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Hyperphosphorylated tau in mesial temporal lobe epilepsy: a neuropathological and cognitive study

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Abstract

Temporal lobe epilepsy (TLE) often courses with cognitive deficits, but its underlying neuronal basis remains unclear. Confluent data suggest that epilepsy share pathophysiological mechanisms with neurodegenerative diseases. However, as most studies analyze subjects 60 years old and

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Author contributions

Eliana Toscano and Érica Vieira designed this study, collected, analyzed, and interpreted clinical data. Eliana Toscano performed the histological examination, immunohistochemical reactions, morphometric evaluation, statistical analyses, and manuscript drafting. Lea Grinberg supported histopathological evaluation and data interpretation. Natalia Rocha and Regina Paradela supported statistical analysis and data interpretation. Joseane Brant applied the neuropsychologist tests. Alexandre Giannetti contributed for collection of sclerotic hippocampi. Claudia Suemoto, Renata Leite, and Ricardo Nitrini provided the control hippocampi. Milene Rachid and Antônio Teixeira coordinated the study and supported data interpretation. All authors contributed and approved the final manuscript.

Statment and Declaration

Competing of interest

The authors have no relevant financial or non-financial interests to disclose.

Consent to participate

All participants have given informed consent for this study. In the case of postmortem samples, the next of kin has given consent for each examination.

older, it is challenging to rule out that neurodegenerative changes arise from age-related mechanisms rather than epilepsy in these individuals. To fill this gap, we conducted a neuropathological investigation of the hippocampal formation of 22 adults with mesial TLE and 20 age- and sex-matched controls (both younger than 60 years). Moreover, we interrogated the relationship between these neuropathological metrics and cognitive performance. Hippocampal formation extracted from patients with drug-resistant mesial TLE undergoing surgery and postmortem non-sclerotic hippocampal formation of clinically and neuropathologically controls underwent immunohistochemistry against amyloid β (Aβ), hyperphosphorylated tau (p-tau), and TAR DNA-binding protein-43 (TDP-43) proteins, followed by quantitative analysis. Patients underwent a comprehensive neuropsychological evaluation prior to surgery. TLE hippocampi showed a significantly higher burden of p-tau than controls, whereas Aβ deposits and abnormal inclusions of TDP-43 were absent in both groups. Patients with hippocampal sclerosis (HS) type 2 had higher immunostaining for p-tau than patients with HS type 1. In addition, p-tau burden was associated with impairment in attention tasks and seizures frequency. In this series of adults younger than 60 years-old, the increase of p-tau burden associated with higher frequency of seizures and attention impairment suggests the involvement of tau pathology as a potential contributor to cognitive deficits in mesial TLE.

Keywords

drug-resistant epilepsy; p-tau; neurodegeneration; cognition

INTRODUCTION

Temporal lobe epilepsy is the most common type of focal epilepsy among adults and shows an early-onset and high drug resistance rate [1]. Two main syndromes have been described in TLE, mesial temporal epilepsy (MTLE) and neocortical temporal epilepsy [2]. MTLE affects mesial temporal limbic structures, mainly the hippocampus [3]. Accordingly, hippocampal sclerosis (HS) is the most common histopathological finding in patients with drug-resistant TLE. HS is characterized by neuronal loss and gliosis within the hippocampus, amygdala, and entorhinal cortex, especially in the Cornus Ammonis (CA) subfields [4,5].

The hippocampus is essential for memory formation and consolidation[6]. In patients with TLE, the loss of neurons in CA1, CA4, and the dentate gyrus (DG) subfields has been associated with impaired preoperative verbal and visual memories [7,8]. The more intense the hippocampal loss, the more severe the deficits in non-verbal memory are [9]. Recently, we demonstrated that microgliosis, but not astrogliosis, is also associated with worse acquisition and consolidation of visual memory in patients with MTLE and HS [10]. Cognitive deficits in TLE are not limited to memory, but also encompass language, executive function, and attention [11]. Clinical parameters of epilepsy, such as disease length and age at onset of seizures, seem to play a role in cognitive deficits [12,13]. However, the mechanisms underlying TLE-related cognitive impairment are still unclear [14,13].

A growing body of evidence has suggested that neurodegenerative diseases and epilepsy share pathophysiological mechanisms [15–18]. Neurodegeneration in TLE mostly occurs in the hilus, CA1, CA2, CA3, and DG regions. This process is associated with neuroinflammation, network reorganization, and molecular changes that contribute to the pathophysiology of TLE and HS [18]. Conversely, epileptic seizures are more common in patients with dementia than in non-demented individuals. Both epileptic individuals with amnestic mild cognitive impairtment and Alzheimers's disease (AD) had symptoms of cognitive decline earlier than patients who did not have epilepsy. Similar results were also observed in patients with AD who had subclinical epileptiform activity [19]. The seizures affect especially patients with early-onset Alzheimer's disease (EOAD) and frontotemporal dementia [20]. In addition, dementia prevalence is higher in adults with epilepsy mainly among patients with drug-resistant TLE, compared with the general population [21,15]. Marked hippocampal atrophy is a common pathological denominator of cognitive decline in AD and TLE [17], and HS is an important feature in both TLE and frontotemporal lobar degeneration (FTLD) with TAR DNA-binding protein 43 (TDP-43) inclusions [22].

