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CORRELATIONS BETWEEN QUALITY OF LIFE, OXIDATIVE STRESS AND METABOLIC FACTORS IN MULTIPLE SCLEROSIS

Abstract

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CONTENT

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1. INTRODUCTION

Multiple sclerosis (MS) is a chronic, severe, inflammatory autoimmune demyelinating disease, affecting the central nervous system (CNS), often debilitating and leading to a progressive alteration of neuronal transmission [1] associated with a wide variety of symptoms. MS is a heterogeneous disease [2], and in young adults it is the most common cause of non-traumatic primary neurological disability [3]. The optimal therapeutic intervention and the etiology of the disease are far from being fully understood, although much research has been done in recent decades [4,5].

The etiology of MS has been attributed to a complex interaction between environmental factors and several genetic factors [6-8]. Vitamin D deficiency is widely considered to be an environmental risk factor for MS [10]. Although much research has been done on the role of vitamin D in the risk and progression of MS, there have been conflicting results.

Oxidative stress involves a key pathway that contributes to many pathological processes, including multiple sclerosis (MS) [17,18]. A balance between the production of reactive oxygen species (ROS) and antioxidant defense is crucial to prevent any structural damage to the central nervous system (CNS). In the progression of MS, oxidative stress promotes neurovascular damage and induces a pro-inflammatory state [23,24].

Because oxidative stress and inflammation correlate in a vicious cycle, recent studies have identified biomarkers of oxidative stress and antioxidant capacity as potential tools for assessing inflammatory status in patients with MS [25-27]. MS has several classifications depending on the severity or clinical course. In the literature, the latest classifications group MS into two forms: recurrent remissive form (RRMS) and secondary progressive form (SPMS).

2. CURRENT STATE OF KNOWLEDGE

Symptoms in MS are unpredictable and variable and include fatigue (80% of patients), paresthesias, pain, Lhermitte's sign, muscle weakness, tremor, gait disturbances, dysphagia, visual dysfunction, diplopia, internuclear ophthalmoplegia, cognitive abnormalities and symptoms [28, 29].

The risk of developing multiple sclerosis is higher for people who have or had family relatives with MS, than for those who did not. In patients with MS, due to cognitive impairments (lack of concentration, memory loss, deficits in information processing and reasoning skills affected), critical thinking and judgment are significantly affected. Patients with MS, compared to the general population, report having more severe depression and anxiety [35].

Oxidative stress is caused by an imbalance between the biological capacity of the system to detoxify reactive intermediates or to easily repair the resulting damage and the production of reactive oxygen species (ROS) [52].

Patients with relapsing-remitting multiple sclerosis (RRMS) had higher levels of oxidative stress markers than healthy individuals and that lipid and protein oxidation were correlated with the disability of patients assessed by the Expanded Disability Status Scale (EDSS) [26] . Oxidative stress biomarkers can be used to assess treatment response or exacerbation prognosis [54].

Vitamin D has important immunomodulatory and anti-inflammatory effects [55, 56] and some studies have reported a possible association between oxidative stress markers and vitamin D [57, 58].

Current treatment focuses on reducing inflammation, but only partially on preventing neurodegeneration. Currently, MS treatment is based on immunomodulatory therapy. It is believed that new immunomodulatory drugs may have an influence on the level of oxidative stress in patients with MS [54].

Vitamin D supplementation is a low-cost, low-risk intervention that can potentiate the effectiveness of certain treatments against MS without the risk of causing serious side effects as with other combination therapies [84]. However, it is not known whether supplementation has a significant impact on MS progression.

Research to date strongly suggests that vitamin D supplementation may be useful in the treatment of MS. However, the exact doses to be prescribed to patients with different clinical symptoms are still awaiting determination [90]. Recent human studies of vitamin D supplementation in MS patients suggest that higher doses of vitamin D are more effective in controlling the symptoms and inflammatory markers of the disease.

However, to determine the ideal dose, it is essential to measure serum vitamin D levels before supplementation and to monitor patients by constantly monitoring for side effects. However, it is important to emphasize that the ideal dose may vary from patient to patient.

The diagnosis of MS is based on neurological symptoms and manifestations, along with evidence of damage to the central nervous system that is made following an MRI-type imaging investigation.

Most treatment options for MS are long-term and they usually aim to suppress the immune system. The literature highlights a reduction in the lifespan of people diagnosed with MS between 6-13 years compared to the lifespan of a healthy person.

For patients diagnosed with MS in Romania, an average decrease of 9.5 years is taken into account, respectively a life expectancy of 65.5 years, compared to 75 years, life expectancy in the case of a healthy person.

