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The Relation between Age, Gender and Apoptosis Regulator FasL Gene Expression in Multiple Sclerosis Patients

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ABSTRACT

Multiple sclerosis (MS) is the most common chronic inflammatory demyelinating disease of the central nervous system (CNS). The exact immunopathogenic mechanisms are not known but there is some evidence that this severe disease is more likely to develop in genetically predisposed individuals, possibly in association with environmental factors Elimination of autoreactive T cells by activation induced cell-death (AICD) is considered to be one of major process in MS. The aim of this study were to evaluate expression level of FasL in whole blood from patients with Relapsing-Remitting (RR) form of MS, and to survey the association of FasL expression with risk, Expanded Disability Status Scale (EDSS) and duration of the disease. We compared FasL expression in 50 RR-MS patients with 50 healthy controls by Taqman Real time PCR technique. Albeit there was an expression decrease, no statistically significant difference was found between total RR-MS patients and controls. However, our results showed a clear association between decrease of FasL expression in females especially older than 40 years with risk of the disease (p= 0.04, 95% CI= 0.387-1.14; p= 0.003, 95% CI= 0.139-3.12, respectively). Moreover, there was not a significant correlation between EDSS and duration of the disease and FasL expression. This finding make a valuable question what is the principal concept for this significant association between FasL expression and risk of RR-MS in females who are older than 40 years. In this study, we failed to draw an exact expression–phenotype correlation which may be due to limited statistical confirmation as a result of the small sample size and needs more investigation. These findings may possibly reflect differences in the pathogenic mechanisms associated with the failure of AICD observed in this group of MS patients.

Key words: FasL, Expression, Multiple Sclerosis, Central Nervous System

1. INTRODUCTION

MS is an autoimmune disorder in which the myelin or insulating covers of neurons in the Central Nervous System (CNS) are damaged. The precise pathogenesis of MS has not been fully understood; however, one of the underlying processes in myelin destruction is caused by the autoreactive immune system (1). Some reasons have been proposed for the hypothesis that MS is an autoimmune disorder such as association of certain genes such as MHC with the disease, Existence of inflammatory plaques in the white matter containing immune cells, Evidences of remedy by immuno-suppressors and immune-modulators such as azathioprine and interferon b (IFN-b). Generally, apoptosis is a key mechanism which determines fate of the cells and mediates tissue degeneration. Dysregulation of apoptosis plays a significant role in many diseases including autoimmune disorders, cancers, developmental disorders, the acquired immunodeficiency syndrome (AIDS) and etc. Therefore, apoptosis failure of autoreactive T cells which

attack myelin antigens specifically, might contribute to the etiopathogenesis of MS (2, 3). Activation-induced cell death (AICD) and stimulation of death receptors are involved in Shutting down of the autoreactive T cells and self-immune responses (4). Interactions between members of the tumor necrosis factor (TNF) superfamily; such as Fas receptor (CD95, APO-1, TNFRSF6) as a 36 kDa transmembrane glycoprotein and Fas ligand (CD95L, TNFSF6, CD178), efficiently mediate apoptosis cascade and triggering immunoregulatory mechanisms including selection of T cell repertoire, deletion of self-reactive cells and -cytotoxicity against target cells or tissues, among others (5-9). Moreover, binding of Fas-FasL can also activate pro-inflammatory signals. FasL is kept under strict control by transcriptional and post-translational regulation and its expression is tightly restricted to activated T cells, natural killer cells, certain tumours and at sites of immune privilege (10). Both genetic and environmental elements contributed to disorder risk. Previously, we studied some genes, especially HLA and cytokine genes, in Iranian patients with MS (11-13). In the present study, we investigated expression of FasL in relapsing-remitting (RR) MS patients group in comparing healthy control individuals. Also, we examined correlation between FasL relative quantitation and EDSS and disease duration in patients.