Other essential pathological hallmarks of AD are the neurofibrillary tangles (NFT) and amyloid plaques. NFT are intracellular aggregates comprised of abnormally hyperphosphorylated tau protein (p-tau) [23]. In AD, p-tau loses its ability to bind to microtubules aggregating in the cytoplasm and causing neuronal injury [24]. Amyloid plaques are extracellular deposits composed mainly of the 40–42 amino acid amyloid-β peptide (Aβ) [25]. Regarding FTDL, abnormal cytoplasmic inclusions of TDP-43 are the most common neuropathological finding in FTLD, evident in approximately 50% of these cases [26]. In addition, FTLD-associated pathologies can be classified into disorders with tau-inclusion bodies (FTLD-Tau) [27,28]. TDP-43 is an ubiquitously expressed nuclear protein that plays a role in DNA transcription and splicing. In FTLD-TDP, the protein is expressed in the cytoplasm instead of the nucleus, where it is hyperphosphorylated and cleaved, causing neurodegeneration [27].

Tau pathology has been observed in hippocampal samples from patients with drug-resistant TLE and associated with post-surgical cognitive decline [29,8]. However, the results regarding Aβ deposition are less clear, with most studies not reporting this protein accumulation in sclerotic hippocampi [29,30], while some showing higher expression of beta amiloyd precursor protein (β-APP) in sclerotic hippocampi of patients with TLE than in control hippocampi [31]. There is no evidence of TDP-43 protein accumulation in TLE-associated HS [32,30,29]. Altogether, the studies above suggest that TLE might have elements of a tauopathy. Nevertheless, pathological accumulation of tau protein is commonly observed in aged brains, and studies investigating inclusions of misfolded proteins in patients with TLE younger than 60 years old are still scarce. In addition, the role played by p-tau depositions in cognitive disorders in drug-resistant TLE remains to be determined [33].

The current study was designed to perform a detailed characterization of brain samples of adults with TLE younger than 60 years compared to age and sex-matched controls for the presence of the main proteins aggregating in neurodegenerative diseases, including Aβ,

p-tau, and TDP-43. We also sought to investigate the relationship between the expression level of these proteins and cognitive performance.

MATERIAL AND METHODS

Participants

This cross-sectional study included 22 patients with drug-resistant MTLE younger than 60 years old and referred to surgery. The diagnosis was based on semiology, temporal seizure onset (confirmed using ictal/interictal electroencephalogram and neuroimaging), and failure to respond to anti-epileptic medication [3]. All patients were followed at the Epilepsy Outpatients Clinic, UFMG University Hospital in Belo Horizonte, Brazil. For inclusion in the study, they should be older than 18 years old and free of other neurological diseases. Hippocampal specimens were obtained by standard *en bloc* resection of the anterior temporal lobe or selective amygdalohippocampectomy. These specimens were fixed in formalin 10% and paraffin-embedded. We confirmed the diagnosis of HS in all samples by routine neuropathological examination [34]. HS classification was based on the scoring system considering neuronal loss (NeuN staining) in CA subfields and GFAP according to the International League Against Epilepsy (ILAE) Consensus Classification System [4]. Briefly, we used a semiquantitative score, where 0 = no obvious neuronal loss or moderate astrogliosis; 1 = moderate neuronal loss and astrogliosis; 2 = severe neuronal loss and fibrillary astrogliosis. Dentate gyrus (DG) was also evaluated using a semiquantitative examination: 0 = normal granular cell layer, 1= generalized or focal dispersion of granular cell layer, 2 = generalized or severe granular cell loss [4]. When samples presented both dispersion and duplication, they were classified in the duplication subgroup [35]. Two blinded observers performed the HS classification using a multi-head microscope, according to the following ILAE criteria [4]:

- HS type 1: pronounced pyramidal cell loss in both CA1 and CA4 sectors (score = 2). Variable and less injury in the CA2, CA3, and DG sectors (score = 0–2).
- HS type 2: predominance of neuronal loss and gliosis in CA1 sector (CA scored 1–2, while the other hippocampal subfields scored 0–1).

Demographic and clinical information of the patients with MTLE were obtained from medical records and presented in Table 1. For comparison purposes, 20 hippocampi from non-epileptic subjects (M/F 13/7, and median [range] age of 46 [25–57] years old) were supplied by the Biobank for Aging Studies, University of São Paulo, Brazil [36]. The control samples were collected within 24h after the death to preserve tissue integrity. For inclusion, control subjects should be older than 18 years old, free of any neurological diseases, Braak stage for neurofibrillary changes ≤ II, none or scarce neuritic plaque density (according to the Consortium to Establish a Registry for Alzheimer's Disease – CERAD) [37], and absence of α-synuclein deposis and abnormal inclusions of TDP-43. Patients and controls had comparable sex and age. The local ethics committee approved this study (protocol number 1.939.783).