3. PERSONAL CONTRIBUTIONS

3.1. WORKING HYPOTHESIS AND GENERAL OBJECTIVES

The research topic is very current because it explores and improves recent discoveries in the field of neurological pathology (multiple sclerosis) based mainly on the evaluation of oxidative stress, the role of vitamin D and personal autonomy.

The aim of our study was to find potential peripheral biomarkers showing levels of oxidative stress and antioxidant capacity in peripheral blood samples collected from MS patients in the remission stage.

The general hypothesis is that the evolution of the disease can be slowed down if possible vitamin deficiencies are identified and corrected as early as possible in high-risk patients. Correction of vitamin status modulates the response of the immune system and potentiates the effect of drug therapy.

Thiobarbituric acid reactive substances (TBARS), carbonyl protein levels (PCO) and total antioxidant capacity (TAC) were analyzed in the peripheral blood sample of healthy patients with low-disability MS (EDSS $\leq$ 4) to demonstrate oxidative stress level using conventional plasma markers. Our aim was also to analyze the correlation between markers of oxidative stress and inflammation in the subclinical stage of neuronal damage.

Personal autonomy (PA) consists in the ability to control one's own life associated with the feeling that it is possible to exercise this control and make an informed decision. Personal autonomy is measured by four dimensions: cognitive, behavioral, emotional and value autonomy.

Another hypothesis of our study is that the monocyte / lymphocyte ratio (MLR) is a psycho-neuro-immunological marker; partially modulated by factors such as depression, stress and isolation / social support. Our hypothesis is that MLR plays a role in MS-related disability, contributing to or reflecting on the pro-inflammatory state.

3.2. RESEARCH METHODOLOGY

3.2.1. APPROACHING THE TOPIC

The study was carried out within the Biochemistry Discipline of UMF Craiova between October 2018 and May 2020, having a prospective character. The study included patients admitted to the Neurology Clinic within the Craiova Clinical Hospital for Neuropsychiatry and diagnosed with multiple sclerosis.

The study was approved by the Ethics Commission of the University of Medicine and Pharmacy of Craiova (registration number 96/2019) in accordance with the guidelines of the European Union (Helsinki Declaration). All patients signed the informed consent before taking biological materials to be included in the study after explaining the details and clarifying any ambiguities.

1. Oxidative stress and the interdependence of inflammation in MS

This study included 41 adult subjects, of whom 26 were diagnosed with MS and 15 were healthy patients. The distribution by groups was made as follows: i) a group was composed of 16 subjects with RRMS (age 38.9 ± 7.08; first 10 years of disease progression and EDSS≤ 4); ii) another group of 10 subjects with SPMS (more than 8 years of disease progression and EDSS> 2); iii) the control group consisted of 15 healthy adult subjects with a median age appropriate (age 37.1 ± 11.2).

2. Personal autonomy - predictor of quality of life for patients with MS

A total of 26 patients with MS were included in this study. The demographic variables were: age, sex, marital status, occupation, level of education, urban / rural environment.

The duration and severity of the disease were considered in this study. EDSS was used to quantify the severity of MS. Patients' activities were monitored before and after standard immunomodulatory therapy, and autonomy was observed and quantified.

3. Oxidative stress and vitamin D as predictors in multiple sclerosis

A total of 36 patients with MS were included in this study. All patients included in our study were non-smokers. The duration and severity of the disease were recorded for this study. The severity of MS was quantified using the Instrumental Activities of Daily Living (IADL) and the Expanded Disability Status Scale (EDSS).

3.2.2. MATERIAL AND METHOD

1. Oxidative stress and the interdependence of inflammation in MS

1.1 Collection and handling of samples

1.2 Test of thiobarbituric acid reactive substances

To analyze the level of lipid peroxidation, we performed plasma analysis of thiobarbituric acid reactive substances (TBARS) using a UV spectrophotometric method [94,95].

1.3 Analysis of carbonylated proteins

Carbonyl proteins can be generated by the irreversible oxidation of several side chains of amino acids (lysine, arginine, threonine and proline) in the structure of proteins or by the increased production of advanced glycation end products. The current successful method used to assess carbonylated protein concentration (PCARB) is a spectrophotometric analysis using 2,4-dinitrophenylhydrazine (DNPH) [95,97,98].

1.4 Analysis of total antioxidant capacity (TAC)

TAC scan is usually used to assess the antioxidant status in human samples associated with various diseases. TAC assessment shows the body's overall ability to fight oxidative stress by making antioxidant compounds. This ability can be easily assessed in human plasma using a spectrophotometric method [95,100].

1.5 Analysis of 25 (OH) serum vitamin D

Serum samples were stored at -40 ° C and protected from direct exposure to sunlight until analyzed. The level of 25-OH colecalciferol was measured in MS patients using automated chemiluminescence immunoassay technology (CLIA, Abbott S.U.A.).

