Caspian Journal of Neurological Sciences
"Caspian J Neurol Sci"
http://cjns.gums.ac.ir

The Serum Amyloid β Level in Multiple Sclerosis: A Case- Control Study

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ARTICLE INFO

Article type:
Original Article

Bullet point:
- ***Serum Aβ level increases in RRMS***
- ***Serum Aβ level increases with MS progression and increasing EDSS scores***

Article history:
Received: 30 Mar 2017
Accepted: 10 Aug 2017
Available online: 1 Oct 2017
CJNS 2017; 3 (11): 214-221
DOI: 10.29252/nirp.cjns.3.11.214

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ABSTRACT

Background: Multiple sclerosis (MS) is one of the most common autoimmune diseases in adults that cause disability in patients. Different studies were conducted on more rapid diagnosis of the disease such as measuring serum or cerebrospinal fluid (CSF) contents.

Objectives: The current study aimed at measuring amyloid β (Aβ) serum levels in patients with relapsing-remitting MS.

Materials and Methods: In the current case-control study, the serum levels of Aβ were measured in 48 patients with RRMS and 33 healthy controls using the enzyme-linked immunosorbent assay (ELISA) technique in Isfahan, Iran, from 2014 to 2016. Data analysis was conducted with SPSS.

Results: The mean serum level of Aβ in the case (patients with RRMS) and control groups were 192.75±125.65 and 128.11±85.20 pg/mL, respectively; so serum Aβ levels in the RRMS group was significantly higher than healthy controls (p=0.02). Also, there was a positive significant correlation between the serum Aβ levels and the expanded disability status scale (EDSS) (r=+0.85, p<0.0001).

Conclusions: Owing to the increase of serum Aβ level in patients with RRMS and its significant increase in severe MS cases (higher EDSS scores), so serum Aβ level can be considered as a marker for MS and its progression.

Keywords: Multiple Sclerosis; Amyloid; Enzyme-Linked Immunosorbent Assay

➢ ***Please cite this paper as:***
Etemadifar M, Ghadimi M, Ghadimi K, Alsahebfosoul F. The Effect of Serum Amyloid B Levels on Multiple Sclerosis: A Case Control Study. Caspian J Neurol Sci 2017; 3(11): 214-221.

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Introduction

Multiple sclerosis (MS) is one of the most common chronic inflammatory diseases in the central nervous system (CNS), which demyelination, destruction or loss of axons, and gliosis are its major characteristics. About 2.5 million people deal with MS worldwide (1) and in spite of a multitude of studies on the disease, its etiology as well as pathophysiology remained unknown.MS is usually considered as a demyelinating while-matter disease and there is substantial evidence on the loss of axons and neurons in clinical stages of the disease based the pathophysiological findings; in other words, there is a direct correlation between the damage to axons and severity of MS (2). Also, neuron destruction plays a major role in the incidence of physical and cognitive disabilities (3). However, MS is diagnosed by the cerebrospinal fluid (CSF) findings as well as magnetic resonance imaging (MRI) analyses, and rejection of other suspicious diseases. The most common type of the disease is called relapsing-remitting MS (RRMS); in addition, other forms of the disease include secondary-progressive MS (SPMS), primary-progressive MS (PPMS), and progressive-relapsing MS (PRMS). Patients with RRMS experience attack and remission episodes, which indicate defective demyelination. The capacity of demyelination is reduced over time, particularly in SPMS cases (4,5). Recently several studies were conducted on the impact of amyloid protein precursor (APP) and its destructive product, amyloid β (Aβ), on Alzheimer's disease (AD) and reported that the reduction of Aβ level in CSF has a diagnostic value in AD. In spite of AD, the level of APP metabolites in CSF, including Aβ peptides, soluble APP (sAPP), α-sAPP, and β-s, are also reduced in CNS inflammatory diseases such as Lime disease, opportunistic infections in patients with HIV, acute bacterial meningitis, systemic lupus erythematosus, and MS (7-11). Some studies indicated that in the active form of MS, active demyelination, the activity of APP is high; however, it is not in chronic MS, the inactive form of the disease. Hence, APP metabolism varies in different stages of the disease (12). In addition, Aβ has positive regulatory effect in chronic and active lesions and is considered as a sensitive immunohistochemical marker in axon damage (13,14). On the other hand, former studies suggested a protective role for the increased serum Aβ in the rat models of MS (15). Hence, due to the role of Aβ in both acute and chronic phases of MS disease and lack of data on the serum level of Aβ in patients with MS, particularly RRMS, the current study aimed at evaluating and comparing the serum level of Aβ between the patients with RRMS and the healthy controls.

