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- **EEG in epilepsy: Sensibility and Specificity**
- **Contralateral hippocampal volume influences surgical outcome in patients with MTLE and similar degree of ipsilateral hippocampal atrophy**
- **Influence of melatonin treatment on the survival and seizures frequency in pilocarpine-induced epilepsy in rats**

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Editorial

Esta edição do JECN apresenta três artigos originais. O primeiro artigo de Dantas e cols. confirma a alta especificidade e baixa sensibilidade do EEG como método diagnóstico auxiliar nas epilepsias em uma amostra de 10.408 exames. O trabalho de Pereira e colaboradores, usando volumetria hipocampal antes e após cirurgia em 67 pacientes com epilepsia de lobo temporal refratária aos fármacos antiepilépticos, mostra que a cirurgia é menos efetiva nestes pacientes quando há dano hipocampal bilateral, mesmo quando não detectado por analise visual. Por último, Barateli e colaboradores apresentam um trabalho em modelo de pilocarpina analisando a influência da melatonina no controle de crises no período crônico deste modelo.

Boa Leitura!

Fernando Cendes

Editor, JECN

Original Article

Journal of Epilepsy and Clinical Neurophysiology

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EEG in epilepsy: Sensibility and Specificity

EEG em epilepsia: sensibilidade e especificidade

Fábio Galvão Dantas[1], Emanuelly Silva de Melo[2], André Pinto Cavalcanti[3], Bruno Diego R. Maciel[3], Clarissa Dantas Ribeiro[3], Gabriela Carvalho Napy Charara[1], Johnnatas Mikael Lopes[4], Paulo Fernando Martins Filho[3], Luiz Ataíde Júnior[1]

ABSTRACT

Objective: the aim of this study was to determine the sensitivity (presence of epileptiform discharges in the EEGs of patients with epilepsy) and specificity (absence of discharges in the EEGs in people without epilepsy) of EEG. **Methodology:** all EEGs performed at the Clinic Santa Vitória, in Campina Grande, PB, from April 2001 to April 2010 were reviewed. All recordings were performed in accordance with international standards for fixing the electrodes, minimum time of registration and methods activation (intermittent photic stimulation and hyperventilation). The reports were divided into 1) patients with epilepsy, previously diagnosed by neurologists, and 2) patients without epilepsy. For both groups, we evaluated the sensitivity and specificity of the EEG. We used SPSS for statistical tests. The study was approved by the Ethics Committee of UEPB. 10,408 EEGs were reviewed. **Results:** epileptiform discharges occurred in 1412 (13.56%). Among those with epilepsy, discharges occurred in 643 (45.57%) - true-positive. Among those who did not have epilepsy, in 54.43% - false positives. From a total of 8,996 (86.44%) EEGs without discharges, 1,276 (14.14%) were from the group of patients with epilepsy - false-negative and 7,720 (85.78%) were from the group of patients without epilepsy - true negative. The positive likelihood ratio test showed that the probability of finding EEG discharges is four times higher among patients with epilepsy compared to those who do not have epilepsy. The negative likelihood ratio test showed no differences between false negative and true negative. In general, a sensitivity of 33.5% and a specificity of 90.9%, with no differences in age and gender was observed. Therefore, EEG showed high specificity but low sensitivity as a diagnostic method in epilepsy.
Keywords: electroencephalogram, epilepsy, sensitivity, specificity

RESUMO

Objetivo: o objetivo deste estudo foi verificar a sensibilidade (presença de descargas em EEGs de portadores de epilepsia) e a especificidade (ausência de descargas em EEGs de sem epilepsia) do EEG. **Metodologia:** foram revisados todos os EEGs realizados na Clínica Santa Vitória, em Campina Grande, PB, no período de abril de 2001 a abril de 2010. Todos os registros foram realizados de acordo com padrões internacionais para a fixação dos eletrodos, tempo mínimo de registro e métodos de ativação (fotoestimulação intermitente e hiperventilação). Os laudos foram divididos em 1) pacientes portadores de epilepsia, previamente diagnosticada por neurologistas, e 2) pacientes sem epilepsia. Para ambos os grupos, estudou-se a sensibilidade e a especificidade do EEG. Foram utilizados testes estatísticos através do programa SPSS. O estudo foi aprovado pelo Comitê de Ética da UEPB. Foram revisados 10.408 EEGs. **Resultados:** descargas epileptiformes ocorreram em 1412 (13,56%). Dentre os portadores de epilepsia, descargas ocorreram em 643 (45,57%) – *verdadeiros-positivos*. Dentre os que não apresentam epilepsia, em 54,43% - *falsos-positivos*. De um total de 8.996 (86,44%) de EEGs sem descargas, 1.276 (14,14%) eram do grupo de portadores de epilepsia – *falsos-negativos* e 7.720 (85,78%) eram do grupo de pacientes sem epilepsia – *verdadeiros-negativos*. O teste de verossimilhança positiva revelou que a probabilidade de ocorrerem descargas é quarto vezes maior dentre os portadores de epilepsia, comparados aos que não apresentam epilepsia. Já o teste de verossimilhança negativa não evidenciou diferenças significativas entre falsos-negativos e verdadeiros-negativos. De modo geral, foi observada uma sensibilidade de 33,5% e uma especificidade de 90,9%, sem diferenças quanto à idade e ao gênero. O EEG apresentou, portanto, alta especificidade, mas uma baixa sensibilidade, como método diagnóstico auxiliar nas epilepsias.
Palavras-chave: eletroencefalograma, epilepsia, sensibilidade, especificidade

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INTRODUCTION

Epilepsy is a neurological condition characterized by repetitive unprovoked seizures[12] duo to excessive and uncontrolled neuronal discharges, which may be registered by scalp or deep EEG[6]. EEG is an easy and low costing exam and in has a very important role on epilepsy diagnosis[12]. Otherwise, EEG may also be used to classify the epileptic syndromes[2]. Nevertheless, EEG may sometimes lead to mistakes. Some patients with epilepsy may not present EEG interictal discharges and others may have discharges, but not epilepsy. Our objective was to investigate sensibility and specificity of EEG as a diagnostic method for epilepsy.

