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ORIGINAL ARTICLE

Association of vitamin D level and *CYP27B1* gene polymorphism with multiple sclerosis in Turkish population

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ABSTRACT

Background: Multiple sclerosis (MS) is a demyelinating disease in which the insulating covers of nerve cells in the brain and spinal cord are damaged. In this study, we investigated the possible association of vitamin D levels and rs703842 in the *CYP27B1* gene with MS.

Methodology: We used blood samples of 99 patients (65 female, 35 male) with an magnetic resonance imaging (MRI)-confirmed definitive diagnosis of MS and 99 controls (70 female, 29 male) between the ages of 18–55 years. We measured their vitamin D levels, isolated their DNA, and scanned rs703842 polymorphism in the *CYP27B1* gene.

Results: Rs703842 polymorphism in the CYP27B1 gene in humans was found as three different genotypes: CC, CT, and TT. Among them, CC genotype was found higher in the patient group and CT genotype was found higher in the control group. The statistical analysis showed that the probability of a C allele having an association with MS to be 1.5189 times of the probability of T allele. Also, the vitamin D levels in the patient group were detected lower than the control group.

Conclusion: Low levels of vitamin D and low expression of CYP27B1 were found to have an association with MS.

Keywords: Multiple sclerosis, vitamin D, 25-Hydroxyvitamin D3 1-alpha-Hydroxylase.

Introduction

Multiple sclerosis, MS (OMIM No. 126200), is an inflammatory disease that is characterized by demyelinating lesions in the brain or spinal cord, as well as by axonal loss and progressive buildup of sclerotic plaques. It has a complex disease etiology, in which both genetic and environmental factors influence disease susceptibility (1). Epidemiologic findings suggest that both genetic susceptibility and some environmental factors in childhood and adolescence may cause the disease after a many-year-long period of latency. In recent years, there have been some speculations that the environmental factor such viral infections could also play a role here. Humoral and cellular immunity dysfunctions against viral infections have been suggested responsible (2,3). If an unknown infection is the first step of developing MS, surely there must be a second factor that plays a role in the manifestation and progression of the disease. Another popular theory is that the second mechanism could be a autoimmune reaction that attacks a component of the myelin sheet and damages neural tissue, predominantly the axons. Many hypotheses support this theory. MS lesions tend to be similar to lesions of acute disseminated encephalomyelitis which is an autoimmune disease with a delayed-type hypersensitivity reaction. Antibodies against myelin proteins, both in serum and cerebrospinal fluid, were detected in support of this theory. As the severity of the disease increases, these antibodies increase with the T cells that are reactive against myelin basic protein (MBP) and myelin proteolipids. Moreover, MBP shows cross-reaction with the antibodies that are against the Measles virus (4). Despite current data, mentioned immune mechanisms in MS, have not been definitely explained and the autoimmunity hypothesis disputes aren't over.

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Even so, nonspecific immunosuppressive treatments change the course of MS consistently (5). In recent years, the immunological effects of vitamin D have been drawing more attention. The beginning of this association started after vitamin D production was showed in activated dendritic cells and vitamin D receptor was showed in immune system cells. After that, it was claimed that vitamin D played a role as an immune regulator. The net effect of vitamin D in the immune system is to suppress it, which is immunosuppression. Vitamin D inhibits Th1 response, therefore, the system shifts toward Th2 direction. Th2 cytokines show anti-inflammatory effects, so it was thought that the immunosuppressive effect of vitamin D could affect the activity of diseases. Many studies about the association of vitamin D with Rheumatoid arthritis (RA), Systemic lupus erythematosus (SLE), Behçet's disease, MS, inflammatory bowel disease, type-1 diabetes have been published.

Studies have shown that 25-hydroxy vitamin D levels are lower in autoimmune diseases than control groups. The vitamin D that is synthesized as previtamin in the skin is then converted to a 25-OHD3 form in the liver. After that, it is converted to its bioactive form, 1,25(OH)2D3 by the enzymes that are coded by CYP27B1 (cytochrome P450 family 27 subfamily B peptide 1) in kidney, skin, and immune cells. Based on this mechanism, some studies have been done on CYP27B1 that codes the bioactive form of vitamin D (6). Those studies showed many functions of CYP27B1 that is localized at chromosome 12q13.1. The main ones are suppressing the immune system, reducing T-cell maturation, maintaining the connection between Th1 and Th2 (6). Polymorphism of rs703842 in the CYP27B1 gene localized at 12q13-14 is the polymorphism point that was found to have an association with the disease by Australia and New Zealand MS consortium.

