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Association of VCAM-1 Gene Polymorphisms with Multiple Sclerosis Susceptibility in the Southeast of Iran

^{1,2}Nima Sanadgol, ³Javad Sanchooli, ¹Abbas Nikraves, ⁴Reza Momeni, ⁵Hamideh Khajeh, ⁶Moein Safari and ⁷Majid Radpour

¹Department of Biology, Faculty of Science, Zabol University, Zabol, Iran

²Cellular and Molecular Research Center, Tehran University of Medical Sciences, Tehran, Iran

³Department of Immunology, Faculty of Medicine, Zabol University of Medical Science, Zabol, Iran

⁴Department of Statistics, Faculty of Science, Zabol University, Zabol, Iran

⁵Biotechnology Research Institute (Biocenter), Zabol University, Zabol, Iran

⁶Department of Biology, Faculty of Science, Ilam University, Ilam, Iran

⁷Department of Biology, Faculty of Science, Islamic Azad University, Sanandaj Branch, Iran

Corresponding Author: Nima Sanadgol, Cellular and Molecular Research Center, Hemmat Campus, Tehran University of Medical Sciences, Tehran, Iran Tel/Fax: 021-88622689

ABSTRACT

Multiple Sclerosis (MS) is an autoimmune nervous system disorder characterized by leukocytes recruitment into nervous system and demyelination. Vascular cell adhesion molecule 1 (VCAM-1) is a cell surface glycoprotein, which is expressed by activated endothelium in a variety of pathologic conditions including MS. The aim of this study (case-control) was to evaluate the probable association of VCAM-1 T-1594C and A-540G single nucleotide polymorphisms (SNPs) with circulating levels of sVCAM-1 and susceptibility to MS. Analysis of VCAM-1 polymorphisms was performed by PCR with Sequence-Specific Primers (SSP) and concentration of sVCAM-1 in serum was performed by ELISA techniques. No significant differences were detected for allele frequencies of VCAM-1 polymorphisms in MS patients than in the controls respectively ($p > 0.05$). Moreover, baseline serum sVCAM-1 concentrations significantly increased among patient in contrast to controls ($p < 0.001$). This study shows that these SNPs in VCAM-1 may not a putative risk predisposing alleles for MS and its clinical covariates in southeast Iran population. The folding rate of the VCAM-1 protein increases for MS patients implicating a potential effect and function of VCAM-1 in immunopathogenesis of MS. Results invites further investigation relevant to understanding the mechanisms underlying the VCAM-1 overexpression in MS patients.

Key words: Vascular cell adhesion molecule-1, heterogeneity, SNP, MS

INTRODUCTION

The etiology of Multiple Sclerosis (MS) are poorly understood but establish autoimmune reaction against myelin in Central Nervous System (CNS) is one of key events in disease development (Hickey *et al.*, 1991). Four major clinical subtypes of MS exist, relapsing remitting (R.R, about 85%), Secondary Progressive (SP), Primary Progressive (PP) and relapsing progressive (R.P, about 5%) (Rossler *et al.*, 1992). The entry of most cells and large molecules into the brain is blocked by the Blood-Brain Barrier (BBB) (Brankin, 1995). The BBB is comprised of highly specialized

