Article

Arq Neuropsiquiatr 2010;68(5):712-715

Traditional biomarkers in narcolepsy

Experience of a brazilian sleep centre

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ABSTRACT

This study was thought to characterized clinical and laboratory findings of a narcoleptic patients in an out patients unit at São Paulo, Brazil. **Method:** 28 patients underwent polysomnographic recordings (PSG) and Multiple Sleep Latency Test (MSLT) were analyzed according to standard criteria. The analysis of HLADQB1*0602 allele was performed by PCR. The Hypocretin-1 in cerebral spinal fluid (CSF) was measured using radioimmunoassay. Patients were divided in two groups according Hypocretin-1 level: Normal (N) - Hypocretin-1 higher than 110pg/ml and Lower (L) Hypocretin-1 lower than 110 pg/ml. **Results:** Only 4 patients of the N group had cataplexy when compared with 14 members of the L group (p=0.0002). **Discussion:** This results were comparable with other authors, confirming the utility of using specific biomarkers (HLA-DQB1*0602 allele and Hypocretin-1 CSF level) in narcolepsy with cataplexy. However, the HLADQB1*0602 allele and Hypocretin-1 level are insufficient to diagnose of narcolepsy without cataplexy.

Key words: narcolepsy, cataplexy, cerebrospinal fluid, hypocretin-1, HLA-DQB1*0602, biomarkers.

Biomarcadores tradicionias em narcolepsia: experiência de um centro de sono brasileiro

RESUMO

Este estudo foi idealizado para avaliar as características clinicas e laboratoriais de uma população de narcolépticos atendidos num centro de referência na cidade de São Paulo (Brasil). **Método:** 28 pacientes realizaram polissonografia e teste de múltiplas latências do sono segundo critérios internacionais. O alelo HLADQB1*0602 foi identificado por PCR. A Hipocretina-1 no líquido cefalorradiano (LCR) foi mensurada por radioimunoensaio. Os pacientes foram divididos em 2 grupos conforme o nível de Hipocretina-1. Normal (N) - Hypocretin-1 >110pg/ml e baixa (B) - Hypocretina-1 <110pg/ml. **Resultados:** Somente 4 pacientes do grupo N tinham cataplexia quando comparados com 14 pacientes do grupo B (p=0,0002). **Discussão:** Estes resultados foram comparáveis com outros autores, confirmando a utilidade do uso de biomarcadores específicos (HLA-DQB1*0602 e nível da hipocretina-1 no LCR) em narcolepsia com cataplexia. Porém, o alelo HLADQB1*0602 e a dosagem da Hipocretina-1 são insuficientes para o diagnóstico da narcolepsia sem cataplexia.

Palavras-chave: narcolepsia, cataplexia, líquido cefalorraquidiano, HLA-DQB1*0602, biomarcadores.

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Support
AFIP & FAPESP- CEPID 98/14303-3
Ségio Tufik and Lia Rita Azeredo Bittencourt have grants from CNPq

Received 8 December 2009
Received in final form 9 March 2010
Accepted 24 March 2010

Narcolepsy is a sleep disorder characterized by excessive sleepiness and in many patients associated to cataplexy and/or hypnagogic hallucinations, and/or sleep paralysis, and sleep fragmentation[1]. The International Classification of Sleep Disorders (ICSD-2) recognizes two main forms of narcolepsy: with and without cataplexy[2]. Patients with narcolepsy with cataplexy have excessive daytime sleepiness

