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ARAŞTIRMA / RESEARCH

Alpha-synuclein levels in multiple sclerosis patients with restless leg syndrome

Huzursuz bacak sendromu olan multipl sklerozlu hastalarda alfa-sinüklein düzeyleri

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Abstract

Purpose: The restless legs syndrome is more frequent and critical in Multiple Sclerosis patients, and it harms their general well-being and life quality. Alpha-synuclein is a synuclein protein that can have an impact on the pathway of signaling, affecting the Dopamin 2 receptor and its receptor trafficking. Studies have shown that the decrease in dopamine 2 receptor and Restless Legs Syndrome disease are correlated. This study is aimed to ascertain the alpha-synuclein level in multiple sclerosis patients with restless legs syndrome.

Materials and Methods: We took blood samples from 40 multiple sclerosis patients and 20 healthy individuals. Half of the patient group had Multiple Sclerosis with restless leg syndrome. In the study groups, the alpha-synuclein level was determined by quantitative real-time polymerase chain reaction (qRT-PCR) and enzyme-dependent immunosorbent assay (ELISA).

Results: Alpha-synuclein gene expression level was found or be significantly lower in restless leg syndrome patients with multiple sclerosis than the Alpha-synuclein gene expression level in the control group.

Conclusion: Alpha-synuclein may have an impact on the pathogenesis of the restless leg syndrome of multiple sclerosis disease. Further investigations are required to determine the impact of alpha-synuclein in the pathogenesis of restless leg syndrome in multiple sclerosis disease.

Keywords:. Restless leg syndrome, multiple sclerosis, alpha-synuclein, qRT-PCR, gene expression

Öz

Amaç: Huzursuz Bacak Sendromu, multipl skleroz hastalığında yaygın olarak görülmektedir, ayrıca hastaların genel sağlığı ve yaşam kalitesi üzerinde olumsuz bir etkisi vardır. Alfa-sinüklein, Dopamin 2 reseptörünün sinyal yolağında önemli bir rol oynamaktadır. Çalışmalar, dopamin 2 reseptörü ve huzursuz bacak sendromu hastalığı arasında ilişki olduğunu göstermiştir. Bu çalışma huzursuz bacak sendromlu multipl skleroz hastalarında alfa-sinükleinin düzeyini belirlemek amacıyla yapılmıştır.

Gereç ve Yöntem: Bu çalışmada 40 multipl skleroz hastasından ve 20 sağlıklı bireyden kan örneği alındı. Hasta grubunun yarısı huzursuz bacak sendromlu multipl skleroz hastalarından oluşmaktadır. Çalışma gruplarında alfa sinüklein düzeyi enzime bağlı immünosorbent kit (ELISA) ve gerçek zamanlı kantitatif polimeraz zincir reaksiyonu (qRT-PCR) metodu kullanılarak belirlendi.

Bulgular: Huzursuz bacak sendromlu multipl skleroz hastalarında alfa-sinüklein gen ekspresyon düzeyi kontrol grubuna göre anlamlı düzeyde düşük bulundu.

Sonuç: Alfa-sinüklein multipl skleroz hastalığında huzursuz bacak sendromu patogenezinde etkili olabilir. Multipl skleroz hastalığında huzursuz bacak sendromu patogenezinde alfa-sinükleinin rolünü belirlemek için daha fazla çalışmaya ihtiyaç vardır.

Anahtar kelimeler: Huzursuz bacak sendromu, multipl skleroz, alfa-sinüklein, qRT-PCR, gen ekspresyonu

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INTRODUCTION

The restless leg syndrome (or Wills-Ekbom disease), is a continuous progressive movement disease that is provoked by the need to propel the legs during sleep and rest[1-3]. Multiple sclerosis (MS) is known as an inflammatory, neurodegenerative, and autoimmune disorder that is caused in the nervous system[4,5]. Clinical conditions related to MS, such as stress, depression, exhaustion, and sleep complications, adversely affect the life quality of patients[6]. In recent years, research has been reported increased RLS pervasiveness in MS patients [7,8]. Giannaki et al. reported that depression, the quality of sleep, and exhaustion were more acute in MS patients (R / S + RLS)[9].

