International Journal of Medical Laboratory 2020;7(1):15-22.

International Journal of Medical Laboratory
Shahid Sadoughi University of Medical Sciences

Original Article

RAGE p.82G>S Polymorphism Is Not Associated with the Risk of Multiple Sclerosis in Iranian Population

Hamideh Valizadeh[1] M.Sc., Gilda Eslami[2,3] Ph.D., Reyhane Rahnama[4] Ph.D., Abolghasem Rahimdel[5] Ph.D., Fateme Sadat Dashti[6] M.Sc., Reza Mansouri[7*] Ph.D.

[1]Department of Immunology, Faculty of Medicine, International Campus of Shahid Sadoughi University of Medical Sciences, Yazd, Iran.
[2]Research Center for Food Hygiene and Safety, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.
[3]Department of Parasitology and Mycology, Faculty of Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.
[4]Department of Immunology, Faculty of Medicine, Isfahan University of Medical Science, Isfahan, Iran.
[5]Department of Neurology, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.
[6]Reproductive Immunology Reseach Center, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.
[7]Department of Immunology, Faculty of Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

Article history
Received 6 Aug 2019
Accepted 13 Jan 2020
Available online 1 Mar 2020

Key words
Advanced glycation end product
Multiple sclerosis
Polymorphism

A B S T R A C T

Background and Aims: Multiple sclerosis (MS) is known as a partially inheritable inflammatory autoimmune disease which involves the nervous system. Different studies suggest that immune dysregulation has an important role in the pathogenesis of MS, but its exact pathomechanism has not yet been explicated. The receptor for advanced glycation end products (RAGE) is a member of the immunoglobulin superfamily of cell surface molecules. In MS patients, the expression of membrane-bound RAGE on peripheral blood mononuclear cells, as well as the serum levels of the RAGE ligands are upregulated.

Materials and Methods: In this case-control study, we evaluated the association between MS incidence and *RAGE* p.82G>S polymorphism (rs2070600) compared with healthy controls in an Iranian population. A total of 158 patients and 156 healthy blood donors were enrolled. This polymorphism was genotyped by polymerase chain reaction-restriction fragment length polymorphism.

Results: Allele frequencies (p=0.319) and genotype distribution (p=0.315) of the *RAGE* p.82G>S gene polymorphism in MS patients and controls were not significantly different.

Conclusions: The present study indicated no relationship between *RAGE* p.82G>S polymorphism and the risk of MS in Iranian population.

[*]**Corresponding Author:** Department of Immunology, Faculty of Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran. **Tel:** +989132538812, **Email:** rezamansouri903@gmail.com

Introduction

Multiple sclerosis (MS) is known as a partially inheritable inflammatory autoimmune disease which involves the nervous system. Based on different symptoms of MS, three types of MS have been described including relapsing-remitting (RR), secondary progressive (SP), or primary progressive (PP) types [1]. The prevalence of MS increases from typically 1 in 1,000 in the normal population to 1 in 4 or so for identical twins where one twin is affected [2]. The main pathological features of MS include local lesions with inflammation and demyelination of nerve fibers, infiltration of immune cells in a perivascular distribution, plus relative sparing of the axons and oligodendroglial death [3-5]. Although it has been frequently confirmed that immune dysregulation has an important role in the pathogenesis of MS, the pathomechanism and the exact causative factors have not yet been explained [6]. Therefore, immunorelevant molecules bear the higher potential to be investigated for clarifying the etiology of this disease. The receptor for advanced glycation end products (RAGE) is a member of the immunoglobulin superfamily of cell surface molecules [7]. It was first recognized as a receptor for non-enzymatic glycosylation products and oxidation of proteins and lipids. These compounds are formed under conditions such as diabetes, kidney failure, aging, and inflammation, which are referred to as advanced glycation end products [8]. Initially, advanced glycation end products were considered to be the main ligands of the RAGE, but later, they were identified as a broad range of ligands such as amyloid β- peptide (accumulates in Alzheimer's disease) and amyloid A (which accumulates in systemic amyloidosis), the S100/calgranulins, (accumulated extracellularly at sites of chronic inflammation due to immune cell activity especially neutrophils) [9]. In MS patients, the expression of membrane-bound RAGE on peripheral blood mononuclear cells [10], as well as the serum levels of the RAGE ligands is up-regulated [11].

