ABSTRACT

Objective: To evaluate the presence of HLA-DQB1*0602 allele, tumor necrosis factor and interleukin-6 in patients with cataplexy and controls. Methods: A prospective controlled study with 22 patients diagnosed as narcoleptic according to DSM4 criteria and 17 healthy control subjects with no sleep disorders. All patients underwent a night-polysonomographic recording and HLA-DQB1*0602 study. Tumor necrosis factor and interleukin-6 levels of controls and patients were quantified. Results: The presence of HLA DQB1*0602 allele was found in 10 patients with cataplexy and in 2 patients with no cataplexy (p = 0.24). A significant increase in tumor necrosis factor in patients with rare cataplexy was observed when compared with controls (p = 0.009), as well as a significant decrease in patients with rare cataplexy versus patients with frequent cataplexy (p < 0.0001). There were no differences in interleukin-6 levels between the groups. Conclusion: Discrepancies in clinical picture of narcoleptic patients may be associated with subtle changes in the pathophysiological mechanism of the disease. The present study findings reinforce the hypothesis of a probable immunological etiology for narcolepsy.

Keywords: Narcolepsy; Cataplexy; Tumor necrosis factor; Interleukin-6; HLA-DQ antigens

INTRODUCTION

Narcolepsy is characterized by excessive daytime sleepiness, cataplexy and it may also be added by sleep paralysis, hypnagogic hallucinations and sleep fragmentation[1,2].

The pathophysiology of narcolepsy is still unknown regarding frequent association with HLA-DQB1*0602[3] allele, which would favor the presence of a genetic...
susceptibility factor. Other environmental, infectious and immunological associations must be taken into account(4).

Decrease of hypocretin in the cerebrospinal fluid has been recently described in narcoleptic patients due to the loss of producing cells in the lateral hypothalamus. Hypocretin is a neuropeptide produced in the lateral hypothalamus and plays a role in vigilance maintenance. The connections between the hypocretin-producing hypothalamus and the dopaminergic pathways in the pre-frontal area and the noradrenergic pathways in the brainstem are well established(5,7).

Recognized abnormalities of the genetic pattern in the histocompatibility class II region, such as HLADQB1*0602 and HLA DR2 alleles, are associated with self-aggressive immune responses as those observed in multiple sclerosis and rheumatic fever, among other conditions(6-9). The theory that supposes an autoimmune pathophysiological pattern related with the finding of HLADQB1*0602 allele in patients with narcolepsy leading to self-aggression of hypocretin-producing cells may be explained by excessive lymphocyte reactivity and cytokines, by loss of natural tolerance to antigens or by presentation of slightly modified antigens after a viral or bacterial infection(10).

Much has been investigated to define narcolepsy as an immune disease, however with little success(11-13). The identification of increased concentration of interleukin-6 (IL-6) and tumor necrosis factor (TNF) was described in patients with narcolepsy and in other sleep disorders, such as the sleep apnea syndrome(12).

IL-6 and TNF play several roles in the immune system as modulators and activators of the cell immunity cascade and act as coadjuvant in antigen presentation. It is well known that changes in the histocompatibility alleles, such as HLADQB1*0602, modify the immune system reactivity with consequent changes in humoral and cell responses(13).

The studies previously mentioned prioritized the comparisons between control groups and narcoleptic groups, and the presence or absence of the histocompatibility allele. No study has evaluated subgroups of patients (with or without cataplexy)(11-13). It is important to emphasize that these studies were carried out before the discovery of decreased hypocretin in the cerebrospinal fluid, which definitely differentiated narcoleptic patients with cataplexy and those without cataplexy.

METHODS

A prospective controlled study carried out between November 2003 and February 2005 at the Outpatient Clinic of Excessive Daytime Sleepiness – Department of Psychobiology – UNIFESP - Escola Paulista de Medicina.

The study was approved by the Research Ethics Committee of the Escola Paulista de Medicina – UNIFESP, under number 1139/03. All patients and control subjects accepted to participate in the study and signed the informed consent form.

