Original Research

Human Leukocyte Antigen-Typing in Patients with Excessive Daytime Sleepiness

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Abstract

Background and Objective: Narcolepsy is a disorder recognized by excessive daytime sleepiness (EDS). Several studies demonstrated its association with human leukocyte antigen (HLA) DQB1*0602, DQA1*0102, and DRB1*15. Our study aimed to perform HLA-typing on patients with EDS. Moreover, we performed HLA-typing for family members of the patients.

Materials and Methods: We studied 83 patients with EDS and 77 of their first-degree relatives. Patients filled out a questionnaire including Epworth Sleepiness Scale (ESS), and underwent polysomnography (PSG) and multiple sleep latency test (MSLT). The whole blood samples were drawn from the patients and their families for HLA typing (class II). International classification of sleep disorders-2 (ICSD-2) criteria was used as the gold standard for diagnosing narcolepsy.

Results: HLA DQB1*0602 was present in 20 (45.5%) of narcoleptic patients and 2 (5.1%) of patients with other causes of EDS. Prevalence of DQB1*0602 in family members of narcoleptic patients were higher than family members of patients with other causes of EDS (38% vs. 11.1%, P = 0.06). DQB1*0602, DQA1*0102, and DRB1*15 were more prevalent in narcoleptic patients with cataplexy than narcoleptic patients without cataplexy and patients with other causes of EDS. The sensitivities of the DQB1*0602 for diagnosing narcolepsy, narcolepsy with cataplexy, and narcolepsy without cataplexy were 40%, 60%, and 20%, respectively; while specificities were 93.9%, 87.9%, and 70.6%, respectively.

Conclusion: HLA typing can be helpful in patients with atypical cataplexy and inconclusive MSLT results. More studies of Iranian narcoleptic patients are required for analyzing their HLA sequences.

Keywords: Narcolepsy; HLA typing; Iran

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Introduction

Narcolepsy is a chronic neurological disorder that affects 0.02% to 0.05% of the general population. Narcolepsy is a sleep disorder known by excessive daytime sleepiness (EDS) accompanied by recurrent, short-term and inevitable sleep attacks, cataplexy, sleep paralysis, and hypnagogic hallucinations. This syndrome is also associated with a shorter sleep onset period, disrupted nighttime sleep, and shorter rapid eye movement (REM) sleep onset in the first stage of sleep (1-3). Cognitive impairment, difficulties in education, occupation, and interpersonal relationships are observed in narcoleptic patients (4).

Molecular studies at the DNA level suggest the presence of human leukocyte antigen (HLA) DQB1*0602 as a marker for narcolepsy in racial groups. The prevalence of DQB1*0602 in different racial groups of a normal population varies from 12% to 38% (5-8). International classifica-

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tion of sleep disorders-2 (ICSD-2) criteria could be used as the gold standard for diagnosing narcolepsy. Patients with narcolepsy are categorized into two groups according to the presence or absence of cataplexy: patients with or without cataplexy. Patients with cataplexy are not required to undergo polysomnography (PSG) and multiple sleep latency test (MSLT); however, if the suspected patient does not have cataplexy, the results of PSG and MSLT can help to diagnose or rule out narcolepsy (9).

A 20 to 40 times increase in the risk of narcolepsy have been observed in first-degree relatives in familial studies (10). Since the patients have not yet been genetically evaluated in Iran, we intended in this study to take a step in the definitive diagnosis of this disorder with HLA typing method.

Materials and Methods

Patients with complaints of EDS referred to sleep clinics of Baharloo and Imam Khomeini hospitals, Tehran, Iran, between 2004 and 2014 were recruited in this cross-sectional study. Patients filled out a questionnaire containing demographic information, history of autoimmune diseases, familial history of narcolepsy, cousin marriages of parents, age of onset of the disease, as well as Epworth Sleepiness Scale (ESS) (11). Patients with ESS score equal or more than 10 were considered to have EDS. Finally, 83 patients with EDS were enrolled in this study. Patients underwent MSLT after the night-time PSG. Genomic DNA samples were purified from each subject's white blood cells (WBCs), and then samples were genetically typed for HLA (class II). Moreover, first-degree family members of the patients were invited for HLA-typing. Written consents were obtained from all the individuals, and those who did not want to participate were excluded. The ethics committee of Tehran University of Medical Sciences approved this study.