1.6 Catalase activity

Catalase activity was analyzed after erythrocyte lysis (EDTA). Catalase activity was measured using the spectrophotometric method.

1.7 Neutrophil/lymphocyte ratio

The neutrophil / lymphocyte ratio (NLR) was calculated by dividing the number of neutrophils by the number of lymphocytes [101].

1.8 Monocyte/lymphocyte ratio

The monocyte / lymphocyte ratio (MLR) was calculated by dividing the number of monocytes by the number of lymphocytes.

1.9 Statistical analysis

Data were analyzed using GraphPad Prism 5.0 software. Comparison of oxidative stress markers between groups was performed using the unpaired Mann - Whitney t-pair test. The non-parametric Pearson was calculated to test the correlation between biochemical and clinical variables (EDSS). P values below 0.05 ($p \leq 0.05$) were selected as significant changes. Data are calculated as mean ± standard error of the mean (SEM).

2. Personal autonomy - predictor of quality of life for patients with MS

2.1 15D-Instrument

Health-related quality of life (HRQoL) was assessed with the general instrument 15D which was validated in patients with chronic pain [102]. In the current study, the Romanian version of 15D was used [104,105]. The generated 15D score is a number with a single index on a 0-1 scale, where 0 indicates death and 1 refers to perfect health.

2.2 Personal autonomy questionnaire (PAQ)

PAQ is a Romanian questionnaire with 36 articles designed to assess four dimensions of PA: cognitive (9 items), behavioral (11 items), emotional (8 items) and value autonomy (8 items), where higher scores reflect autonomy greater personal [106].

2.4 Statistical-mathematical data processing

The data were analyzed and processed using Microsoft Excel (Microsoft Corp., Redmond, WA, USA), together with the XLSTAT 2014 add-on for MS Excel (Addinsoft SARL, Paris, France) and IBM SPSS Statistics 20.0 (IBM Corporation, Armonk, NY, USA).

The data obtained were recorded in Microsoft Excel files, in order to analyze the relationships between clinical and paraclinical data of patients and then processed statistically.

In MS Excel the data were processed using the descriptive analysis of the batch according to different parameters, and their graphical representation was made using the commands Functions-Statistical, Chart, Pivot Tables and functions in the Data Analysis menu. To perform complex statistical tests (Z test for proportions, Kruskal-Wallis test, Chi square test, calculation of rho Spearman correlation coefficient), and data normality tests (Shapiro-Wilks and Anderson-Darling) we used commands from the XLSTAT module using the SPSS program.

To characterize the numerical data used in this paper, we used the fundamental statistical indicators: standard deviation and arithmetic mean, as well as scattering indicators, minimum, maximum, median, quartiles (percentiles).

3.3. RESULTS

Clinical and biological characteristics

Patients with MS included in this study are in the recurrent-remissive stage of diseases with low level of disability (EDSS = 1-4). Hyperactivation of the immune system response can be monitored using a blood marker such as erythrocyte sedimentation rate (ESR) and neutrophil/lymphocyte ratio (NLR).

Neurophilic/lymphocyte ratio (NLR)

Currently, NLR has been described as a new biomarker associated with inflammation, which is easy to achieve and accurate. NLR was significantly higher in the MS group than in the control group ($p = 0.0025$).

The plasma oxidative stress marker was significantly increased in MS patients compared to the control group. In contrast, total antioxidant capacity (TAC) decreased significantly ($p = 0.04$) in patients with MS compared to the control group.

Correlation of oxidative stress status with EDSS and inflammatory status

Interestingly, we found a significant positive correlation between TAC level and NLR ($p = 0.02$) in the plasma of the MS patient. We did not find any significant correlation between EDSS and NLR ($p = 0.05$) or ESR and NLR ($p = 0.79$) in patients with low-disability MS (EDSS≤4).

ROC curve

We performed an analysis using the ROC curve to evaluate the value of some biomarkers of inflammation and oxidative stress in the group of patients with MS. Interestingly, we found TBARS as the most specific marker of oxidative stress with the highest accuracy (AUC = 0.94) followed by the PCARB level (AUC = 0.86). NLRs have a moderate accuracy (AUC = 0.82) in MS in the early stage. In contrast, CT scan has a low diagnostic accuracy (AUC = 0.71) at this stage of the disease.

The mean duration of MS was 9.5 years (SD = 5.12), with no significant differences between the two groups.

The mean QoL of MS patients was 0.66 (SD = 0.18) before a new treatment and 0.71 (SD = 0.16) after the new treatment, with no significant differences between the two groups. The same level of personal autonomy was described by both RRMS patients and those with SMSP (p = 0.357).

