Clinical and Experimental Health Sciences

Activation of NLRP1 and NLRP3 Inflammasomes in Multiple Sclerosis and Clinically Isolated Syndrome

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ABSTRACT

Objective: Multiple sclerosis (MS) is a chronic, inflammatory and neurodegenerative disease characterized with demyelination and axonal damage in central nervous system (CNS). Inflamasomes, which are important part of this inflammatory process, regulate maturation of proinflammatory cytokines. Infamazom complexes are thought to increase in MS attacks. We investigated role of inflammasome complexes (nod-like receptor protein 1 and 3) in serum and cerebrospinal fluid (CSF) levels for MS development.

Methods: Eighteen clinically isolated syndrome (CIS), 19 relapsing remitting multiple sclerosis (RRMS) and 20 healthy control cases were included in the study. Nod-like receptor protein 1 and 3 (NLRP1, NLRP3), inflammasome complex levels and oligoclonal band (OCB) patterns of all the groups were measured in serum and CSF samples using Enzyme-Linked Immuno Sorbent Assay (ELISA) method.

Results: Although NLRP1 and NLRP3 levels in both RRMS and CIS patients measured in serum and CSF were significantly higher than healthy control group, there was no statistically significant difference between RRMS and CIS patients. On the other hand, the levels of NLRP1 and NLRP3 in CSF were significantly higher in OCB pattern positive patients compared to the OCB pattern negative patients.

Conclusion: In this pilot study, it is shown that NLRP1 and NLRP3 inflammasome complexes increased in CSF samples of MS cases and that this tendency occurred during or maybe before the first MS attack. As a result, it was thought that these complexes may have an effect on the formation of the OCB band.

Keywords: Multiple sclerosis, Inflammasome complex, Clinically isolated syndrome

1. INTRODUCTION

Multiple sclerosis (MS) is a chronic, inflammatory and neurodegenerative disease characterized with demyelination and axonal damage in central nervous system (CNS) (1) Multiple sclerosis promotes especially among young women (women/men:2/1) and it was reported that it affects more than 2 million people in the world. Genetic factors, infectious and other enviromental factors play role in pathogenesis of multiple sclerosis (2). Multiple sclerosis causes neurologic dysfunction and neurodegeneration due to myelin loss and inflamations. Magnetic resonanse, spinal fluid analysis and evoked potantial recordings can be used to support diagnosing multiple sclerosis but they are not spesific for MS(3, 4).

Multiple sclerosis usually begins as a relapsing, episodic disorder. This, relapsing remitting multiple sclerosis (RRMS), evolves into a chronic neurodegenerative disease characterized by progressive neurologic disability(5). CIS occurs in the first episode of neurologic symptoms that lasts at least 24 hours. CIS may or may not go on developing MS. Central nerveus system MRI findings are one or more subclinical white matter lesions(4).

Inflammasomes are multi-oligomeric subunits whose primary duty is the activation of caspase-1 and they regulate maturation of proinflammatory cytokines such as interleukin 1β (IL-1β) and interleukin 18 (IL-18) (6). Inflammasome complex is frequently composed of a pattern recognition receptor (member of nod-like receptor (NLR) family), an adaptor protein and active form of caspase-1. Upon ligand binding, NLR undergoes oligomerization and is bound to adaptor protein with protein region interactions. Thus, it transforms pro-caspase-1 to active caspase-1 biologically. Finally, active caspase-1 breaks down the initial forms of

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IL-1β, IL-18 and IL-33 cytokines and transforms them into their mature forms (6, 7).

Inflammasome complexes are named and function according to the structure of pattern recognition receptor (PRR) they contain. Although over 20 NLR under four different categories were shown, there are still four different inflammasome complexes identified (8).

Inflammasome complexes, which are known to be activated in the early stages of inflammation, are thought to increase in MS attacks (9). This study aims to determine the predictive value of serum and cerebrospinal fluid (CSF) levels of nod – like receptor protein 1 and 3 (NLRP1, NLRP3) molecules in MS development.

2. METHODS

2.1. Study Group

The research included 18 CIS, 19 RRMS and 20 healthy control cases followed between November 2016 and July 2017 in University of Health Sciences Istanbul Haydarpasa Numune Research and Training Hospital. The patients were chosen according to McDonald criteria revised in 2015.

The study was approved by the University of Health Sciences Istanbul Haydarpasa Numune Research and Training Hospital Ethics Committee (dated on 28.11.2016 and with approval KAEK2016 /KK/110)and the participants were enrolled after having signed the written informed consent. Serum and CSF samples of 18 CIS, 19 RRMS and 20 healthy control group patients were used.

