro-inflammatory activity, considered to be related to the chronic systemic inflammatory environment of this disease [45,46].

In our study, we found no significant difference between RRMS and SPMS group of MLR. These results show that the MLR alone, cannot predict the diseases progression. A limitation of our study is due to a small number of cases included in the study and this can be an explanation. However, our results need to be further validated on a large cohort.

Vitamin D plays a role in innate and adaptive immunity [47].

Longitudinal studies utilizing magnetic resonance imaging have described a correlation between disease activity and low serum levels of vitamin D [48-50], a higher risk of recurrence [51,52] and an increased progression of disability [53,54].

Low levels of vitamin D have also been associated with higher disabilities, assessed by the EDSS score, in some studies [55,56]. van der Mei et al. [57], showed in a study on 136 patients with MS and 272 controls from Australia, that cases with more severe disease (EDSS>3) showed higher vitamin D deficiency compared to the patients with low disabilities.

Thouvenot et al. [54], on a group of 181 MS patients from France, found an indirect correlation between the EDSS scores and the levels of vitamin D for the whole patients' group, however, for the patients with RRMS the levels of vitamin D did correlate only for calculated disability EDSS scores lower than 4.

Long-term oral administration of vitamin D in therapeutic doses might have severe side effects, such as nephrolithiasis, hypercalcemia, or metastatic calcifications [58,59].

Effective therapy may require increased doses of vitamin D or an extension of treatment to achieve a significant effect on clinical symptoms in MS.

Blood levels of vitamin D above 30ng/ml are adequate in opinion of many experts, but for health the best level of vitamin D is uncertain [60].

Levels of vitamin D above 30ng/ml may further help protect patients with MS like suggested by several studies [60].

In the study published by Burton et al., It was found that a high dose (10,000IU/day) of vitamin D3 is safe, with evidence of immunomodulatory effects [61].

Several authors have reported an association between 25 (OH) D levels and the risk of recurrence [62].

Overall, this may indicate that vitamin D does play a role in the onset and activity of the disease in young patients with early MS, inflammatory diseases, but not at a later stage of disability progression.

In our study we showed no significant correlation between IADL and vitamin D status in the later stage of diseases (SPMS).

Interestingly, we found a modest negative correlation of EDSS score and vitamin D status in SPMS group. These results suggest that the vitamin D sufficiency did not improve de EDSS score in the later stage of diseases. Also, we showed that in the early stage (RRMS) the vitamin D status can significantly improve the EDSS and IADL score and may slow down the disease progression.

In addition, vitamin D might show beneficial consequences on the CNS in late stages of MS, either by exhibiting a direct neuroprotective effect, for example by maintaining the epithelial integrity of the blood-brain barrier, decreasing axonal degeneration, or improving nerve fiber myelination. [63-65].

The CNS response to vitamin D may be increased due to neuroinflammation. It remains to be seen whether vitamin D treatment in MS is beneficial for both brain's health and the immune system, bones and muscles of these patients. Anti-oxidant molecule can improve the functional outcome in many other diseases including MS [66-69].

However, started with the early stage of diseases (RRMS) we found that catalase activity, an enzyme that act as anti-oxidant, is significantly decreased compare with healthy people, and can be associated with a low level of vitamin D.

Conclusions

Prospective studies are still needed to assess the best levels of vitamin D that lead to clinical and immunological benefits for patients with MS.

Long-term follow-up studies on high-dose vitamin D supplementation are needed to confirm the preliminary results of the studies.

It may be useful to consider preventive vitamin D supplementation in young patients with MS.

Our findings suggest that a pro-oxidative and anti-oxidative balance is an important player in the multifactorial mechanism of MS diseases progression.

The main limitation of the present study was the small number of patients, and this work should be further expanded by studies based on larger cohorts.

However, MS is a diseases that affect mostly young and active population (age mean of 39 in RRMS group in our study) and here is crucial to identify new strategies in order to slow down the diseases progression and maintain a good functional outcome.

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Conflict of interests

None to declare.

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