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Evaluation of Serum Interleukin-10, Tumor Necrosis Factor Alpha Level and Cardiovascular Autonomic Functions in Multiple Sclerosis Patients

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Abstract **Research Article**

In relapsing-remitting multiple sclerosis (RRMS), the autonomic nervous system (ANS) can be affected and may deteriorate the disability of patients. Evaluation and management of autonomic dysfunction may improve quality of life of patients. We aimed to assess the autonomic dysfunction via heart rate variability (HRV) along with serum tumor necrosis factor-α (TNF-α) and interleukin-10 (IL-10) levels and disclose the correlations between HRV and levels of these two chief biomarkers in the immunopathology of MS, if any. Thirty-six consecutive RRMS patients who had an EDSS score of 0-3 EDSS, not in the relapse period and receiving interferon beta treatment were compared with age and gender-matched 37 healthy subjects in terms of HRV, serum TNF- and IL-10 levels. ANS was evaluated by frequency-based HRV analysis. TNF-α and IL-10 levels in serum were measured by the ELISA method. The mean serum TNF-α level was found to be higher in the RRMS group compared to the controls (p=0.010) but not the IL-10 (p=0.726). HRV parameters were significantly lower in the RRMS patients compared to the controls. No correlation was found between the inflammatory markers and HRV parameters in patients with MS. We found high levels of TNF-α, known to correlate with the severity and progression of MS, along with low levels of HRV in our patient group when compared with controls. Our results show that neurodegeneration and autonomic dysfunction can be present even in patients with RRMS with a low level of disability.

INTRODUCTION

Multiple sclerosis (MS) is an inflammatory and neurodegenerative disease that involves an intricate interaction between the central nervous system and the immune system [1]. The pathological course of the disease is heterogeneous and involves an early, predominantly inflammatory demyelinating disease phase of relapsing–remitting MS (RRMS), which, over a variable period, evolves into a progressively degenerative stage associated with axonal loss and scar formation, causing physical and cognitive disability [2]. In the majority of MS patients, the disease initially takes a relapsing–remitting course, characterized by acute symptomatic relapses followed by periods of variable recovery. In the absence of treatment, more than 50 % of patients with RRMS will develop progressive disability after approximately 15 years [3,4]. Some assessment tools have been developed to assess the clinical severity and progression to disability in MS patients. These assessments have been used as clinical endpoints in MS clinical trials. Two frequent assessment tools, the Expanded Disability Status Scale (EDSS) and the Multiple Sclerosis Functional Composite (MSFC), are commonly used by clinicians for reporting primary and secondary endpoints in MS. The MSFC is considered more sensitive than EDSS in detecting the progression of MS disease. However, when both assessment tools were used in the same clinical trial, EDSS seemed to change more frequently than MSFC [5].

One of widespread and important cause of disability of MS patients is autonomic dysfunction (AD) presented in 45-84%. Activity of the disease seems to affect the parasympathetic and sympathetic parts of the autonomic system in different patterns. AD including sweating abnormalities, urinary dysfunction, orthostatic dysregulation, gastrointestinal symptoms, and sexual dysfunction are frequent complications that reduce the quality of life of affected patients [6,7]. In clinical trials, assessment of heart rate variability (HRV) as a non-invasive test can be used for the evaluation of status of autonomic nervous system. It is an indirect measurement of

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R-R intervals between successive pulses. It represents the amount of variability that occurs between pulses [8].

Biomarkers in MS might assist with diagnosis, prediction of disease course, or identification of response outcome to treatments. Despite the need for biomarkers and extensive research to identify them, validation and clinical application of biomarkers is still an unmet need in multiple sclerosis and the biomarker research field is very active in MS. Disease activity biomarkers can be used in conjunction with clinical and radiological information to identify patients in need of treatment because of severe disease courses or, conversely, patients who can be left untreated because of benign or mild disease courses. Despite the large numbers of candidate molecular biomarkers proposed, very few biomarkers have been rigorously validated and used in clinical practice [9]. Tumor necrosis factor-alpha (TNF-α) and interleukin-10 (IL-10) as immunomodulatory cytokines play important roles in the development of MS. Based on strong basic science data implicating TNF signaling in contributing to MS disease severity, the effects of manipulation of the TNF pathway were investigated in several studies. TNF-α-triggered disease can also exhibit classical features of autoimmunity, specifically infiltration of the CNS by CD4+ and CD8+ T-cells and T-cell autoreactivity to myelin antigens, a finding that may have important implications for our understanding of the pathogenesis of human diseases such as MS [10]. The anti-inflammatory role of IL-10 in the CNS has been extensively studied in experimental autoimmune encephalomyelitis model of MS [11]. IL-10 is a pleiotropic cytokine that displays suppressive activity toward several cell types in the immune system and appears to be important in regulating the severity and duration of inflammatory responses [12]. IL-10 has been shown to exert its immunosuppressive activity on macrophages, dendritic cells, neutrophils, eosinophils, and T helper 1 cells [13-15].