2. MATERIALS AND METHODS

2.1. Subject and control group

This study was performed on fifty unrelated patients with sporadic MS and fifty healthy controls. The patients were diagnosis and recruited from Tehran's Hospitals and MS society of Iran. The clinical inclusion criteria were a diagnosis of clinically definitive MS. All the patients in time of sampling were in remitting phase. All of our patients were responsive to interferon-beta and were treating with Cinnovex™. Moreover, Vitamin D level was normal for both patients and control groups. As the control group had normal serum level of Vitamin D, for matching the patients to controls, the patients with Normal Vitamin D were selected. The clinical diagnosis was supported by the MRI criteria. All the patients that were positive for HLA-DRB1*15, as a highest susceptible allele, were excluded from investigation. *Our HLA typing was performed by Combi Tray™ kit from Olerup Company.*

2.2. Blood sampling

3 ml peripheral blood was taken in an EDTA tube. All individuals gave their informed written consent. This study was approved by a local Ethical Committee (IR.SBMU.SM.REC.1394.91).

2.3. Real-time quantitative RT-PCR

Geneall Hybrid-R™ blood RNA extraction Kit (cat No.305-101) was used to isolate total RNA. Total RNA was reverse transcribed into single-strand cDNA using Applied Biosystems High-Capacity cDNA Reverse Transcription Kits (PN: 4375575). They used according to the manufacturer's instructions. The cDNA was stored at -80°C until further use for Real-Time PCR. The specific primers and probes were designed Allele ID 7 for x64 windows software (Premier Biosoft, Palo Alto, USA) and order for synthesis. HPRT1 was used as the reference gene. The primers and probes sequences and PCR product length have been shown in Table 1.

Table 1. The primers and probes sequences and PCR product length

property	*HPRT1*	*Fas Ligand*
Forward primer	AGCCTAAGATGAGAGTTC	ATGCACACAGCATCATCTTTGG
Reverse primer	CACAGAACTAGAACATTGATA	ATGGGCCACTTTCCTCAGCT
Probe	Fam-CATCTGGAGTCCTATTGACATCGC-Tamra	Fam-AAGCAAATAGGCCACCCCAGTCCACC-Tamra
Amplicon length	101bp	96bp

Real-time quantitative polymerase chain reaction was performed by using Applied Biosystems TaqMan® Universal PCR Master Mix (PN: 4304449) in Corbett Rotor gene 6000 (rotary analyzer) machine. The negative control was simultaneously carried out for quality control of the method and detection of possible cases of contamination.

2.4. Statically analysis

Differences between both groups were analyzed using the independent samples t test. In the case of a higher number of groups, the one way ANOVA test was used instead. In order to evaluate the correlation between variables, Pearson correlation coefficient was used. P values of <0.05 were considered to be statistically significant. The data were analyzed by SPSS 18 (Chicago, IL, USA).

3. RESULTS AND DISCUSSION

3.1. Stratification of the study population

Demographic and clinical profiles of MS patients and healthy controls have been listed in Table 2.

Table 2. Demographic and clinical data of the MS patients and healthy controls

Variables	MS patient	Control
Female/Male [no. (%)]	**31 (62%)/19(38%)**	**29 (58%)/21 (42%)**
Age (mean ± SD, Y)	**37.4 ± 5.2**	**36.9 ± 4.6**
Age range (Y)	**17-69**	**19-63**
Age at onset (mean ± SD, Y)	**31.36 ± 2.4**	-
Relapsing-remitting course (no. %)	**100 (100%)**	-
Duration (mean ± SD, Y)	**6.1 ± 3.1**	-
EDSS[a] (mean ± SD)	**2.72 ± 2.3**	-

[a] Expanded Disability Status Scale (EDSS). nKurtzke, J.F., 1983. Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). Neurology 33, 1444–1452.

Their characteristics show that both groups are well matched in age and sex. Moreover, we categorized our patients and controls in three different classes based in their ages (>30, 30-40, 40<).

3.2. FasL expression and Risk of RR-MS

We compared the expression level of FasL among patients and healthy control groups and our results are presented in Table 3.