endothelial cells that regulate the entry of cells into the brain. The entry of immune cells is primarily controlled by the expression of adhesion molecules on endothelial cells lining the BBB (Springer, 1994). Under healthy conditions, expression of adhesion molecules on BBB endothelial cells is low to none, but can be up regulated during inflammatory brain diseases such as multiple sclerosis (Wuthrich, 1992). Vascular cell adhesion molecule-1 (VCAM-1) expressed by active endothelial cells and facilitate entry of leukocytes into inflamed tissues (Springer, 1994). Endothelial cell expression of VCAM-1 has been demonstrated in MRL/lpr non-neural tissues suggesting an increased interaction of leukocytes with endothelium, associated with the autoimmune response (Wuthrich, 1992). Studies using human brain microvascular endothelial cells has been shown that VCAM-1 is important in the both adhesion and migration of the T lymphocytes across the cerebral endothelial barrier (Wong *et al.*, 1999; Wu *et al.*, 2000). Furthermore, soluble VCAM-1 (sVCAM-1) is present at higher concentrations in the Cerebrospinal Fluid (CSF) and serum of MS patients (Baraczka *et al.*, 2001). The human VCAM1 gene is located on chromosome 1p32-p31 (www.ncbi.nlm.nih.gov/entrez/query). Two nonsynonymous single nucleotide polymorphisms (SNPs) sites T-1594C (rs1041163) and A-540G (rs3783605) of VCAM-1 have been previously described (Hoppe *et al.*, 2004). Endothelial Cell Adhesion Molecules (ECAMs) Gene polymorphisms have been defined and studied extensively in diseases with multifactorial etiology (Vora *et al.*, 1994; Wenzel *et al.*, 1996). Genetic polymorphisms of VCAM-1 has been implicated in susceptibility to a number of degenerative and inflammatory diseases (Taylor *et al.*, 2002; Zameer and Hoffman, 2003; Kloda, 2011; McDonnell *et al.*, 1999; Miyoshi *et al.*, 2008). Despite the large body of literature on the pathology and molecular biology of the CAMs in a range of disease, function of the circulating form of these molecules remain unclear (Gearing and Newman, 1993). Therefore, new investigation in role of circulating CAMs in autoimmune disease such as MS is necessarily speculative. Based on this introduction, it is hypothesized that VCAM-1 gene variations modulate cell to cell interactions and may contribute to Pathogenesis and exacerbation of MS. The present study was designed to test, for the first time, the relationship between SNPs in VCAM-1 gene and concentration of sVCAM-1 in southeast Iranian patients with MS.

MATERIALS AND METHODS

Study population: The study approved by the University of Zabol Multiple Institutional Review Board. Clinical samples obtained from MS patients and Healthy blood donors that voluntary submitted for research from December 2008 through July 2009. MS patients (in southeast of Iran) who had been diagnosed with magnetic resonance imaging (MRI) and McDonald criteria were collected (Polman *et al.*, 2005). Two hundred and one different samples were analyzed; 78 were from patients and 123 were from healthy controls. In the patient group, there were 22 men (mean age, 28.8 years; age range, 17-48 years) and 56 women (mean age, 30.3 years; age range, 16-52 years). In the control group of healthy blood donors, there were 34 men (mean age, 26.4 years; age range, 17-42 years) and 89 women (mean age, 26.0 years; age range, 17-50 years). Patients adjusted in definite RRMS (n = 46; 10 men and 36 women), SPMS (n = 11; 2 men and 9 women), PPMS (n = 10; 5 man and 5 woman) and RPMS (n = 11; 5 man and 6 woman) subtypes.

Genotyping: Genomic DNA was extracted and purified from whole blood lymphocytes using a blood DNA Kit (Takara, Japan) according to the manufacturer's instructions. PCR with sequence-specific primers (SSP) was used to detect the VCAM-1 polymorphisms (rs1041163 and

rs3783605) as described elsewhere (Saiki *et al.*, 1989; Idelman *et al.*, 2007). For the positive internal control, the primers 5'-GAAGGTGAAGGTCTGGAGT-3' (forward) and 5'-GAAGATGGTGATGGGATTTC-3' (reverse) coding for the 225 bp fragment of human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene were used. DNA was amplified by one rounds of PCR performed with 5 µL of DNA in a 25 µL reaction mixture (1U of Smar Taq DNA Polymerase (Takara, Japan), 0.5 µM of each primers, 240 µM of each dNTPs, 20 mM of Tris-HCl 3 mM MgCl₂, 50 mM KCl and 20 mM Ammonium Sulfate). Thermal cycling conditions were as follows: denaturation of 95°C for 5 min followed by 30 cycles of denaturation at 94°C for 30 sec, annealing at 60°C for 30 sec and extension at 73°C for 30 sec. The amplification was followed by a final extension step at 73°C for 5 min. PCR products purified by precipitation with 2.5 M ammonium acetate and isopropyl alcohol and subsequently washed with 70% ethanol to remove the dNTPs and excess primers. The pellet was diluted in 20 µL of DNase-free sterile water and a 10 µL aliquot was analyzed by conventional (1.5% agarose) gel electrophoresis containing ethidium bromide (Sigma, Germany).

Quantitative determination of sVCAM-1 levels using ELISA Serum was separated from 5 mL whole blood by centrifuge for 15 min at 150 g at 20°C and then stored at -20°C until the experiment was performed. Soluble VCAM-1 in serum was quantified using the ELISA kit (invitrogen Corp. USA), which based on a sandwich format (Sanadgol *et al.*, 2010). The data presented were means of triplicate determinations.

