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Original Article

Is there an association between the Expanded Disability Status Scale and inflammatory markers in multiple sclerosis?

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Abstract

Background: Multiple sclerosis (MS) is a chronic autoimmune demyelinating disease characterized by inflammation of white matter in the central nervous system. It has been indicated that this inflammation causes increased levels of proinflammatory cytokines. Therefore, we aimed to evaluate if there is a possible association between inflammatory markers and the Expanded Disability Status Scale (EDSS) score in patients with MS.

Methods: We reviewed the data of 127 patients (91 women and 36 men) who were retrospectively diagnosed as MS according to the revised Mc Donald's criteria who were seen at our facility between January 2007 and December 2012. Patients were divided into two groups according to EDSS score: Group 1, EDSS < 5; and Group 2, EDSS ≥ 5. The risk factors that were evaluated included age and sex of the patients, duration of MS, drugs, thyroid function tests, vitamin B12 levels, homocysteine levels, immunoglobulins (Ig) A, G, and M, rheumatoid factor, complement 3 and 4, antistreptolysin O, C reactive protein (CRP), white blood cell count, and neutrophile-lymphocyte ratio (NLR).

Results: There was a statistically significant difference between the groups in terms of age, duration of the disease, drug received, Ig M, free T3, serum homocysteine levels, CRP, and NLR ($p < 0.05$). Pearson's correlation analysis showed a significant correlation between age, duration of MS, IgM, serum homocysteine levels, CRP, and NLR. According to the receiver operating characteristic curve analysis, IgM and NLR were discriminative factors in patients in Group 2.

Conclusion: According to this study, inflammation may have a role in the pathogenesis of MS and in patients with EDSS > 5. Additionally, NLR and CRP levels may be discriminative factors of adverse clinical outcomes.

Keywords: expanded disability status scale; inflammation; multiple sclerosis

1. Introduction

Multiple sclerosis (MS) is a chronic degenerative disease of the central nervous system with multiple focal demyelinated lesions of the white matter.[1] The initial and precise etiology of MS remains unknown and is probably multifactorial.[2] It is debatable whether inflammation initiates neurodegeneration or neurodegeneration occurs independently of inflammation.[3] This inflammatory process is thought to be due to an interaction between T cells, B cells, macrophages, dendritic cells, and endothelial cells.[4] Previous studies reported increased levels of proinflammatory cytokines in MS lesions such as interferon-gamma, interleukin (IL)-17, IL21, IL23, and tumor necrosis factor-alpha.[5–7] The inflammation biomarker neutrophile-lymphocyte ratio (NLR) has been reported to be cost-effective, readily available, and easy to calculate.[8] Cytokines including growth factors or ILs may also contribute to the accumulation of neutrophils.[9] Increased neutrophil levels inhibit the lymphocyte activity and stimulate lymphopenia by

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increasing lymphocytes apoptosis.[10] This is the physiological immune response of circulating leukocytes to various stressful events such as inflammation or malignancy which is characterized by an increased neutrophil count and decreased lymphocyte count.[11] Expanded disability status scale (EDSS) is a scale that is used to evaluate the levels of MS. EDSS is a 20-step scale of disease severity ranging from 0 (normal) to 10 (death due to MS).[12] Although EDSS is the most frequently used scale to assess MS severity, the Multiple Sclerosis Severity Score combines the EDSS and disease duration, and is also used to determine the different EDSS scores in patients with similar disease duration.[13]

The aim of the study was to clarify the relationship between patient NLR and its association between EDSS and MS.

2. Methods

2.1. Ethics statement

The study was approved by the Ethics Committee of Ankara Numune Research and Education Hospital. Owing to the retrospective study design, informed consent was not necessary.

2.2. Study population and design

The present study was a retrospective review of MS cases between January 1, 2007 and December 31, 2012 in the Ankara Numune Research and Education Hospital. This is a tertiary referral hospital in the capital city of Turkey. A total of 127 patients (91 women and 36 men) with MS were included in the study. A diagnosis of MS was made according to the revised Mc Donald's criteria.[14] Patients with autoimmune diseases, acute disseminated encephalomyelitis, and chronic inflammatory diseases such as brucellosis and stroke were excluded from the study.

Clinical information included age and sex of the patients, duration of MS, drugs, thyroid function tests, vitamin B12 levels, homocysteine levels, immunoglobulin (Ig) A, G, and M, rheumatoid factor, complement 3 and 4, antistreptolysin O, C reactive protein (CRP), white blood cell count, and NLR. The blood samples were obtained during patient MS flare-up events. Thereafter, patients were divided into two groups according to their EDSS score: Group 1 patients had an EDSS score < 5, and Group 2 patients had an EDSS score ≥ 5.

