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Association Study of HLA-DQB1*0602 Allele in Iranian Patients with Narcolepsy

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ABSTRACT

Narcolepsy is a rare, disabling disorder characterized by excessive daytime sleepiness, cataplexy, hypnagogic hallucinations and sleep paralysis. Several studies demonstrated its association with HLA-DQB1*0602 in various ethnic groups. Our study aimed to determine the prevalence of HLA-DQB1*0602 allele in Iranian patients with narcolepsy and assess its predictive parameters for diagnosing narcolepsy. In addition, car accidents and job problems were assessed among narcoleptic patients.

We studied 44 narcoleptic patients, 30 patients with other types of excessive daytime sleepiness (EDS) and 50 healthy age and sex matched individuals in this case-control study. Patients and controls filled out a questionnaire including items about car accidents due to sleepiness and job problems. International classification of sleep disorders-2 criteria was used as the gold standard for diagnosis of narcolepsy. The DNAs isolated from whole blood samples were collected from the patients and controls to assess the presence of HLA-DQB1*0602.

The results showed that HLA DQB1*0602 was present in 4 (8%) individual of controls and 20 (45.5%) patients with higher prevalence in patients with cataplexy (78.9%) than patients without cataplexy (p<0.001). The sensitivities of the DQB1*0602 for diagnosing narcolepsy with cataplexy and narcolepsy without cataplexy were 78.9 and 20; specificities were 88 and 72.4, respectively. 18.2% of patients had car accidents due to sleepiness and 68.2% suffered from job problems.

Our study shows that evaluation of DQB1*0602 in patients suspected to narcolepsy could be helpful especially in complex cases with atypical cataplexy and indistinguishable multiple sleep latency test MSLT results. Moreover, high rates of car accidents and job problems are found among narcoleptic patients.

Keywords: Cataplexy; HLA-DQB1*0602; Narcolepsy; Sleep disorder

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INTRODUCTION

Narcolepsy is an autoimmune debilitating sleep disorder. The main symptoms are excessive daytime sleepiness, cataplexy (i.e. sudden loss of muscle tone triggered by strong emotions during wakefulness), hypnagogic hallucination and sleep paralysis. Autoimmunity and the lack of cerebrospinal fluid (CSF)-hypocretin are the probable cause of this disease.[1-3] Several studies offer the association of HLA-DQB1*0602 and narcolepsy especially in patients with cataplexy. About 90% of narcoleptic patient with cataplexy are DQB1*0602 positive while only 30% to 50% of cases without cataplexy are positive for DQB1*0602. Moreover 12% to 38% of general population across ethnic groups are DQB1*0602 positive.[4-7]

International classification of sleep disorders-2 (ICSD-2) criteria is used as the gold standard for diagnosis of narcolepsy. There are two types of narcolepsy: type 1 narcolepsy (narcolepsy with cataplexy) and type 2 narcolepsy (narcolepsy without cataplexy). The distinctive attribute of type 1 narcolepsy is sudden loss of muscle tone elicited by positive emotions such as laughter and surprise.[8] There is not necessitate to perform polysomnography (PSG) or multiple sleep latency test (MSLT) in patients with cataplexy but if the cataplexy does not exist, findings of the PSG and MSLT helps to rule out or in the narcolepsy.[8]

Narcolepsy is associated with both medical and psychiatric disorders. Major depressive disorder and social anxiety disorder affecting nearly 20% of patients with narcolepsy.[9] Clinical symptoms of narcolepsy cause adverse effects on social and personal life of the patients such as job, school, driving and interpersonal relationships.[10] Previous studies showed that patients with narcolepsy had increased risk of driving accidents compared to general population.[11-13] Long-term treatment of narcolepsy protects patients against the car accidents.[13]

There is no data on HLA-typing of Iranian narcoleptic patients. In this study we aim to evaluate the predictive parameters of the HLA-DQB1*0602 for diagnosing narcolepsy by using ICSD-2 criteria. Also, car accidents and job problems were assessed among these patients.

MATERIALS AND METHODS

Patients and Controls

We studied a group of 44 narcoleptic patients, 30 patients with other types of excessive daytime sleepiness (EDS) and 50 healthy age and sex matched individuals in this case-control study. Patients and controls filled out a questionnaire including demographic data, items about car accidents due to sleepiness and job problems and the Epworth sleepiness scale (ESS). Subjects were assigned to control group according to the ESS score. ESS is a well-known questionnaire for evaluating daytime sleepiness.[14] Patients with ESS score≥10 considered having excessive daytime sleepiness. All of the patients underwent the night-time PSG in the sleep disorders clinic, Baharloo hospital, Tehran, Iran from 2010 to 2015 whereas 32 patients agreed to undergo MSLT following their night-time PSG. Subjects who did not want to participate in the study nor had other disorders explaining sleepiness such as delayed sleep phase syndrome were excluded. Written consents were obtained from all the patients and controls after the procedures and goals of the study were fully explained. This study was approved by the ethics committee of the Tehran University of Medical Sciences (No: IR.TUMS.REC.1394.868).