Materials and Methods

Subjects:

The current case-control study was authorized by the Vice President of Research of Isfahan University of Medical Sciences (code number: 395371) and a total of 48 eligible patients with RRMS (30 females and 18 males, mean ages 34.45±9.52 years) who met the inclusion criteria were selected out of the patients who referred to MS Center of Isfahan, Iran, from 2014 to 2016. The inclusion criteria were patients with RRMS within the age range of 16 to 60 years who were diagnosed based on the McDonald's

criteria (2010) (16) as well as clinical signs and symptoms, brain magnetic resonance image (MRI) and CSF findings, expanded disability status scale (EDSS) <5 with a stable conditions a month prior to the study, being under the treatment with the first line immunomodulatory drugs, and being in recovery period. All subjects should sign the written informed consent form. The subjects who were in the relapsing period during the last 3 months or had the history of inflammatory diseases, except MS, were excluded. In addition, 33 healthy controls (22 females and 11 males, 35.78±10.85 years) were recruited out of 220 healthy people with normal neurologic status and lack of the history of inflammatory, autoimmune or neurologic diseases who referred to the Iranian Blood Transfusion Organization (IBTO) in 2014. All neurologic experiments were performed by a neurologist in the both patients and healthy controls. Case and control groups were matched by age and gender.

MS severity was assessed by EDSS; it has direct correlation with MS severity and lower scores indicate mild MS and all patients with lower EDSS scores show normal symptoms. But, by increasing EDSS scores the severity of symptoms also increases in such extents that score 10 causes death (17). In addition, demographic data of the patients including age and gender were also recorded.

Four-mL blood specimens were taken from the case and control subjects by a routineblood collection method without adding anti-coagulant agent. The serum samples of the subjects were separated and stored at -20°C until it was tested. The serum levels of Aβ (pg/mL) were measured by the enzyme-linked immunosorbent assay (ELISA) technique according to the instructions of the manufacturer (Convance, Princeton, NJ, USA).

Statistical analysis:

Data of the current study was entered into SPSS version 24. The Kolmogorov-Smirnov test was used to assess the normality of data. To compare the case and control groups, chi-square and independent *t* test were used. In addition, the Pearson correlation was used to evaluate the correlation between the data. The qualitative data were expressed as numbers or percentages and the quantitative data as mean±standard deviation (SD); $p<0.05$ was considered as the level of significance.

Results

In the current study, there were no significant differences between RRMS patients and healthy control regarding to gender ($p=0.70$) and age ($p=0.46$).The mean of EDSS and duration of disease in the RRMS patients were 2.04±1.40 and 4.15±3.34 years, respectively (table 1).

Table 1. Clinical and Para-clinical Characteristics

Characteristics	RRMS	Healthy control	*p-value*
Number of subjects	48	33	-
Gender M/F	18/30	11/22	0.70
Age (Mean±SD) (years)	34.45±9.52	35.78±10.8	50.46
EDSS (Mean±SD)	2.04±1.40	-	-
Amyloid β (Mean±SD) (Pg/ml)	192.75±125.65	128.1±85.20	0.02
Duration of disease (Mean±SD) (years)	4.15±3.34	-	-

RRMS: Relapsing-Remitting Multiple sclerosis, EDSS: Expanded Disability Status Scale

The means of serum Aβ level were 192.75±125.65 and 128.11±85.20 pg/mL in the RRMS and control groups, respectively; it was significantly higher in the RRMS group compared with the controls (p=0.02) (figure 1).