Yan et al. showed that the expression of RAGE and S100/calgranulins increase in spinal cord samples from MS patients as compared with controls. They declared that the interaction between RAGE and S100/calgranulins can enhance the production of chemokines, cytokines, and adhesion molecules of T $CD4^+$ cells infiltrating the central nervous system [12]. Another isoform of RAGE called soluble RAGE (sRAGE) acts as a decoy and prevents the noxious effects of RAGE [13]. Studies have demonstrated lower levels of sRAGE in MS patients as compared to healthy controls along with an inverse relationship between the severity of MS disease and serum level of sRAGE [14, 15].

In 1998, a single nucleotide polymorphism (SNP) was identified within the ligand-binding domain of RAGE, where at codon 82, serine replaces glycine (p.82G>S; rs2070600) [16]. In the cells expressing the *RAGE82S* allele compared with the *RAGE*

82G expressed cells, enhanced binding of RAGE with S100/calgranulin and in turn increased cytokine/MMP generation were displayed [17].

As *RAGE 82S* allele has the potential to be a highly conceivable genetic risk factor for MS, the aim of this study was to evaluate the association between susceptibility to MS and *RAGE p.82G>S* polymorphism compared with healthy controls in Yazd, Iran.

Materials and Methods

A total of 158 patients with clinically definite MS disorder, referring to the Neurology departments at Shahid Sadoghi hospital of Yazd, participated in this research. The diagnosis was made based on the criteria of McDonald and colleagues [18]. In our study, all the enrolled patients had the relapsing-remitting clinical type (RR-MS). The RR-MS form was defined as the occurrence of exacerbations followed by complete or partial remissions [1]. The control group consisted of 156 healthy blood donors, who were matched for age, gender, and ethnicity. Moreover, their first-degree relatives had no history of MS or any autoimmune disease. All participants signed the informed consent for this research. This study was approved by the Ethical Committee of Shahid Sadoughi university of medical sciences, Yazd, Iran. Both groups were also categorized based on individual genders to compare the distribution of the genotypes among men and women.

Genomic DNA was extracted from ethylenediaminetetraacetate-treated peripheral blood using AccuPrep ® Genomic DNA Extraction (Bioneer, Korea) according to the manufacturer's instructions. Each DNA sample was quantitatively and qualitatively analyzed by a spectrophotometer and electrophoresis on an agarose gel, respectively. All DNA samples were stored at -20°C for further use.

The *RAGE* p.82G>S polymorphism was genotyped by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). The oligonucleotide primer sequences for the target gene *(RAGE*) were F: 5'-ACTGGTGCTGAAGTGTAAGG-3' and R: 5'-AGAGGCTGTAATTGTGAAGGTT-3'. PCR was carried out in a final volume of 25 µl containing 25 pmol of forward and reverse primers,100 ng of template DNA, 100 µmol/l of each deoxynucleotide phosphate, 1.125 mmol/l MgCl2, 1 U Taq polymerase (Fermentas, Vilnius, Lithuania), and 2.5 µl of reaction buffer. Further, 10 µl of the PCR product was digested with 0.25 µl AluI (Fermentas), for 16 h at 37°C, followed by electrophoresis on 2% agarose gels. It was finally visualized in UV light after gel red staining.