Cognitive assessment

A comprehensive neuropsychological assessment was performed by a neuropsychologist in patients with MTLE between 3 and 6 months before their surgery. Patients completed the Logical Memory I and II, Visual Reproduction I and II, Digit Span Forward, and Boston Naming (BNT) tests. All these tests are commonly used to evaluate the cognitive performance in patients with TLE [13,11,10].

In the Logical Memory test, the patients listened to two short stories and should reproduce them immediately (Logical Memory I) and after 30 min (Logical Memory II). In the Visual Reproduction test, the examiner showed pictures of simple geometric forms to the patients who should reproduce them immediately (Visual Reproduction I) and after 30 min (Visual Reproduction II). Both tests are part of the Wechsler Memory Scale (WMS). The time I evaluates the immediate memory and time II, the consolidation of memory [38]. The Digit Span test is a subtest of both the Wechsler Adult Intelligence Scale (WAIS) and the WSM [38,39]. The Digit Span forward was used to assess the attention efficiency and capacity [40]. The patients had to repeat strings of digits in increasing length, following the same order said by the examiner. The forward performance was reported as a score based on the maximum number of digits correctly produced by the subjects [41]. To evaluate visual naming abilities (language function), the participants completed the BNT [42]. Briefly, the neuropsychologist showed line drawings of objects to the patients, who should recognize and name them. The drawings were presented at increasing difficulty levels. The total score was calculated as the sum of the number of spontaneously correct responses and the number of correct responses after a semantic or phonemic cue [43].

The absolute scores obtained in the cognitive tests were corrected by age and expressed as z-scores. According to normative data, a z-score (corrected by age) ≤ -0.9 (percentile 16) is regarded as an impairment [43]. Regarding to the control subjects, since the hippocampal samples were collected postmortem, cognitive fuctions was evaluated using the Clinical Demantia Rating Scale (CDR) corresponding to a time point of 3 months before death, that is recognized as reliable informant based assessment [44,45]. All the control subjects had CDR=0.

Immunohistochemical and morphometric analyses

Paraffin-embedded hippocampal fragments were sectioned, fixed on silanized slides, and submitted to immunohistochemical reactions for detection of $A\beta_{42}$, total TDP-43, and p-tau. The sections were submitted to antigen retrieval with sodium citrate buffer (pH=6.0), moist heat, and pressure. Endogenous peroxidase and unspecific proteins were blocked, respectively, with 0.3% hydrogen peroxide solution and a solution of 0.5% bovine serum albumin and 0.5% casein (Spring Bioscience, Pleasanton, USA). Sections were incubated overnight at 4°C with monoclonal antibody anti-$A\beta_{42}$ (D9A3A, 1:1600 dilution, Cell Signaling, Danvers, USA), rabbit polyclonal antibody anti-total TDP-43 (10782–2-AP, 1:1000 dilution, Proteintech, Rosemont, USA), and mouse monoclonal antibody anti-phospho-Serine 202-tau (CP-13, 1:600 dilution, gift from Peter Davies, NY, USA). A secondary antibody conjugated to *horseradish peroxidase enzyme* (Spring Bioscience) and diaminobenzidine chromogen (DAB) (Spring Bioscience) was used for

reaction visualization. Finally, sections were counterstained with hematoxylin. In parallel with each reaction, sections incubated without primary antibodies were used as negative controls to check the specificity of the reaction.

The slides were submitted to a comprehensive histopathological analysis. NFT was classified in pretangle and mature tangle, according to the morphological features of tau inclusions. Neurons with normal morphology, and diffuse, granular and/or perinuclear tau deposits were classified as pretangles. While mature tangles are composed of tightly packed bundles of fibers, which assumes the neuronal shape [46]. Since both control and TLE groups exhibited p-tau positive cells, the digital morphometry for this marker was performed. Fifteen frames (231,539 μm^2 each one) of the hippocampal formation at the level of the lateral geniculate body, including whole CA and DG subfields, were captured from each slide using an ×20 objective (Axiolab Microscope – Carl Zeiss, Oberkochen, Germany) and digitalized using a Samsung SDC 415 microcamera (Seoul, SKO). Brown pixels of DAB-stained cells were selected for making a binary image and the percentage of the immunostained area was calculated using the Image J 1.53e software (National Institute of Health, USA). The threshold applied was 0–206 pixels. In addition, the percentage of p-tau positive neurons for calculated ([number of p-tau positive neurons / total number of neurons] x100). Both histopathological and morphometric analyses were performed blinded to clinical parameters.