2.3. Statistical analysis

Mean and standard deviation were calculated for continuous variables, and the normality of the variables was analyzed by use of the Kolmogorov–Smirnov test. We also used the independent samples *t*-test and Chi-square test to evaluate the associations between the categorical and continuous variables. All variables were included in the backward stepwise regression procedure. Additionally, Pearson's correlation coefficient was used for the association between clinical parameters. The receiver operator characteristic (ROC) curve analysis was used to establish the cut-off values for CRP and NLR. Two-sided *p* values were considered statistically significant at $p < 0.05$. Statistical analyses were carried out using the statistical packages for SPSS 15.0 for Windows (SPSS Inc., Chicago, IL, USA).

3. Results

The patients were divided into two groups according to their EDSS score. Group 1 had 80 patients, with a mean age of 40.18 ± 9.25 years. In Group 2, there were 27 patients with a mean age of 42.43 ± 9.78 years ($p < 0.05$). Table 1 shows the demographic and clinical characteristics of the patients. The mean duration of MS was 4.78 years in Group 1 and 8.5 years in Group 2; this difference was statistically significant between the groups ($p < 0.001$). There was no statistically significant difference between the groups in terms of sex, thyroid stimulating hormone levels, vitamin B12 levels, complement 3 and complement 4, antistreptolysin O, and rheumatoid factor ($p > 0.05$). The mean IgM, homocysteine, CRP, and NLR levels were significantly higher in Group 2 ($p < 0.005$). In Group 1, 27 (72.9 %) patients and in Group 2, 83 (95.4 %) patients took medication. There was a statistically significant difference between the groups in terms of medications (Table 1, $p < 0.05$). Correlation analysis showed that there was a correlation between EDSS and age, IgM, homocysteine, CRP, and NLR (Table 2). It also appeared that ROC curve analysis (Fig. 1) demonstrated that CRP and NLR levels may be

Table 1
The demographic and clinical differences between the groups.

	Patients with EDSS ≤ 5 ($n = 90$)	Patients with EDSS > 5 ($n = 37$)	p*
Age mean ± SD	40.18 ± 9.25	42.43 ± 9.78	0.054
Sex			0.137
Male	33	17	
Female	57	20	
Duration of MS (y)	4.78 ± 2.66	8.50 ± 5.02	<0.01
Drugs			0.026*
No drugs	22	4	
Interferon-β 1a	33	7	
Glatiramer acetate	14	4	
Interferon-β 1b	11	5	
Mitoxantrone	0	7	
Prednisolone	3	0	
IgA (mg/dL)	228.77 ± 109.74	203.42 ± 48.01	0.987
IgM (mg/dL)	177.38 ± 80.90	120.00 ± 52.93	0.041
IgG (mg/dL)	1149.08 ± 258.96	1081.75 ± 341.02	0.427
TSH (μIU/mL)	1.90 ± 1.31	1.83 ± 1.19	0.799
FT3 (pg/mL)	3.00 ± 0.67	2.62 ± 0.83	0.038
C3 (mg/dL)	130.52 ± 20.96	126.25 ± 27.60	0.901
C4 (mg/dL)	27.20 ± 9.86	26.87 ± 9.43	0.453
Homocysteine (μmol/mL)	9.26 ± 5.65	15.64 ± 6.06	0.003
CRP (mg/dL)	3.42 ± 1.56	10.62 ± 18.76	<0.001
NLR	2.12 ± 0.87	4.88 ± 3.71	<0.001

C = complement; CRP = C-reactive protein; EDSS = Expanded Disability Status Scale; FT3 = free T3; Ig = immunoglobulin; MS = multiple sclerosis; NLR = neutrophil-lymphocyte ratio; SD = standard deviation; TSH = thyroid stimulating hormone.
* p was calculated using Chi-square test.

Table 2
Correlation between Expanded Disability Status Scale and age, immunoglobulin-M, homocysteine, C-reactive protein, and neutrophil-lymphocyte ratio.

	Age	IgM	Homocysteine	CRP	NLR
EDSS	CC = 0.299 $p < 0.001$	CC = −0.322 $p = 0.033$	CC = 0.401 $p = 0.006$	CC = 0.224 $p = 0.021$	CC = 0.346 $p < 0.001$

CC = correlation coefficient; CRP = C-reactive protein; EDSS = Expanded Disability Status Scale; IgM = immunoglobulin-M; NLR = neutrophil-lymphocyte ratio.

discriminative parameters. The area under curve, cut-off values, and sensitivity and specificity are shown in Table 3. The cut-off value (sensitivity % − specificity %) of CRP was 6.63 mg/dL (96.1−71.4) and for NLR was 4.52 (96.1−57.1).

4. Discussion

In the current study, we hypothesized that inflammation may have a role in the pathogenesis of adverse clinical outcome in MS prognosis. Therefore, we designed this study to indicate if inflammation in MS patients in Group 2, which is an adverse clinical form of the disease, is higher than the Group 1 patients by detecting inflammatory markers such as CRP and NLR. We divided our patient population into two groups according to EDSS number, and there was no statistically significant difference between the groups in terms of demographic features. Overall, Group 2 levels were demonstrated to be significantly higher. IgM and homocysteine levels were higher in Group 2. In correlation analysis, we found a positive correlation between age and EDSS. This showed that as the duration of MS and age increases, the EDSS score also increases. As expected, CRP and NLR levels were also higher in this group, and ROC curve demonstrated that these markers may be discriminative for adverse clinical outcomes.