Statistical analysis

The genotype frequencies for this polymorphism were tested for deviation from the Hardy–Weinberg equilibrium by the χ^2 test, with 1 degree of freedom. The significance levels of the genotype frequencies were analyzed by χ^2 test or the Fisher exact test as required. The probability levels of $p<0.05$ indicated statistical significance. The relationship between the genotypes and the disease was presented as the odds ratio (OR), with a 95% confidence interval (CI) (95% CI).

All statistical calculations were performed by the statistical package for social sciences (SPSS) software (version 17, SPSS Inc, Chicago, IL, USA).

Results

A total of 156 DNA samples from the control group (112 females, 44 males; mean age 32/76±9/95 years) and 158 samples from the MS patients (115 females, 43 males; mean age 34/48±9/93) were investigated for G82S SNP.

The distribution of the genotypes was in accordance with the Hardy-Weinberg equilibrium in both the case (p=0.95) and the control groups (p=0.93). After electrophoresis of the digested PCR product, genotypes were scored according to the patterns of DNA bands on agarose gel (Fig. 1). The digestion provided fragments of 209 and 322 bp lengths for GG homozygotes as well as 255, 209, and 67 bp lengths for AA homozygotes. For AG heterozygote, four bands reflecting fragment lengths of 209, 255, 322, and 67 were detected. The allele frequencies and genotype distribution of the *RAGE* p.82G>S SNP for two groups are presented in Table 1. The frequencies of the GG and GS genotypes were similar in the two groups. On the other hand, the SS homozygote genotype was not detected in either the case or the control group. This polymorphism demonstrated no significant difference between women and men. The data are reported in table 2.

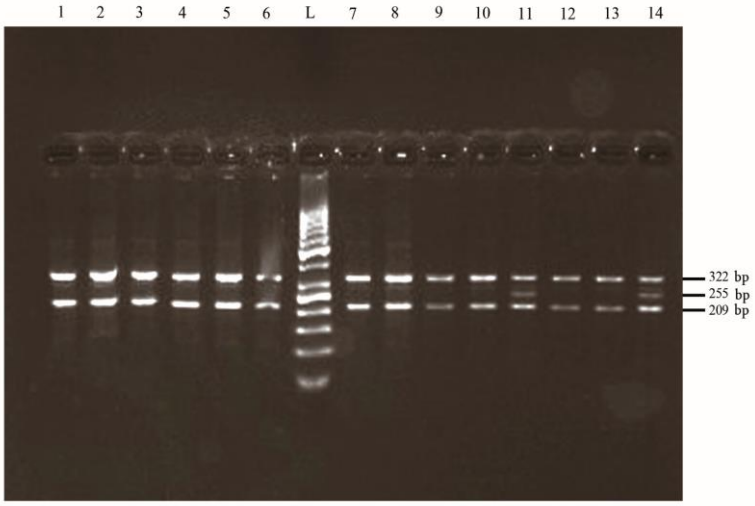

Fig. 1. RFLP genotyping of rs2070600 G>A polymorphism
1: GG homozygotes, 11: AG heterozygote

Table 1. Genotype and allele frequencies of the *RAGE* p.82G>S gene polymorphism in MS patients and controls

	Patients (N=158)	**Controls (N= 156)**	**OR (95% CI)**	**P-value**
Alleles				
G	312 (98.7)	306 (98.1)	0.492	0.319
A	4 (1.3)	6 (1.9)	(0.122-1.98)	
Genotypes				
GG (82G/82G)	154 (97.5)	150 (96.2)	0.487	0.315
AG (82S/82G)	4 (2.5)	6 (3.8)	(0.120-1.983)	
AA (82S/82S)	0.0	0.0		

CI= Confidence interval; OR= Odds ratio

Table 2. Distribution of rs2070600; AG genotype based on the study population gender

Gender	Patients (N)	Controls (N)	OR (95% CI)	P-value
Female	3	4	0.72 (0.16-3.3)	0.68
Male	1	2	0.5 (0.04-5.72)	0.57

CI= Confidence interval; OR= Odds ratio,

Discussion

MS is an inflammatory disease of the central nervous system, characterized by myelin degradation, immune cell infiltration, loss of oligodendrocytes, and axonal damage. It is thought that both genetic and environmental factors contribute to MS disease [19].