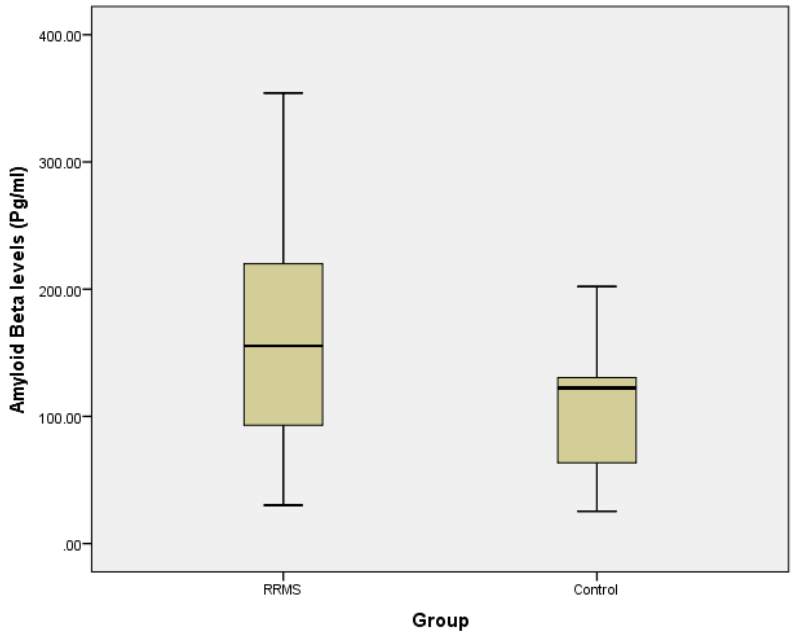

Figure 1: Boxplot of amyloid β concentrations in serum of RRMS patients and control group.

In addition, a positive significant correlation was observed between EDSS and serum Aβ level (r= +0.85, p<0.01) (figure 2), although there were no significant correlation between the serum Aβ level with age (p=0.81), gender (p=0.89), and duration of illness (p=49).

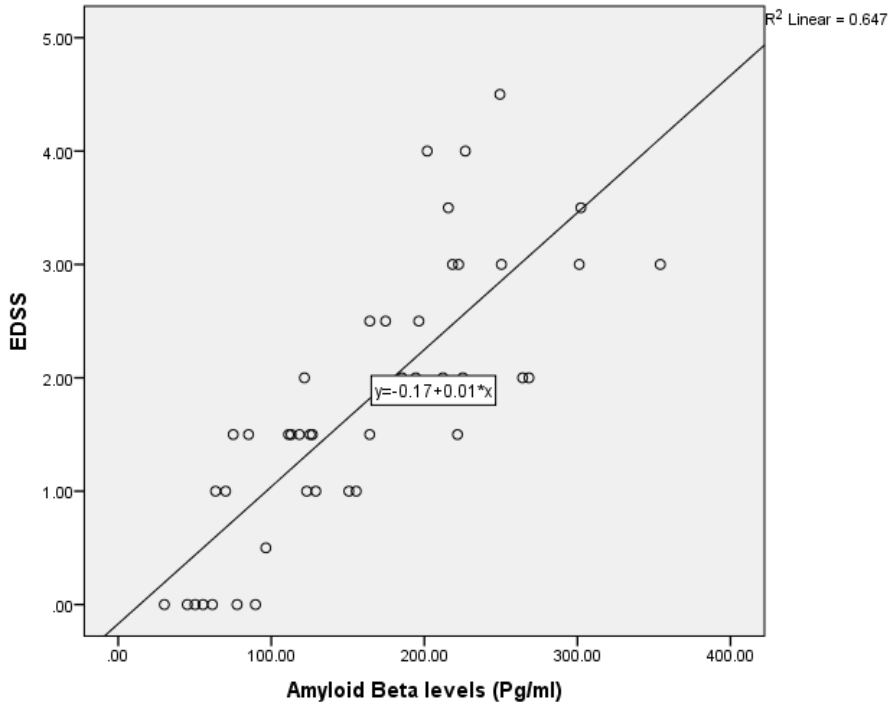

Figure 2. Positive significant correlation between serum levels of amyloid β and EDSS.