Narcoleptic patients were categorized into patients with cataplexy and those without cataplexy according to the ICSD-2 criteria. In patients without cataplexy, positive MSLT was defined as mean sleep latency ≤ 8 minutes, and two or more sleep onset REM periods (SOREMPs) are required for diagnosing narcolepsy.

Using sensitivity analysis, predictors of HLA DQB1*0602, DQA1*0102, and DRB1*15 (sensitivity, specificity, positive and negative likelihood ratio, and positive and negative predictive value) were calculated for the diagnosis of narcolepsy.

Statistical analysis: SPSS software (version 21, IBM Corporation, Armonk, NY, USA) was used for data analysis. Mean, standard deviation (SD), frequency, and percent were used to describe the data. Normality of data was checked by the Kolmogorov-Smirnov test (K-S test). T-test and chi-square tests were used to compare the means and frequencies. Sensitivity analysis was used to calculate predictive indices. The results with $P < 0.05$ were considered statistically significant.

Results

Forty-four patients with narcolepsy (19 with cataplexy and 25 without cataplexy) and 39 patients with other causes of EDS were studied. In addition, 77 family members of the patients participated in this study, 50 of whom were family members of patients with narcolepsy and 27 were family members of those suffering from other causes of EDS. Table 1 shows the characteristics of the subjects participating in the study.

There was no difference in age, the onset age of narcolepsy, body mass index (BMI), and gender between patients with narcolepsy and those with other causes of EDS.

Table 1. Characteristics of the subjects participating in the study

	Narcolepsy (n = 44)	Other EDS (n = 39)	P-value
Age (Mean ± SD)	32.5 ± 9.9	40.4 ± 12.2	0.0900
Onset age of narcolepsy symptoms (Mean ± SD)	23.4 ± 10.2	29.4 ± 13.8	0.0600
BMI (Mean ± SD)	27.2 ± 4.1	27.1 ± 4.7	0.6200
Male gender [n (%)]	30 (76.9)	39 (81.6)	0.1500
Cataplexy [n (%)]	19 (43.2)	0	< 0.0001
Sleep paralysis [n (%)]	19 (43.2)	15 (38.5)	0.6600
Hypnagogic hallucination [n (%)]	24 (54.5)	14 (35.9)	0.0800
Sleep attack [n (%)]	40 (90.9)	28 (71.8)	0.0200
History of autoimmune disease [n (%)]	3 (8.3)	1 (2.3)	0.2100
Family history of sleep disorders* [n (%)]	9 (25.0)	10 (22.7)	0.8100
Cousin marriage of parents [n (%)]	9 (25.0)	10 (22.7)	0.8100

EDS: Excessive daytime sleepiness; BMI: Body mass index

*Sleep disorders referred to narcolepsy, insomnia, obstructive sleep apnea

Table 2. Comparison of ESS, PSG, and MSLT tests in patients with narcolepsy and patients with other causes of EDS

		Narcolepsy (n = 44)	Other EDS (n = 39)	P-value
ESS		16.7 (4.9)	15.6 (5.5)	0.3600
	AHI (events/hour)	5.4 (4.8)	19.1 (24.1)	< 0.0001
PSG	Sleep latency (minute)	21.5 (18.5)	21.1 (19.9)	0.8000
	REM Sleep latency (minute)	97.8 (56.4)	134.5 (88.5)	0.2300
	Mean Sleep Latency (minute)	5.5 (3.3)	12.3 (8.1)	0.0200
MSLT	SOREMP ≥ 2 (%)	84.4	21.4	< 0.0001
	MSL ≤ 8 and SOREMP ≥ 2 (%)	71	8.7	< 0.0001

ESS: Epworth sleepiness scale; PSG: Polysomnography; MSLT: Multiple sleep latency test; EDS: Excessive daytime sleepiness; AHI: Apnea hypopnea index; REM: Rapid eye movement; SOREMP: Sleep onset rapid eye movement period; MSL: Mean sleep latency

Cataplexy and sleep attack were significantly more observed in narcoleptic patients than those with other causes of EDS. Three patients (8.3%) with narcolepsy had autoimmune diseases, while only 1 of the other patients (2.3%) suffered from autoimmune disease (P = 0.2100). Nine patients (25.0%) with narcolepsy were children of cousin marriages, while this proportion was 10 (22.7%) in other patients (P = 0.8100).