Narcoleptic patients were categorized into two groups according to the ICSD-2 criteria: (1) narcolepsy with cataplexy was defined as chronic excessive daytime sleepiness and definite history of cataplexy (2), narcolepsy without cataplexy was defined as chronic excessive daytime sleepiness in the absence of cataplexy, plus MSLT mean sleep latency ≤8 minutes and two or more sleep onset rapid eye movement periods (SOREMPs).

DNA Extraction and HLA-DQB1*0602 Genotyping

The whole blood sample were collected from the patients and controls to assess the presence of HLA-DQB1*0602. Genomic DNA samples were purified from whole blood using salting out method, and then samples were genetically typed for HLA-DQB1*0602 locus using a commercial kit, HLA-ready gene narcolepsy, HLA-DQB*0602 test (Inno-train, Germany).

Statistics

Descriptive statistics are presented as mean (SD).

Normality of data was analyzed by the Kolmogorov-Smirnov test. MannWhitney U test was used for comparing continuous variables, and the $\chi 2$ test was used to assess differences in the categorical variables. We calculated predictive parameters of the DQB1*0602 including sensitivity, specificity, positive and negative predictive values, positive and negative likelihood ratio for narcolepsy, narcolepsy with cataplexy and narcolepsy without cataplexy. $p<0.05$ was considered statistically significant. Predictive Analytics SoftWare (PASW) (IBM, USA version 18 was used for data analysis.

RESULTS

In our study, 44 patients with narcolepsy (36 male), 30 patients with other types of excessive daytime sleepiness (EDS) and 50 healthy subjects (38 male) were evaluated. Cataplexy was present in 19 patients. There is no significant difference in gender between patients with cataplexy (15 male, 4 female) and those without cataplexy (21 male, 4 female). PSG and MSLT results of patients are shown in Table 1. MSLT was available for 32 patients, 20 patients (62.5%) had MSL≤8 min and SOREMP≥2. There was not a significant difference in MSLT result between patients with cataplexy and those without ($p=0.7$).

HLA DQB1*0602 was present in 20 patients (45.5%) with higher prevalence in patients with cataplexy (n=15, 78.9%) than patients without cataplexy (n=5, 20%) ($p<0.001$). Also we found a significant difference in the presence of DQB1*0602 between the patients with narcolepsy and the control group ($p<0.01$). DQB1*0602 was positive in 4 individuals (8%) of control group and 1 patient (3.3%) with other types of excessive daytime sleepiness (EDS).

Table 1. Demographic and clinical characteristics of patients and controls

Characteristic	Narcolepsy with cataplexy (n=19)	Narcolepsy without cataplexy (n=25)	All Patients (n=44)	Controls (n=50)
Age (years)	30.1 (11.4)	35.6 (12)	33.2 (11.9)	32.3(9.6)
ESS score	16.9 (4.3)	16 (6)	16.4 (5.3)	5.5(3.4)
PSG				
AHI (events/h)	6.8 (4.3)	2.7 (2.6)	4.4 (3.9)	
Sleep latency (min)	18 (14.5)	20.4 (19.1)	19.4 (17.2)	
REM Sleep latency (min)	97.7 (57.1)	86 (52.2)	91.2 (53.6)	
MSLT				
Mean Sleep Latency (min)	6.1 (3.2)	5.1 (3.2)	5.5 (3.2)	
SOREMP≥2 (%)	8 (66.7)	16 (80)	24 (75.8)	
MSL ≤8 and SOREMP≥2	7 (58.3)	13 (65)	20 (62.5)	

Data are presented as mean (SD) or %.

ESS: Epworth sleeping scale, PSG: polysomnography, AHI: apnea-hypopnea index, REM: rapid eye movement, MSLT: multiple sleep latency test, SOREMP: sleep-onset REM periods

Table 2. Predictive parameters of the DQB1*0602 for diagnosing narcolepsy, narcolepsy with cataplexy and narcolepsy without cataplexy

Variables	Narcolepsy	Narcolepsy with cataplexy	Narcolepsy without cataplexy
Sensitivity	45.5 (30.3-61.1)	78.9 (54.4-93.9)	20 (6.8-40.7)
Specificity	92 (80.7- 97.7)	88 (78.4-94.3)	72.4 (60.3-82.5)
Positive likelihood ratio	5.68 (2.1-15.3)	6.5 (3.4-12.6)	0.73 (0.3-1.7)
Negative likelihood ratio	0.59 (0.45-0.79)	0.24 (0.1-0.57)	1.1 (0.8-1.41)
Positive predictive value	83.3 (62.6-95.2)	62.5 (40.5-81.2)	20.8 (7.1-42.1)
Negative predictive value	65.7 (53.4-76.6)	94.2 (86-98.4)	71.4 (59.3-81.6)

