Am J Clin Exp Immunol 2019;8(3):16-20
www.ajcei.us /ISSN:2164-7712/AJCEI0091697

Original Article

Serum level measurement of progranulin in relapsing-remitting multiple sclerosis and neuromyelitis optica patients

Niloufar Nasr Azadani[1], Faezeh Norouzi[2], Mahsa Hajizadeh[3], Sara Parsa[4], Farnaz Khalighinejad[5]

[1]Department of Pharmacy, Rajiv Gandhi University of Health Sciences, Bengaluru, India; [2]Medical Student, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran; [3]Isfahan Research Center of Multiple Sclerosis, Isfahan University of Medical Sciences, Isfahan, Iran; [4]School of Medicine, Islamic Azad University of Najafabad, Isfahan, Iran; [5]Postdoc Associate, University of Massachusetts Medical School, Worcester, United States

Received January 23, 2019; Accepted May 1, 2019; Epub June 15, 2019; Published June 30, 2019

Abstract: Background: Multiple sclerosis (MS) is a complex autoimmune disease of the central nervous system (CNS) with unknown etiology and variable clinical evolution. Although the role of serum progranulin levels in the pathogenesis of MS remains unclear, it is well known that progranulin is involved in several physiological and pathophysiological process of CNS including modulation of neurite outgrowth, neuronal differentiation, and neuronal survival. Therefore, in this study, we aimed to measure serum levels of progranulin in patients with neuromyelitis optica (NMO) and relapsing-remitting multiple sclerosis (RRMS) in comparison with healthy control subjects. Methods: In a case-control study, plasma was collected from healthy controls (n = 37) and also patients with RRMS (n = 115) and NMO (n = 33). Serum level measurement of progranulin was performed using a sandwich ELISA method. Results: The serum levels of progranulin were 65.07 ± 11.64, 56.81 ± 10.34, and 47.73 ± 10.37 in NMO and MS patients and healthy controls, respectively, showing a statistically significant difference between them (P = 0.00). Furthermore, we found a positive correlation between serum levels of progranulin and EDSS of patients (r = 0.79 and P = 0.00). Conclusion: The present study demonstrated that progranulin is up-regulated in MS patients and our findings strengthen the evidence for progranulin being involved in the pathogenesis of MS. However, further studies will be required to establish progranulin as an important marker for MS.

Keywords: Multiple sclerosis, neuromyelitis optica, progranulin, ELISA, inflammation

Introduction

Multiple sclerosis (MS) is considered predominantly an inflammatory autoimmune disease of the central nervous system (CNS) that normally conduces to temporary neurological impairments followed by disability progression [1]. Although the cause underlying this disease is still obscure, extensive research on different aspects of MS has resulted in a markedly improved understanding of its pathomechanisms. Although the role of serum progranulin levels in the pathogenesis of MS remains unclear, it is well known that progranulin is involved in several physiological and pathophysiological processes of CNS including modulation of neurite outgrowth, neuronal differentiation, and neuronal survival [2, 3]. Since progranulin interacts with TNF-a receptor, it is not surprising that elevated serum levels of progranulin can be detected in neuroinflammatory disorders [4]. Progranulin is an extracellular glycoprotein, containing seven and a half repeats of cysteine-rich motifs which are secreted from cells mediated by its 17 amino-acid signal peptides. Proteinase 3 and elastase are two possible enzymes that are presumed to cleave progranulin to granulin, an antagonist of progranulin with opposite effects [5]. Since granulin is involved in inflammation, Proteinase 3 and elastase can increase neutrophil-dependent inflammation by eliminating the local anti-inflammatory activity of progranulin.

Furthermore, it has been shown that progranulin can be administrated as a therapeutic target against inflammatory arthritis. Therefore, progranulin is a potential target for the treatment of autoimmune disorders [6]. As reported earlier by Etemadifar et al. [7], Isfahan, a central

province of Iran, is one of the highest areas in the region that MS prevalence has sharply increased.