METHODS

We retrospectively examined EEG recording refereed to Santa Vitoria EEG laboratory in Campina Grande, state of Paraiba, Brazil, from April, 2001 to April, 2010. The records were scalping surface routine EEG, EEG following sleep deprivation and they were done with a 20-channel 420 Meditron EEG-recorder. Twenty-one electrodes were placed according to the international 10-20 system. EEGs lasted 20 to 30 minutes including hyperventilation and photic stimulation. Bipolar, longitudinal, transverse, referential and average montages were used. All the EEGs were reported and reviewed by a board-certified neurophysiologist and neurologist. According to the clinical aspects, EEGs were classified into A) patients with diagnosed epilepsy and B) patients with other clinical or neurological conditions or routine examination. For both groups, we determined EEG sensibility (patients with epilepsy with interictal discharges (ID) and EEG specificity (patients with no epilepsy and no ID). This research was approved by the Ethical Committee on Research of the State University of Paraiba. We used a 2x2 contingency table to verify EEG accuracy, sensitivity, specificity, positive and negative likelihood ratio. Sensitivity was determined by the *true positive* rate among epileptic patients. Specificity refers to *true negative* rate among non-epileptic patients. Positive likelihood ratio estimated the occurrence of EEG discharges among epileptic and non-epileptic patients, while negative likelihood ratio estimated the occurrence of the absence of discharges among non-epileptic and epileptic patients. All data were processed by using the Statistical Package for Social Science (SPSS), International Business Machine ® version 20.0.

RESULTS

We reviewed 10,408 EEGs. In general, discharges occurred in 1,412 (13.56%). In group A, discharges were seen in 643 (45.57%) – *true positive*, and in group B, they occurred in 54.43% - *false positive*. Out of 8.996 (86.44%) of non-discharges EEGs, 1,276 (14.14%) were from group A – *false negative* and 7,720 (85.78%) were from group B – *true negative*. Positive likelihood ratio revealed that discharges are four times more likely to appear in patients with epilepsy, when compared to non-epileptic. Negative likelihood ratio showed that there was no significant difference between *false-negative* and *true-negative* EEGs. In general, EEGs exhibited a sensibility of 33.5% and a specificity of 90.9%. Age and gender did not influence the results.

DISCUSSION

SENSIBILITY: EEG sensibility in patients with epilepsy is related to the presence of discharges, while EEG specificity reflects the absence of discharges in non-epileptic patients. In our research, EEG had a low sensibility (33.5%), as discharges occurred similarly among group A and group B patients.

Ajmone-Marsan and Zivin (1970) reviewed 1,824 EEGs from 308 patients with epilepsy[1]. Discharges were seen in 55.5% in the first EEG. After repetitive EEGs, the sensibility raised up to 82.5%, mainly among young patients with temporal lobe epilepsy with frequent seizures. Goodin and Aminoff (1984) found a general sensibility of 52% in the first EEG of 764 patients with epilepsy. Salinsky, Kanter and Dasheiff (1987) analyzed the sensibility of serial EEGs in 429 patients with epilepsy. In the first EEG, they found discharges in 50%; in the third EEG, 84%; in the fourth, 92%. Sleep may also increase EEG sensibility in epilepsy diagnosis[13]. Binnie, Elwes and Polkey (1994) observed that the sensibility rose from 49% to 81% after including sleep EEG[3]. The age of diagnosis and/or the first EEG may decrease EEG sensibility, although we did not find any differences[8]. Dantas et al. (2005) analyzed 259 EEGs of epileptic patients. They found discharges in 30.1%[7]. González de La Aleja et al. (2008) studied 137 patients with epilepsy. Focal discharges were seen in 42% and diffuse spikes, in 14.6%[11]. Both results were similar to ours.

SPECIFICITY: in our research, EEG had a high specificity, as 90.9% (non-epileptic patients with no EEG discharges). Others have reported similar results. Zivin and Marsan (1968) analyzed 6,361 EEGs of non-epileptic patients. Discharges were seen only in 2.2%[14]. Gregory, Oates and Merry (1993) studied 13,658 EEGs of healthy persons. Only 0.3% had epileptic discharges[10]. Bridgers (1987) studied 3,000 EEGs of patients with psychiatric diseases with no epilepsy. Only 2.6% of them had discharges[4]. Cavazzuti, Capella and Nalin (1980) found discharges in 3.5% of 3,716 healthy children[5]. González de La Aleja et al. (2008) reviewed 99 EEGs of patients with non-epileptic ictal features. Only 4% had epileptic discharges[11]. Discharges in epileptic patients seem to be more prevalent in temporal lobe epilepsy[1,7,11]. We conclude that EEG has a low sensibility and a high specificity for epilepsy diagnosis. It emphasizes the needing of a complete previous clinical evaluation.

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Original Article

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Contralateral hippocampal volume influences surgical outcome in patients with MTLE and similar degree of ipsilateral hippocampal atrophy

O volume hipocampal contralatral influencia o desfecho cirúrgico de pacientes com ELTM e grau semelhante de atrofia hipocampal ipsilateral

Amanda Régio Pereira[1], Livia Conz[1], Elio Barbosa Belfiore[1], Jarbas Emílio de Moraes Neto[1], Helder Tedeschi[2], Marcia Elisabete Morita[1,2], Fernando Cendes[1,2], Clarissa Lin Yasuda[1,2]