Changes in 15D scores before and after a new treatment

In the post-treatment group, we observed a statistical improvement of QoL (p <0.001). The overall QoL was better after treatment than before it started. After treatment, patients with MS had a significantly higher average 15D clinical score than before treatment. Age and disease severity were negatively correlated, while education was positively correlated with QoL. Moreover, no type of autonomy was significantly linked to QoL.

Vitamin D and catalase

In our study groups we did not find any significant correlation between IADL status and vitamin D (p = 0.29), but, a significant difference (p = 0.04) between EDSS and SPMS subgroups. In the RRMS group, we showed a significant correlation of both IADL (p = 0.04 *) and EDSS (p = 0.06 **). We found that catalase activity is significantly low in the RRMS group compared to the control group (p = 0.04 *).

4. DISCUSSIONS

As far as we know, this is the first study to evaluate the association of NLR and oxidative stress in patients with MS. In this study, we found that patients with MS show a significant increase in plasma oxidative stress markers (TBARS and PCARB) suggesting that the induction of oxidative stress began in the RRMS stage with a low level of irreversible neuronal damage.

According to other research studies [27,51], we found a low antioxidant capacity that exposes brain tissue to neuronal damage. We also showed that the plasma CT marker is significantly correlated with the NLR inflammation marker in patients with RRMS and may be useful in the clinical management of diseases.

We found that NLR did not correlate with EDSS and has a single mean specificity in MS diseases, but these findings should be validated on a large cohort. Elevated levels of TBARS were found in patients with RRMS, but did not correlate with inflammation.

Interestingly, the TBARS test displayed the highest specificity in RRMS and may be a useful tool in the management of MS disease. In contrast, we found a significant correlation of EDSS with TAC in patients with SPMS and we did not find a significant correlation with PCARB and TBARS in the stage of this disease. One possible explanation is due to the limited number of patients included in this study.

To support these findings, another PCARB oxidative stress marker was significantly increased in patients with MS. In this study, we found that PCARB has an increased specificity (after TBARS) in MS and did not correlate with NLR.

The SMSP form represents the transformation of the RRMS form after about 10 years. However, there are no criteria to determine when RRMS will turn into SPMS. In this study, patients with RRMS and SPMS reported the same level of quality and personal autonomy.

The literature review showed a lack of research studies on the differences between RRMS and SPMS patients in terms of quality of life or personal autonomy. Little is known about the quality of life and personal autonomy of MS patients in relation to the subtype of the disease.

Our study is a pilot study designed to identify a valuable tool that can be used in assessing QoL in a particular cultural setting to improve health policy strategies.

According to the classification by Alanne et al. from differences in 15D scores [125], our study demonstrated an improvement with a much better change of 0.05 in the 15D score, although not for all dimensions evaluated by the 15D instrument.

In our study, we used two of the few valid instruments available, namely the 15D instrument and the PAQ, which allow a large number of measurements with a single instrument. The main finding of this study is that after starting a new treatment that increases QoL, the score for patient autonomy does not influence QoL.

At the same time, personal autonomy was only partially associated with value autonomy with better results for regular activities such as employment, studies, household chores, leisure activities.

In our study, we did not find any significant difference between RRMS and SPMS compared to MLR. These results show that MLR alone cannot predict disease progression. A limitation of our study is due to a small number of cases included in the study and this may be an explanation. However, our results need to be further validated on a large cohort.

In our study we did not find any significant correlation between IADL and vitamin D in patients with SPMS. Interestingly, we found a modest negative correlation of EDSS score and vitamin D status in the SPMS group. These results suggest that vitamin D adequacy did not improve the late-stage EDSS score. We also showed that in the early stage (RRMS) the status of vitamin D can significantly improve the score of EDSS and IADL and can slow the progression of the disease.

However, starting with the early stage of the disease (RRMS), we found that the activity of catalase, an enzyme that acts as an antioxidant, is significantly lower compared to healthy people and can be associated with low levels of vitamin D.

5. CONCLUSIONS

Improved protection of the CNS through a sustained antioxidant capacity, plays a crucial role in the management of the patient with MS which aims to limit irreversible neuronal damage.

We found an increased level of markers of oxidative stress and a low level of TAC starting from subclinical neuronal damage.

NLR is an easy-to-achieve biomarker that is accurate and can show the proinflammatory status of patients with MS.

Vitamin D levels in MS patients were significantly lower than in healthy subjects, a significant association was also found between vitamin D level and degree of disability.

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

In our opinion, a multimodal approach is needed to be added to MS management starting with the first 10 years of disease progression to improve functional outcome.

However, our study is limited to a small number of patients and needs to be further explored in a large cohort.

Value autonomy, which contributes to QoL, influences the normal activities of patients with MS.

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