Alpha-Synuclein (αSyn) is a neuronal cytoplasmic protein, and its physiological function is uncertain[10]. It has an impact on the handling of dynamics in presynaptic vesicle in neuronal plasticity and learning[11]. Extracellular αSyn can also produce neuroinflammation as it increases microglial activation and astrocyte [12]. Other studies found that dysfunctional immune responses and inflammation were associated with declined α-Syn levels [13-15]. Research has shown that αSyn was considerably upregulated in glia of the spinal cord and neurons of animal model of MS[16].

The objective of this research is to identify the association between αSyn and MS patients with RLS.

MATERIALS AND METHODS

Between April 2018 and July 2019, a total of 40 MS patients (20 patients had MS with RLS, 20 patients with MS) from the MS Clinic of Neurology at the Çanakkale Onsekiz Mart University of Medical Sciences and 20 healthy control subjects (16 females, 4 males) participated in our study. Disease type was defined as primary progressive (PPMS), secondary progressive (SPMS), or relapsing-remitting (RRMS). We diagnosed all of the patients via the revised McDonald criteria[17]. Inclusion criteria were (1) age≥20 years or≤85 years, and (2) patients with MS with RLS or without RLS. Exclusion criteria were as follows: (1) age of<20 years or>85 years (2) Patients with chronic diseases, such as diabetes mellitus, chronic kidney disease, rheumatoid arthritis, systemic lupus erythematosus, malignancies, gastrointestinal diseases, pregnancy and (3) only RLS disease. Controls were chosen among healthy subjects who had no patients with MS and RLS or MS (gender and age-matched).

The RLS diagnosis was established based on the minimum criteria defined by the International Restless Legs Syndrome Study Group (IRLSSG): (1) an urge to move the legs, usuallyaccompanied or caused by uncomfortable and unpleasant sensa-tions in the leg; (2) the urge to move or unpleasant sensations beginor worsen during periods of rest or inactivity, such as lying or sitting;(3) the urge to move or unpleasant sensations are partially or totallyrelieved by movement, such as walking or stretching, for at leastas long as the activity continues; and (4) the urge to move or un-pleasant sensations are worse in the evening or at night[18].

All experiments were performed in compliance with the relevant laws and institutional guidelines. Our study protocol is designated in accordance with Helsinki Decleration (revision 3). Canakkale Onsekiz Mart University Faculty of Medicine, Clinical Research Ethics Committee (2011-KAEK-27/2017-E.124369) has approved the experiments and informed consents were obtained from each participant before enrollment.

ELISA test for the evaluation of αSyn serum level

We collected blood samples by vacutainer tubes, and after allowing them to clot for 30 minutes at room temperature, centrifuged them at 1000xg for 10 minutes. We measured the serum levels of αSyn through an enzyme-linked immunosorbent assay (ELISA) kit (Product KHB0061, Invitrogen, Frederick, MD) based on the instructions of the manufacturer. The analytical (linear) detection range was 0.23-15 ng/mL. For the human alpha-synuclein ELISA assay kit, the minimum detection limit was 0.20 ng/mL, and the reported interassay and intraassay CVs were 5.70% and 6.40%, respectively.

Gene expression analysis of αSyn

Blood samples were collected MS patients who were treated at the Faculty of Medicine, Department of Neurology, and healthy volunteers into EDTA tubes and stored at -80 ^{o}C. We extracted total RNA from blood samples with a commercial kit (Bioneer), and the quantity of RNA was assessed using the NanoDrop ND-1000 spectrophotometer. We synthesized cDNA from the total RNA using the