Table 3. Car accidents and job problems in DQB1*0602 positive or negative patients suspected to narcolepsy with or without cataplexy

Topics	Cataplexy		HLA-DQB1*0602		All Patients (n=44)
	Present (n=19)	Not present (n=25)	Positive (n=20)	Negative (n=24)	
Car accidents					
Yes	3 (15.8)	5 (20)	5 (25)	3 (12.5)	8 (18.2)
No	16 (84.2)	20 (80)	15 (75)	21 (87.5)	36 (81.8)
Job problems					
Yes	13 (68.4)	17 (68)	12 (60)	18 (75)	30 (68.2)
No	6 (31.6)	8 (32)	8 (40)	6 (25)	14 (31.8)

Data are presented as n (%).

The sensitivities of the DQB1*0602 for diagnosing narcolepsy, narcolepsy with cataplexy and narcolepsy without cataplexy were 45.5, 78.9 and 20; specificities were 92, 88 and 72.4, respectively. Predictive parameters of the DQB1*0602 for diagnosing narcolepsy, narcolepsy with cataplexy and narcolepsy without cataplexy are shown in Table 2. In our study, 18.2% of patients had car accidents due to sleepiness and 68.2% suffer from job problems. Table 3 shows car accidents and job problems in narcoleptic patients according to the cataplexy and DQB1*0602. There is no significant difference in car accidents and job problem between patient with cataplexy and without cataplexy as well as between DQB1*0602 positive patients and negative ones.

DISCUSSION

In this study we used the ICSD-2 criteria as the gold standard for diagnosing narcolepsy. HLA DQB1*0602 was assessed in patients and controls to identify its utility for detecting narcolepsy. Especially car accident due to sleepiness and job problem were asked from patients to establish the adverse outcomes of narcolepsy.

We found that the prevalence of DQB1*0602 in Iranian general population is 8%, which is lower than other ethnics such as African American (31.3%), Caucasian (23.3%), Korean (12.8%) and Japanese (12.2%). We also report lower rate of DQB1*0602 positivity in narcoleptic patients with and without cataplexy in comparison with other ethnics. Our results are comparable with previous studies that showed the high positivity of DQB1*0602 in narcoleptic patients with cataplexy. In our study, DQB1*0602 was positive in 45.5% and 8% of patients and controls, respectively. Narcoleptic patients with cataplexy were 78.9% DQB1*0602 positive while DQB1*0602 was positive in only 20% of narcoleptic patients without cataplexy. In relevant previous results, more than 90% of patients with cataplexy were DQB1*0602 positive. Okun et al. evaluated 482 multiethnic patients with cataplexy and showed that 92% of patients with typical cataplexy were DQB1*0602 positive.[15] Patients without cataplexy have lower rate of DQB1*0602 positivity in studies. Mignot et al. in a study of 509 narcoleptic patients showed that DQB1*0602 was positive in 40.9% of patients without cataplexy.[7]

Hong et al. in a study on 163 patients with excessive daytime sleepiness and 282 controls evaluated the diagnostic accuracy of DQB1*0602 and reported the sensitivity of 92.4 and 36.2 in patients with cataplexy and without cataplexy, respectively. The specificity of DQB1*0602 was 72.8 for diagnosing both narcolepsy with cataplexy and narcolepsy without cataplexy.[1] In our study, we report lower sensitivity for diagnosing narcolepsy with cataplexy due to lower positivity of DQB1*0602 among our patients with cataplexy in comparison with Hong et al. study. On the other hand, due to lower prevalence of DQB1*0602 in our controls than their control group, the specificity of DQB1*0602 in our study is higher than Hong et al. study.

In this study, we found that patients with narcolepsy are in greater risk for car accidents and job problems. Cataplexy and DQB1*0602 status did not vary the risk of car accidents and job problem in patient with narcolepsy. Our results are consistent with previous

studies which showed that quality of life is deteriorated in patients with narcolepsy.[10,12] Pizza et al. in a cross sectional study reported a significant difference between patients with narcolepsy and healthy subjects for driving accidents in last 5 years (22.7% vs. 14%).[13]

The study's limitation was low number of patients available or diagnosed, which was due to the low incidence of disease.

In conclusion, according to the predictive parameters of the DQB1*0602 for diagnosing narcolepsy in Iranian narcoleptic patients, the evaluation of DQB1*0602 in patients suspected to narcolepsy could be helpful in complex cases with atypical cataplexy and indistinguishable MSLT results. Moreover, our data shows the high rate of car accidents and job problems among Iranian narcoleptic patients.

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