Better understanding of the underlying mechanisms that drive disease progression will lead not only to discovery of new therapeutic targets but also to identification of biomarkers to measure disease progression, enabling more effective management of progressive disease to achieve optimal outcomes. However, the heterogeneity of the disease, and the complexity of the underlying biological mechanisms, can render this challenging. A lack of understanding of the cause of MS, as well as disease heterogeneity, make it unlikely that one single biomarker will satisfy the needs for disease monitoring in MS. Identification and validation of predictive biomarkers of therapeutic response are urgently needed to help guide optimal treatment management strategies in MS patients. At present, the clinical parameters that are used to assess disease activity and therapeutic efficacy of administered drugs depend on relapse rates, MRI outcomes, and changes in disability scores [16]. These assessments have limited sensitivity with respect to subclinical disease activity. Thus, there is a need for sensitive, specific, and relatively inexpensive biomarkers that can detect disease activity. Ultimately, accurate and sensitive biomarkers of subclinical disease activity will provide neurologists with more objective tools, in addition to magnetic resonance imaging (MRI), to better assess and predict therapeutic outcomes in individual patients with MS [2]. Within this context, with thought that it would be valuable a reliable method to evaluate the changes in the function of autonomic nervous system with the help of HRV with serum TNF-α and IL-10 biomarkers. The aim of this study was to evaluate the autonomic dysfunction with serum IL-10 and TNF-α biomarkers in RRMS patients with EDSS up to 3.

MATERIAL and METHODS

Ethical approval

This study protocol was approved by the Clinical Research Ethics Committee of Cumhuriyet University (Sivas, Turkey; approval no. 2019-12/04).

Patient selection, evaluation of cardiac autonomic parameters and blood inflammatory biomarkers

Two hundred consecutive patients applying to the MS outpatient service of our university hospital between January 2017 and September 2017 were pre-evaluated for compliance with the study. Of them, 36 RRMS patients who were diagnosed with MS in line with McDonald 2010 diagnostic criteria, volunteered to participate in the study, and met the inclusion criteria were included in the study. Inclusion criteria contained the following: patients must be clinically and radiologically in remission, patients must be taking interferon beta treatment as immunomodulator agent, and must have an EDSS score of lower than 3. Individuals who were using other immunomodulators or immunosuppressive agents, who were in a clinical relapse state when the study was conducted, patients who has active infection, a history of previous head trauma,

chronic alcohol use, a chronic disease or drug use effective on the autonomic nervous system, and cardiac disease were excluded from the study. Thirty-seven healthy volunteers that were age and gender matched with the patient group were selected as the control group. The disability status of the patients was evaluated according to the Expanded Disability Status Scale (EDSS) [17].

Autonomic nervous system functions were assessed by frequency-based heart rate variability (HRV) analysis. For HRV analysis, recording was performed by using 7 electrodes. The recording was made at X, Y and Z planes. X electrode was positioned on the intercostal space in the armpit, Y+ electrode was positioned on the 5th costal area on the midclavicular line, Y- electrode was positioned on the interclavicular area, Z+ electrode was positioned at interventricular septum level; and Z - electrode was positioned on dorsal side at the level of Z+ electrode. Spectrum fluctuations were computed using Kardiosis ArsLP Analysis system program. Average reference signals were determined with HRV analysis. When the reference signal was evaluated, R-R intervals were measured which was made by moving the reference signal on the recording for the finding the maximum point of the correlation coefficient, which was taken as 0.98. When the maximum values were over the threshold value (0.98), it was taken as the R wave. When the process ended, a graphic was obtained to show the beat rate at horizontal axis and R-R interval between each beat vertical axis in millisecond. The HRV parameters were defined regarding spectrum analysis. This includes total power frequency (TP), very low frequency power (VLF), low frequency power (LF), high frequency power (HF), and LF/HF parameters.

For TNF-α and IL-10 analysis, patient and control group blood sera were stored at -80 °C until the study. Once the samples reached room temperature, they were analyzed using a Trithium brand (Spain) fully automatic Elisa device system by following the manufacturer's protocol of Dia Sourge (Belgium) TNF-α and IL-10 ELISA test kits.