In recent years, genome-wide association studies (GWAS) have revolutionized the genetics of MS. To date, after a number of GWAS screenings, 110 MS risk variants have been discovered outside the MHC locus in European populations [20].

As a member of the immunoglobulin superfamily of cell surface molecules, RAGE comprises one V-type domain, which binds diverse ligands, followed by two C-type immunoglobulin-like domains [21]. It has been shown that when ligands, such as S100/calgranulins, engage the V-domain of RAGE, they modulate gene expression by activating signal transduction. In that context, a study in 2002 suggested that a Gly-Ser substitution in the V-type Ig domain because of p.82G>S polymorphism could have noticeable effects on the role of RAGE molecules in inflammatory adjustment. Serine is more hydrophilic and reactive than glycine; therefore, Gly-Ser replacement may induce increased ligand-binding affinity and improved production of inflammatory intermediaries [17]. Infiltration of T lymphocytes into the central nervous system plays a crucial role in the progression and relapse of MS. Further, migration of T lymphocytes to the central nervous system of MS patients is facilitated by RAGE as a receptor of leukocyte integrins such as Mac-1 [22]. Regarding the role of RAGE in MS, Yan et al. found that in the spinal cord tissue of MS patients, the expression of RAGE and its inflammatory ligands, including S100-calgranulins, were increased [12]. The receptor-ligand interactions can modify the properties of infiltrated $TCD4^{+}$ cells to the central nervous system due to increased cytokine, chemokine production, and expression of adhesion molecules. This belief was later confirmed by other studies. According to these studies on a rodent model for MS, blockage of the RAGE on the cell surface by sRAGE interrupts the entrance of encephalitogenic T-cell into the central nervous system [23].

In 2019, Safari et al. identified that rs184003 and rs1800625 polymorphism of RAGE fail to be associated with the risk of MS in Iranian populations [24]. In this study, for the first time, rs2070600 G> A polymorphism of the RAGE gene was evaluated in Iranian MS patients. This polymorphism was located in exon 3 of the RAGE gene at position 32259421 of chromosome 6. The main and mutated alleles were G and A respectively, and the allele with less frequency, in our results, belonged to the

mutant A allele. We also found no relationship between this polymorphism and the risk of MS. Our study was consistent with that of Tiszlavicz et al. where there was no significant association between rs2070600 and MS patients in a Hungarian population [14]. However, Li et al. detected an elevated MS risk in RAGE p.82G>S in a Chinese population. Their study suggested the serum level of sRAGE in this group of patients being significantly lower than that of the healthy subjects, which was obvious in individuals with genotype 82 (GS-SS). Regarding the role of sRAGE in neutralizing RAGE ligands, it seems that genotype-associated reduction of serum sRAGE levels could trigger an increase in the amount of ligand which encounters RAGE receptors on the cell surface, thereby potentially causing intensified post-receptor signaling and consequent cellular responses. From this perspective, in MS patients, *RAGE* 82GS or 82SS genotype, in comparison with the wild type will make them more susceptible to the RAGE-S100-calgranulins-induced cellular disturbance. This can then determine a peculiar mechanistic definition for the dissimilarity in the course and intensity of MS [25].

