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

Masoomeh Rahmanian 1; Mohammad Kargar 2,*

1Young Researchers’ Club, Department of Microbiology, Jahrom Branch, Islamic Azad University, Jahrom, IR Iran
2Department of Microbiology, Jahrom Branch, Islamic Azad University, Jahrom, IR Iran
*Corresponding Author: Mohammad Kargar, Department of Microbiology, Jahrom Branch, Islamic Azad University, Jahrom, IR Iran. Tel: +98-9173149203, Fax: +98-7116262102, E-mail: m.kargar@jia.ac.ir

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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
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) of 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 further use. 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'-GCCCTCAGATTTCATCCAGCCA-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 MgCl₂, 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 allele, genotype and haplotype 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 each population. 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 status 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 RMS, 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 populations (P < 10-4) (Table 2). TNF-α*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 was particularly high in this study (0.8); the most frequent alleles were TNF-α*2 (0.47) and TNF-α*11 (0.38). The other alleles were found in lower frequencies (Table 2).

| 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

| 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          |

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 II region on chromosome 6p21.3. 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 the 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 statistically 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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