Statistical analysis

The distribution of characteristics of participants was presented as percentage and mean with SD as appropriate. Mann-Whitney and Spearman correlation tests were performed for demographic and selected clinical data of study groups as appropriate. IBM SPSS Statistics (version 23.0) was used for all analyses. A p value of less than 0.05 was accepted as significant

RESULTS

Table 1 presents demographic features of RRMS patients and controls. No significant difference was detected between the patient and control groups regarding the mean age (37.1±8.8 and 35.1±6.2, respectively) and female male ratio (58.3% and 56.8%), (p=0.34,p=0.89). Mean disease duration was 5.4±4.4 years in the patient group, and mean EDSS score was 1.5±0.9. Table 2 presents the serum TNF-α and IL-10 values of two groups. The serum TNF-α value of RRMS patients was significantly higher than that of the controls (p=0.010). The serum IL-10 values of RRMS patients and controls were found as similar (p=0.726). Table 3 expresses HRV parameters including TP, LF, HF, and LF/HF values of RRMS patients and controls. The TP, LF, and HF values of RRMS patients were significantly lower than those of the controls (p<0.05). The LF/HF, since LF and HF values have reduced at same ratio separately at patient group, values of RRMS patients and control group were insignificant (p>0.05).

Table 1. Demographic characteristics results of the MS patients and control groups.

	Patients	Controls	
Results	Mean	Mean	P value
Mean age (year)	37.1 ±8.8	35.1±6.2	0.348
Female/Male	21/15	21/16	0.892
Disease duration (year)	5.44±4.43	-	

Table 2. Serum TNF-α and IL-10 values results of the MS patients and control groups.

	Patients	Controls	P value
IL-10, pg/mL, (median (IQR))	3.65 (0.01-7.06)	0.01 (0.01-8.10)	0.726
TNFα, pg/mL, (mean ± SD)	5.89 ± 2.01	4.82 ± 1.38	0.010

Table 3. HRV parameters of patients and control groups.

	Patients	Controls	P value
TP, ms2 median (IQR)	1243 (658-2118)	1947 (1631-4523)	<0.001
VLF, ms2, median (IQR)	333.50 (129.50-616.50)	376 (278-987)	0.045
LF, ms2, median (IQR)	478.50 (323.50-1011.50)	882 (808-2253)	<0.001
HF ms2, median (IQR)	280.50 (149.50-926.50)	515 (364-1550)	0.008
LF/HF ratio, median (IQR)	1.50 (0.85-2.90)	1.70 (1.10-3.10)	0.191

Table 4. Correlation analysis of the patients group serum TNF-α and IL-10 values with HRV parameters

Patients	IL-10	TNFα	TP	LF	HF	LF/HF
IL-10 Person Correlation	1	.174	.009	-.168	.111	-.085
Sig. (2-tailed)		.309	.960	.329	.520	.623
N	36	36	36	36	36	36
TNF α Person Correlation	.174	1	.013	-.060	.076	-,078
Sig. (2-tailed)	.309		.938	.730	.658	.650
N	36	36	36	36	36	36

Table 4 shows correlation analysis the serum TNF-α and IL-10 values with HRV parameters in patients group. In the RRMS patients, no correlation was found between both serum TNF-α and IL-10 values with HRV parameters including TP, LF, HF, and LF/HF values ($p>0.05$). The clinical significance of the VLF parameter is not known, so it was not discussed in our study[18].

DISCUSSION

ANS is a very significant section of the central nervous system, which works involuntarily and enables homeostasis of the body. It consists of two components, sympathetic and parasympathetic, the transmitters of which are norepinephrine and acetylcholine, respectively. ANS distributed throughout the peripheral and central nervous system, mediating functions of smooth muscle cells, glands, and cardiac tissue, immune system. In classical terms, the two constituents of ANS display antagonistic but complemental functions. To regulate natural immunity, sympathetic and parasympathetic systems show an effect which suppresses proinflammatory cytokine expression[19,20]. When inflammation is at early stage, afferent vagus nerve fibers transmit signals to the brain for inducing immunomodulatory responses. Afferent vagal nerve has the duty of regulating inflammation by inhibiting proinflammatory cytokine release. This function of afferent vagal nerve is referred to as the cholinergic anti-inflammatory role [21]. Sympathetic nervous system acts through the natural and acquired immune system. It regulates cellular and humoral immune functions. Beta-adrenergic receptors suppress inflammatory T helper-1 (TH-1) functions and inhibit the production of TH-1 cytokines such as interleukin-12, TNFα and interferon-γ by antigen presenting cells while supporting the anti-inflammatory T helper-2 (TH-2) response by activating TH-2 cytokines interleukin-10 and transforming growth factor beta (TGF-β)[22].