ABSTRACT

Objective: to investigate the relationship between hippocampal atrophy (HA) and surgical outcome in patients with mesial temporal lobe epilepsy (MTLE). **Methodology**: we compared 34 patients free of seizure (GroupA) with 33 patients with persistent seizures after surgery (GroupB). All had preoperative diagnosis of unilateral MTLE by EEG and MRI evidence of unilateral hippocampal sclerosis (HS) by visual analysis. We performed hippocampal volumetry using high resolution T1 MRI (1mm) in all patients and in 30 healthy controls. **Results**: Z-score (Mean±SD) of affected hippocampus was -2.58±1.29 in GroupA and -2.57±1.47 in Group-B (p=0.98). The Z-Score of contralateral hippocampus was significantly lower in GroupB, compared to GroupA (p=0.038). Grouping all patients, smaller hippocampal volumes in the affected side were associated with history of meningitis (p=0.049), febrile seizures (p=0.049) and absence of family history of epilepsy (p=0.049). **Conclusions**: Ipsilateral HA was more severe in patients who had febrile seizures and meningitis, and in those without family history of epilepsy, supporting the notion that in the absence of genetic predisposition, more severe cerebral insult is necessary to induce epileptogenesis. Less favorable surgery outcome for unilateral MTLE was associated with smaller hippocampal volumes contralateral to the operated side, suggesting that surgery is less effective when bilateral damage exists, even when it is not detectable by visual MRI analysis.

Keywords: MRI, volumetry, epilepsy surgery, refractory epilepsy, temporal lobe epilepsy

RESUMO

Objetivo: investigar a relação entre atrofia hipocampal (AH) e resultado cirúrgico de pacientes com epilepsia de lobo temporal mesial (ELTM). **Methodology:** comparamos 34 pacientes livres de crises (grupoA) com 33 pacientes que permaneceram com crises após cirurgia (GrupoB). Todos apresentavam o diagnóstico pré-operatório de ELTM unilateral por EEG e RM com sinais de atrofia hipocampal (AH) unilateral na análise visual. Realizamos volumetria do hipocampo utilizando imagens T1 de RM de alta resolução (1mm) em todos os pacientes e em 30 controles sadios. **Resultados**: o Z-score (Média±DP) dos hipocampos afetados foi -2.58±1.29 no GrupoA e -2.57±1.47 no GrupoB (p=0.98). O Z-score dos hipocampos contralaterais foi significativamente menor no grupoB comparado ao grupoA (p=0.038). Agrupando todos os pacientes, volumes hipocampais menores no lado afetado foram associados à história de meningite (p=0.049), crises febris (p=0.049) e ausência de história familiar de epilepsia (p=0.049). **Conclusão:** AH ipsilateral foi mais acentuada em pacientes com antecedente de crises febris e meningite, e naqueles sem história familiar de epilepsia, reforçando a ideia de que na ausência de predisposição genética, um maior insulto cerebral seria necessário para induzir epileptogenesis. Um resultado cirúrgico menos favorável na cirurgia para ELTM unilateral foi associado a menores volumes hipocampais no lado contralateral ao lado operado, sugerindo que a cirurgia é menos efetiva quando há dano bilateral, mesmo quando não detectado por analise visual.

Palavras chave: RM, volumentria, cirurgia de epilepsia, epilepsia refrataria, epilepsia de lobo temporal

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INTRODUCTION

Mesial temporal lobe epilepsy (MTLE) is a chronic condition associated with complex partial seizures of presumed origin in the mesial temporal lobe. The most common pathological substrate is hippocampal sclerosis (MTLE-HS), described as selective hippocampal neuronal loss and gliosis (cellular loss is more severe in CA1 and CA3 sectors of hippocampus)[3,4,26]. MTLE-HS is the most frequent epilepsy syndrome in surgical series and is refractory to antiepileptic drugs (AED) in 58-89% of patients in tertiary centers[33]. Surgical treatment – selective amygdalohippocampectomy or anterior temporal lobectomy – is a safe and effective treatment option for these patients, yielding 70-80% seizure-freedom outcome[16,39,44]. The reason for the 20-30% surgical failure in MTLE-HS is still unclear[17]. MRI has become an essential tool in the preoperative evaluation of patients with MTLE candidates for surgery. MRI allows the identification of underlying pathological substrate, differentiating HS from other conditions and in addition, may help to predict the outcome after surgery[10]. The detection of HS is of great importance as these patients have a substantial chance of becoming seizure free with surgery [19, 39].

MRI allows the diagnosis of HS, based on hippocampal atrophy (HA), loss of internal architecture and increased hippocampal T2-weighted signal[9,40]. Qualitative MRI interpretation and quantitative analysis of hippocampal volume are both sensitive in detecting HS. Although MRI is the "gold standard" for in vivo detection of HS, qualitative methods may fail do detect mild alterations that are subsequently identified on histopathological examination[40] Mild HA may not be visually identifiable, although it can be disclosed by manual volumetry of high resolution MRI[12].

Quantification methods such as MRI volumetry may achieve sensitivity as high as 80-90% in detecting HS[12,33], but is time consuming (approximately 60 min per patient) and requires trained operators, workstation and software. Usually, manual quantification has been restricted for research purposes in tertiary centers with trained personnel, therefore leaving many potentially surgical candidates with more subtle HA without appropriate diagnosis[19].

Previous studies confirmed the presence of HS on preoperative MRI as predictor of better surgical outcome [21,22,27,32,35,37]. Most of these studies consider the diagnosis of HS based on visual analysis of MRI, without a volumetric quantification. Hippocampal volume, as a continuous variable, has not been frequently correlated with outcome measures and has shown conflicting results[1,29]. In this study we aimed to investigate clinical factors associated with more severe hippocampal atrophy (HA) as well as differences in hippocampal volumetry, ipsilateral and contralateral to the surgery, between patients who became seizure free and those who remained with seizures after surgery.

METHODS

Patient Selection:

We evaluated a group of surgically treated patients with refractory unilateral MTLE. All patients underwent our routine investigation that includes detailed neurological examination, magnetic resonance imaging (MRI), series of electroencephalography (EEG), neuropsychological and psychological evaluation. Seizures were lateralized according to the medical history, interictal EEGs and comprehensive neurological examination. When necessary, we performed in hospital video-EEG and ictal SPECT for those patients with unclear origin of ictal discharges[44].