Statistical analyses

All variables were tested for distribution (parametric or non-parametric) using the Shapiro-Wilk test. The continuous sociodemographic and clinical data were presented using measures of central tendency and dispersion, and categorical ones, through measures of frequency. For two-group comparisons of numeric and parametric variables, t-test was appplied; while the Mann-Whitney test was used for numeric and non-parametric ones. For comparison of categorical variables, we used Fisher's exact test. Pearson correlation was performed to ascertain the relationship between clinical and cognitive data.

The association between neuronal expression of p-tau (continuous independent variable) and the z-scores of the neuropsychological tests was evaluated by linear regression models adjusted for sociodemographic (age, sex, and education) and clinical variables (seizure frequency and HS type). In addition, we verified the association between frequency of seizures and p-tau expression, as well as how the HS type (dichotomic independent variable) was associated with the z-scores of the neuropsychological tests, neuronal expression of p-tau, and immunostaining area for p-tau in models adjusted for age, sex, and education. Normal quantile-quantile (Q-Q) plots of the residuals and plots of the residuals versus the predicted values, as well as histograms of the residuals were used to assess the assumptions of linear regression.

Statistical significance was assumed at an alpha of 0.05 in two-tailed tests. The analyses were performed using the GraphPad Prism 8.4.2 software (La Jolla, San Diego, USA) and the R 4.0.0 software (Vienna, Austria). Based on a power of 90% and an alpha level of 5%, we estimated that a sample size of 10 subjects/group would be required. To calculate

the magnitude of the effect, we used data from a quantitative study evaluating the total tau expression in hippocampal samples of control and patients with TLE [16].

RESULTS

Demographic and clinical data

Patients and controls had comparable age and sex (age: $p = 0.27$, sex: $p = 0.37$). All patients had uncontrolled focal seizures and were on treatment with at least two antiepileptic drugs (AEDs), including iminostilbenes, benzodiazepines, barbiturates, topiramate, valproate derivatives, and lamotrigine. Demographic and clinical variables of patients with MTLE are shown in Table 1.

Cognitive assessment

Approximately 20% of the patients with MTLE had impairment in both acquisition and consolidation of verbal memory (Logical Memory tests I and II, respectively). More than 60% of them had impaired visual memory (Visual Reproduction tests I and II), and more than 50% had language dysfunction (BNT). Regarding attention, 45% of the patients exhibited deficits in the Digit Span test. The performance at each cognitive test was classified as normal, mild, moderate, or severe impairment according to normative data. The distribution of the patients according to cognitive performance is described in Table 2.

Scores in the neuropsychological tests did not correlate with clinical parameters, except Logical Memory II test scores that correlated negatively with epilepsy duration ($r = -0.444$, $p=0.034$, Pearson correlation coefficient).

Immunohistochemical and morphometric analyses

Hippocampi of control subjects did not show HS, whereas MTLE hippocampi showed diffuse loss of pyramidal cells and gliosis in CA1 and CA4 subfields, as previously reported [34].

$A\beta_{1–42}$ peptide was deposited neither in control nor in sclerotic hippocampi (Fig. 1 A–F). The morphology and cellular localization of total TDP-43 were normal (nuclear only) in both control and sclerotic hippocampi (Fig. 1 G and H). Glial cells and pyramidal and granular neurons showed normal nuclear TDP-43 expression (Fig. 1 I–L).

P-tau was absent or presented low expression in pyramidal neurons of control hippocampi (Fig. 2 A, C, and D). These samples did not have NFT. Hippocampi of patients with MTLE displayed marked p-tau pathological changes, including cytoplasmic inclusions in pyramidal neurons, granule cells of the DG, and axons in the alveus (Fig. 2 B, E, F, and H). Neuronal deposits of p-tau were found in 95% of the sclerotic hippocampi (21 patients): mature NFT was visualized in 66.7% (14 patients, Fig. 2 I) and neuronal pre-tangles in all of them (Fig. 2 J). Neuropil threads were also found (Fig. 2 I). In addition, extracellular aggregations were observed in periventricular areas (Fig. 2 K), as well as axonal immunostaining of p-tau in CA4 (Fig. 2 L). Glial depositions of p-tau in both CA and stratum radiatum areas were also observed in 95% of MTLE hippocampi (Fig. 2 M). Interestingly, patients with MTLE had neuronal inclusions of p-tau in the subpial band of the hippocampal sulcus (Fig. 2 N).

