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Evaluation of plasma soluble CD137 level in relapsing-remitting multiple sclerosis patients in comparison with healthy controls in Isfahan Province, Iran

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Abstract

Objective: Multiple sclerosis (MS) is a chronic neuroinflammatory disease, characterizes by demyelination in the central nervous system (CNS). Co-stimulatory molecules such as CD137 (4-1 BB) play a major role in the activation of lymphocytes in CNS. The exact immunopathogenesis of MS is unknown. Hence, detection of specific biomarkers in the process of MS disease can lead to new therapeutic approaches. This study aimed to compare plasma sCD137 levels in relapsing-remitting multiple sclerosis (RRMS) patients with healthy controls in Isfahan province. *Methods:* Plasma sCD137 level was measured by enzyme-linked immune sorbent assays (ELISA) in 36 RRMS patients as well as 52 (age and sex-matched) healthy controls and the results were compared. *Results:* The plasma sCD137 level in studied RRMS patients was significantly higher in the patient group compared to the healthy controls (P- value=0.027). In addition, there was no significant association between age, sex, job and education level, with plasma sCD137 level in both the control and the case groups (P value>0.05). There was no correlation between mean of sCD137 and EDSS score, age of onset, duration of disease as well as serum 25 (OH) D concentrations of the patients.
Conclusion: High plasma sCD137 level was detected in RRMS patients when compared with the controls, which may indicate the possible role of this biomarker in the immunopathogenesis of MS. Since CD137 can affect T lymphocytes activation and apoptosis, further studies are needed to elucidate its exact role in the pathogenesis of MS.

Keywords: Multiple sclerosis, relapsing-remitting multiple sclerosis, CD137, autoimmune disorders

INTRODUCTION

Multiple sclerosis (MS) is a high-prevalence chronic neuroinflammatory disease, affecting the central nervous system.[1,2] The cause of MS is still unknown, but studies have shown that environmental and genetic factors, such as human leukocyte antigen (HLA) (eg, HLA DRB1*15:01), smoking, vitamin D deficiency or ultraviolet B light (UVB) exposure can contribute to the disease progression.[3-5] It has been shown that 50-300 per 100,000 population and about 2.3 million people are living with MS in the world.[6,7] In Iran, Isfahan province has one of the highest prevalence of MS.[8,9] MS is more common amongst females and its sex ratio is 3:1 (F:M), characterized by demyelination of neurons in the CNS.[2] The defect in the regulation of immune responses is responsible for neuronal damage. Also, there is significant role of $TCD4^+$ cells and their cytokines in the stimulation of macrophages and demyelination of neurons.[10]

CD137, also known as 4-1 BB or tumor necrosis factor receptor 9 (TNFR9), is a member of TNFR superfamily and is expressed as a homotrimer. CD137 is expressed as an inducible molecule

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on the surface of activated $CD4^+$ and $CD8^+$ T cells.[11] In addition, to activated T cells, CD137 also express on myeloid cells, B cells, activated natural killer cells (NKCs) and regulatory T cells (Tregs).[12] CD137 ligand (CD 137L), or 4-1 BBL, expresses on the antigen-presenting cells (APCs) as a homotrimer.[13] Engagement of CD137 with its ligand on APCs augments proliferation, cytokine secretion, and survival; thus, enhances the effector function.[14] The soluble form of CD137 (sCD137) is a 16 KDa protein, secreted by activated lymphocytes. It was reported that the sCD137 level increased in some autoimmune diseases, such as MS and rheumatoid arthritis.[15,16] Studies on CD137 have also been performed in non-autoimmune diseases, such as cancer and suggested that anti-CD137 antibodies have anti-cancer effect via the activation of T lymphocytes and NKCs by reducing the activation of regulatory T cells.[17] Based on the role of sCD137 in autoimmune diseases, in this study, we aimed to assess sCD137 in peripheral blood from patients with MS in Isfahan province and compare it with healthy controls.

METHODS

Study participants

In this study, we enrolled two groups of participants, consisting of 36 patients with relapsing-remitting multiple sclerosis (RRMS) (mean age: 30.87±8.61) according to 2017 revised McDonald's criteria [18] in remission. The patients were recruited from the Isfahan MS and Neuroimmunology Research Centre. The mean age of the 52 age and sex-match healthy controls was 34±5.18 years. The patients' functional status was evaluated by using the Expanded Disability Status Scale (EDSS) assessment and brain MRI by a neurologist who was blind to the sCD137 levels. None of the patients had relapses one year before the onset of study. The exclusion criteria for selecting RRMS patients are as follows: other types of MS, other neurodegenerative disorders, history of organ transplantation, malignancies, cardiovascular or renal disease, and other types of autoimmune or inflammatory diseases. None of the patients have received treatment with corticosteroid agents for at least 6 months from the sample collection, but some patients had received IFN-ß as their routine treatment. Written informed consent was obtained from all participants in this study. This study was approved by the research ethics committee of Isfahan University of Medical Sciences (Grant No: 194067). Table 1 presents the demographic characteristic of the participants.