All patients presented clinical and EEG features of MTLE as described previously[17], including clear-cut interictal EEG epileptiform discharges in anterior-infero-mesial temporal regions and absence of EEG abnormalities outside temporal lobe regions. We only included patients with simple partial or complex partial seizures (with or without secondary generalization), or both, with features of mesial temporal lobe origin and no suggestion of any other partial or generalized epilepsy syndrome. Seizure were described as rising epigastric sensation, unexpected fear, and other psychic phenomena such as *déjà vu* and *jamais vu*, and complex partial seizures with staring, automatisms, dystonic posturing of one hand and post-ictal confusion. Anti-epileptic drugs (AED) refractoriness was considered when patients had failure of seizure control with at least two AEDs regimens[45].

The diagnosis of unilateral signs of HS was carried out by visual analysis of our MRI diagnostic protocol for epilepsy that consists of T1- and T2-weighted MRIs in three orthogonal planes, axial fluid-attenuated inversion recovery, as well as thin coronal (3 mm) T1 inversion recovery (IR) and T2 images as described below. One of the investigators with experience in neuroimaging in epilepsy (F.C.) performed visual analyses of routine MRI protocol to confirm unilateral hippocampal atrophy and abnormal shape, with or without hyper intense FLAIR/T2 signal. In addition, he certified there were no abnormalities on the contralateral hippocampus or other suspected MRI abnormalities, thus, excluding MTLE patients with dual pathology[11], bilateral hippocampal atrophy and normal MRI[5,38]. Therefore, this group of operated patients does not represent our entire surgical series of MTLE.

We included 67 patients, (40 women, mean age±SD, 34.1±10.8 years). Unfortunately, we excluded 23 patients with unilateral hippocampal atrophy who presented artifacts or did not have available high resolution T1 (3D) MRI sequence for volumetry. The control group was composed of 30 healthy subjects (18 women, 32.4±9.4years).

The study was approved by Ethics Committee of our institution and patients signed a written informed consent.

SURGICAL PROCEDURE

All surgical procedures were performed between 1997 and 2006 in our university hospital. The surgical approach based on the surgeon's experience and consisted of anterior cortical resection with amygdalohippocampectomy (16 patients) and selective trans-Sylvian amygdalohippocampectomy (51 patients). Surgical outcome was similar for both surgical approaches[2].

Histological analysis confirmed the presence of HS for all patients by detecting the typical pathological findings of presence of gliosis and neuronal loss (predominant in dentate gyrus, CA1 and CA3, with sparing of CA2)[4].

Post-surgical Follow-up and Group Categorization:

Patients were followed-up by routine visits after surgery and were instructed to build up a seizure diary. Clinical assessment was performed each month during the first 3 months after surgery, every 2 months in the following 6 months and every 4-6 months after that. All data about pre and postoperative outcome were prospectively included in our epilepsy surgery database.

After post-surgical follow-up, patients were divided into two groups according to Engel's outcome scale[16]. Group-A included 34 patients completely free of seizures after surgery (Engel IA, with19 right MTLE, 28 women) and Group-B was composed of 33 patients with seizures after surgery (8 patients

IB, 3 patients IC, 6 patients ID, 8 patients IIA, 5 patients IIIA and 3 patients IVA, with 21 female,12 right MTLE).

MRI Acquisition and Processing:

The MRIs for diagnostic purposes were acquired on a 2T scanner (Elscint Prestige,Haifa, Israel) with the following parameters: (1) *sagittal* T_1 spin echo; (2) *coronal images*, perpendicular to long axis of hippocampus, defined on the sagittal images: (a) T_2-weighted and proton density fast spin echo; (b) T_1-weighted inversion recovery; (3) *axial images* parallel to the long axis of the hippocampi: (a) T_1-weighted gradient echo; (b) FLAIR (inversion recovery fast spin echo). For the manual volumetry we obtained an additional sequence, *T_1-weighted 3-dimensional* gradient echo with 1-mm isotropic voxels, acquired on the sagittal plane[7,42,45].

Volumetric Analysis

Volumetric analysis of preoperative MRI was performed by one of the investigators (A.R.P.), who was initially blind to the affected side and the surgical outcome of each patient.

Different algorithms from the McConnel Brain Imaging Centre (Montreal, Canada) were used to prepare raw MRI volume for quantitative and qualitative analysis. Initially, raw images were converted into the "mnc" electronic file format (2014b)(See http://www.bic.mni.mcgill.ca/software/minc/minc.html). Then, we used the N3 software program(2014c) (http://www.bic.mni.mcgill.ca/software/N3) to correct for field inhomogeneity and automatically register these images into Talairach stereotaxic space to adjust for differences in brain volume and orientation, as well as to minimize variability in slice orientation[7,34]. All anatomical boundaries of the hippocampus were delineated manually in the MRI slices, one by one, following previous validated protocols[7,31] and anatomical atlases[14,15].

For manual segmentation of the hippocampi we used the interactive software package Display (2014a), developed at the McConnel Brain Imaging Centre (Montreal, Canada) (http://www.bic.mni.mcgill.ca/software/Display/Display.html). This program allows simultaneous viewing of structures in coronal, sagittal and horizontal orientations. Volumes of labeled structures are calculated automatically by the software[31].

Quantitative analysis of hippocampal volumes of a control group was also performed, to make possible the transformation of hippocampal volumes into Z-Scores (standardized scores defined by the number of SDs away from the mean of control group). This group was composed of 30 healthy subjects, with proportion of gender and mean age similar to the patients.

In addition to hippocampal volume, the asymmetry index (AI) was obtained for each patient and control. AI was defined as the ratio of the smaller by the larger hippocampus.

Clinical Data

Volumetry was compared to clinical data extracted from complete revision of our epilepsy surgery database. In case of absent or dubious information, patients were contacted by phone or in person, during medical appointments.

The following information was collected: duration of post-surgical follow-up, side of the surgery, age at seizure onset, age at surgery, duration of the epilepsy until surgery, history of febrile seizure in childhood, history of meningitis, history of tonic-clonic generalized seizures (TCGS) and family history (FH) of epilepsy.

