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Tumor Necrosis Factor-Alpha Polymorphism and Susceptibility to Multiple Sclerosis in the Iranian Population

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Background: Multiple sclerosis (MS) is an immune-mediated disease of polygenic etiology. Tumor necrosis factor-α (TNF-α) microsatellite as a proinflammatory cytokine is believed to play an important role in the etiology of this disease.
Objectives: The aim of this study was to investigate the association of TNF-α microsatellite sequence variation in patients with MS and its risk factor in the southern Iranian population.
Patients and Methods: This polymorphism was investigated in an Iranian population of 163 native southern people [81 patients with MS according to the poser criteria and 82 healthy controls (HC) with the same age, sex, social, ethnical and geographical features (Hormozgan and Fars provinces)]. All the controls were nonimmunological, neurological patients. All the cases and controls were chosen randomly and genotyped for polymorphism of TNF-α microsatellite.
Results: The frequencies of TNF-α*11 (0.25, $P < 0.005$) and TNF-α*10 ($P < 0.005$) alleles increased in patients with MS compared with controls, showing a significant difference among the studied population.
Conclusions: The current study adds evidence to the association of TNF-α gene polymorphism and MS in this southern south Iranian population which is consistent with the genetic analysis of MS in Europeans (GAMES) project reports and these two alleles reported in this study may be one of the genetic risk factor for MS. Furthermore, this data can be used to build the Iranian gene bank for future studies.

Keywords: Tumor Necrosis Factor-Alpha; Multiple Sclerosis; Sclerosis

1. Background

Multiple sclerosis (MS, OMIM 126200) is the most common cause of nontraumatic chronic neurological disability of the central nervous system (CNS), characterized by demyelination, axonal degeneration, and inflammation (1-5). The disease onset usually occurs in young adults and it is more common in females, with prevalence rates varying across ethnic groups and depending on geographic latitude (6-8). Most MS patients (85%) present a relapsing-remitting MS (RRMS) clinical course at the onset, while the remaining groups of patients present primary-progressive MS (9). Epidemiological analysis shows that MS results from unknown environmental factors, acting on genetically susceptible individuals (10). Susceptibility to MS is held to have a strong genetic component in a large degree, as shown by the increased risk of the disease seen among relatives of affected individuals (11). Genetic contributions are clearly included at least in 20% of affected individuals, indicating the family background of the disease. Concurrence rates comprise 26% monozygotic twins compared to 3% in dizygotic twins (12). Although the etiology of MS is unknown, epidemiological data recommend that predisposition to the disease is approximately genetically found out and linked to a number of genetic loci. An association with human leukocyte antigen (HLA) class II is well recorded (13). Tumor necrosis factor α (TNF-α) gene is located between the HLA-B (class I) locus and the class III region in tandem in the major histocompatibility complex (MHC) region. The location of the TNF locus within the MHC region has generated notion about the emphasis of TNF in the etiology of MHC-associated diseases, especially those with an inflammatory or autoimmune history. MS is not figured as a hereditary disease; although, a lot of genetic variations have been exhibited to increase the risk of developing the disease (8, 13). The role of TNF-α in a demyelinating disease such as experimental allergic encephalomyelitis (EAE) is well understood and increasing evidence exists to recommend a role for TNF in the pathogenesis of MS (13).

2. Objectives

Considering the significant differences between the TNF-α microsatellite polymorphism gene in patients with MS and healthy controls in Europeans, we studied the role of TNF-α genotype in MS by determining the correlation between the TNF-α microsatellite located in the HLA

region and MS in patients of two southern provinces of Iran (Hormozgan and Fars).