Overall, there is rarity of information on the association between MS and *RAGE* gene polymorphism except two studies conducted in 2009 [14] and 2011 [25] respectively in Hungry and China and a recent study which has been performed in an Iranian population [24]. Nevertheless, due to the effect of this gene on inflammatory responses and the properties of infiltrating immune cells to the central nervous system, we investigated the relationship between RAGE polymorphism and MS incidence. Based on the results, it can be concluded that rs2070600 polymorphism of the *RAGE* gene has no significant relationship with the risk of MS in the Iranian population. The probable weakness of this research can be the low sample size and the inability to examine the rarer subtypes of MS due to the low number of patients. It is important to note that MS is a multifactorial illness and many factors, such as genetics, environment and the interactions between them are involved in the development of the disease. Various genes may play a role in the development of MS, and yet not all genetic risk factors and interactions between genes or the environment along with the genes involved in the incidence of multiple sclerosis have been identified. Although our study did not specify the role of rs2070600 single-nucleotide polymorphism of the *RAGE* gene as a risk factor, for more accurate results, an increase in sample size, study of MS subgroups, and analysis of haplotypes are suggested.

Conclusions

Our finding showed that there was no relationship between *RAGE* p.82G>S polymorphism and the risk of MS in Iranian population.

Conflict of Interest

The authors declare they have no conflict of interest.

Acknowledgment

The authors gratefully acknowledge the financial support for this work provided by international campus of Shahid Sadughi University of medical sciences.

References

[1]. Poser CM, Paty DW, Scheinberg L, McDonald WI, Davis FA, Ebers GC, et al. New diagnostic criteria for multiple sclerosis: guidelines for research protocols. Annals of Neurology: Official J Am Neurol Associ Child Neurol Society 1983; 13(3): 227-31.

[2]. Parnell GP, Booth DR. The multiple sclerosis (MS) genetic risk factors indicate both acquired and innate immune cell subsets contribute to MS pathogenesis and identify novel therapeutic opportunities. Frontiers Immunol. 2017; 8(1): 425.

[3]. Barnett MH, Prineas JW. Relapsing and remitting multiple sclerosis: pathology of the newly forming lesion. Ann Neurol. 2004; 55(4): 458-68.

[4]. Trapp BD, Peterson J, Ransohoff RM, Rudick R, Mörk S, Bö L. Axonal transection in the lesions of multiple sclerosis. New Eng J Med. 1998; 338(5): 278-85.

[5]. Lucchinetti C, Brück W, Parisi J, Scheithauer B, Rodriguez M, Lassmann H. A quantitative analysis of oligodendrocytes in multiple sclerosis lesions: a study of 113 cases. Brain 1999; 122(12): 2279-295.

[6]. Grigoriadis N, Grigoriadis S, Polyzoidou E, Milonas I, Karussis D. Neuroinflammation in multiple sclerosis: evidence for autoimmune dysregulation, not simple autoimmune reaction. Clinical Neurol Neurosurg. 2006; 108(3): 241-44.

[7]. Hori O, Brett J, Slattery T, Cao R, Zhang J, Chen JX, et al. The receptor for advanced glycation end products (RAGE) is a cellular binding site for amphoterin mediation of neurite outgrowth and co-expression of rage and amphoterin in the developing nervous system. Journal of biological chemistry. 1995; 270(43): 25752-5761.

[8]. Lindsey JB, Cipollone F, Abdullah SM, Mcguire DK. Receptor for advanced glycation end-products (RAGE) and soluble RAGE (sRAGE): cardiovascular implications. Diabet Vascul Dis Res. 2009; 6(1): 7-14.

[9]. Herold K, Moser B, Chen Y, Zeng S, Yan SF, Ramasamy R, et al. Receptor for advanced glycation end products (RAGE) in a dash to the rescue: inflammatory signals gone awry in the primal response to stress. J Leukocyte Biol. 2007; 82(2): 204-12.

[10]. Sternberg Z, Chiotti A, Tario J, Chichelli T, Patel N, Chadha K, et al. Reduced expression of membrane-bound (m) RAGE is a biomarker of multiple sclerosis disease progression. Immunobiol. 2016; 221(2): 193-98.

[11]. Sternberg Z, Hennies C, Sternberg D, Bistulfi GL, Kazim L, Benedict RH, et al. Plasma pentosidine: a potential biomarker in the management of multiple sclerosis. Multiple Sclerosis J. 2011; 17(2): 157-63.

