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Increased Level of Caspase-1 in the Serum of Relapsing-remitting Multiple Sclerosis (RRMS) Patients

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ABSTRACT

Multiple sclerosis (MS) is an inflammatory autoimmune disease of the central nervous system, in which proinflammatory cytokines play a critical role in the pathogenic formation of lesions. Caspase-1 is a cysteine protease that proteolytically cleaves precursors of interleukin (IL)-18 and IL-1β and turns them into their active forms. These inflammatory cytokines play an important role in the development of MS. The aim of the present study was the investigation of caspase-1 and its downstream products, IL-18 and IL-1β, in relapsing-remitting MS (RRMS) patients.

In this study, we used an ELISA assay to measure serum and cellular caspase-1 and serum levels of IL-18 and IL-1β in RRMS patients in the relapse phase (n=23) and healthy age-and gender-matched controls (n=19).

We observed that the caspase-1 level was significantly increased in the serum of MS patients compared to the healthy controls (p=0.03). Although caspase-1 concentration in the lysate of peripheral blood mononuclear cells (PBMCs) was higher than serum among patients and controls (p<0.001), no significant difference was found in cellular levels of caspase-1 between the two groups. There was no significant difference in serum levels of IL-18 and IL-1β between patients and controls.

In this study, we found an elevation of extracellular caspase-1, as a reflection of its intracellular level, in the serum of RRMS patients during the relapse phase. Therefore, it suggests being a suitable peripheral biomarker of disease activity in multiple sclerosis.

Keywords: Caspase 1; Inflammasomes; Interleukin-1 beta; Interleukin-18; Multiple sclerosis

INTRODUCTION

Multiple sclerosis (MS) is an inflammatory demyelination disease of the central nervous system (CNS) which occurs mainly in 20-40-year-old adults and is more prevalent in women. In a recent meta-analysis study, the prevalence and incidence of MS in Iran were estimated 29.3/100,000 and 3.4/100,000 respectively.[1] A variety of immunological factors have been investigated to understand the etiology of the

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disease but it has remained unknown thus far.

The nucleotide-binding oligomerization domain (NOD), leucine-rich repeat (LRR)- and pyrin domain-containing protein 3 (NLRP3) forms an inflammasome in response to a wide variety of bacterial and viral components. The NLRP3 inflammasome formation activates caspase-1 through the proteolytic cleavage of procaspase-1. Activation of caspase-1 leads to the processing and secretion of the pro-inflammatory cytokines, interleukin-1β (IL-1β) and IL-18. IL-1β and IL-18 play important roles in the induction of adaptive immune responses by regulating the differentiation and activation of T-helper 1 (Th1) and T-helper 17 (Th17) cells which are pathogenic in multiple sclerosis. IL-18 also promotes Th1 cell polarization via inducing interferon-γ (IFN-γ) production by activated T cells and natural killer (NK) cells. Recent studies have shown that IL-1 and IL-18 synergized with IL-23 to induce IL-17 production from pre-committed Th17 cells.[2]

The evidence for the role of caspase-1 in MS was mainly provided by studies on experimental autoimmune encephalomyelitis (EAE), an animal model of MS. Caspase-1 deficient mice have shown a reduced level of IL-18 cytokine and are protected from EAE incidence and severity. Moreover, during EAE, caspase-1 is up-regulated at the protein and transcriptional levels in the blood, also in inflammatory and neuronal cells, which is parallel with the upregulation of pro-inflammatory cytokines such as IL-1β, IFN-γ, Tumor necrosis factor (TNF)-α, and IL-6. Nlrp3, caspase-1, or IL-18 knockout mice exhibited delayed neuroinflammation, demyelination, and oligodendrocyte loss in the EAE.[3] IL-1β and IL-18 were demonstrated to promote Th17 cells differentiation and contribute to Th17-driven exacerbation of EAE.[4] Interestingly, IL-18 knockout mice showed enhanced re-myelination, over-expression of IL- 18 base pair (bp), an endogenous inhibitor of IL-18, and reduced Th17 responses in the CNS and EAE severity as well.[5] Furthermore, Nlrp3 and IL-18 knockout mice had similarly reduced IFN-γ/IL-17 production and EAE disease severity.[6]

Consistent with the results in a mouse model of multiple sclerosis, it was reported that the expression of caspase-1, IL-1β, and IL-18 were elevated in MS plaques and/or cells from MS patients.[7] Caspase-1-positive neurons and inflammatory cells were also observed during the relapse phase of the disease. Increased caspase-1 mRNA levels have been shown in PBMCs from MS patients in a week preceding an acute attack and it was correlated with the number of new but not persistent gadolinium-enhancing brain Magnetic resonance imaging (MRI) lesions.

Since the activation of NLRP3 exacerbates CNS inflammation probably via caspase-1, IL-18, and IL-1β, we measured caspase-1, IL-1β, and IL-18 levels in RRMS patients in the relapse phase. We believe that the results can be very helpful to understand the pathogenesis of the disease.