The study group was composed of 6 patients without cataplexy, 10 patients with rare cataplexy, 6 patients with frequent cataplexy and 17 control subjects. All subjects underwent clinical interview and narcoleptic patients answered the Epworth excessive sleepiness questionnaire(14).

All patients met the diagnostic criteria for narcolepsy according to the International Classification of 2005(2), as well as the electrophysiological criteria with two or more episodes of REM sleep in the Multiple Sleep Latency Test (MSLT)(15,10).

The presence HLA DQB1*0602 allele was evaluated and TNF and IL-6 levels were measured in all subjects. No control subject presented daytime sleepiness or family history of narcolepsy. The study excluded patients and control subjects who were presenting acute or uncontrolled chronic disease; those taking immunosuppressive, pro-stimulant or depressant drugs; alcohol or drug abusers at the moment of blood collection.

Patients were separated into subgroups according to presence and frequency of cataplexy(6), patients with rare cataplexy(10) (up to one episode of cataplexy per month) and patients with frequent cataplexy(6) (more than one episode of cataplexy per month).

Polysomnography (PSG) and Multiple Sleep Latency Test (MSLT) were performed and analyzed according to international criteria(14).

Evaluation of HLADQB1*0602

It was performed at the Genetics Laboratory of the Sleep Institute - UNIFESP. The presence of HLA DQB1*0602 was determined through the following procedure: genomic DNA was extracted from blood white cells and the region where the DQB1*0602 allele is found was amplified by means of polymerase chain reaction (PCR) using the primers DBQF (5'-CCGCGAGAGTTCGTGTT - 3) and DBQR (5'-AATCTCGCCCCGGGTCCC - 3). These primers amplified DQB1*0602, as well as the rare alleles.
DQB1*0610, DQB1*0613 and DQB1*0614, as a product of 218 base pairs. As an internal control for certification of amplification, the following primers that amplify only exon 3 of DRB1 gene were used in the same PCR: EX3f (5-TGCCAAGTGAGCACCCA - 3) EX3r (5-GCATCTTGCTCTGCGAT - 3). For PCR, we used 35 cycles at 95°C, for 30 seconds, at 63°C for 30 seconds and at 72°C for 60 seconds.

Measuring TNF and IL-6

The measurement of serum cytokines was performed by ELISA with specific capture and detection antibodies for TNF and IL-6 (R&D Systems). The concentration of each cytokine in the supernatant was calculated based on the linear regression equation of the standard curve obtained with recombinant cytokines, according to the kit manufacturer instructions. The reading was carried out in a Tecan Genius instrument.

Statistical analysis

The distribution of variables was verified using the Kolgomorov-Smirnoff test and the values were presented as means and standard deviation. The chi-square test was used to confirm that the groups of narcoleptic patients and control subjects were homogeneous and the Fischer’s test was used in other analyses, whenever necessary. The Student’s t test for independent samples was used to compare the results of TNF and IL-6 between the groups. Statistical significance was considered as p < 0.05. The statistics program “Statistica” (Stafsoft 1984-1997) was used.

RESULTS

The groups of narcoleptic patients and control subjects were comparable in regards to age and gender (table 1), as well as the subgroups of patients with narcolepsy (table 2). All patients answered the Epworth questionnaire with a maximum and minimum of 22 and 9 points, respectively, with a mean of 18.31 ± 3.15. There was no difference in the sleepiness scale between the subgroups of patients. The prevalence of HLA DQB1*0602 allele was 20% in control subjects, 40% in patients with cataplexy, 66% in patients with rare cataplexy and 100% in patients with frequent cataplexy. There was no difference in the measurements of IL6 and TNF levels in narcoleptic patients when compared in terms of presence or absence of HLA DQB1*0602 allele (tables 3 and 4). There was an increase in TNF levels in narcoleptic patients with rare cataplexy when compared with the group of control subjects (p = 0.009) and patients with frequent cataplexy (p = 0.0001).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>Narcoleptic patients</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>35.2 ± 14.6</td>
<td>40.9 ± 14.8</td>
<td>0.20</td>
</tr>
<tr>
<td>Sex</td>
<td>M 9</td>
<td>12</td>
<td>0.79</td>
</tr>
<tr>
<td>F 8</td>
<td>10</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Age and sex characteristics of patients with no cataplexy, rare cataplexy and frequent cataplexy