[12]. Yan SS, Wu ZY, Zhang HP, Furtado G, Chen X, Yan SF, et al. Suppression of experimental autoimmune encephalomyelitis by selective blockade of encephalitogenic T-cell infiltration of the central nervous system. Nat Med. 2003; 9(3): 287-93.

[13]. Park IH, Yeon SI, Youn JH, Choi JE, Sasaki N, Choi IH, et al. Expression of a novel secreted splice variant of the receptor for advanced glycation end products (RAGE) in human brain astrocytes and peripheral blood mononuclear cells. Mol Immunol. 2004; 40(16): 1203-11.

[14]. Tiszlavicz Z, Gyulai Z, Bencsik K, Szolnoki Z, Kocsis ÁK, Somogyvári F, et al. RAGE gene polymorphisms in patients with multiple sclerosis. J Mol Neurosci. 2009; 39(3): 360.

[15]. Sternberg Z, Sternberg D, Drake A, Chichelli T, Yu J, Hojnacki D. Disease modifying drugs modulate endogenous secretory receptor for advanced glycation end-products, a new biomarker of clinical relapse in multiple sclerosis. J Neuroimmunol. 2014; 274(1-2): 197-201.

[16]. Hudson BI, Stickland MH, Grant PJ. Identification of polymorphisms in the receptor for advanced glycation end products (RAGE) gene: prevalence in type 2 diabetes and ethnic groups. Diabetes 1998; 47(7): 1155-157.

[17]. Hofmann MA, Drury S, Hudson BI, Gleason MR, Qu W, Lu Y, et al. RAGE and arthritis: the G82S polymorphism amplifies the inflammatory response. Gene Immun. 2002; 3(3): 123-35.

[18]. Mantero V, Abate L, Balgera R, La Mantia L, Salmaggi A. Clinical application of 2017 McDonald diagnostic criteria for multiple sclerosis. J Clinic Neurol. 2018; 14(3): 387-92.

[19]. Dendrou CA, Fugger L, Friese MA. Immunopathology of multiple sclerosis. Nat Rev Immunol. 2015; 15(9): 545-58.

[20]. Didonna A, Oksenberg JR. The Genetics of Multiple Sclerosis. In: Zagon IS, McLaughlin PJ, editors. Multiple Sclerosis: Perspectives in Treatment and Pathogenesis [Internet]. Brisbane (AU): Codon Publications; 2017 Nov 27. Chapter 1. Available from: https://www.ncbi.nlm.nih.gov/books/NBK470155/.

[21]. Neeper M, Schmidt AM, Brett J, Yan SD, Wang FE, Pan YC, et al. Cloning and expression of a cell surface receptor for advanced glycosylation end products of proteins. J Biologic Chem. 1992; 267(21): 14998-5004.

[22]. Frohman EM, Filippi M, Stuve O, Waxman SG, Corboy J, Phillips JT, et al. Characterizing the mechanisms of progression in multiple sclerosis: evidence and new hypotheses for future directions. Archiv Neurol. 2005; 62(9): 1345-356.

[23]. Sternberg Z, Weinstock Guttman B, Hojnacki D, Zamboni P, Zivadinov R, Chadha K, et al. Soluble receptor for advanced glycation end products in multiple sclerosis: a potential marker of disease severity. Multiple Sclerosis J. 2008; 14(6): 759-63.

[24]. Safari MR, Noroozi R, Azari I, Mazdeh M, Taheri M, Ghafouri-Fard S. RAGE polymorphisms are not associated with risk of multiple sclerosis in Iranian population. Gene Rep. 2019; 15(1): 100400.

[25]. Li K, Zhao B, Dai D, Yao S, Liang W, Yao L, et al. A functional p. 82G> S polymorphism in the RAGE gene is associated with multiple sclerosis in the Chinese population. Multiple Sclerosis J. 2011; 17(8): 914-21.