A positive FH was defined when we identified at least one other individual (first-or second-degree relative) with epilepsy in the family of the operated patient, and a negative FH was considered if the operated patient was the only individual with seizures in the 3 most recent generations of the entire family[43].

Statistical Analysis

We used SPSS 21® to analyze clinical variables from patients and controls. General Linear Model with multivariate analyses with 2 dependent variables (Z-score of ipsilateral and contralateral hippocampus) was applied to investigate differences between the two groups of patients. Univariate analyses were used to compare other unrelated variables between patients and controls and between groups of patients. To control for multiple comparisons, we applied FDR (False Discovery Rate)[18].Fisher's exact test was used to analyze categorical variables.

Results

Groups of patients and controls were balanced for gender (p=1) and age (p=0.44) (Table 1). In the control group, right hippocampal volume was 4532.27±516.63mm^3 (maximum: 5537mm^3; minimum: 3335mm^3) while the left hippocampal volume was 4594.27±578.88mm^3 (maximum: 5755mm^3; minimum: 3338mm^3). AI was 1.05±0.05.

Patients grouped all together had a mean hippocampal volume of 3144.7±752.07mm^3 in the affected side and, in the contralateral hippocampus, 4569.67±606.11mm^3. The AI was 0.68±0.14. Post-surgical follow-up duration was (Mean±SD) 61.3±31 months, ranging from 15 to 120 months.

Groups A and B differed on gender (p<0.001) and volume of non-atrophic hippocampus (p=0.026). No statistical difference was observed between groups concerning to: side of surgery, age at seizure onset, duration of disease, febrile seizure, meningitis and presence or absence of family history for epilepsy. Baseline characteristics are shown in Table 2.

Table1. Data from patients and controls

	Control Group (n=30)	Patients (n=67)	
Female	60%	59.7%	p=1
Age (years)	32.4±9.4	34.1±10.8	p= 0.44
Asymmetry index	1.05±0.05	0.68±0.14	p<0.001

Table 2. Clinical data from patients in Group A and B

	Group A (n=34)	Group B (n=33)	Significance
Z-Score affected hippocampus	-2.58±1.29	-2.57±1.47	p=0.98
Z-Score contralateral hippocampus	0.34±0.97	-0.32±1.17	**p=0.038**
Asymmetry Index	0.66±0.13	0.69±0.15	p=0.37
Contralateral hippocampus (in mm^3)	4760.91±528.49	4452.09±580.17	p=0.026
Female	82%	36%	**p<0.001**
TCGS (% of patients with)	74%	91%	p=0.11
Resected side (% of right side)	56%	38%	p=0.15
Duration of disease (years)	24.3±10.9	28.9±10.4	p=0.086
Age at seizure onset (years)	7.5±5.8	7.6±5.8	p=0.92
Age at surgery (years)	31.8±11.2	36.5±9.9	p=0.074
Febrile seizure (n)	12 (35%)	12 (36%)	p=1
Meningitis (n)	4 (12%)	2 (6%)	p=0.67
Family history of epilepsy (n)	19 (56%)	18 (58%)	p=1

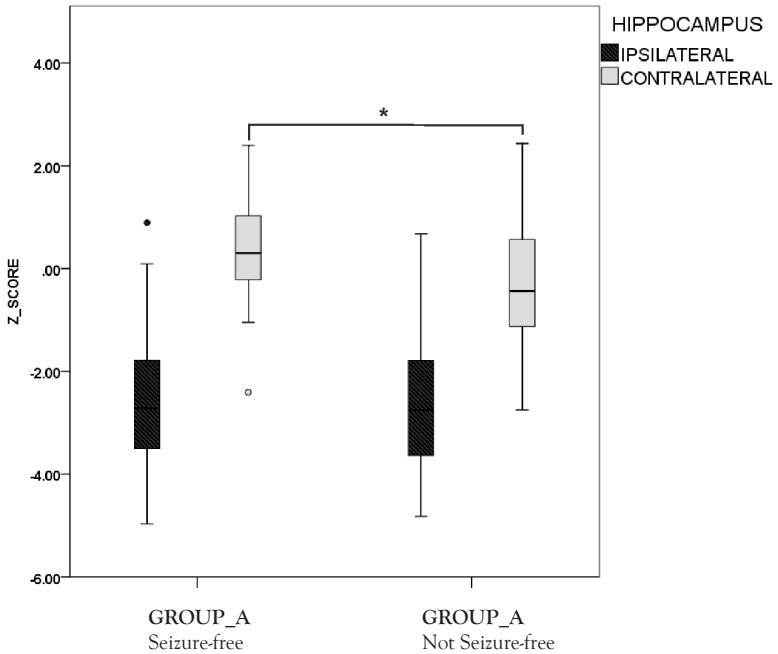
TCGS (Tonic clonic generalized seizure).

The multivariate analyses revealed significant differences between Groups A and B, on the dependent variables ($F(2,64)=3.05$, p=0.05; Wilks´ Lambda =0.913, partial η^2 =0.09). The analyses of each dependent variable separately, the only difference to reach statistical significance, using FDR adjustment was the contralateral hippocampus (Z-Scores: Group A: 0.34±0.97, Group B: -0.32±1.17; p=0.038). The affected hippocampus was similarly reduced in both groups (p=0.98) (Figure 1).

We also investigated a possible relationship between hippocampal volumes and gender, as Group A included more women than Group B. We did not identify significant differences on the AI (p=0.41), Z-score of affected (p=0.91) or the contralateral hippocampus (p=0.13). In addition, we analyzed each group separately to search for differences in the Z-score of contralateral hippocampus depending on gender. Neither Group A (female 0.3±0.988, male 0.499±0.652, p=0.64), nor Group B (female 0.088±1.412, male -0.549±0.978, p=0.136) presented significant differences.