MATERIALS AND METHODS

Twenty-three RRMS patients (18 Females, 5 Males; mean age: 32.3±9.6 years old; EDSS: 2±0.5) with clinically definite MS were examined in this study. The diagnosis has been performed according to McDonald's criteria.[8] All patients met our assumed inclusion criteria of not being treated with any kind of IFN-β and corticosteroid for at least three previous months and had a history of at least two relapses at the time of initial sampling. All Patients were in the relapse phase and disease activity was assessed by an experienced neurologist. All of the patients were referred to Amiralam general hospital affiliated to Tehran University of Medical Sciences (TUMS).

Nineteen ethnically sex and age-matched healthy controls (14 Female, 5male, mean age: 29.3±7.2 years old), were examined in this study. By completing the questionnaire, we could rule out any history of MS or autoimmune/inflammatory diseases in healthy controls.

All patients and controls were of Iranian origin. The study was conducted according to the ethical guidelines of our institution (Grant No. 90-03-30-15044). Informed consents were obtained from all participants before the study.

Peripheral blood mononuclear cells (PBMCs) were isolated using Lymphodex (inno-train, Germany). Isolated serum and PBMCs were stored at -80°c until the testing day.

Caspase-1, IL-1β, and IL-18 Assays

Caspase-1, IL-1β, and IL-18 were investigated in the serum of patients and controls by using the ELISA kit (Uscn Life Science Inc., PRC). It was a sandwich enzyme immunoassay method. According to the manufacturer's instruction, the intended biomarkers (if present) bonded to its specific antibody (pre-coated in the wells), biotin-conjugated antibody, and avidin

conjugated to horseradish peroxidase (HRP) was added respectively. After adding the substrate (3,3′,5,5′-Tetramethylbenzidine, TMB), the reaction was terminated by the addition of a sulphuric acid solution. The plates were then read at 450 nm using an ELISA reader (spectraMax microplate reader, USA). For the measurement of cellular caspase-1, PBMCs were frozen and thawed three times and centrifuged. Caspase-1 was then measured in the lysate. Furthermore, the concentrations of caspase-1, IL-1β, and IL-18 were determined by comparing the O.D of the samples to the standard curve. The detection limits of IL-18, IL-β and Caspase-1 were 5.9 pg/mL, 6.6 pg/mL and 0.115 ng/mL, respectively.

Statistical Analysis

Data are expressed as mean±SD. Independent T-test and Pearson correlation tests were used to test the differences between the groups and correlation analysis respectively. All Variables were quantitative and analyzed as parametric data using the statistical package for social sciences (SPSS); version 21.0 (SPSS Inc; Chicago, IL, USA). The difference between groups was considered statistically significant when *p* values were less than 0.05.

RESULTS

Our findings showed that the caspase-1 level was significantly higher in the serum of RRMS patients in comparison with healthy controls (p=0.030). However, there was no significant difference in cellular caspase-1 between patients and healthy controls (p=0.9) (Figure 1A). Caspase-1 was higher in cells than sera in each studied group (p=0.022 for RRMS patients and p=0.004 for healthy controls) (Figure 1A). Interestingly, our data revealed a negative correlation between serum and cellular caspase-1 in all studied individuals (p=0.049) (Figure 1B). There was no correlation between serum and cellular caspase-1 within each group (p=0.08 for RRMS patients and p=0.20 for healthy controls). We also identified no significant differences in IL-1β and IL-18 levels between patients and controls (p=0.13 and p=0.28 respectively) (Figure 1C). There was a positive correlation between IL-18 and serum caspase-1(p=0.010) (Figure 1D). IL-1 and IL-18 were also measured in serum of 8 SPMS and 5 primary-