Table 3. FasL Expression in comparing of RR-MS patients and control group based on gender and age category

FasL expression		Control no.	RR-MS patient no.	p value	Expression ration	Std.Error	95% CI
Total		50	50	0.09	0.64	0.481	0.99-1.2
Male		21	19	0.18	0.75	0.914	0.53-1.146
Female		29	31	**0.04**	0.42	0.378	0.387-1.14
<30	Male Female	9 13	5 11	0.31 0.08	0.94 0.61	1.992 0.619	0.202-6.588 0.274-1.243
30-40	Male Female	7 9	6 9	0.17 0.07	0.78 0.53	1.272 0.744	0.033-1.46 0.404-2.656
>40	Male Female	5 7	8 11	0.08 **0.003**	0.54 0.12	1.862 0.525	0.593-8.594 0.139-3.12

Totally, we could not find significant statistical difference between all patients and all healthy controls (p= 0.09, 95% CI= 0.99-1.2). After separating the individuals into male and female group, there was a significant association between female RR-MS patients and healthy controls (p= 0.04, 95% CI= 0.387-1.2). Therefore, we categorized our samples based on age and sex to find any association. Among male samples (patients and healthy controls), neither of the age classes are nevertheless statistically significant (p>0.05). However, we could find a clear association between RR-MS and healthy females older than 40 (p= 0.003, 95% CI= 0.139-3.12). Females with RR-MS who are older than 40 years express FasL about ten times fewer than healthy females with the same age (Expression ration= 0.12). Other females did not show any significant association of FasL expression and risk of RR-MS.

3.3. FasL expression and EDSS

Among 50 RR-MS patients, we investigated whether expression level of FasL influences on the disability status or not. So, we compared the expression level of FasL with Expanded Disability Status Scale (EDSS) of our patients (Figure 1).