Statistical analysis: Each polymorphism was tested in controls to ensure the fitting with Hardy-Weinberg equilibrium. To test the hypothesis of association between genetic polymorphisms and MS, multivariate methods based on logistic regression analyses were used. Allele and genotype frequencies in all subjects were calculated by direct counting. Hardy-Weinberg equilibrium was tested using the Fisher's exact test. The strength of the gene-MS associations was measured by Odds Ratio (OR) and its 95% Confidence Interval (CI). The $p < 0.05$ was considered statistically significant. The SPSS was used in the statistical analysis.

Ethical considerations: The study conformed to the Helsinki Declaration and was reviewed and approved by the local Research Committee; written informed consent was obtained from all subjects.

RESULTS

VCAM-1 polymorphisms: No significant differences in allele were observed for VCAM-1 polymorphisms between MS patients and controls ($p < 0.1$). The frequencies of the three possible genotypes of the VCAM-1, T-1594C polymorphisms in these populations are shown in Table 1. The frequency of VCAM-1 T1594 homozygosity was relatively lower in MS patients than in the controls (42.53 vs. 65.85% subsequently, $p < 0.05$). Moreover, VCAM-1 C1594 homozygosity was more frequent in MS patients than in controls (31.04 vs. 17.88% subsequently, $p < 0.05$). The frequencies of the three possible genotypes of the VCAM-1, A-540G polymorphisms in these populations showed in Table 2. The VCAM-1 A-540G genotype and allele frequencies of MS patients and healthy volunteers were approximately similar (Table 2). The distribution of T-1594C and A-540G, SNPs between MS and controls was not significantly different ($p < 0.1$).

Soluble VCAM-1 concentrations: Higher levels of sVCAM-1 were observed for the patients with T1594 homozygosity (1170 ng mL⁻¹) and lower levels of sVCAM-1 were observed for those with

Table 1: Allele and genotype frequencies of the VCAM-1 T-1594C polymorphisms in MS cases and controls

	MS (n = 87)	Controls (n = 123)	p-value	OR (95%CL)
Genotype				
TT	37 (42.53)*	81 (65.85)	0.017	0.479
TC	23 (26.43)	20 (16.27)	0.083	1.851
CC	27 (31.04)	22 (17.88)	0.031	2.066
Allele				
T	48 (55.17)	91 (73.98)	0.005	0.432
C	39 (44.83)	32 (26.02)	0.005	2.311

OR: Odd ratio, CL: Confidence interval, *Values in bracket are percentage

Table 2: Allele and genotype frequencies of the VCAM-1 A-540G polymorphisms in MS cases and controls

	MS (n = 87)	Controls (n = 123)	p-value	OR (95%CL)
Genotype				
AA	31 (35.63)	45 (36.58)	1.000	0.959
AG	32 (36.79)	48 (39.03)	0.774	0.909
GG	24 (27.58)	30 (24.39)	0.632	1.181
Allele				
A	47 (54.02)	69 (56.50)	0.779	0.919
G	40 (45.98)	54 (43.50)	0.779	1.087

OR: Odd ratio, CL: Confidence interval, *Values in bracket are percentage

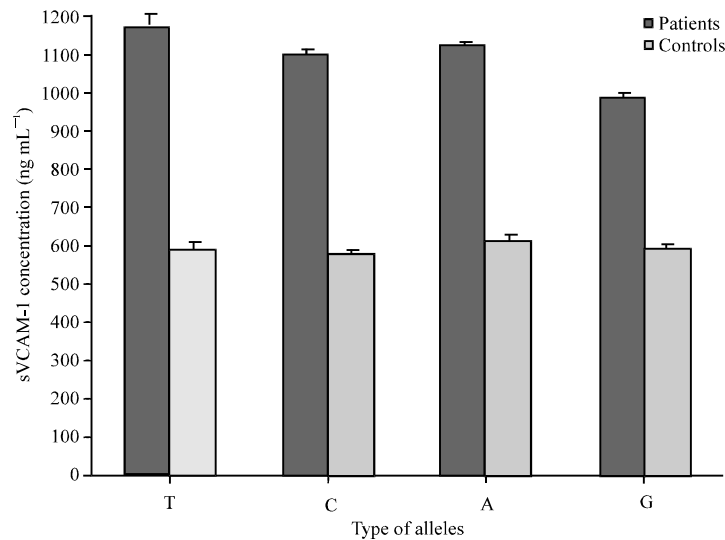


Fig. 1: Means of sVCAM-1 concentration among individuals carrying T, C, A and G alleles in MS cases and controls. Expression of sVCAM-1 was analyzed with ELISA assay, Each column shows the Mean±SD of triplicate determinations