Discussion

According to the results of the current study, the serum Aβ level was above normal limits in MS patients. Also, the positive significant correlation between EDSS and serum Aβ level indicated that by increasing the severity of MS (increased EDSS or disability) the serum Aβ level also increases. As mentioned before, the level of Aβ decreases in the CSF of patients with MS (10). According to the study conducted by Augutis *et al.* (18) the levels of Aβ and sAPP in CSF were assessed in 87 patients with MS, including 54 RRMS and 33 SPMS cases, and 28 healthy controls using ELISA technique. The patients were also received natalizumab and mitoxantrone for 1-2 years. They indicated that the level of Aβ and sAPP in CSF of the patients with MS reduced, but the serum level of Aβincreased in patients with MS following the treatment with natalizumab. They concluded that treatment with natalizumab may neutralize and make changes in the metabolism of APP in patients with MS. Also, they found an isoform distribution profile for Aβ in the CSF of the ones with SPMS, which differentiated them from the healthy controls. In another study by Pietroboni *et al.* (19) on 48 recently diagnosed MS subjects, the serum Aβ level was measured in the beginning of the study in all subjects; the test was also repeated after a 3-year follow-up and their EDSS scores were recorded at 6-month intervals. Results showed that the Aβ level in CSF was lower in MS patients compared with the healthy controls; based on the results of a 3-year follow-up, lower levels of Aβ in CSF of the patients with MS implied a prognostic factor for disability. In addition, they concluded that decrease of Aβ in CSF can be considered as a

diagnostic marker for neurodegeneration in MS disease, which may occur prior to clinical symptoms; hence, Aβ can affect the prognosis of the disease.

In a study by Mai (20), the level of Aβ42 and the soluble form of αAPP or αsAPP in CSF were measured in 42 patients with MS, 10 subjects with neuromyelitisoptica, and 25 subjects with clinically isolated syndrome, as well as 21 healthy controls. Results of his study indicated no significant correlation between the patients and healthy controls in the level of Aβ42 and soluble form of αAPP in CSF. Also, the level of αsAPP in CSF of the patients undergone statin therapy was significantly higher than the ones not received such treatment; hence, the authors suggested a neuroprotective role for statin. Another study evaluated the association between cognitive disorder and cortical plasticity with the changes of Aβ level in CSF of patients with MS and the results indicated that the inflammation of CNS in MS disease makes changes in the metabolism of Aβ, which results in the reduction of Aβ in CSF as well as cognitive and synaptic plasticity impairments (21). Claner (22) in a study showed that APP particularly caused demyelination in MS studies in vitro; in addition, they indicated that astrocytes play a key role in the pathogenesis mechanism of demyelination. Some studies measured the serum level of amyloid A (AA) in patients with MS. For example, in a study by Yokote (23) who suggested AA as factor for T-helper 17 (Th17), which can participate in the pathogenesis of the disease in critical conditions and its amount was high in neuromyelitis optica cases compared with the patients with RRMS and healthy controls, respectively. He suggested the association between serum level of AA and clinical phenotypes. In a study by Ristori *et al.* (24) the serum level of AA was measured in patients with RRMS. They concluded that the increase of serum AA level is attributed to the progressive peripheral inflammation; in fact, serum AA increase was one of the prognostic signs of progressive peripheral inflammation in their study. A case report also indicated the amyloid in the demyelinated plaques in MS (25).

Conclusion

Since according to the results of similar studies, the CSF level of Aβ decreases in patients with MS, which some studies attributed it to the inflammation of CNS or the progression of peripheral inflammation, based on the results of the current study, the serum level of Aβ increases in patients with MS compared with the healthy controls; serum Aβ level also increases in the ones with more severe MS or disabilities (higher scores of EDSS or critical inflammatory conditions following the demyelination) and play a pro-inflammatory role in the pathogenesis of MS disease, particularly RRMS. Owing to the limitations of the current study, such as small sample size and not including other types of MS to measure serum level of Aβ, further studies seems necessary. The current study was the first in Iran that evaluated the serum level of Aβ in patients with RRMS.

Conflict of Interest

The authors have no conflict of interest.

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