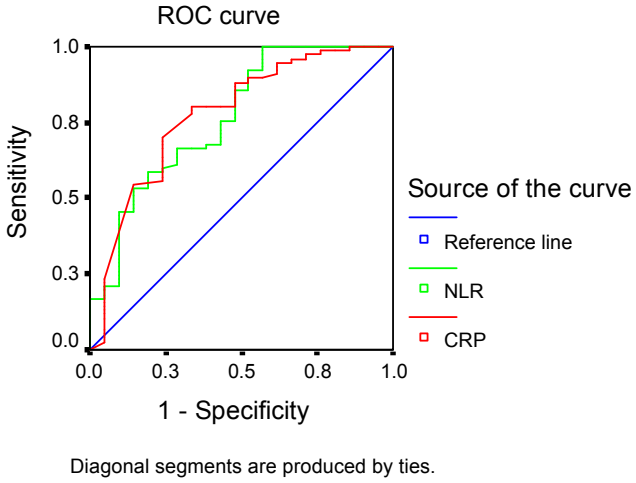

Fig. 1. The area under the curve for C-reactive protein and neutrophil-to-lymphocyte ratio levels in patients with Expanded Disability Status Scale > 5. CRP = C-reactive protein; NLR = neutrophil-lymphocyte ratio; ROC = receiver operating characteristic.

Table 3
The area under the curve, cut-off values, and sensitivity and specificity for C-reactive protein and neutrophil-lymphocyte ratio levels in patients with Expanded Disability Status Scale > 5.

	AUC	SE	95% CI	Cut-off value	Sensitivity (%)−Specificity (%)
CRP	0.778	0.061	0.658−0.897	6.63	96.1−71.4
NLR	0.766	0.062	0.645−0.887	4.52	96.1−57.1

AUC = area under the curve; CI = confidence interval; CRP = C-reactive protein; NLR = neutrophil-lymphocyte ratio; SE = standard error.

4.1. Limitations

There were limitations to our study. The primary limitations were the retrospective design of the study, and the lack of cerebrospinal fluid evaluation.

To the best of our knowledge this is the first study evaluating the association between EDSS and inflammatory markers in MS. The etiology of MS is currently unsettled and presumably multifactorial. Similar to other multifactorial diseases, there is no comprehensive overview of the events that lead to affliction with MS. Currently, the most widely accepted etiological factors are immune-mediated response, genetic transformation, and neuroinflammation that causes neurodegeneration.[3] Previous studies have demonstrated that proinflammatory cytokines are increased in progressive MS.[5–7] Therefore, it is hypothesized that neuroinflammation may be a key factor for disease progression and axonal damage.[14] Our results also may indicate a role of inflammation in adverse outcomes of MS.

In this study, we hypothesized that inflammation may be a factor in MS and should be a discriminative factor in MS patients with higher EDSS scores. The clinical information of 127 patients with MS was evaluated and classified according to patient EDSS scores. As a result, we found that the mean IgM and homocysteine levels, and CRP and NLR levels were significantly higher in patients with EDSS $\geq$ 5 (Group 2). The ROC curve analysis demonstrated that both CRP and NLR levels were discriminative factors for disease progression.

The association between MS and inflammation has been previously expanded upon in many earlier studies. Clark and Coker[15] reported generalized inflammation in MS patients with cortical demyelinization and white matter injury. Slavin et al[16] described that multifocal inflammation in white matter and spinal cord caused relapse remitting episodes, and generalized inflammation caused neurodegeneration and axonal loss.

In order to evaluate the association between MS and inflammation, various studies have assessed the role of proinflammatory cytokines. Transforming growth factor-β is a proinflammatory cytokine;[16] in animal models of neuroinflammation and neurodegeneration, the presence of this cytokine has been demonstrated.[17] Serum amyloid A is an inflammatory marker, and has been evaluated in relapse remitting MS patients; elevated serum amyloid A levels were found in these patients.[18,19] We also found that inflammatory markers such as CRP and NLR were higher in MS and in patients with an EDSS score > 5, and these markers were discriminative for adverse outcomes.

CRP, an acute-phase protein in blood, rises in response to inflammation.[20] Yoon et al[11] reported that CRP may be a useful marker for the detection of inflammation-mediated MS relapses. Physiological immune response of circulating leukocytes to various stressful events such as inflammation or malignancy which is characterized by an increased neutrophil count and decreased lymphocyte count and as a result increased NLR.[11]

Overall, our study suggests that inflammation may have a role in MS pathogenesis. In patients with adverse outcomes, proinflammatory components such as CRP and NLR may be discriminative and promising markers. However, further studies involving more participants of a randomized and controlled nature will further strengthen this hypothesis.

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