Age of onset, number of attacks, number of lesions detected on cranial MRI during serum and CSF samples taking, oligoclonal band positivity (OCB) and expanded disability status scale (EDSS) scores were recorded for all patients.

Healthy CSFs were taken from patients with a complaint of headache, without feature in cranial MR, complete blood count and extensive biochemistry studies, with normal neurological and systemic examinations and normal CSF cells, glucose, and protein values and whose headaches were treated with non-steroidal anti-inflammatory therapy without relapse.

2.2. Serum And CSF Measurement

Serum and CSF samples were taken from all the cases between 8.00-10.00 a.m. while CSF sample was taken, all the cases were in remission and none of them were on corticosteroid treatment. Samples of CIS cases were collected short after their first attacks, when they were in remission period two weeks after completion of steroid treatment. The samples were aliquoted and kept in – 80C freezer until tested. In serum and CSF samples NLRP1 and NLRP3 levels were measured. These measurements were conducted with ELISA method in accordance with the instructions of the producing company (Abcam, Cambridge, England) and for each sample optical density (OD) values were obtained. OD values were converted into concentration values as pg/ml utilizing the standard curve formed with standard protein solutions.

2.3. Statistics

Demographic and clinical characteristics of the cases included in the study were compared with chi-square, Student t-test, ANOVA and Mann-Whitney U test. NLRP1 and NLRP3 levels measured in serum and CSF were compared with ANOVA or t test. Correlation studies were carried out with Pearson and Spearman methods for parametric and non-parametric values, respectively. ($p<0.05$ was considered significant.)

4. RESULTS

4.1. Clinical And Demographic Characteristics

There was no significant difference between the age and gender of the CIS, MS and healthy controls. As expected, disease duration, number of attacks, number of cranial lesions and EDSS scores of MS cases were higher than the CIS cases. There no significant difference between the OCB pattern positivity of CIS and MS groups (Table 1).

Table 1. *Clinical and demographic characteristics of the clinically isolated syndrome (CIS) patients, multiple sclerosis (MS) patients and healthy controls (HS)*

	CIS (n=18)	MS (n=19)	HC (n=20)	p value
Gender: Female/Male	10/8	12/7	11/9	0.850*
Mean age±SD	26.4 ± 7.9	29.9 ± 7.1	28.6 ± 6.3	0.512**
Mean disease duration±SD	-	5.9 ± 1.3	-	-
Number of attacks	1.0 ± 0.0	6.4 ± 3.1	-	Non-applicable
Lesion number on MRI	1.8 ± 1.0	7.1 ± 1.9	-	<0.001***
Oligoclonal band (+) patient number pattern 2 or 3)	10	13	-	0.640*
EDSS score	1.7 ± 0.7	3.1 ± 1.0	-	<0.001†

EDSS, expanded disability status scale, SD, standard deviation. Numerical values are defined in mean ±standard deviation., chi-square test; **, ANOVA; ***, Student t-test; †, Mann-Whitney U test*

4.2. Serum And CSF Measurements

As a result of the measurements performed with ELISA method for CIS, MS and healthy control cases, there was no statistically significant difference between the serum NLRP1 ($p=0.369$) and NLRP3 ($p=0.241$) values with Student t test. In the measurements done in CSF samples with ANOVA method, NLRP1 ($p=0.031$) and NLRP3 ($p=0.027$) values of the CIS and MS cases were significantly higher than the values of the healthy control cases.

There was a significant difference between the healthy control and patient groups in the paired comparisons carried out with Tukey's post hoc test ($p<0.05$ for all parameters).

There was no statistically significant difference between the CIS and MS groups for all parameters (Figure 1a and 1b).

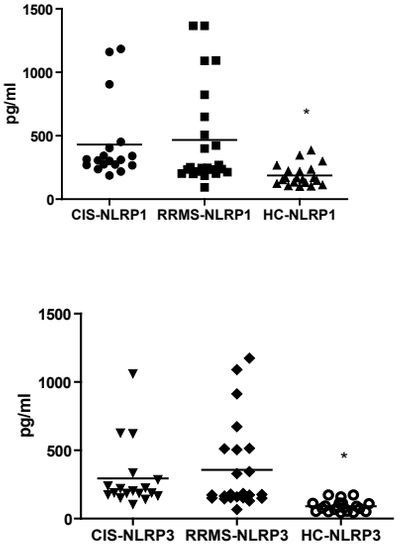

Figure 1a. *Serum NLRP1 – NLRP 3 levels of clinically isolated syndrome (CIS) patients, relapsing remitting multiple sclerosis (RRMS) patients and healthy control cases. Horizontal lines indicate mean values.*