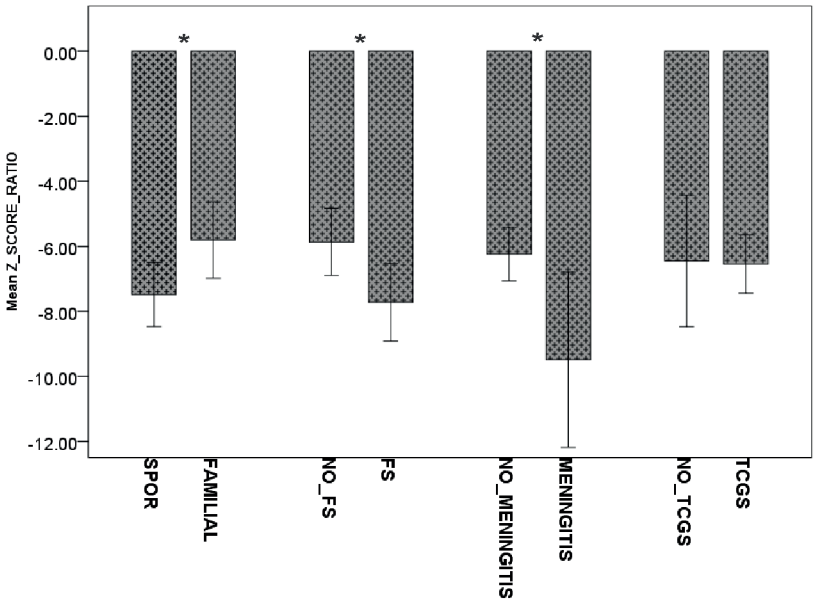
Grouping patients all together, smaller hippocampal volumes in the affected side was associated with history of meningitis (meningitis present: 2450.17±640.70mm^3; meningitis absent: 3185.32±670.73mm^3; p=0.015) and patients without positive family history of epilepsy (patients with positive family history: 3258.76±803.55mm^3; patients without family history: 2904.59±475.82 mm^3; p=0.028). The analysis of Z-score of AI confirmed significant lower scores for sporadic patients (without family history for epilepsy, p=0.049), patients with history of meningitis (p=0.049) and febrile seizures (p=0.049). (Figure 2, Table 3).

Figure1. Differences of volume of contralateral hippocampus on pre operatory MRI scans, determined by manual volumetry.



Footnote: The * indicates a statistically significant comparison (p=0.038). Although visually normal for both groups, subject who persisted with seizures after surgery presented reduced volume of contralateral hippocampus, detected only by manual volumetry.

Figure 2. Comparisons of *z*-score of AI ratio between subgroups of patients, according to clinical characteristics.



Footnote: the * indicates the statistically significant comparisons. Subjects with previous history of febrile seizures and meningitis presented more severe ipsilateral hippocampal atrophy, as well as those with sporadic epilepsy (without family history of epilepsy). FS= febrile seizures, SPOR=sporadic, TCGS = tonic-clonic generalized seizures

Table3. Comparisons of Z-scores between groups according to IPIs and occurrence of TCGS

Characteristics	Z-score of AI	Significance P uncorrected	Significance P corrected (FDR)
Positive family history	-5.80±3.58	p=0.037	p=0.049
Negative family history	-7.48±2.59		
TCGS present	-6.55±3.32	p=0.93	p=0.93
TCGS absent	-6.45±3.18		
Meningitis present	-9.49±2.57	p=0.019	p=0.049
Meningitis absent	-6.24±3.20		
Febrile seizure present	-7.72±2.83	p=0.025	p=0.049
Febrile seizure absent	-5.86±3.34		

TCGS (Tonic clonic generalized seizure), IPI (Initial precipitating injury).

DISCUSSION

The initial hypothesis was that patients with more atrophic hippocampus would present better surgical outcome, presuming that the resection of well-defined affected structure would more likely include the seizure focus.

This hypothesis was confirmed by[20], who also analyzed the relationship between volumes of hippocampal formation and seizure outcome after anterior temporal lobectomy, in 50 consecutive patients. That study demonstrated a worse outcome related to a larger hippocampal volume on the side of resection (p=0.012). In addition, smaller hippocampal volume on the contralateral side was also associated with poor outcome, although statistical significance was borderline (p=0.057).

Another study including 453 patients submitted to amygdalohippocampectomy revealed a positive relationship between severity of hippocampal sclerosis, in terms of neuronal loss and gliosis, and better surgical outcome[41].

Contrary to that study, our results revealed no differences in the affected hippocampal volume between groups with and without post-operative seizure control. However, patients with poorer surgical outcome presented significant smaller z-scores of contralateral hippocampus compared to seizure-free patients. This finding suggests the existence of other affected regions that could be implicated in seizure generation, which would explain the persistence of the seizures after surgery. It is possible that this is related to a more diffuse pattern of structural abnormalities in these patients with unilateral signs of hippocampal sclerosis on MRI[45]. Other previous studies revealed the association between hippocampal sclerosis and extra-hippocampal brain abnormalities[6,8,28]. One of these studies[24] identified 26 brain regions with significant volumetric reduction in patients with MTLE.

Our group has published one study with Voxel Based Morphometry showing that widespread pattern of gray matter

atrophy on pre-operatory MRI was associated with failure of seizure control after surgery. Patients who were seizure-free after surgery had a more restricted pattern of pre-operatory gray matter atrophy[45].These data are also in accordance with another study that demonstrated a relationship between extra-hippocampal gray matter atrophy and a poor outcome in patients submitted to amygdalohippocampectomy[23].

More recently, functional-MRI(fMRI) studies have been used to investigate factors associated with surgical outcome, indicating that a pre-operatory widespread pattern of BOLD change[36] and/or functional connectivity[30] can be associated with a poorer surgical outcome. One EEG-fMRI study revealed a poorer surgical outcome when BOLD signal change was not concordant with the area of resection[36]. Another recent study[30] demonstrated a significantly less lateralized functional connectivity in patients with surgical failure compared to patients who became free of seizures after surgery

These evidences reinforce the need for more sensitive methods to analyze images from surgical candidates, in order to detect subtle abnormalities that could help to predict surgical outcome, avoiding the risks of surgery for those less likely to benefit from the intervention.