Subjects and Methods

In this study, we used blood samples of 99 patients (65 female, 35 male) with an MRI-confirmed definitive diagnosis of MS and 99 controls (70 female, 29 male) between the ages of 18–55 years. While determining the control group, we selected the individuals without any autoimmune disease or multiple sclerosis history in their families. Also in the patient group, we investigated any autoimmune disease other than multiple scleroses, such as diabetes mellitus type 1, Crohn's disease, ulcerative colitis, ankylosing spondylitis, autoimmune thyroiditis, and we excluded the patients with those diseases. Patients and controls all lived in the southern part of Turkey (coordinates 38°45.48′N 30°32.32′ E). We isolated genomic DNA from the peripheral blood samples of our patient group and control group. Each isolated DNA molecule was evaluated for CYP27B1 gene polymorphisms. After isolation, the amount and purity of each DNA sample were measured by spectrophotometer (Nanodrop ND-1000). Real-time PCR was used as the genotyping method. 25-OH Vitamin D total ELISA study was made on microplates with a solid phase immunosorbent Assay method. During the 2-hour incubation period, proteins in the serum contacts with monoclonal antibodies with the presence of a total of 25 OH (D2-D3) calibrator, controls, and samples at room temperature. After the first washing phase, a fixed amount of biotin-labeled vitamin D competes with the marked Vitamin D2 and D3 in the presence of horseradish peroxidize. Statistical Package for the Social Sciences 15.0 for Windows (Inc., Chicago, IL) was used to analyze the data. Genotype and allele frequencies were compared between patients and controls using the χ^2 test. Logistic regression analysis was performed to calculate odds ratios (OR) and 95% confidence intervals (95% CI). A p-value < 0.05 was considered as statistically significant. The study was approved by the ethics committee of our university, which covered the participating hospitals.

Results

The average age was 37.6 ± 9.5 for the patient group and 33.6 ± 7.3 for the control group. There was not a significant difference between the average ages of the two groups ($p = 0.55$). When the patient and the control groups were evaluated for smoking, it was detected that 38 patients smoked (38%), while 62 patients did not (62%) and 44 controls smoke (44.4%), while 55 controls did not (55.6%). The average duration of the MS disease in the patient group was 14.6 ± 9.9, the average number of the times they were hospitalized was 3.3 ± 3.1. Rs703842 polymorphism in the CYP27B1 gene in humans can be found as three different genotypes: CC, CT, TT. In our study, the aforementioned polymorphisms in the CYP27B1 gene were evaluated in patients with MS and the controls. The genotypes are compatible with the Hardy–Weinberg principle in both groups. Among the 99 individuals in the control group, the number of individuals with CC genotype was 16 (16.2%), the number of individuals with CT genotype was 40 (40.4%) and the number of individuals with TT genotype was 43 (43.4%). Among the 99 individuals in the patient group, the CC genotype frequency was 36 (36.4%), the CT genotype frequency was 20 (20.2%) and the TT genotype frequency was 43 (43.4%). The frequencies of rs703842 polymorphisms in the CYP27B1 gene in the patient and the control groups are shown in Table 1. When the rs703842 polymorphism genotype frequencies were statistically compared between the patient and control groups by using the χ^2 test; the difference for CC, CT, and TC genotypes between two groups was determined ($p < 0.05$). When the statistical analysis was evaluated, CC genotype was found higher in the patient group and CT genotype was found higher in the control group. In the control group, the number of C alleles was found 72 (36.36%) and the number of T alleles was found 126 (63.64%). In the patient group, the number of C alleles was found 92 (46.46%) and the number of T alleles was found 106 (53.54%). When the rs703842 polymorphism allele frequencies were statistically compared between the patient and control groups by using the χ^2 test; the

***Table 1**. The frequencies of rs703842 polymorphisms in the CYP27B1 gene in the patient and the control groups.*

Genotype	Control group *n* = 99 (%)	Patient group *n* = 99 (%)	*p*	OR (95% CI)
CC	16 (%16.2)	36 (%36.4)		
CT	40 (%40.4)	20 (%20.2)	0.001	
TT	43 (%43.4)	43 (%43.4)		
Allele				
C	72 (36.36%)	92 (46.46%)	0.0414	1.5189 (1.0158–2.2711)
T	126 (63.64%)	106 (53.54%)		
	HWE p-0,2064	**HWE p-0**		

***Table 2**. The vitamin D levels of the patient and the control groups.*

	Group	*n*	Average	Standard deviation	Standard error of the average	*p*
Vitamin D	Patients	90	28.8046	14.22101	1.49903	0.005
	Controls	90	34.5426	12.68765	1.33740	

difference for C and T allele between two groups was determined ($p < 0.05$). The statistical analysis shows that the probability of the C allele having an association with MS is 1,5189 times of the probability of T allele (1.5189; %95 Cl, 1.0158–2.2711).