Sclerotic hippocampi had a higher immunostaining area and percentage of positive neurons (mean = 5.69 μm^2 ± 3.83 and 58.86% ± 25.32) for p-tau than control hippocampi (mean = 0.26 μm^2 ± 0,34 and 7.48% ± 6.36) - $p<0.0001$, Man-Whitney and t-test, respectively (Fig. 3 A and B). In addition, we assessed the possible association between p-tau expression in sclerotic hippocampi and cognitive performance. Using a simple univariate analisys, we found that a higher percentage of p-tau neuronal expression was associated impairtment in the BNT (p=0.033, t-test) and Digit Span Forward (p=0.048, t-test) tests (Supplemmentary figure). Howerver, when we investigated this association using a multivariate model (adjusted for age, sex, education, seizure frequency, and type of HS), neuronal p-tau was associated only with lower z-scores in the Digit Span Forward, a cognitive test for attention (β = −0.03, 95% CI = −0.05 to −0.005, p = 0.02). Expression of p-tau was not associated with the z-scores of the Logical Memory I, Logical Memory II, Visual Reproduction I, Visual Reproduction II tests, and BNT in the maultivariate model (Table 3).

Interestingly, HS type 2 was associated with a higher immnostainig area for p-tau compared to HS type 1 in a model adjusted for age, sex, and education (β = 8.06, 95% CI = 1.65 to 14.47, p = 0.02) (Table 4). Given that seizure frequency and p-tau expression correlated moderately (seizure frequency *versus* neuronal expression of p-tau: r=0.45, p=0.04; seizure frequency versus p-tau immunostained area: r=0.43, p=0.04), we adjusted a multivariate model and found a association between neuronal p-tau and seizure frequency (β = 3.09, 95% CI = 0.21 to 5.97–0.05, p = 0.04) (Table 5).

DISCUSSION

In the current study, we evaluated the expression of Aβ, p-tau, and TDP-43 in sclerotic hippocampi of patients with MTLE in comparison with control hippocampi. Despite the growing evidence of an association between chronic epilepsy and neurodegenerative diseases, the role of misfolded protein deposits in the TLE-related pathophysiology and cognitive decline has been poorly studied, especially in patients younger than 60 years old. We demonstrated that the immunostaining of Aβ and pathological inclusions of TDP-43 were absent in sclerotic hippocampi, while p-tau expression was higher in patients with TLE than in controls. Moreover, the increased neuronal expression of p-tau was associated with pre-surgery impairment of attention function (but not with episodic memory and language) in patients with MTLE.

Chronic epilepsy has been associated with accelerated brain aging, suggesting that degenerative mechanisms in the hippocampus may be responsible, at least in part, for epilepsy-related cognitive impairment [16,17]. In MTLE, the neurodegenerative process mostly occurs in the hilus, CA, and DG areas. This process is associated with neuroinflammation, network reorganization, and molecular changes that contribute to the pathophysiology of TLE and have also been implicated in cognitive impairment [47,18].

Although TLE and FTLD share HS as a remarkable neuropathological finding [22], we found neither aberrant mislocalization nor cytoplasmic and nuclear accumulation of TDP-43 in sclerotic hippocampi of patients with MTLE, corroborating previous studies [32,30,29]. Of note, TDP-43 inclusions are not observed in ischemic stroke, anoxic encephalopathy, and

necrotic tumors [32]. Altogether, these results suggest that pathological mechanisms such as hypoxia, neuronal hyperexcitability, and neuroinflammation are not sufficient to result in mislocalization or accumulation of TDP-43, reinforcing the view that FTD-related HS is different from the MTLE one [32].

We did not find any evidence of Aβ-related pathology in the hippocampi of patients with MTLE. A larger retrospective study evaluating 101 surgical samples of individuals with TLE found senile plaques in only 10% of them, and the presence of senile plaques correlated positively with patients' age [48]. Similarly, Tai et al. (2016) observed Aβ positive cases in 15% of people with TLE aged 50–65 years old [29]. The majority of these cases (10%) presented sparse plaque scores. Depositions of Aβ, reported for AD patients, were not associated with worse cognitive performance in adult and older patients with chronic epilepsy [16,29]. The sample size and the younger age of our study patients may explain why we did not observe any hippocampal immunostaining for $A\beta_{1-42.}$ In fact, the current evidence does not support a major role for Aβ in the pathogenesis of MTLE-associated cognitive impairment.

Regarding p-tau, 95% of the participants with MTLE showed neuronal, glial, and/or extracellular expression of the protein in CA, DG, and subiculum, corroborating previously reported neuropathological findings in temporal and extra-temporal drug-resistant epilepsy [30,29,33]. Importantly, we assessed a younger cohort of patients, demonstrating a consistent pattern of tau inclusions.

The detection of pathologic tau protein in the brain of epileptic individuals suggests the role of tau pathology in the pathophysiology of TLE. However, the mechanisms underlying tauopathy in epilepsy remain to be determined [18]. For instance, reduction of p-tau seems to decrease seizure susceptibility in mutant murine and Drosophila models of epilepsy [49]. In parallel, seizures can promote abnormal p-tau expression in murine models of epilepsy [50,17]. There is also evidence that tau contributes to experimental epileptogenesis by increasing the glycogen synthase kinase 3b (GSK3b) and cyclin-dependent kinase 5 (CDK5) activity [15], which can phosphorylate tau protein at specific epitopes [51]. In humans, Sen et al. found an association between occurrence of HS and increased activity of CDK5 in adults with drug-resistant epilepsy [52].