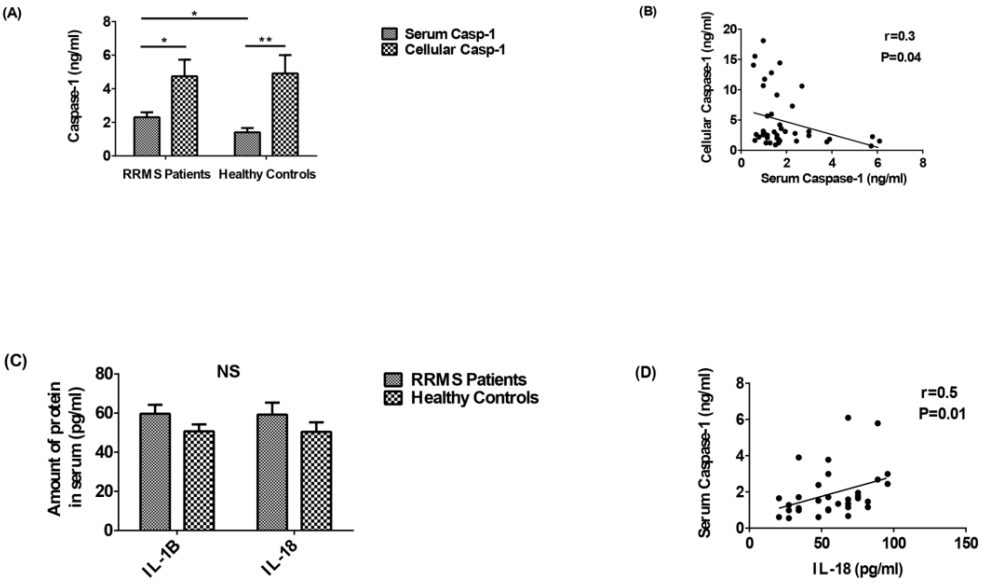

Figure 1. The cytokine level of caspase-1, interleukin (IL)-18, and IL-1β in relapsing-remitting multiple sclerosis (RRMS) patients and normal subjects. (A) Serum and cellular caspase-1 level in multiple sclerosis (MS) patients compared to controls, (B) Correlation of serum and cellular caspase-1 level, (C) IL-18 and IL-1 β serum concentration in study groups, (D) Correlation of serum caspase-1 and IL-18 level. Independent T-test and Pearson correlation tests were used to test for differences between the groups and correlation analysis respectively. *p*-value<0.05 was considered statistically significant. *: p<0.05, **: p<0.01, ***: p<0.001.

progressive multiple sclerosis (PPMS) patients but there was no significant difference between patients and controls (data not shown).

DISCUSSION

Activation of caspase-1 following the NLRP3 inflammasome formation leads to the processing and secretion of IL-1β and IL-18, which are critical in the early steps of the inflammatory events. An important role for Nlrp3 components in multiple sclerosis was provided by studies showing that mice lacking these inflammasome components were protected from EAE development because of reduced Th1 and Th17 responses.[6,9] Previous studies showed that caspase-1 absence in knockout mice mainly affects the secretion of pro-inflammatory cytokines such as IL-1β, IL-1α, IL-6, TNF-α, IL-18, and IFN- γ.[10] Results also showed that caspase-1 transcription was up-regulated during mouse EAE, reaching a peak at maximal disease severity.

A study on MS also showed that the level of the NLRP3 in patients at the remission phase after IFN-B therapy was significantly reduced in comparison with patients at the relapse phase and healthy controls.[11]

Additionally, in a very recent study, NLRP3 inflammasome was investigated in PPMS. The results revealed an increased expression of NLRP3, Caspase-1, and the adaptor protein apoptosis-associated speck (ASC) in PPMS.[12]

In the present study, we found that serum caspase-1 level was significantly higher in RRMS patients in comparison with that in healthy controls, but no significant difference was detected in cellular caspase-1, IL-1β, and IL-18 levels between patients and healthy controls. IL-1 and IL-18 were also measured in serum of 8 SPMS and 5 PPMS patients but there was no significant difference between patients and controls.

Caspase-1, IL-1β, and IL-18 have been measured in several studies in cerebrospinal fluid (CSF) or serum of MS patients using different techniques with conflicting results so far. Some studies showed reduced levels of IL-1 and IL-18 whereas increased levels were reported by others. According to our knowledge, there is only one report regarding the measurement of caspase-1 in serum and CSF of MS patients by ELISA. This study showed an increase in caspase-1 level only in the CSF of the patients in comparison with controls.[13] In another study, caspase-1, IL-1β, and IL-18 were measured at the protein and mRNA levels by western blot and real-time PCR techniques in serum and CSF of patients who were not under chronic therapy of immunomodulatory drugs. Caspase-1 increased in all multiple sclerosis subtypes but IL-18 increased in the remission phase of RRMS and chronic progressive MS.[7] A recent study has reported that mRNA expression levels of IL-1β and caspase-1 were significantly higher in PBMC from RRMS patients, while IL- 18 mRNA levels did not differ and in the presence of inflammasome culture supernatant, Th17 cells frequencies and IL-17 protein levels increased.[14]

As far as we know, there is no other study investigating serum and cellular caspase-1 simultaneously. Our data revealed a negative correlation between serum and cellular caspase-1 in all studied individuals. This remarkable finding is possibly explained by knowing the fact that cytokines have a short half-life and they are generally consumed at the site of production or action.

Caspase-1 is the best-described inflammatory factor. Caspase-1 processes the cytokines IL-1β and IL-18 and after activation induces pyroptotic cell death. In response to CNS inflammation in Multiple Sclerosis and EAE, Caspase-1 is up-regulated in oligodendrocytes and initiates pyroptosis in these cells, a pro-inflammatory cell death. Some reports were suggesting that Caspase-1 may exert effects independent of IL-1β and IL-18 secretion.[15]

In conclusion, our findings suggest that the level of extracellular caspase-1 which is a reflection of its intracellular level can be elevated in the serum of RRMS patients in the relapse phase and its extracellular level might indicate a suitable peripheral immunological marker of disease activity in multiple sclerosis.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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