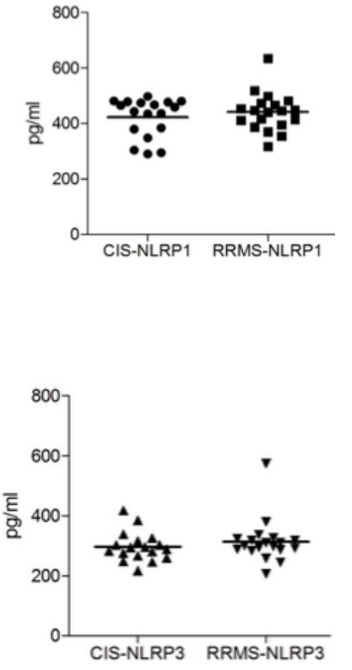

Figure 1b. *Cerebrospinal fluid NLRP1 – NLRP 3 levels of clinically isolated syndrome (CIS) patients and relapsing remitting multiple sclerosis (RRMS) patients. Horizontal lines indicate mean values.*

4.3. Correlation Studies

In order to reveal the possible correlations between the NLRP1 and NLRP3 levels measured in serum and CSF samples and age, disease duration, number of attack, number of lesions on MRI and EDSS scores of the CIS and RRMS cases, Pearson (parameters except for EDSS) and Spearman (only for EDSS) tests were applied.

As a result of this assessment, there was no statistically significant difference between serum and cerebrospinal fluid NLRP1 and NLRP3 levels measured in both CIS and RRMS cases, and clinical and demographic characteristics of the patients. ($p>0.05$ for all comparisons).

4.4. Comparison Of OCB Pattern Positive and Negative Cases

In order to investigate the effects of inflamazome complexes on oligoclonal band positivity, statistical analysis was performed with student t test. There was no significant difference between the serum NLRP1 ($p=0.169$) and NLRP3 ($p=0.308$) values of the OCB positive and OCB negative MS cases (Figure 2a) while NLRP1 ($p=0.043$) and NLRP3 ($p=0.017$) values were found significantly high in OCB positive cases in the measurements carried out in CSF samples of RRMS cases (Figure 2b).

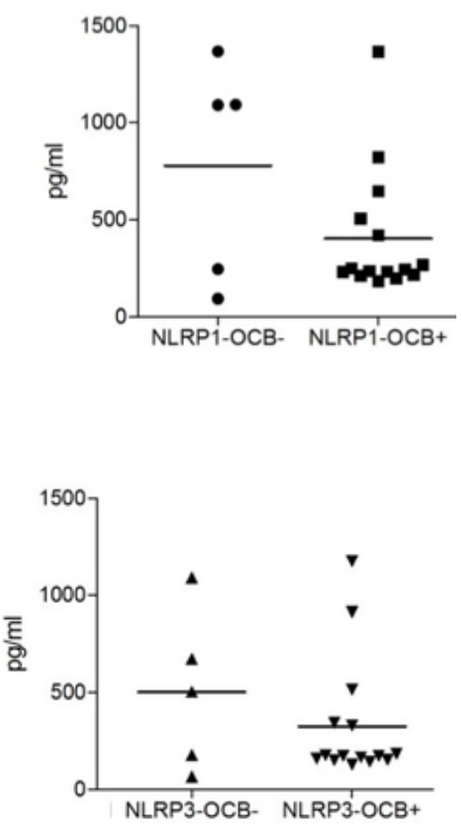

Figure 2a. *Serum NLRP1 – NLRP3 levels of oligoclonal band (OCB) positive (pattern 2 or 3) and OCB negative relapsing remitting multiple sclerosis (MS) patients. Horizontal lines indicate mean values. *p>0.05 by Student's t-test*

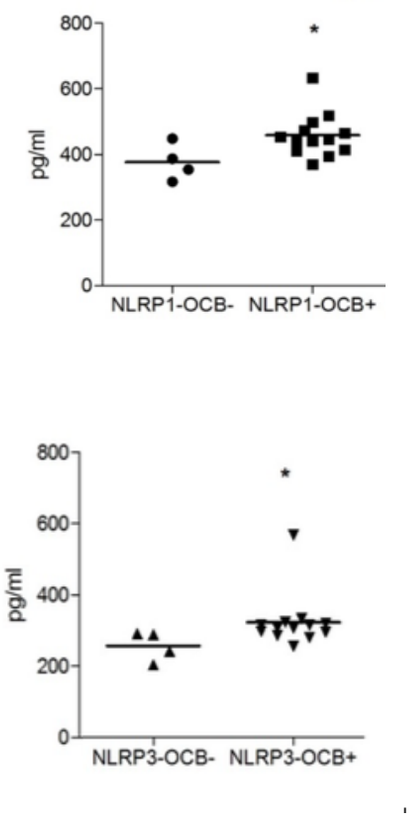

Figure 2b. *Cerebrospinal fluid NLRP1 – NLRP3 levels of oligoclonal band (OCB) positive (pattern 2 or 3) and OCB negative relapsing remitting multiple sclerosis (MS) patients. Horizontal lines indicate mean values. *p<0.05 by Student's t-test*