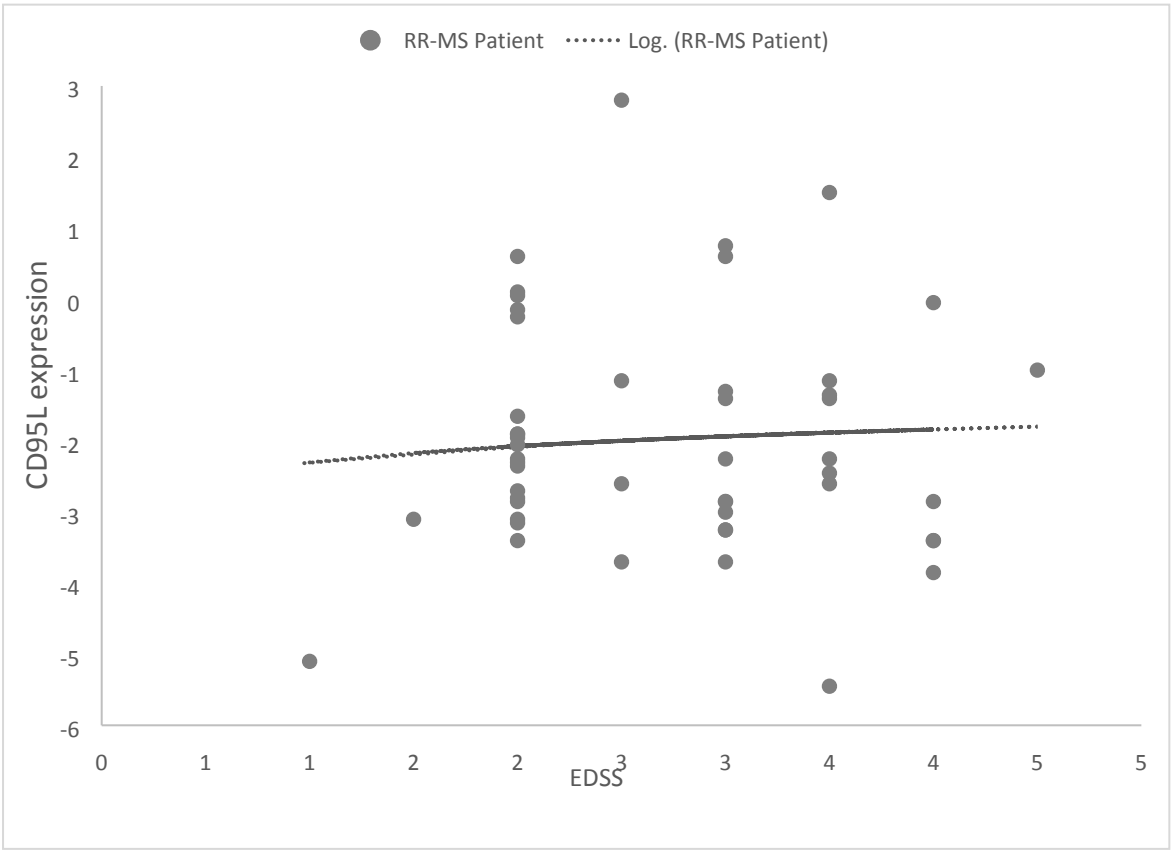

Figure 1. Correlation between FasL relative quantitation and EDSS in RR-MS patients

But the analysis did not show a significant statistical correlation (R^2= 0.0061).

3.4. FasL expression and disease duration

Our results illustrate that disease duration of most of our patients are fewer 15 years. However, quantified FasL expression was different in each of them (Figure 2).