Therefore, in this study, we aimed to determine progranulin levels in the serum of well-characterized patients with relapsing-remitting multiple sclerosis (RRMS) and neuromyelitis optica (NMO).

Methods

The study population of the study comprised of 115 RRMS (45 men and 70 women) and 33 NMO (13 men and 20 women) patients who all were resident of Isfahan province of Iran. All of the patients were chosen from the multiple sclerosis private clinics of Isfahan. RRMS and NMO patients were diagnosed according to the Mcdonald's and Wingerchuk criteria. We also selected 37 healthy subjects (16 men and 21 women) as controls group who were referred to Isfahan Transfusion Organization (ITO) for blood sampling. They were matched well with patients regarding age and sex. Patients signed informed written consents.

Blood samples were collected from both patients and healthy controls according to the usual puncture method. Then samples were centrifuged, and plasma samples were stored at -80°C. Serum level measurements of progranulin were performed using a sandwich ELISA method. The procedure of progranulin detection was specified for only full-length of progranulin. In this regard, we used anti-progranulin monoclonal antibody and anti-progranulin rabbit antibody (R&D systems, Minneapolis, MN, USA). The HRP goat anti-rabbit IgG antibody reaction and Colorimetric reaction were performed, and the optical density was measured at 450 nm.

Statistical analysis of the study was carried out by SPSS for hardware (ver.24). Kolmogorov-Smirnov Z test was used to evaluate the normal distribution of serum levels of progranulin, age, duration of disease, and EDSS. ANOVA test was used for comparison of variables mentioned above between groups of study also Pearson correlation was used for correlation between serum level of progranulin with EDSS, duration of disease, replace number and age. Also we used of Chi Square test. Data were shown as mean ± SD and number (percent). All tests were two-tailed, and P-value ≤ 0.05 was considered as the significant threshold.

Results

The mean age of patients with NMO and RRMS were 36.06 ± 8.28 and 36.22 ± 9.77, respectively, which did not show a significant difference compared with that of healthy controls (39.18 ± 10.05 and P = 0.23). In addition to age, patients and healthy subjects were also matched well concerning their genders (P = 0.84). Since distributions of serum levels of progranulin, age, duration of disease, number of relapses, and EDSS were significantly different with normal distribution, we used non-parametric tests for these variables as their results are shown in **Table 1**. The serum levels of progranulin were 65.07 ± 11.64, 56.81 ± 10.34, and 47.73 ± 10.37 in NMO and MS patients and healthy controls, respectively, showing statistically significant differences between them (P = 0.00) (**Figure 1**). Furthermore, we found a positive correlation between serum levels of progranulin and EDSS of patients (r = 0.79 and P = 0.00) (**Figure 2**). Our results also show that serum level of progranulin was significantly correlated with duration of disease, relapse number, and age (respectively, r = 0.82/P = .00, r = .57/P = .00, and r = -0.19/P = 0.001).

Discussion

In this study, we determined serum levels of progranulin in RRMS and NMO patients and healthy controls. Serum levels of progranulin in the healthy group of our study were lower than of those that reported previously. We supposed that this difference is probably due to a different method of progranulin detection which we selected for this study. However, the range of progranulin which we found for our healthy controls was similar to the results of Yamamoto et al. [8], as they also used a similar sandwich ELISA for measuring progranulin levels. Our findings showed that serum levels of progranulin are significantly higher in RRMS and NMO patients than in healthy controls. The concentration of progranulin was significantly higher in the serum of NMO patients than those of RRMS patients. Also, we found that the concentration of progranulin is significantly correlated with EDSS and duration of disease which can propose the implication of this marker in pathogenesis and severity of MS.