Given the observations above, there is the debate whether TLE can be considered a tauopathy, similar to AD and chronic traumatic encephalopathy (CTE) [53]. Tauopathies are traditionally defined as a group of conditions that result from tau hyperphosphorylation, abnormal tau splicing, or mutations in the microtubule-associated protein tau (*MAPT*) gene [15]. In the current study, we reported CTE- and AD-like inclusions of p-tau, as well as a novel pattern of tau pathology in middle-aged adults with MTLE. We observed NFTs and neuropil threads within the CA1 and subiculum areas; and non-pyramidal and pyramidal cells of CA3 and CA4 areas, which is remarkable in the Braak stages II/III and IV/V of AD, respectively [54]. In addition, we observed p-tau immunostaining in granular neurons (a late finding in AD) in 73% of the patients with MTLE [54]. The presence of pre-tangles was also a frequent finding in the sclerotic hippocampi evaluated in our study. Tai et al. (2016) described similar neuropathologic findings in older patients with

drug-resistant focal epilepsy (aged 50–65 years) [29]. We also found axonal expression of p-tau, especially in the CA4 and subiculum, as well as glial immunostaining in people with MTLE. These data suggest a CTE-like tau pattern. Conversely, neuronal inclusions of tau in perivascular regions, a feature of CTE neuropathology, were not observed [55]. Repetitive head injury due to seizure-related falls can act together with epilepsy-related pathophysiological factors, contributing to a unique distribution of p-tau in TLE, an issue that must be addressed in future studies. Interestingly, we also found unusual patterns of p-tau expression characterized by Tai et al. (2016) [29] in patients with focal epilepsy. These patterns seem to be typical of focal epilepsy and comprise a subpial band of p-tau deposition and granular extracellular aggregates of p-tau next to the ventricle.

Aging is an important factor associated with tau deposition [56]. Given the multiple pathological findings of p-tau expression in the hippocampi of adults with MTLE, our results suggest an accelerated brain aging profile associated with the disease. Corroborating this, we found an association between neuronal expression of p-tau and deficits in attention function in patients with MTLE. However, visual memory deficits observed in roughly 70% of the patients were not associated with p-tau immunostaining.

Pharmacological interventions reducing p-tau levels had both anti-seizure and anti-epileptogenic effects in preclinical models of epilepsy [57,58]. Herein, we reported that positive neurons for p-tau was associated with seizures frequency in the MTLE group. Moreover, the p-tau immunostaining area was increased in HS type 2 in comparison with HS type 1. We have demonstrated previously that the patients with HS type 2 have a higher frequency of seizures than those with type 1 [34], indicating a possible relationship between seizure mechanisms and tau-related cognitive decline in MTLE.

Our results should be interpreted taking into account the limitations of the study. Our sample size was small, but relatively homogenous comprising adult patients with refractory MTLE. While this homogeneity might limit the generalization of the findings, the results for this subset of patients were consistent and supported by careful histopathological, morphometric, and neuropsychological tests. The small sample size also hindered the assessment of the potential mediating role of clinical variables, such as age, disease duration, and medical comorbidities. We did not perform unbiased stereology since the paraffin blocks with resected hippocampus were previously cut for routine histopathological evaluation.

CONCLUSION

The current study corroborates the emerging role of p-tau in the pathophysiology of seizures and cognitive decline in MTLE. We demonstrated a similar expression of p-tau in adults with MTLE to those previously reported in elderly epileptic patients. Since the neuropathological pattern of p-tau in MTLE had particular features, diverging from AD, FTLD-TDP, and CTE, our data also support the emerging concept that TLE might be seen as a subtype of tauopathy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Ethics approval

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee in Research of the Universidade Federal de Minas Gerais (COEP-UFMG), protocol number 1.939.783.

Data availability

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

Abbreviations

Aβ	amyloid beta
AEDs	antiepileptic drugs
AD	Alzheimer’s disease
β-APP	beta amyloid precursor protein
BNT	Boston naming test
CA	Cornus Ammonis
CDK5	cyclin-dependent kinase 5
CDR	Clinical Demantia Rating Scale
CTE	chronic traumatic encephalopathy
DAB	diaminobenzidine
DG	dentate gyrus
EOAD	early-onset Alzheimer’s disease
FTLD	frontotemporal lobar degeneration
GSK3b	glycogen synthase kinase 3b
ILAE	International League Against Epilepsy
MAPT	microtubule-associated protein tau

MTLE	mesial temporal lobe epilepsy
NFT	neurofibrillary tangles
p-tau	hyperphosphorylated tau
TDP-43	TAR DNA-binding protein-43
TLE	temporal lobe epilepsy
WAIS	Wechsler Adult Intelligence Scale
WMS	Wechsler Memory Scale

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Key Points

- P-tau burden in hippocampal formation is significantly higher in young/middle age individuals with MTLE compared to matched controls.
- Patients with MTLE had both Alzheimer's disease like- and divergent patterns of hyperphosphorylated tau deposits.
- Increased neuronal expression of p-tau was associated with impaired attention, but not with memory deficits in MTLE.