3. Patients and Methods

From February to November 2012, all individuals involved in this comparative case-control study gave written informed consents for the genetic analysis according to the Iran Medical Committee. The 81 unrelated patients with MS (26 males and 55 females) considered in this study lived in these two southern areas of Iran (Hormozgan and Fars provinces) and had Iranian origins dating back at least three generations from both maternal and parental sides. They were classified according to the poser criteria (14) and diagnosed with either clinically definite (90%), laboratory supported definite (7%) or clinically probable (3%) MS. All the controls were free from acute or chronic internal and neurological diseases, determined by physical examinations. HLA typing had been previously performed for HLA class II alleles: increase of DRB1*15 allele ($P < 0.005$) in the patients was the most important point. Genomic DNA was extracted from peripheral blood using DNA extraction kit (DNPTM, CinnaGen Co., Iran) according to the manufactures protocol and stored at -20°C until used. Using spectrophotometry, the DNA quantity was evaluated in each sample. The microsatellite marker used in this study contained AC/GT repeats and had 13 alleles. The amplification was carried out in a PCR reaction using 5'-GCCTCTAGATTTCATCCAGCCACA-3' and 5'-CCTCTCTCCCCTGCAACACACA-3' primers. Of the genomic DNA, a 100 ng sample was amplified in a total volume of 10 μL containing 10 mM Tris-HCl, 50 mM KCl, 1.5 mM $MgCl_2$, 250 μM of each dNTP, 1 μM of each primer and 0.5 U of Taq DNA polymerase (Fermentas, Germany). The following thermal-cycling conditions were used: initial denaturation at 94°C for two minutes, 30 cycles of 94°C for 45 seconds, 65°C for 30 seconds and 72°C for 45 seconds, and final extension at 72°C for 10 minutes. The amplified products were loaded into a 9% polyacrylamide gel with a standard ΦX174/Hinf1 marker (15). All the data were analyzed by SPSS program version 18 (SPSS Inc., Chicago, IL, USA) in which all the averages were reported as mean ± SD and frequencies as percentage. Allele, genotype and haplotype the frequencies were analyzed using Gene Pop program (http://genepop.curtin.edu.au) and the comparison of intergroup differences were conducted by the chi-square and t tests. Test of Hardy-Weinberg (HW) equilibrium was carried out for TNF-α microsatellite gene in the case and control populations to check any significant deviation. P value less than 0.05 was considered statistically significant.

4. Results

Our study was performed in a case-control design consisting of 163 people (81 patients with MS and 82 HCs). Clinical characteristics of the patients group are presented in (Table 1). Basic demographic details of the patients were unremarkable with mean age [mean ± SD] of 31.20 ± 9.11 years, mean expanded disability statue score (EDSS) of 4, mean disease duration of 8.55 years, mean age at diagnosis of 27.49 ± 9.07 years, and female: male ratio of 2.12. In total, 83.95% of the patients had RRMS, 11.11% had secondary progressive disease (SPMS), and 4.94% had primary progressive multiple sclerosis (PPMS) at the time of assessment. A total of 82 unrelated healthy controls (27 males and 55 females; mean age 34 ± 4.7 years) with almost the same ages, sexes and geographical origins as cases were selected for this study. Most of the case group had a family history of MS (89.9%, $P < 0.005$). All the 13 alleles were identified for TNF-α microsatellite and genotypic distribution of TNF-α microsatellite differed significantly between the two population ($P < 10\text{-}4$) (Table 2). TNF-a*11 and TNF-α*10 alleles increased significantly in patients with MS (0.25, $P < 0.005$ and 0.19, $P < 0.005$, compare to 0.06 and 0.08, $P < 0.005$, respectively). In contrast, TNF-α*1 and TNF-α*2 allele frequencies decreased in patients (0.01 and 0.02 respectively, $P < 0.005$), compared to HCs (0.15 and 0.21, $P < 0.005$). The heterozygosity for the TNF-α microsatellite gene was particularly high in this study (0.8); the TNF-α genotypes in studied population were in Hardy-Weinberg equilibrium ($P = 0.002$ for cases and $P = 0.05$ for controls).

Table 1. The most Prevalent Clinical Characteristics of Patients With Multiple Sclerosis [a]

Symptom	Females	Males	Total
Complete blindness	1 (1.8)	2 (7.7)	3 (3.7)
slight blindness	10 (18.1)	5 (19.2)	15 (18.5)
Pain during movement of eyeballs	8 (14.5)	2 (7.7)	10 (12.3)
Diplopia(double vision)	8 (14.5)	4 (15.4)	12 (14.8)
Blurred vision	21 (38.2)	5 (19.2)	26 (32.1)
Dysphagia	9 (16.4)	1 (3.8)	10 (12.3)
Poor memory	19 (34.5)	1 (3.8)	20 (24.7)
Tremor	7 (12.8)	5 (19.2)	12 (14.8)
Fatigue	25 (45.5)	15 (18.5)	40 (49.4)
Vertigo	17 (30.9)	8 (30.8)	25 (30.9)
Imbalance	26 (47.3)	10 (38.5)	36 (44.4)
Bladder symptoms	13 (23.6)	8 (30.8)	21 (25.9)
Speech problems	15 (27.3)	3 (11.5)	18 (22.2)
Sensory symptoms	11 (20.0)	5 (19.2)	16 (19.8)
Hearing problems	8 (14.5)	5 (19.2)	13 (16.0)
Electric shock-like sense	8 (14.5)	5 (19.2)	13 (16.0)

[a] Data are presented as No. (%).