Table 1. Clinical and paraclinical features of RRMS and NMO patients

Characteristics		RRMS	NMO	Healthy control	*P*-value
Number of subjects		115	33	37	-
Gender M/F		45/70	13/20	16/21	0.90*
Age (Mean ± SD)		36.22 ± 9.77	36.06 ± 8.28	39.18 ± 10.05	0.23**
Duration of disease (Mean ± SD)		3.71 ± 3.34	4.81 ± 2.80	-	0.86**
EDSS (Mean ± SD)		1.51 ± 1.32	2.07 ± 1.36	-	0.03**
Relapse number (Mean ± SD)		0.73 ± 0.79	0.81 ± 0.76	-	0.61**
Brain MRI	Positive	113 (98.2%)	15 (45.4%)	0 (0.0%)	0.00*
	Negative	2 (1.8%)	18 (54.6%)	37 (100%)	
Spinal MRI	Positive	91 (79.2%)	33 (100%)	0 (0%)	0.00*
	Negative	24 (20.8%)	0 (0%)	37 (100%)	
CSF-OCB	Positive	84 (73.04%)	4 (12.12%)	-	0.00*
	Negative	31 (26.96%)	29 (87.88%)	-	
NMO-Ig-G	Positive	6 (5.2%)	15 (45.45%)	-	0.00 *
	Negative	109 (94.78%)	18 (54.56%)	-	
Progranulin (Mean ± SD)		56.81 ± 10.34	65.07 ± 11.64	47.73 ± 10.37	0.00**

*Chi Square, **One-Way ANOVA.

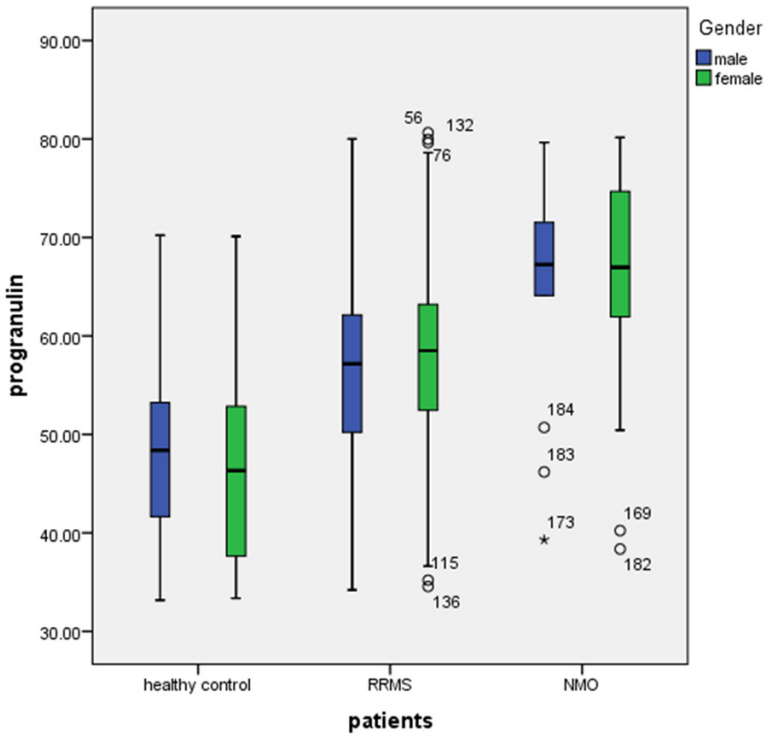

Figure 1. Boxplot of progranulin concentrations in serum of different types of MS.

It should be mentioned that progranulin plays an anti-inflammatory role in the body while, its cleavage leads to the production of granulin with pro-inflammatory properties. However, progranulin and granulin are implicated in the regulation of inflammation; the association between MS and serum levels of these two markers has not been investigated enough so far. It has been shown that progranulin and granulin both are implicated in neural growth and survival suggesting neurotrophic properties of them which lack of them can contribute in neural death [2]. In this study, we only investigated the serum level of progranulin which showed significant elevation of this marker in patients compared to healthy controls supporting neural survival in MS and NMO. This anti-inflammatory role of progranulin is due to binding to tumor necrosis factor receptor (TNFR) which leads to inhibition of signal transduction. It is well known that TNF is implicated in the pathogenesis of many autoimmune disorders so its inhibition by TNFR inhibitors such as progranulin can reduce some related clinical problems. In a study that was performed by De Riz et al. [9] CSF level of progranulin but not granulin was measured in 55 patients with MS