<table>
<thead>
<tr>
<th>Variable</th>
<th>Patients with no cataplexy A</th>
<th>Patients with rare cataplexy B</th>
<th>Patients with frequent cataplexy C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>38.8 ± 15.6</td>
<td>47 ± 5.3</td>
<td>32 ± 5.1</td>
</tr>
<tr>
<td>Sex M F</td>
<td>24</td>
<td>7</td>
<td>15</td>
</tr>
</tbody>
</table>

A vs. B (sex p = 0.80 and age p = 0.11); B vs. C (sex p > 0.50 and age p = 0.69) and A vs. C (sex p > 0.50 and age p = 0.47).

<table>
<thead>
<tr>
<th>Lymphocytes</th>
<th>Control</th>
<th>Narcoleptic patients with no cataplexy</th>
<th>Narcoleptic patients with rare cataplexy</th>
<th>Narcoleptic patients with frequent cataplexy</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6</td>
<td>3.51 ± 1.08</td>
<td>2.54 ± 0.43</td>
<td>2.61 ± 0.58</td>
<td>2.73 ± 1.46</td>
<td>NS</td>
</tr>
<tr>
<td>TNF</td>
<td>1.93 ± 0.56*</td>
<td>2.38 ± 1.35</td>
<td>2.64 ± 2.02* **</td>
<td>1.68 ± 0.51**</td>
<td>* p = 0.009** p ≤ 0.0001</td>
</tr>
</tbody>
</table>

NS - p > 0.05 – Student’s t test.

DISCUSSION

There has been much development in knowledge about narcolepsy. The higher prevalence of HLA DQB1*0602 allele in patients with narcolepsy and cataplexy was established(3). The finding of hypocretin with a function of maintaining wakefulness and its decreased levels due to cellular loss in the lateral hypothalamus started to be thought as a possible pathophysiological mechanism(5-7).

Our study showed that the prevalence of HLA DQB1*0602 allele was increased in the total population of narcoleptic patients studied when compared with the overall population; and it was even more pronounced
in patients with more frequent cataplexy. These findings were comparable with the findings reported in the literature, which describe a prevalence of 90-95% for the HLA DQB1*0602 allele in patients with frequent cataplexy and about 40% for the HLA DQB1*0602 allele in patients with rare or absent cataplexy(3).

The measurement of IL-6 was not different between the control group and narcoleptic patients, and the values were kept within the normal limits. However, the levels of TNF were increased in the population of narcoleptic patients with rare cataplexy when compared with the levels found in control subjects, and decreased in patients with frequent cataplexy when compared with patients with rare cataplexy.

The idea to separate narcoleptic patients according to different clinical presentations with possible differences in the intensity of self-aggression in hypocretin-producing cells is reinforced by our findings.

In our theory, a patient with frequent cataplexy would present intense inflammatory reaction at the beginning of the disease with destruction of hypothalamic cells at the beginning of adolescence, whereas patients with less frequent cataplexy would present a milder but more persistent autoimmune reaction with continuously increased TNF levels.

This hypothesis reinforces the idea of destruction of hypocretinergic cells of hypothalamus mediated by an immune process of self-aggression. A patient with frequent cataplexy could be regarded as an individual presenting sequels caused by the destruction of hypocretinergic cells of hypothalamus, whereas a patient with rare cataplexy or absent cataplexy would have suffered a milder autoimmune reaction, with preservation of part of the hypothalamus cell population. However, other immunological studies are necessary in larger patient groups to reinforce or confirm this hypothesis.

CONCLUSION
Discrepancies in clinical picture of narcoleptic patients may be associated with subtle changes in the pathophysiological mechanism of the disease. The present study findings reinforce the hypothesis of a probable immunological ciliology for nacolepsy.

REFERENCES