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and typical cataplexy is defined as short and sudden loss of muscle tone that occurs frequently after intense emotions. Narcoleptic patients without cataplexy present excessive daytime sleepiness without cataplexy, and could present hypnagogic hallucinations, and/or sleep paralysis. The electrophysiological diagnosis of narcolepsy is characterized by a latency of sleep lower than 8 minutes and two or more sleep onset REM periods (SOREMP) during Multiple Sleep Latency Test (MSLT). The HLA-DQB1*0602 allele is prevalent in 95% of patients with cataplexy, and has been highlighted in less than 50% of patients without cataplexy[3]. The Hypocretin-1 level in the cerebrospinal fluid (CSF) is lower than 110 pg/ml in almost all patients with typical cataplexy (narcolepsy/cataplexy syndrome)[4,5]. Only 5% of all patients with narcolepsy without cataplexy or atypical cataplexy would have this characteristic of CSF. Nowadays, the association among clinical presentation, electrophysiological features and the biological markers (HLA-DQB1*0602 allele and Hypocretin-1) is the gold standard to diagnostic of narcolepsy[2]. Worldwide, there are a limited number of places with a large experience in working with narcoleptic patients. No study, as far as we know, has described complete presentation of clinical and laboratory patients with narcolepsy, in a specific medical service in South America. The main objective of this study was to describe the relationship between clinical characteristics, polysomnography, MSLT, HLADQB1* 0602 allele prevalence, and CSF Hypocretin-1 level in a group of outpatients with narcolepsy from the Sleep Institute, UNIFESP, São Paulo SP, Brazil.

METHOD

The study was approved by the Ethics and Research Committee of UNIFESP, code number 1802/07 and all

Table 1. Clinical and laboratorial features of the patients with narcolepsy.

Age (years)	Sex	Hypocretin-1 (pg/ml)	HLA	Latency of MLST (min)	SOREMP	Cataplexy	Sleep paralysis	Hypnagogic Hallucinations	Sleep paralysis
33	M	0.0	P	0.1	4	yes	no	no	no
38	M	0.0	P	0.2	4	yes	no	no	no
44	M	0.0	P	2	4	yes	yes	yes	yes
30	M	0.0	P	3	4	yes	yes	yes	yes
27	M	0.0	P	3	4	yes	yes	yes	yes
32	F	0.0	P	3	4	yes	yes	yes	yes
34	F	0.0	P	2	5	yes	yes	yes	yes
23	M	0.0	P	1	5	yes	yes	yes	yes
66	F	0.0	P	4	4	yes	yes	yes	yes
65	M	0.0	P	2	5	yes	yes	yes	yes
32	F	0.0	P	2	5	yes	yes	yes	yes
44	M	50.8	P	2	5	yes	no	no	no
40	F	58.2	P	2	3	yes	yes	yes	yes
33	M	93.2	P	1	4	yes	yes	yes	yes
58	M	414.5	N	3	2	no	yes	yes	yes
32	M	491.1	N	5	2	no	no	no	no
27	F	529.2	N	3	5	no	yes	no	yes
32	M	491.1	N	5	2	no	no	no	no
37	M	135.4	P	3	5	yes	yes	yes	yes
30	F	286.5	P	3	2	no	yes	yes	yes
30	F	527.2	P	5	3	no	yes	yes	yes
32	F	602.9	P	2	3	no	no	no	no
43	F	504.1	P	2	2	yes	yes	yes	yes
45	M	685.1	P	3	2	no	no	no	no
28	M	530.3	P	2	3	yes	yes	no	yes
42	F	546.8	P	4	2	no	no	no	no
43	F	554.3	P	5	3	no	yes	no	yes
28	M	669.0	P	1	5	yes	yes	yes	yes

MLST: multiple sleep latency test; SOREMP: sleep onset REM periods.

Table 2. Signals and symptoms of patients with narcolepsy according level of Hypocretin-1.

Hypocretin-1 below 110 pg/ml (14)		Hypocretin-1 above 110 pg/ml (14)	(p)
Cataplexy	14	4	0.0002
Hypnagogic hallucinations	11	6	0.12
Sleep paralysis	11	9	0.67
Gelineau's syndrome	11	3	0.02