5. DISCUSSION

Inflammasomes increasingly gain interest in multiple sclerosis and this is shown in experimental autoimmune encephalomyelitis animal model. In experimental autoimmune encephalomyelitis animal model, there was a decrease in disease severity and Th1 ve Th17 cells in peripheral lymphoid tissues and spinal cord (10) of the mice having NLRP3-deficiency. Similarly, in a cuprizone-induced demyelination model, NLRP3 gene expression was significantly upregulated and the mice having NLRP3-deficiency showed delayed demyelination and oligodendrocyte loss (9). It is shown that NLRP1 and NLRP3 inflammasome complexes have a role in MS physiopathology and that they were highly expressed in MS lesions (11-14).

In this study examining the serum and CSF levels of inflammasome complexes it is observed that there was no significant difference between the CIS, RRMS and healthy control cases in terms of serum NLRP1 and NLRP3 levels. This finding suggests that the mentioned serum levels of inflammasome complexes are not important as biomarker. NLRP1 and NLRP3 levels in CSF samples were significantly higher in CIS and MS cases (patient group) compared to the healthy controls. However, there was no significant difference between the CSF levels of the CIS and MS cases. These results support the opinions that intrathecal levels of the inflammasome complexes (NLRP1-3) increase in MS cases. The fact that there was no difference between the CIS and MS cases indicates that the increase in NLRP1 and NLRP3 inflammasome complexes started to occur before the first clinical attack. For this reason, it is not possible to use the inflammasome complexes examined in this study as a biomarker to foresee the CIS-MS transformation.

In our study, no correlation was found between serum and cerebrospinal fluid infamazom complex levels and clinical parameters such as disease duration disability level and number of attacks. This finding suggests that the increasing of the NLRP1 and NLRP3 inflammasome complex levels did not have a significant effect on the clinical course of MS.

An important result of the study is that NLRP1 and NLRP3 levels were higher in the CSF samples of the OCB pattern positive RRMS cases than the OCB pattern negative cases. This finding suggests that inflammasome complexes facilitate the formation of oligoclonal band with an unknown mechanism. It may be related that OCB development depends on the antibodies produced by B lymphocytes accumulated in intrathecal compartment (15). It seems possible that the mentioned inflammasome complexes change the blood-brain barrier (BBB) permeability and facilitate B lymphocyte transmission and thereby increase the accumulation of immunoglobulin G (IgG) molecules forming OCB in CSF.

NLRP1 and NLRP3 basic inflammasomes are pathway factors and it is known that the activation of these two factors lead to the release of various proinflammatory cytokines. In MS cases, NLRP1 and NLRP3 production increases and with immunomodulatory treatment, expression levels of these two molecules decrease (16, 17). It is also indicated that NLRP1 and NLRP3 also release the cytokines that will increase the survival of B lymphocytes in CSF and brain parenchyma (16). Therefore, it is an expected finding that NLRP1 and NLRP3 levels increased in OCB pattern positive patients.

Although OCB pattern positivity is a finding frequently encountered in MS, it is not diagnosed in some MS cases. There are few studies conducted on physiopathological differences of the OCB pattern positive and negative patients. Association of OCB with increased lesion burden and disability is well known (18). The increase of CSF NLRP1 and NLRP3 levels in OCB pattern positive patients is possibly associated with the fact that myeloid cells could easily pass the BBB in these patients. The results suggest that BBB dysfunction developing as a result of this might be one of the underlying factors of OCB pattern positivity.

This study has some limitations; firstly, the number of patients for our research was not sufficient, secondly, we have limited budget for the test materials.

Consequently, in this pilot study, it is shown that NLRP1 and NLRP3 inflammasome complexes increased in CSF samples of MS cases and this tendency occurred in the first MS attack or maybe even before the attack. Some evidence was presented regarding that the mentioned inflammasome complexes may

have a role in OCB development. In case the number of the serum and CSF samples examined is increased in the future studies, whether inflammasome complexes have an effect on CIS-MS transformation will be more clearly understood.

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