Table 2. Allelic Frequencies of Tumor Necrosis Factor-Alpha Microsatellite Genetic Marker [a]

TNF-α	Patients	Controls	Probability
α-1	0.01	0.15	P < 0.005
α-2	0.02	0.21	P < 0.005
α-3	0.04	0.05	NS
α-4	0.05	0.06	NS
α-5	0.06	0.07	NS
α-6	0.03	0.05	NS
α-7	0.04	0.03	NS
α-8	0.07	0.03	NS
α-9	0.09	0.05	NS
α-10	0.19	0.08	P < 0.005
α-11	0.25	0.06	P < 0.005
α-12	0.13	0.09	NS
α-13	0.03	0.06	NS

[a] Abbreviations: TNF-α, tumor necrosis factor-alpha NS, not significant.

5. Discussion

Linkage analysis is a main method for recognition of genetic factors which can cause diseases. However, this procedure is not enough for finding the etiology of complex diseases such as MS. To date, Crohn's disease is the only one that can be diagnosed successfully using linkage analysis (16). We screened TNF-α microsatellite polymorphism in patients with MS to find their association in an Iranian population. To our knowledge, this is the first study on TNF-α microsatellite performed in a tropical area of Iran, showing association with pathogenesis of MS. The position of TNF-α gene within the HLA region has led to paying more attention to the role of TNF-α alleles in etiology of MS (15). The TNF-α gene is located tandemly and maps within the MHC region centromeric to HLA-B and telomeric to the class III region on chromosome 6p2l.3. Its chromosomal region and the immunomodulatory influence of this gene have caused much more speculation about the role of the TNF locus in MHC-linked diseases (17). It has been repeatedly suggested that genetic predisposition to MS might be influenced by TNF gene polymorphism (3, 18). The investigation of polymorphic markers (including microsatellites) within the TNF locus has resulted in a lot of studies which have shown the relationship between TNF haplotypes and pathogenesis of the disease (17). To find the association of TNF-α polymorphism and MS, Goertsches et al. performed a research on 200 patients with MS (67 males and 133 females) and 200 HCs in a Spanish Caucasian population. All the patients had clinically definite MS according to poser and McDonald criteria (14, 19, 20). Performing PCR according to the genetic analysis of MS in Europeans (GAMES) consortium (http://www-gene.cimr.cam.ac.uk/MSgenetics/GAMES), TNF-α polymorphism showed a significant correlation, which confirmed the association of this microsatellite marker and the etiology of this disease (20). As a part of the GAMES collaborative project, Godde et al. screened microsatellite markers in 198 Germans with MS and 198 HCs to identify any susceptible region to MS. Performing PCR and statically analysis, TNF-α marker residing in the HLA region on chromosome 6p21 showed the most significant relationship (12). Comparing gene frequencies of TNF-α alleles in a French population of 74 patients with MS and 75 HCs, Lucotte et al. showed a strongly significant positive association between TNF-α*11 allele and MS (15). Our results were consistence with those of a study on Europeans patients with MS in the huge GAMES project using 6000 microsatellite markers. The GAMES collaborative screened 19 case-control cohorts and 10 trio family-based cohorts (8, 21), as described in the original publications: Australia (22), Belgium (23), Finland (24), France (25), Germany 1 (6), Germany 2 (26), Hungary (27), Iceland (28), Ireland (29), Italy (30), Poland (31), Portugal 1 (32), Portugal 2 (33), Sardinia (34), Scandinavia (35), Spain (20), Turkey (36), and UK (37). It was found that some special alleles of these markers were noticed significantly more usual in MS groups, as compared with the HC groups. In the GAMES project altogether involving 13896 individuals, meta-analysis determined by the Fisher's method for the six markers was validated. Three non-MHC markers and three MHC markers (including TNF-α marker) were identified to be potentially associated with MS (10). Our findings also demonstrated that the TNF-α*11 allele was more frequent in RRMS, PPMS and SPMS groups than the HCs, suggesting a possible genetic predisposition to MS in Iranians patients with MS. A large number of microsatellites from the human MHC region presented the polymorphism information content (PIC) of around 0.75. For the TNF-α microsatellite locus, PIC values around 0.85 have been observed (38). In the Iranian population (current study), PIC for TNF-α was 0.84.

In summary, the findings indicated the association between TNF-α microsatellite polymorphism in the HLA region and the risk of developing MS in the native Iranian population. Our findings also confirmed the relationship between TNF-α microsatellite and predisposition to MS, as reported previously by the GAMES project. However, achieving the exact results need similar studies in other parts of Iran as well as in other parts of Asia.

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