Assessment of sCD137 plasma level

In brief, peripheral blood of patients with MS and healthy individuals as the control was collected in a vacuum tube, containing EDTA. Plasma was isolated by centrifugation at 2400 g for 10 min and stored at -80 °C until further evaluation. After collecting the samples, plasma sCD137 level was assessed by enzyme-linked immune sorbent assay (ELISA) technique, using commercial kit (Human sCD137 EASTBIOPHARM ELISA kit, cat.NO:CK-E91545, China) according to the manufacturer's instructions.

Statistical analysis

The Kolmogorov- Smirnov Z test was performed to check for normal distribution of sCD137 level. Independent t-test, one-way ANOVA and chi-square tests were used to compare the groups in

Table 1: Demographic characteristic of subjects

Characteristics	RRMS	HC
n	36	52
Female / Male	20/16	28/24
Age (years)[a]	30.87±8.61	34±5.18
Serum 25 (OH) D (ng/ml)[a]	18.73±1.72	20.5±2.42
Age at onset (years)[a]	27±8	-
Duration of disease (years)[a]	2.5±1.15	-
EDSS[a]	1.3±0.7	-
Number of relapses in the previous year [a]	1.2±0.6	-

EDSS: Expanded Disability Status Scale
[a] Data are expressed as mean±SD.

terms of continuous and categorical variables. The logistic regression model was performed to evaluate the association of sCD137 with MS patients, adjusting for confounding variables. All data are shown as mean (Standard deviation) and all P values less than 0.05 were considered to be statistically significant. All statistical analyses were performed using IBM SPSS Statistics for Windows (Version 24.0. Armonk, NY: IBM Corp).

RESULTS

We assessed plasma sCD137 level in 88 individuals (36 RRMS patients and 52 healthy controls) in Isfahan province. Mean sCD137 level and various factors including age, sex, education level, and job in both groups were compared.

Assessment of plasma sCD137 level showed that the mean of sCD137 in RRMS patients was significantly higher than the control group (1021.75±296.12 ng/L versus 892.18±184.21 ng/L) (P value= 0.027) (Figure 1).

Table 2 shows the comparison of mean plasma sCD137 level in relation to various factors including age, sex, education level, and job in the control group as well as the comparison of age, sex, education level, job, and treatment with IFN- ß in the case group. As shown in Table 2, there was no significant association amongst these factors with the plasma level of sCD137 in both the control and the case groups.

The logistic regression model was performed by adjusting for the confounding variables, such as age, education level, and job in both groups. The results showed that the risk of MS increases by 30% for every 100-unit increase in sCD137, which is statistically significant. The similarity between the two OR, indicates that the underlying variables were not able to distort the overall result (sCD137 effect on MS) (Table 3).

Our results also showed no correlation between mean of sCD137 and EDSS score, age of onset, duration of disease as well as serum 25 (OH) D concentrations of patients.

DISCUSSION

MS is a type of neurodegenerative disease, characterized by demyelination and inflammation caused by immune cells in CNS.[19] $TCD4^+$ and $TCD8^+$ lymphocytes play a major role in the stimulation of macrophages in CNS that leads to demyelination.[20] Activation of T lymphocytes depends on several factors; including co-stimulatory molecules, such as B7-1, B7-2, CD28, CD40, and CD137.[21] CD137 is a co-stimulatory molecule that stimulates T lymphocytes in the absence of CD28.[22] Several studies assessed soluble and non-soluble CD137 in some autoimmune diseases. Sharief *et al*. assessed soluble CD137 in patients with MS and their results showed a high concentration of intrathecal and systemic levels of sCD137 in patients with clinically active MS.[16] Liu *et al*. also examined the expression of CD137 and soluble sCD137 protein levels in the peripheral blood of MS patients. Their result showed reduced expression of CD137 on $CD4^+CD25^+$ Treg cells in MS patients, this reduction was thought to be associated with decrease in the function of Treg cells. In addition, they reported increase in plasma sCD137 level in MS patients.[23] According to these findings, we decided to assess the plasma sCD137 level in patients with RRMS in Isfahan province.