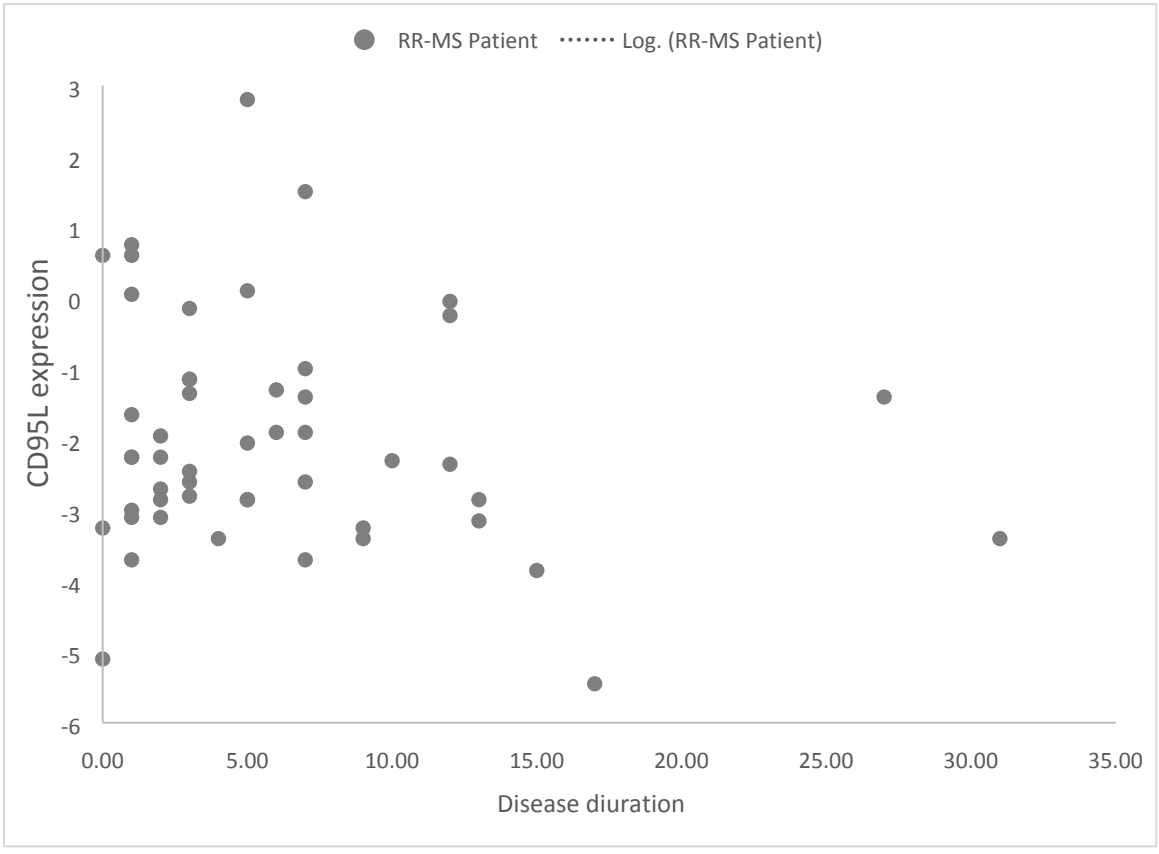

Figure 2. Correlation between FasL relative quantitation and disease duration in patients

There was no significant correlation between expression status of FasL and duration of the disorder (R^2=0.0256). Recent investigations have clarified that defects in interaction of CD95/Fas with CD95L/FasL can lead to impaired apoptotic process in activated lymphocytes of patients with relapsing-remitting multiple sclerosis (14, 15). Previous studies have suggested that the expression levels of Fas and FasL are increase in MS plaques (16) and on peripheral blood lymphocytes (17, 18). High systemic (19, 20) and CSF (21) levels of soluble Fas protein have been detected in MS patients. But Lopatinskayaa et al, showed that the levels of FasL mRNA, decrease during the development of clinical exacerbations (22). The goal of our study was to assess the expression of mRNA encoding FasL, in relation to risk of relapse-remitting multiple sclerosis (RR-MS). We evaluated expression level of FasL in whole blood of healthy and affected individuals. Overall expression of FasL has decreased in patients group, but it is not significant statistically. Actually, our results in comparing all patients and all healthy controls did not show statistical significant association between FasL expression and risk of RR-MS. However, females and specially females who are older than 40 years showed a clear association in risk of the disorder in regarding FasL expression. This finding make a valuable question which it should be answered in future investigations, what is the principal concept for this significant association between FasL expression and risk of RR-MS in females who are older than 40 years. Although, we could not find significant correlation between EDSS and duration of the disease and FasL expression which is agreement with the previous report (23), but this may pave the way to a novel approach. Base on this approach, reduction of FasL expression leads to decrease in apoptosis of autoreactive lymphocytes. Also, we analyzed FasL expression level of patients who were in remission stage, we hypothesize that expression level of FasL will decrease more in relapse period due to more reduction of apoptosis occurrence. We suggest evaluating expression level of other apoptotic factors such as Fas, sFasL, TRAIL and etc. A significant association between FasL expression and risk of RR-MS in females who are older than 40 years was founded in this study. As this finding was not detected in other studies, unfortunately, comparisons among studies were impossible. For other sections of results, we have effort to compare the results and references to them. We believe that the ratio of these proteins can be determinant of age of onset and severity of multiple sclerosis. In this study, we failed to draw an exact expression–phenotype correlation which may be due to limited statistical confirmation as a result of the small sample size and needs more investigation. These findings may possibly reflect differences in the pathogenic mechanisms associated with the failure of AICD observed in this group of MS patients. In addition, It is recommended to consider in future studies non-responder patients comparing to responder ones.

4. CONCLUSION

As apoptosis failure of autoreactive T cells which attack myelin antigens specifically, might contribute to the etiopathogenesis of MS, the expression level of FasL in MS patients was evaluated by Taqman Real time PCR. Our results showed a clear association between decreases of FasL expression in females especially older than 40 years with risk of the disease. This finding make a valuable question what is the principal concept for this significant association between FasL expression and risk of RR-MS in females who are older than 40 years. In this study, we failed to draw an exact expression–phenotype correlation which may be due to limited statistical confirmation as a result of the small sample size and needs more investigation. These findings may possibly reflect differences in the pathogenic mechanisms associated with the failure of AICD observed in this group of MS patients.

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AUTHORS CONTRIBUTION

This work was carried out in collaboration among all authors.

CONFLICT OF INTEREST

The authors declared no potential conflicts of interests with respect to the authorship and/or publication of this paper.

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