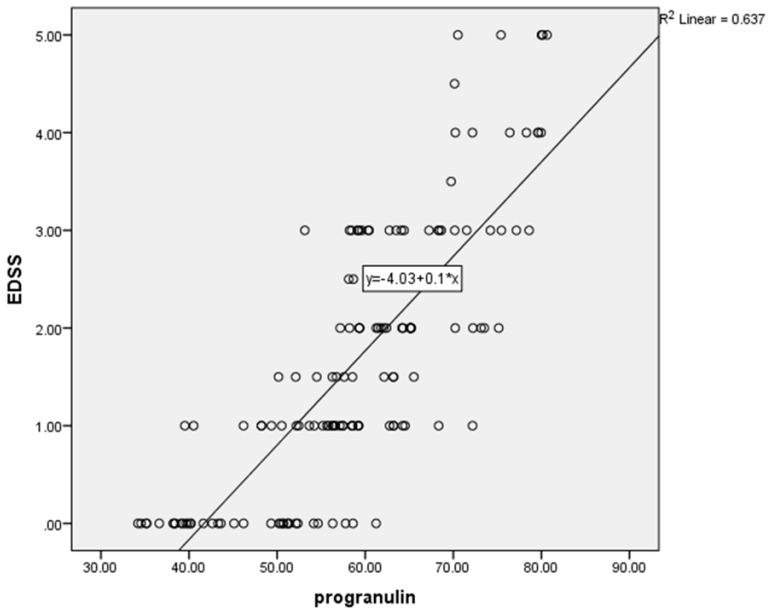

Figure 2. Correlation between serum concentration of progranulin and EDSS.

as well as in 35 subjects with non-inflammatory neurological diseases, 7 individuals with another inflammatory neurological disease, and 8 controls. They failed to show a significant difference between patients and controls, even after further analysis by stratifying samples according to gender or disease subtype. They also found a positive correlation between CSF levels of progranulin and age of patients, while we found a positive correlation between serum levels of progranulin and duration of disease, but not the age of patients. These findings can be due to the small size of control groups as well as the heterogeneity of controls; therefore, further studies will be required to exclude any involvement of progranulin in the pathogenesis of MS. Serum levels of progranulin was also studied in other autoimmune disorders such as rheumatoid arthritis and systemic lupus erythematosus (SLE) [8, 10]. Yamamoto et al. [8] earlier investigated this marker in serum of 417 healthy subjects, 56 patients with rheumatoid arthritis and 31 patients with osteoarthritis. They used similar methods for measurements of progranulin in serum and in addition to the establishment of a new range of progranulin for healthy people, they showed increases serum level of progranulin in rheumatoid arthritis and osteoarthritis patients compared to those in age-matched healthy controls suggesting the role of progranulin in the pathogenesis of rheumatoid arthritis. Another study in this regard was carried out by Qiu et al. [10] who evaluated serum levels of progranulin and some inflammatory biomarkers in 30 newly diagnosed severe SLE patients and 30 healthy subjects. They found that serum levels of progranulin are statistically significantly higher in patients compared to controls. Also, they showed a significant correlation between serum level of progranulin and serum concentrations of other studies on inflammatory biomarkers. Pre-treatment and post-treatment level measurement of progranulin of patients who received prednisone showed the considerable effect of glucocorticoid treatment on the down-regulation of progranulin.

Conclusion

The present study demonstrated that progranulin is up-regulated in MS patients and our findings strengthen the evidence for progranulin being involved in the pathogenesis of MS. However, further studies will be required to establish progranulin as an essential marker MS.

Acknowledgements

We appreciate from Isfahan University of Medical Sciences personnel collaborated with us in this study.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Farnaz Khalighinejad, University of Massachusetts Medical School, Worcester, MA 01655, United States. Tel: +1 857-269-0020; E-mail: farnaz.khalighinejad@gmail.com

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