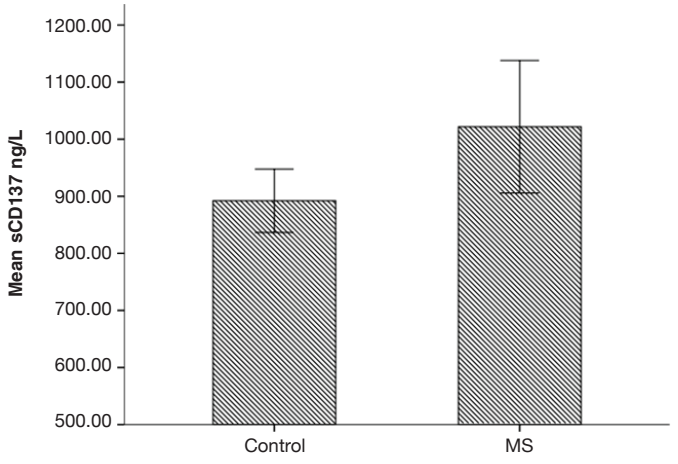

Figure 1. Mean of sCD137 in RRMS patients and healthy control group. Mean of sCD137 was significantly higher in RRMS patients (1021.75±296.12 ng/L) compared with control group (892.18±184.21 ng/L) (P-value= 0.027)

Table 2: Comparison of mean sCD137 in various factors within case (RRMS patients) and control groups

Groups	Factors		sCD137 (ng/L) Mean ±SD	P-value
Control	Age (years)	Less than 25 (n=14)	902.89±136.50	0.913
		25-35 (n=17)	902.60±235.22	
		Upper than 35 (n=21)	878.30±180.09	
	Sex	Female (n=28)	887.59±188.70	0.606
		Male (n=24)	938.16±142.80	
	Education	School education (n=45)	885.31±192.66	0.576
		University education (n=7)	928.49±136.54	
	Job	No employee (n=50)	896.46±185.65	0.487
		Employee (n=2)	802.36±170.59	
Case (RRMS)	Age (years)	Less than 25 (n=0)	-	0.129
		25-35 (n=9)	859.89±302.36	
		Upper than 35 (n=27)	1070.31±283.84	
	Sex	Female (n=20)	1015.10±313.61	0.924
		Male (n=16)	1026.62±293.72	
	Education	School education (n=17)	1103.83±317.63	0.100
		University education (n=19)	909.81±232.55	
	Job	No employee (n=25)	1089.01±282.74	0.054
		Employee (n=11)	839.17±268.93	
	Treated with IFN-ß	No (n=14)	1022.10±318.72	0.936
		Yes (n=22)	1031.62±285.65	

Our result showed that plasma sCD137 plasma level in patients with RRMS was significantly higher in comparison with healthy controls, which was a confirmation of previous studies that reported the increased expression of CD137 in MS patients. CD137 appears to have a dual role. CD137 expression on resting T lymphocyte leads to apoptosis in the helper and cytotoxic T lymphocyte population, but it has been shown that CD137 expression on active T lymphocytes inhibits T cell apoptosis, by inducing Bcl-xL and c-FLIP[24,25]

In the current study, we did not compare sCD137 level in RRMS patients during remission with those with relapse; hence, it cannot be said that high plasma sCD137 level in MS patients is an indication for its role in the inflammatory process of the disease. Hence, more studies are needed to discuss this issue. Future studies should investigate the association between lymphocyte apoptosis and sCD137 expression in different stages of the disease. Targeting this molecule is another way to understand its function in the pathogenesis of MS. Further studies might be able to eliminate the uncertainties regarding the function of this molecule in the pathology of MS.

On the other hand, the role of CD137 in other autoimmune diseases is of interest. Jan Michel *et al*. detected a large amount of sCD137 in the sera of patients with rheumatoid arthritis compared

Table 3: The logistic regression model adjusted for confounders: age, sex, job, and education

	Odds Ratio	P-value
Crude	1.003 (1.001 – 1.005)	0.040
Adjusted	1.003 (1.001 - 1.006)	0.043

with healthy donors.[15] According to previous studies and the current one, it can be suggested that sCD137 increases in autoimmune diseases and affects the function of immune cells. Since the elevation of sCD137 have been confirmed in autoimmune diseases such as rheumatoid arthritis, systemic lupus erythematous, Behcet's disease[26] and also is associated with inflammatory and metabolic parameters[27], sCD137 is not a specific marker for MS. Further studies are necessary to understand the exact role of increased expression in MS pathology.

In conclusion, we evaluated the plasma sCD137 level in RRMS patients and compared with healthy controls in Isfahan province. Our result showed that the mean plasma sCD137 level were higher in RRMS patient than the healthy controls. Since the elevation of sCD137 has been confirmed in other autoimmune diseases, it is not a specific marker for MS. Further investigations might be able to clarify its role in the pathogenesis of MS.

DISCLOSURE

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Conflict of interest: None.

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