commercial kit (Bioneer). Real-time RT-PCR was performed by the GreenStar qPCR Master Mix and Bioneer system. We performed real-time PCR amplification for SNCA using a total volume of 20 μL that contained 10 μl qPCR mix, 1.0 μM of each primer, 2μl cDNA, and distilled water. The PCR statuses were as follows: 94 °C for 3 min, and then 40 cycles of 94 °C for 30 s, 58 °C for 30 s, and 72 °C for 60 s, and finally 72 °C for 5 min. The primers set were as follow: αSyn, 5'-GTGTGGCAACAGTGGCTGAG-3' (forward) and 5'-TGGGGCTCCTTCTTCATTCTTG-3' (reverse); GAPDH, 5'-TGCACCACCAACTGCTTAGC-3' (forward) and 5'-GGCATGGACTGTGGTCATGAG-3'. The intersection point is the threshold cycle (Ct). The variation in threshold cycles among the reference and target is calculated by Delta Ct. We used the values of Delta Ct used for statistical analysis.

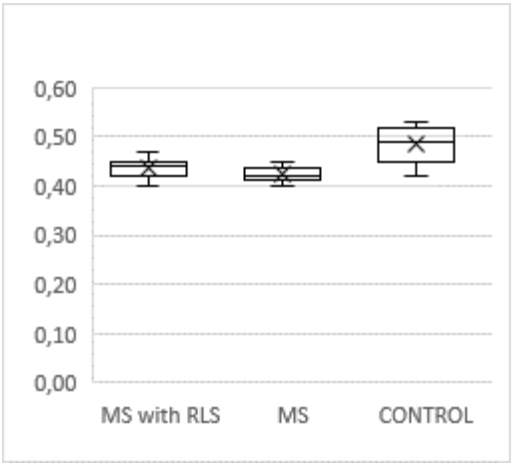

Figure 1. Alpha-synuclein relative expression level

Statistical analysis

We used SPSS, version 19.0. (SPSS, IBM Company) for our statistical analysis. Data were expressed as mean±SD or median (interquartile range) as was appropriate. Continuous variables were compared using Student's t-test or Mann–Whitney U test and categorical variables were compared by the chi-square or Fisher's exact test as was appropriate to the data distribution. For statistical analysis and data description, patients were stratified according to the presence or absence of RLS. MS patients with RLS (RLS subgroup) were compared to the control group and patients with MS (non-RLS subgroup). In addition, the control group was compared to patients with MS (non-RLS subgroup). We considered P-values ≤0.05 as statistically significant.

RESULTS

Serum samples of 40 MS patients, including 36 RRMS, 2 SPMS, and 2 PPMS subjects, were analyzed for the presence of αSyn and compared with the control group. Twenty MS patients were diagnosed with restless leg syndrome. The information of all patients is shown in Table 1. We did not find any meaningful differences across groups regarding age and gender. Mean serum levels of αSyn were 33.97±5.61 in RLS, 33.02±7.20 in the MS and 36.95±5.59 in the control group. We did not find any statistically significant differences between the two groups ($p > 0.05$).

After comparison with the control group, there was a decrease of αSyn relative expression level in the MS group ($p = 0.01$). In MS patients with RLS, we also have seen significantly lower relative levels of αSyn mRNA in peripheral blood compared with the control group ($p = 0.01$) (Figure 1) (Table 1).

DISCUSSION

RLS is a movement disorder associated with the need to involuntarily move the legs during sleep[18]. RLS adversely affects the general quality of life and well-being of MS patients[19]. Liu et al. found that RLS is highly correlated with MS, and the results of their analysis showed a notable impact of RSL on the MS patients' sleep quality[20]. Despite the fact that the biological mechanism associating with RLS and MS is not known, recent genetic studies have contributed to the explanation of these mechanisms[21]. Some in vitro immunocytochemical studies have shown that α-synuclein has an impact on the repair of DNA[22].

Lu et al. in their study, showed an increased αSyn level in activity MS lesions, which was absent in the control group and chronic inactive lesions[23]. Mejia et al. showed that the levels of αSyn in the epidermis were lower in patients with MS-relapse[24]. Lahut et al. reported that αSyn levels were decreased in the genetic examination of patients with Parkinson compared to the control group[25]. This study found no evidence for differences in the concentrations of serum αSyn and gene expression among MS patients, RLS cases, and healthy cases.