Patients with narcolepsy had a higher ESS score than those with other causes of EDS, but this difference was not significant (P = 0.3600). Apnea Hypopnea Index (AHI) was 5.4 in patients with narcolepsy, but in patients with other causes of EDS was 19.1, which was statistically significant (P < 0.0001). Sleep latency and REM sleep latency in PSG was not significantly different among patients with narcolepsy and patients with other causes of EDS. In the MSLT, the mean sleep latency in patients with narcolepsy was 5.5 minutes, which was significantly lower than that of other patients (12.3 minutes) (P = 0.0200). 84.4% of patients with narcolepsy and 21.4% of other patients had SOREMPs ≥ 2 (Table 2).

The results of the HLA-typing in the studied patients are shown in table 3. The prevalence of DQB1*0102, DQA1*0602, and DRB1*15 in patients with narcolepsy was significantly higher than that of other patients. Considering their prevalence, these 3 HLAs were categorized in group 1 and 45.5% of the patients with narcolepsy had all 3 HLAs, while 5.1% of the other patients had all 3 HLAs. DQA1*11, DRB1*0505, and DQB1*03 were also categorized in group 2, of which, only the difference of DQB1*03 between patients with narcolepsy and other patients was not significant. The prevalence of DQB1*0602 and other HLAs in group 1 in patients with cataplexy was significantly higher than patients without cataplexy, while HLAs of group 2 were more prevalent in patients without cataplexy than in patients with cataplexy.

DQB1*0602 sensitivity for the diagnosis of narcolepsy, narcolepsy with cataplexy, and narcolepsy without cataplexy were calculated at 40%, 60%, and 20%, respectively. DQB1*0602 had a higher specificity for diagnosing narcolepsy, with a specificity of 93.9% for diagnosing narcolepsy, 87.9% for diagnosis of cataplexy, and 70.6% for diagnosis of narcolepsy without cataplexy. Sensitivity and specificity of DQA1*0102 for diagnosis of narcolepsy were 40.0% and 81.8%, respectively. Sensitivity and specificity of DRB1*15 for diagnosis of narcolepsy was 50.0% and 75.7%, respectively (Table 4).

Table 3. Comparison of HLA-typing results in studied patients

	Narcoleptic patients			Other EDS	P-value*
	All (n = 44)	with cataplexy (n = 19)	without cataplexy (n = 25)	(n = 39)	
DQB1*0602 [n (%)]	20 (45.5)	15 (78.9)	5 (20.0)	2 (5.1)	< 0.0001
DQA1*0102 [n (%)]	20 (45.5)	15 (78.9)	5 (20.0)	6 (15.4)	0.0030
DRB1*15 [n (%)]	23 (52.3)	16 (84.2)	7 (28.0)	10 (25.6)	0.0130
Group 1 [n (%)]	20 (45.5)	15 (78.9)	5 (20.0)	2 (5.1)	< 0.0001
DQA1*0505 [n (%)]	25 (56.8)	7 (36.8)	18 (72.0)	12 (30.8)	0.0170
DRB1*11 [n (%)]	22 (50.0)	7 (36.8)	15 (60.0)	11 (28.2)	0.0400
DQB1*03 [n (%)]	24 (54.5)	6 (31.6)	18 (72.0)	17 (43.6)	0.3100
Group 2 [n (%)]	21 (47.7)	6 (31.6)	15 (60.0)	10 (25.6)	0.0380

HLA: Human leukocyte antigen; EDS: Excessive daytime sleepiness
*P-values were calculated for comparing all patients with narcolepsy and those with other causes of EDS.

Table 4. Predictive factors of DQB1 * 0602, DQA1*0102, and DRB1*15 for diagnosis of narcolepsy

	DQB1* 0602 (%)	DQA1*0102 (%)	DRB1*15 (%)
Sensitivity	40 (26.4-54.8)	40 (26.4-54.8)	50 (35.5-64.4)
Specificity	93.9 (79.7-99.2)	81.8 (64.5-93)	75.7 (57.7-88.9)
Positive likelihood ratio	6.6 (1.6-26.8)	2.2 (0.9-4.8)	2.06 (1.06-4)
Negative likelihood ratio	0.64 (0.5-0.81)	0.7(0.56-0.97)	0.66 (0.47-0.93)
Positive predictive value	90.9 (70.8-98.8)	76.9 (56.3-91)	75.7 (57.7-88.9)
Negative predictive value	50.8 (37.7-63.8)	47.3 (33.9-61)	50 (35.5-64.4)

HLA-typing results in family members of patients with narcolepsy and other patients showed that the difference of none of the investigated HLAs were significant among them. The prevalence of all six investigated HLAs in family members of patients with narcolepsy was higher than that of family members of patients with other causes of daytime sleepiness. HLA DQB1*0602 was found in 30% of family members of patients with narcolepsy, while only 11.1% of family members of patients with other EDS had HLA DQB1*0602 (P = 0.0600) (Table 5).