patients had signed the informed consent form. Twenty-eight patients with narcolepsy willingly agreed to participate in the study. The ICSD-2 was used to diagnose narcolepsy: all patients complained of excessive daytime sleepiness, scored higher than 10 on the Epworth Sleepiness Scale, some of them experienced REM-related sleep events (sleep paralysis, hypnagogic hallucination and/or cataplexy) and highlighted a sleep latency of less than 8 minutes plus 2 or more SOREMP during MSLT[6,7]. No other sleep diseases had been identified. Neurological examination was normal in all patients. All patients underwent standard PSG recordings: with electroencephalogram, eletro-oculogram, chin electromyogram, electrocardiogram, oral/nasal airflow and nasal/canula, chest/abdominal respiratory effort, oxymetry, snoring, and tibial electromyogram. Respiratory events were evaluated according to the recommendations of the 1999 Task Force of the American Academy of Sleep Medicine. Arousals and periodic leg movements were analyzed according to ASDA (1992) and ASDA (1993) criteria, respectively[8-11]. The MSLT was analyzed according to standard criteria[7]. The presence of DQB1*0602 was determined by PCR using the following primers: DQBF (5'- CCCGCAGAGGATTTCGTGTT - 3') and DQBR (5'- AACTCCGCCCGGGTCCC - 3') (Bioneer, Inc., Daejeon, Korea)[12]. After the lumbar puncture, 10 ml of CSF was kept frozen (–80ºC), until used for Hypocretin-1 determination by a radioimmunoassay kit (Phoenix Pharmaceuticals, St. Joseph, MO, USA)[13]. Patients were divided in two groups according Hypocretin-1 level: Normal (N), Hypocretin-1 higher than 110 pg/ml and Lower (L) Hypocretin-1 lower than 110 pg/ml. Statistical analysis was carried using the Chi-squared test for qualitative data like clinical and demographic features, and the t test or Mann-Whitney were used for quantitative data like sleep features.

RESULTS

The two groups of 14 patients were compared for sex and age after CSF analysis (Table 1). Only 4 patients (28.5%) of N group had cataplexy when compared with 14 (100%) of L group (p=0.0002). There were no differences between N and L groups when compared with hypnogogic hallucinations, and sleep paralysis. Gelineau's tetrad (sleepiness, cataplexy, hypnagogic hallucinations, and sleep paralysis) was more prevalence in L group (Table 2). Mean sleep latency was lower in L group (1.95±0.29 vs. 3.23±0.3 minutes, p=0.008) and the SOREMP was higher in L group (4.28±0.16 vs. 2.92±0.33, p=0.01). HLA-DQB1* 0602 allele was present in 14 patients (100%) of L group and in 10 patients (71%) of N group (p=0.20). Thirteen patients belonging to N group had Hypocretin-1 CSF level higher than 200 pg/ml and one patient had Hypocretin-1 level between 110 to 200 pg/ml.

DISCUSSION

Fourteen of 18 (77%) patients with narcolepsy/cataplexy syndrome have presented sleepiness characterized by the MLST, the prevalence of HLA-DQB1*0602 allele increased, and the Hypocretin-1 CSF level scored below 110 pg/ml. Other narcoleptic features like hypnagogic hallucinations and sleep paralysis were no more frequent in patients according Hypocretin-1 level. The prevalence of the HLA-DQB1*0602 allele was higher than expected in patients with Hypocretin-1 level above 110 pg/ml when compared with other studies[14,15]. A possible explanation about these results is the prompt acceptation to be part of the study by the more affected patients. However, the higher prevalence of cataplexy in our L group of patients is comparable with other authors[13]. Interestingly, one patient with Hypocretin-1 level of 135.4 pg/ml and two patients with Hypocretin level above 200 pg/ml had narcolepsy/cataplexy syndrome with Gelineau's tetrad. The cut-off value of Hypocretin-1 level for diagnosis in CSF patients has been discussed[13]. Other author has been described narcolepsy/cataplexy syndrome in patient with Hypocretin-1 between 110 and 200 pg/ml, for example[15]. The differential diagnosis of some patients with sleepiness is a challenge, especially patients who have no narcolepsy/cataplexy syndrome and when they have other comorbidities[14]. Although the important and with higher sensibility and specificity for narcolepsy/cataplexy syndrome, our findings confirm that these biological markers are insufficient to help for the diagnosis of narcoleptic patients without cataplexy.

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