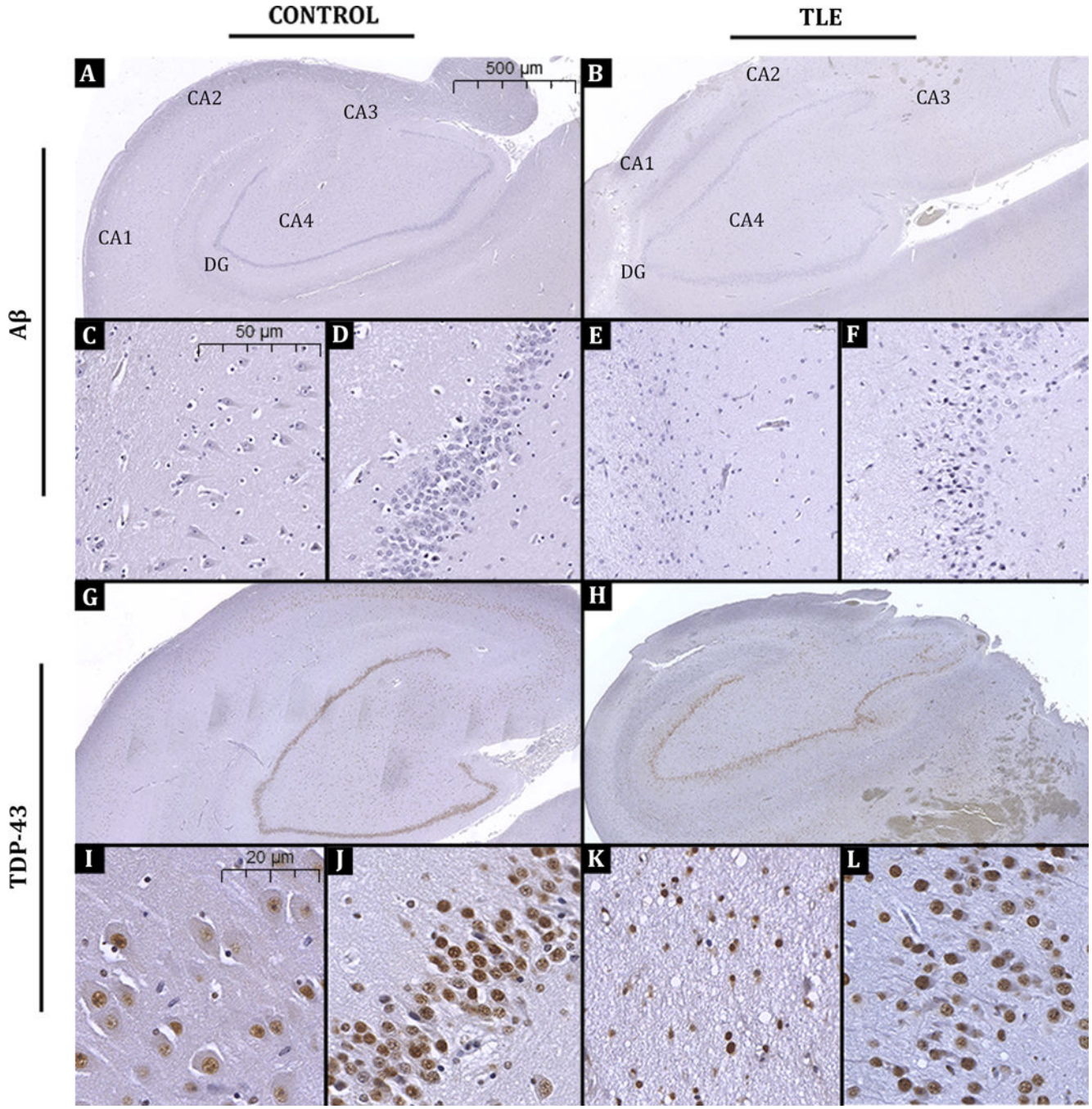

Fig. 1. Representative histopathological images of control hippocampi (left column) and sclerotic hippocampi from patients with MTLE (right column) submitted to immunohistochemical reaction for $A\beta_{42}$ and total TDP-43.

Control and sclerotic hippocampi exhibited absent immunostaining for $A\beta_{42}$ (A-F). Abnormal cytoplasmic inclusions were not observed neither in neurons nor glia of the both groups (G-L). Note the neuronal loss and gliosis in the CA1 area (E and K), as well as dispersion of granular cell layer (F and L) of sclerotic hippocampi.