Table 1. Demographic and clinical details of study subjects

	MS (n=20)	MS with RLS (n=20)	Control (n=20)	P-value
Age (mean years ± SD)	38.2 ± 11.2	37.8 ± 12.8	38.5 ± 14.7	0.94[a], 0.89[b], 0.94[c]
Sex (n (%))				0.47[a], 0.64[b], 0.80[c]
Male	6 (30.0)	5 (25.0)	4 (20.0)	
Female	14 (70.0)	15 (75.0)	16 (80.0)	
MS-Type (n (%))				
RRMS	18 (90.0)	18 (90.0)		
PPMS	1 (5.0)	1 (5.0)		
SSMS	1 (5.0)	1 (5.0)		
Serum alpha-synuclein (ng/ml)	33.02±7.20	33.97±5.61	36.95±5.59	0.20[a],0.17[b],0.86[c]
alpha-synuclein relative expression level	0.44±0.02	0.42±0.02	0.48±0.03	0.01[a], 0.01[b], 0.03[c]

SD: Standard Deviation; RRMS: Relapsing-remitting MS; PPMS: Primary progressive MS; SPMS: Secondary progressive MS a: Control vs. MS, b: Control vs. RLS, c: MS vs. RLS

We found a lower αSyn relative expression level in the MS group compared to the control group. Tan et al. reported that the examination of mRNA expression of peripheral lymphocytes indicated no notable difference in αSyn-mRNA expression between the spPD and control group[26]. The protein levels of α-syn have shown some conflicts in data. Although elevated αSyn levels in the plasma of patients with PD is reported in some studies[27], some others have indicated a significant decline[28], or no change in this parameter[29]. The reasons for these inconsistencies can be attributed to the different testing methodologies and designs of these studies, as well as the possible effects specific to the cell or tissue type.

The pathophysiology of RLS remains uncertain; however, studies on dopamine-related mechanisms have been carried out in recent years. In cultured cells, αSyn is shown to adjust the dopamine creation[30].

This study was designed to evaluate the possible role of αSyn in the occurrence of MS patients with RLS related complications. relatively small, this situation limits the reliability of comparison of these parameters between subgroups in MS patients with RLS. For the validity of our results, larger sample studies are needed.

MS and RLS have only recently been recognized as comorbid conditions. RLS incidence is increasing in MS patients. Although in one study in Spain the association between MS and RLS was not significant, a large multicenter case-control study was conducted in Italy and found a 5.4 times greater odds of RLS in patients with MS than that for control subjects. In a recent meta-analysis study provides evidence that MS could be a risk factor for RLS and suggests that demyelination in the CNS may have an important role in RLS etiology. If the association between MS and RLS is confirmed in later studies based on objective measurements, also supported by future treatment studies, then we would suggest that clinicians should screen for RLS and other sleep disorders among patients with MS. Early RLS diagnosis in these patients may avoid ineffective chronic drug treatment in favor of more efficacious therapies[21,31,32].

In conclusion, αSyn relative expression level decreased in group MS with RLS when compared to the control group, but serum αSyn levels have not changed. The change in serum αSyn level should be considered not only in relation to gene expression but also in environmental, age, and gender factors. This study may be a reference for future research. Finally, it should be noted that our findings were based on examinations performed on a limited number of patients., and studies on a larger population are needed for αSyn search as a new therapeutic target.

Yazar Katkıları: Çalışma konsepti/Tasarımı: SÇ; Veri toplama: SÇ, SY, CÇP; Veri analizi ve yorumlama: SÇ, SY; Yazı taslağı: SÇ, SY, ŞÖ, CÇP; İçeriğin eleştirel incelenmesi: SÇ, SY, ŞÖ, CÇP; Son onay ve sorumluluk:SÇ, SY, CÇP, ŞÖ; Teknik ve malzeme desteği: SY, CÇP; Süpervizyon: SÇ, SY; Fon sağlama (mevcut ise): yok.
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