The CNS establishes communication in a bi-directional manner with the immune system by modulating the ANS. The ANS shows interaction in a pathological way with immune components in MS. Diverse immune cell subsets demonstrate different pathological changes which depend on factors like disease stage (relapsing or progressive) and use of drugs that modulate the autonomic nervous system (e.g., beta-interferons, beta-blockers) [22].

The sympathetic and parasympathetic section of the cardiovascular autonomic system are affected by MS [10,22,23]. AD in MS is explained by presence of lesions in regions responsible for autonomic regulation, such as nuclei in the periventricular region of fourth ventricle in the brainstem as well as medullar lesions [24,25] . The total MRI brain MS lesion load is another pathologic substrate related to AD incidence as demonstrated by Saari et al [26]. Given the weak relationship between the position of the lesions and the autonomic dysfunction presence, these causes may at least partly explain the presence of cardiovascular autonomic dysfunction in MS. Besides, another cause of autonomic dysfunction in MS patients may be the effects of environmental factors on the lymphocyte base of the autonomic receptors on the lymphocyte base on the hyperreactive immune system, such as disruption of interaction or viral infection, vitamin D deficiency [22], except that areas responsible for autonomic control in the CNS are involved.

The relationship between proinflammatory receptors (for example, the contrast to the inverse relationship in normal conditions) may be thought of as an abnormal response in the form of a cardiac response to lymphocyte dysfunction due to atypical release of catecholamines may alter cardiovascular functions [27].

In the current study, we performed HRV test with the measurement of serum TNF-α and IL-10 in the RRMS patients and controls in the neurology outpatient service of tertiary care hospital. We determined that ANS activity in the RRMS patients was reduced compared to healthy controls by HRV analysis (although increased inflammatory activity, as supported by increased serum TNF-α levels, is expected to activate the ANS in normal conditions).

The serum level is important factor as anti-inflamatuar factor. There are IL-10 releated many studies

in literature. It has even been reported in one of the investigations that low levels of serum IL10 may be a predictor of a second clinical symptom from clinical isolated syndrome [28]. We didn't find anydifference in our patient and control group's datas. TNF, which is one of the inflammatory markers accused in the pathogenesis of MS [29], was found to be statistically higher in our study group than the control group . As expected, the high rate in the patient group indicates that the inflammatory process continues.

In MS, immunomodulatory drugs are particularly effective during the initial phase of RRMS, in which EDSS score is below 3 and the inflammatory process predominates, and in the period so-called the opportunity window [33]. For this reason, we included in our study patients who had an EDSS score below 3 and were receiving interferon beta as immunomodulatory treatment. Interferons used in patients increase anti-inflammatory cytokines and repress proinflammatory cytokines [34]. Proinflammatory TNF-α level was found to be high at a significant in the our RRMS patient group. In additionally our study results, anti-inflammatory agent, IL-10 serum levels were similar in the both groups. The autonomic function was expected to be active in this case to suppress inflammation with both sympathetic and parasympathetic activity (HF values reflecting parasympathetic and LF values reflecting sympathetic activities were significantly lower in the patient group). These findings indicate that there is autonomic dysfunction in the RRMS patient group. Although interferon therapies are used in treatment increase anti-inflammatory cytokines and suppress pro-inflammatory cytokines, but our results were not as expected. This phenomena may be due to lack of ability to suppress of pro-inflammatory cytokines and increase in anti-inflammatory cytokines of drug molecules and could be the underlying cause of 30% clinical efficacy of beta interferon treatment [35]. However, this may be elucidated by the comparison of inflammatory cytokine levels between a group of patients with the same characteristics that do not accept treatment and patients receiving interferon treatment.

CONCLUSION

In conclusion, our study revealed that inflammation persists in the patient group below EDSS 3. In these conditions, HRV increase reflecting autonomic functions was not observed due to inflammation and no correlation with biomarkers were detected. This situation supports autonomic dysfunction. In patients, it may be thought to add sensitive methods such as the follow-up of autonomic functions as biomarkers that can be used in routine.

Limitations

Several limitations should be considered when interpreting the results of this study. Firstly, we could not conduct further subgroup analyses such as by gender, EDSS values, and disease duration because of insufficient original data. This study has significant strengths including neurological evaluation performed in the same outpatient service with similar setup; use of only Mc Donald 2010 criteria for the final diagnosis of MS patients; small rate of migration in our city and opportunity of enrolling subjects with same genetic and environmental background. The clinical significance of the VLF parameter is not known, so it was not discussed in our study.

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Conflict of interest

The authors declare that they have no conflict of interest.

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