The difference related to gender seems to be an incidental statistical finding, caused by a major female sample. There is no logical supposition to explain this finding and all studies revised by McIntosh[27], did not identify a correlation between gender and surgical outcome.

We also identified lower z-scores of AI in non-familiar MTLE (sporadic patients) and patients with past history of meningitis and febrile seizures. These data raises an important discussion regarding the pathogenesis of MTLE, which is not yet fully understood[42].

Retrospective studies from surgical series have demonstrated a higher incidence of "initial precipitating injury" (IPIs) in the past history of patients with MTLE-HS. These IPIs include febrile seizures, trauma, hypoxia and intracranial infection, and usually, but not always, occur before age of 5 years[40]. Therefore, MTLE-HS would probably result from cerebral insult secondary to these IPIs, which would lead to neuronal loss and epileptogenesis. In this sense, it is unlikely that HS would result from repeated habitual seizures[25].

In familial MTLE, the antecedent of febrile seizure in childhood is less frequent, as compared to sporadic MTLE[13]. It is thought that in individuals with a familiar predisposition to MTLE, the impact of the IPI in the pathogenesis of HS is less important. Our findings support this theory, showing smaller hippocampal volume in the affected side in non-familiar MTLE.

In conclusion, contralateral hippocampal damage, even when it is subtle, may influence surgical outcome in patients with MTLE.

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Disclosure of Conflicts of Interest

None of the authors has any conflict of interest to disclose

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Influence of melatonin treatment on the survival and seizures frequency in pilocarpine-induced epilepsy in rats

Influência do tratamento com melatonina na sobrevivência e frequência das crises epiléticas em ratos induzidas por pilocarpina

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RESUMO

Objetivo: verificar se o tratamento com melatonina no período crônico pode alterar a frequência de crises e sobrevida de ratos Wistar submetidos ao modelo de epilepsia induzido por pilocarpina. **Métodos:** os animais foram divididos em dois grupos: Epi+MEL (n=8), animais tratados com melatonina (10 mg/kg) no período crônico; Epi+VEI (n=5), animais tratados com solução veículo no período crônico. Para analisar a duração e a frequência de crises os animais foram vídeo-monitorados antes do tratamento no 5 º e 7º mês de vida e após o início do tratamento no 9°, 11° e 16° mês de vida. **Resultados:** os animais tratados com melatonina não apresentaram alterações na duração e frequência de crises. Embora tenhamos observado uma taxa de sobrevida de 87,5% nos animais tratados com melatonina e 40% nos animais tratados com veículo, não observamos diferença estatística. **Conclusão:** o tratamento com melatonina não foi eficaz no controle da frequência e duração das crises, bem como não alterou a sobrevida dos animais. Contudo, acreditamos que a melatonina tenha forte potencial no aumento da expectativa de vida, porém mais estudos são necessários para uma melhor compreensão da sua ação neuroprotetora, bem como seu papel na expectativa de vida.

Palavras-chave: epilepsia, melatonina, status epilepticus, neuroproteção, modelo experimental

ABSTRACT

Objective: to verify if treatment with melatonin in the chronic period can modify the seizures frequency and survival in Wistar rats submitted to pilocarpine-induced model of epilepsy. **Methods:** animals were divided in two groups: Epi+MEL (n=8) animals treated with melatonin (10 mg / Kg) in the chronic period; EPI+VEH (n=5) animals treated with vehicle solution in the chronic period. To analyze duration and frequency of seizure, all animals were video-monitored during the 5th and 7th month of life and during the treatments in the 9th, 11th and 16th month of life. **Results:** the animals treated with melatonin in the chronic phase not presented changes in the duration and frequency of seizures. Although, the animals treated with melatonin have shown a survival rate of 87.5% and the animals treated with vehicle 40%, this finding was not statistically significant. **Conclusion:** Chronic treatment with melatonin was not effective in the control of frequency and duration of seizures, as well did not modify the survival of the animals. Nevertheless, we believe that melatonin has strong potential to increase life expectancy, however, more studies are needed for a better understanding of its neuroprotective action, as well as their role in life expectancy.

Keywords: epilepsy, melatonin, status epilepticus, neuroprotection, experimental model

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INTRODUCTION

Epilepsy is a chronic disorder characterized by recurrent seizures. This condition presents cognitive, neurobiological, psychosocial and social consequences that can affect the quality of life[1]. Approximately 1% of the population has epilepsy, the equivalent of 50 million people in world[2], and the temporal lobe epilepsy (TLE) is the most common epilepsy in adults, accounting for 40% of all cases[3,4].

Neuroprotective substances have been studied in epilepsy with the objective to prevent or decrease characteristics like inflammation, excitotoxicity and neuronal death in the brain. In this context, the melatonin receives attention due to present anti-inflammatory and antioxidant action[5,6] for its inhibitory effects on the central nervous system and prevent neuronal death[7,8,9].

In the last decades several studies showed a relationship between epilepsy and melatonin. In humans, the melatonin in addition to their antiepileptic drug (AED) can decrease the seizures frequency in children with severe intractable seizures[10]. In addition, patients with epilepsy present lower levels of melatonin salivary in the interictal period and higher levels after a seizure[11].

Studies in animal models of epilepsy can contribute to better understanding of behavioral, physiological and molecular aspects of this condition in human. In this context, our team's recent data showed that melatonin treatment after status epilepticus improves behavioral and morphological aspects of TLE and the pinealectomy promotes increased neuronal excitability facilitating the epileptogenic process. This facilitation can be reversed by subsequent melatonin administration[12].

Thus, the aim of this study was to verify if melatonin treatment can modify the seizure frequency and survival in chronic phase of pilocarpine-induced epilepsy.

METHODS

1. Animals

All experimental protocols were approved by the Ethical Committee of the Federal University of São Paulo (Unifesp) nº 1390/07 and all efforts were made to minimize animal suffering following the proposal of International Ethical Guideline for Biomedical Research[13].