In our study, vitamin D levels between the patient and control groups were compared to evaluate the connection between MS and 25 hydroxyvitamin D which is thought to reduce the risk of the disease. The vitamin D levels obtained from 90 MS patients and 90 controls that participated in the study were compared statistically by using the *T*-test and the difference was determined ($p < 0.05$). The vitamin D levels in the patient group were detected lower than the control group. The vitamin D levels of the patient and control groups are shown in Table 2.

Discussion

Vitamin D levels in MS have been a subject of controversy for many years. At first, because the prevalence is higher in higher latitudes, sunlight exposure was thought to be related to the etiology of the disease. Since sunlight plays a role in vitamin D activation, it was thought that people lacking enough sunlight exposure would have decreased vitamin D levels and would be more vulnerable to developing MS. Our study was based on this theory. Serum vitamin D levels of the patient and control groups were measure by ELISA method. Then, an SNP called rs703842 in the CYP27B1 gene, which codes an enzyme in vitamin D metabolism, was scanned. Vitamin D is essential to utilize dietary calcium efficiently. A serum 25(OH)D level of at least 20 ng/ml is necessary to minimally satisfy the body's vitamin D requirement, and maintenance of a 25(OH)D serum level of 30 to 50ng/ml is recommended. Adequate exposure to sunlight is the cheapest and most efficient method of preventing vitamin D deficiency. However, heat intolerance is a well-recognized feature of MS that causes many patients to protect themselves from sunlight exposure. Many MS patients are homebound or limit their outdoor activities. Furthermore, 1,25-dihydroxy vitamin-D, the active form of 25(OH)D, is a potent inhibitor of cellular growth, stimulator of insulin secretion, and modulator of immune function; therefore, vitamin D-deficient people are prone to autoimmune disorders, including multiple sclerosis. As vitamin D supplementation alone may not be enough, some researchers recommend giving additional treatment with a bisphosphonate, calcitonin, or calcitriol, or a combination of these drugs, to prevent osteoporosis and reduce fracture risk in MS patients. Additionally, maintenance of adequate sunlight exposure is also recommended. We found a significant correlation between lower serum vitamin D and MS patients (7) and to evaluate factors influencing bone mineral density (BMD) (8). Epidemiologic studies have shown that there is an association between vitamin D and MS. Likewise, a study made among young soldiers in the United States Army showed an increased risk for developing MS in soldiers with a low vitamin D serum levels (2). In a study made in Canada with 302 children who had Acute Demyelinating Syndrome, a higher risk of developing MS in the following 3 years were found if they had low vitamin D levels. According to the last reports in the United States after the association between vitamin D and MS was put forward, 7% of the patients used vitamin D before MS diagnosis and 66% of the patients used after the diagnosis (9). In our study, we obtained a similar result with literature. Serum vitamin D levels of the patient group were found significantly lower

than the levels of the control group ($p < 0.05$). Recently, The Australian New Zealand MS Genetics Consortium (ANZgene) published the results of a large study with 2900 patients and 5700 controls. Besides confirming the previously known association of a group of genes; they scanned a region that contains 17 known genes on chromosome 12q13-14 and 20q13, including CYP27B1 (cytochrome P450, family 27, subfamily b, polypeptide 1) on SNP.12q13-14 region. Eventually, in the combined cohort analysis, rs703842 in CYP27B1 was found to be associated with MS ($p = 5.4 \times 10^{-11}$). Also, it was found that the same region might be associated with Hashimoto's thyroiditis, Graves' disease, and Diabetes Mellitus type-1 as well (6).

Furthermore, the study of the third consortium from Sweden was put together with the Australia and New Zealand group and a large GWAS study was made with 6032 patients and 7482 controls. The results obtained by meta-analysis was rs703842 (% 95 CI 0.79-0.88) 0.83. It showed that the SNP was generally associated with MS ($p = 5.1 \times 10^{-11}$) (6). Previously mentioned studies evaluated C allele in rs703842 in CYP27B1 as wild type and T allele as high risk for MS. In our study, we found the exact opposite. T allele had a higher frequency in the control group. Therefore, it contradicts with the previous results in the literature. This study was a case-control study although it has been performed in the fairly homogeneous Turkish population, it is possible, although unlikely, that our observed association is due to population stratification between cases and controls.

Conclusion

MS is an autoimmune disease; therefore, we should keep in mind that other autoimmune diseases may accompany. Lower exposure to sunlight and low expression of CYP27B1 will likely affect the amount of active vitamin D3 in the body. Considering the effects of vitamin D on the immune system, this could lead to the development of MS as well as other autoimmune diseases.

List of Abbreviation

ANZgene	The Australian New Zealand MS Genetics Consortium
MBP	Myelin basic protein
MS	Multiple sclerosis
RA	Rheumatoid arthritis
SLE	Systemic lupus erythematosus

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Ethical approval
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Consent for publication
Written consent was obtained from the patients.

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