Discussion

In this study, the existence of HLA DQB1*0602, DQA1*0102, DRB1*15, DQA1*0505, DQB1*03, and DRB1*11 was studied in the patients and their families. Definite diagnosis of narcolepsy was based on ICSD-2 criteria.

AHI score in narcoleptic patients was lower than patients with other causes of EDS, because many of the patients with other causes of EDS suffer from moderate to severe obstructive sleep apnea (OSA). REM latency in narcoleptic patients was lower than patients with other causes of EDS, but the difference was not statistically significant.

Current study, similar to Mignot et al., showed a high incidence of DQB1*0602, DQA1*0102, and DRB1*15 in narcoleptic patients with cataplexy (5). In our study, 45.5% of patients with narcolepsy carried HLA DQA1*0102 and DQB1*0602, and 52.3% carried HLA DRB1*15. In Mignot et al. study, 57.6% of Japanese patients were positive for HLA DQB1*0602, 60.0% for HLA DRB1*15, and 61.0% for HLA DQA1*0102 (5). Alaez et al. in a case-control study of 32 narcoleptic patients with cataplexy and 203 controls found a positive association of HLA-DRB1*1501 (Odds ratio: 8.2) and DQB1*0602 (Odds ratio: 8.4) with narcolepsy (12).

Similar studies reported higher prevalence of HLA DQB1*0602 in narcoleptic patients with cataplexy than those without cataplexy and general population (13).

In our study, the prevalence of DQA1*0102 and DRB1*15 in patients with narcolepsy were almost the same as DQB1*0602; and when all three HLAs were categorized in one group, patients with narcolepsy had a significantly higher prevalence of these HLAs compared to patients with other causes of EDS. Prevalence of HLA DQA1*0505, DRB1*11, and DQB1*03 were also relatively similar and were categorized in group 2, which among them, only DQB1*03 difference was not significant between patients with narcolepsy and other patients. In patients with cataplexy, the prevalence of HLAs in group 1 was significantly higher than patients without cataplexy, while group 2 HLAs in patients without cataplexy were more prevalent than patients with cataplexy.

Table 5. HLA-typing in family members of patients with narcolepsy and those with other causes of EDS

	Family of patients with narcolepsy (n = 50)	Family of patients with other EDS (n = 27)	P-value
DQB1*0602 [n (%)]	15 (30.0)	3 (11.1)	0.0600
DQA1*0102 [n (%)]	21 (42.0)	7 (25.9)	0.1600
DRB1*15 [n (%)]	24 (48.0)	8 (29.6)	0.1100
Group 1 [n (%)]	15 (30.0)	3 (11.1)	0.0600
DQA1*0505 [n (%)]	20 (40.0)	7 (25.9)	0.2100
DRB1*11 [n (%)]	25 (50.0)	10 (37.0)	0.2700
DQB1*03 [n (%)]	17 (34.0)	8 (29.6)	0.6900
Group 2 [n (%)]	15 (30.0)	6 (22.2)	0.4600

HLA: Human leukocyte antigen; EDS: Excessive daytime sleepiness

In our study, the prevalence of all six investigated HLAs in family members of patients with narcolepsy were higher than family members of patients with other causes of EDS, but the difference between them was not statistically significant. Chen et al. in a case-control study of 12 narcoleptic patients, 34 first-degree relatives, and 30 controls showed a familial aggregation and HLA susceptibility of narcolepsy. They found that 61.8% and 44.1% of the first-degree relatives were DQB1* 0602 and DRB1*1501 positive, respectively (10).

We had problem in taking blood sample of patients because some of them were not available. More studies with greater sample size are needed for HLA typing.

Conclusion

Given the predictive value of DQB1*0602, DQA1*0102, and DRB1*15 for diagnosing narcolepsy in Iranian patients, this test can be helpful in patients with atypical cataplexy and inconclusive MSLT results. More studies on Iranian narcoleptic patients are required for analyzing their HLA sequences.

Conflict of Interests

Authors have no conflict of interests.

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