Wistar adult male rats, (200–250g – two months of age), were used under a 12:12-h light–dark cycle (light on 7:00h), room temperature of 21±2°C, and granted free access to food and water for all the period of the experiment. These animals were divided in two groups: 1 - Group - (Epi+MEL) - Animals submitted to pilocarpine model and treated with melatonin (n=8). 2 - Group (Epi+VEH) - Animals submitted to pilocarpine model and treated with vehicle solution (n=5).

2. Pilocarpine administration

All animals received a systemic injection of pilocarpine HCl (350 mg/kg, ip, Merck S.A.) 30 min after scopolamine methylnitrate administration (1 mg/kg s.c., Sigma Co., MO, USA to prevent the peripheral cholinergic effects).

3. Treatment

The animals were treated with melatonin (10mg/Kg) or vehicle solution (ethanol 1%)[14] during the night. These solutions were prepared daily, the melatonin 10mg/Kg was diluted in 100% ethanol followed by dilution in drinking water (final ethanol 1%). Treatment started in 9th month of life of the animals, overnight solutions were available to the animals and during the day they received only water.

4. Frequency and duration of seizures

Following 45 days SE onset the animals were continuously monitored 24h/day until to be established the chronic phase of this model and the frequency and duration of seizures were video-recorded (Stella system) during the 5th and 7th month of life, to observe of spontaneous seizures. After this period, the animals were divided into two groups (Epi+MEL and Epi+VEH) and started treatment with melatonin, which lasted until the end of life. These animals were video-recorded during the treatment in the 9th, 11th and 16th month of live to observe the frequency and duration of seizures.

5. Statistical Analysis

To analyze the frequency and duration of seizures it was performed a nonparametric statistical test Friedman test followed by Dunn's post for multiple comparisons. For survival analysis was performed chi-square test statistic with Yates correction. A value of $p < 0.05$ was accepted as significant in all cases. The values were expressed as a mean ± standard deviation (SD).

RESULTS

1. Seizures frequency

The animals of Epi+MEL and Epi+VEH groups were monitored before treatment at 5th and 7th months of life and during the treatment with melatonin or vehicle solution in the 9th, 11st and at 16th months of life. The Table 1 shows the frequency of seizures before and during treatment in each group.

2. Seizures Duration

Animals were video-monitored and the duration of seizures was recorded and accounted through chronometer. There was no significant difference in duration of seizure in both groups (Table 2).

Table 1: No significant differences were founded in seizure frequency before and during treatment in both groups. Friedman non-parametric statistical test followed by Dunn's post test for multiple comparisons, (Epi+MEL Fr = 3.700 p=0,448) and (Epi+VEH Fr = 1.570 p=0,814).). Values expressed as a mean ± standard deviation and considered significant at p <0.05.

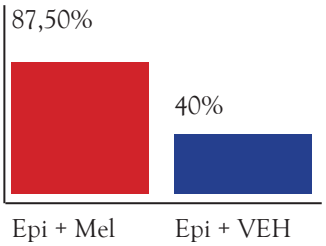
Groups	Before treatment		During treatment		
	5º month	7º month	9º month	11ºmonth	16ºmonth
EPI+MEL					
Mean	7.3	9.7	9.2	8.1	25.2
DP	9.1	8.9	10.8	11.7	33.3
EPI+VEI					
Mean	2.9	3.9	5.8	4.3	3.1
DP	2	2.1	3.3	3.7	0.3

Table 2: No significant differences in duration of seizures before and during treatment in both groups were observed. Friedman non-parametric statistical test followed by Dunn's post-test multiple comparisons test Epi+MEL (Fr = 2,189 p = 0.70) and Epi+VEH (Fr= 4.600 p=0,37). Values expressed as mean ± standard deviation and considered significant at p <0.05.

Groups	Before treatment		During treatment		
	5º month	7º month	9º month	11ºmonth	16ºmonth
EPI+MEL					
Mean	42.3	38.5	33.5	33.6	29.8
DP	15.54	10.35	8.35	10.15	10.22
EPI+VEI					
Mean	46.9	36.9	32.5	40.5	35.1
DP	15.13	5.76	1.15	13.73	6.17

3. Survival rate

Animals were kept until completed the 20th month to assess the life expectancy. In Epi + MEL group 7 animals survived until the 20th month of life while only one animal died before completing this period. In Epi+VEH group, two animals survived until the 20th month of life, while three died before completing this period. Thus, 87.50% of the animals treated with melatonin survived until the 20th month of life, while 60% of the animals treated with the vehicle died before this time.



Epi+MEL: Animals submitted to pilocarpine model and treated with melatonin. Epi+VEH: Animalssubmitted to pilocarpine model and treated with vehicle solution.

Figure 1: The chi-square test with Yates correction showed no significant difference in the survival of animals in the both groups (X^2 = 1.411 fixed, p = 0.235).

CONCLUSION

Several studies in experimental models and humans suggest that melatonin is a neuroprotective molecule, due to anticonvulsant, anti-inflammatory and scavenging properties[5,6,15], although some studies have shown that melatonin may exert proconvulsant activity in humans[16].

Our results demonstrated that the animals treated with melatonin did not present significant changes in the duration and frequency of seizures. The animals presented 2-60 seizures per month, and the duration of seizures remained similar throughout the life of the animal.

In the same context, a clinical study evaluated 11 children with epilepsy that received melatonin treatment (0.1 mg / day) in addition to their AED, and showed no change in the frequency of seizures[22].

In respect to the survival time only 40% of the Epi + VEI group animals survive until the 20th month. On the other hand, in the Epi + MEL group, 87.5% of the animals survive until the 20th month. This finding was not statistically significant, but this result leads us to believe the treatment with melatonin can extend animal survival.

Despite the beneficial effects of melatonin, treatment with melatonin was not effective in the control of the frequency and duration of seizures. Besides that, our data show no statistical differences in survival rate between the groups. However, the melatonin-treated group presented a higher survival rate compared to the control group indicating that melatonin may have a good potential to increase life expectancy.

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