G540 homozygosity (990 ng mL⁻¹) but the sVCAM-1 distributions were not notably different between controls with different VCAM-1 polymorphisms (Fig. 1). Patients who are carrying the polymorphic T allele showed relatively higher sVCAM-1 serum levels than carriers of the homozygous CC wild type (p<0.01). In contrast, no significant difference in the sVCAM-1 serum levels was seen regarding the VCAM-1 genotypes distribution in controls (Fig. 1). Generally observed increase sVCAM-1 levels in MS patients in contrast with controls (p<0.001).

DISCUSSION

The Cell Adhesion Molecules (CAMs) of the immunoglobulin superfamily adhere with high affinity to integrins expressed on inflammatory and endothelial cells. A critical member, the vascular cell adhesion molecule 1 (VCAM-1), coordinates the inflammatory response by recruiting leukocytes and in turn, activating lymphocytes (Springer, 1995). VCAM-1 is a cell surface sialoglycoprotein highly expressed on endothelial cells following cytokine stimulation with interleukin 1 alpha (IL-1 α), tumor necrosis factor alpha (TNF- α) and IL-4 (Swerlick *et al.*, 1992). It also constitutively expressed on the bone marrow stroma where it has recently been implicated as a possible determinant of hematopoietic cell mobilization in response to granulocyte colony-stimulating factor (G-CSF) (Levesque *et al.*, 2001). A potential role in the regulation of circulating leukocytes is further supported by targeted disruption of the murine VCAM1 gene, in which it has been observed that VCAM-1-deficient mice have elevated peripheral leukocytes 18. Endothelial vascular cell adhesion molecules are key mediators of leukocyte recruitment. Vascular cell adhesion molecule-1 (VCAM-1) has been shown to be important in the development of Experimental Autoimmune Encephalomyelitis (EAE) and MS, mediating both leukocyte movements across the BBB and their retention within the parenchyma during a relapse (Graesser *et al.*, 2000).

Selective blockade of the interaction between VCAM-1 and its ligand, integrin $\alpha 4 \beta 1$ (VLA-4), on leukocytes has been shown to abolish leukocyte recruitment and the associated neurological deficit in EAE models (Yednock *et al.*, 1992). Similarly, selective VLA-4 inhibitors reduce the number of MRI-detectable lesions in MS (Polman *et al.*, 2006). Although, marked increases in soluble VCAM-1 have been reported (Rieckmann *et al.*, 1998; Hellings *et al.*, 2002), there has been considerable controversy as to the cellular localization of vascular VCAM-1 in MS, with reports of both positive (Allavena *et al.*, 2010; Cannella and Raine, 1995; Serres *et al.*, 2011) and negative (Peterson *et al.*, 2002; Barcellos *et al.*, 2002) endothelial VCAM-1 immunoreactivity in MS plaques. Clinical trials in patients with MS have demonstrated clear clinical benefits arising from inhibition of the interaction between VCAM-1 and its ligand VLA-4 (Polman *et al.*, 2006). Although, it is unclear how the VCAM-1 polymorphism contributes to the pathogenesis of MS, results showed that the increase in sVCAM-1 expression was not accompanied by these VCAM-1 polymorphisms at least in southeast of Iran. With attention to my previous study, sVCAM-1 beside sICAM-1 might be a useful tool to evaluate disease progression in MS patients (Sanadgol *et al.*, 2011). These findings did not provide a possible reason why increasing of sVCAM-1 expression occurs in MS patients and need more investigations in this area.

CONCLUSION

Study herein showed significantly over expression of sVCAM-1 in MS patients independent of gene polymorphisms. However, present study unable to define the association of the VCAM-1 polymorphisms with MS risk owing to the limitations of the development of the MS and control populations. Moreover, there is a possibility that a different linked gene may contribute to the phenotype and that the observed association might be a secondary one resulting from linkage disequilibrium. Thus, traditional approaches, including construction and analysis of congenic, knockout or transgenic rodents, may still be needed to validate the phenotypic association. Future findings may help to evaluate the prognosis of MS according to the individual genetic background.

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