A, B, G, H: x2 magnificence, scale bar 500 μm; C-F and I-L: x40 magnificence, scale bar 50 μm. CA: Cornu Ammonis; DG: dentate gyrus; MTLE: mesial temporal lobe epilepsy; $A\beta_{42}$: amyloid β 42; TDP-43: Tar binding DNA protein 43 kDA

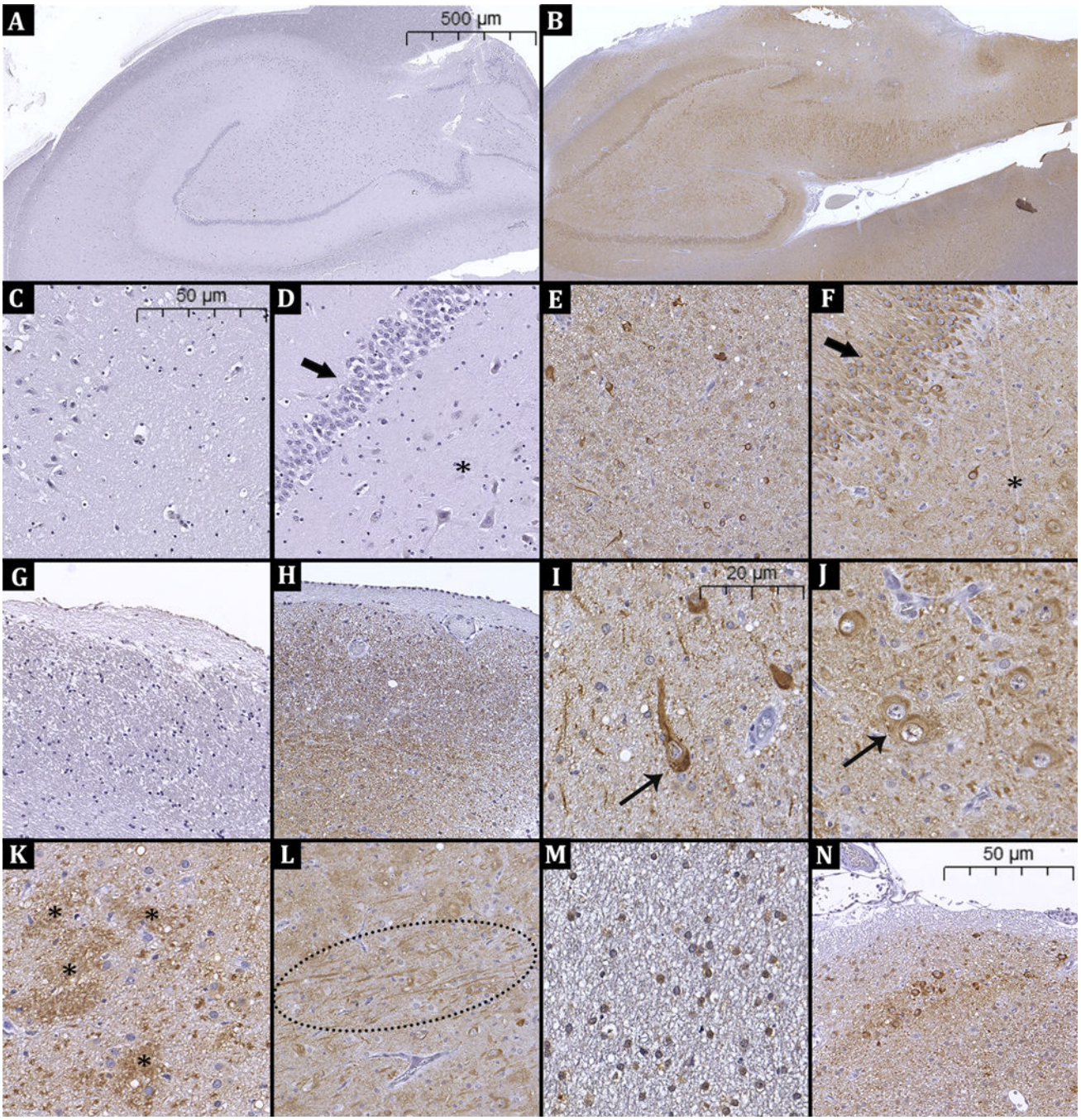

Fig. 2. Representative histopathological images of control and sclerotic hippocampi submitted to immunohistochemical reaction for p-tau.

Control hippocampi exhibited absent or low immunostaining for p-tau (A) in CA (C and asterisk in D), DG (arrow in C), and alveus (G). Patients with MTLE displayed p-tau immunostaining along the hippocampus, including CA (asterisk in F), DG (arrow in F) areas, and alveus (H). NFT was observed in pyramidal neurons (arrow in I), as well as *p-tau* in *neuropil* threads (I). Pretangles were visualized (arrow in J), especially in the CA4 and subiculum. Axonal bundles (circle in L) were also observed in these regions. Different from

the p-tau pattern found in Alzheimer's disease, we noted spread p-tau positive glia (M), extracellular aggregates in the peri-ventricular region (K), and a subpial positive layer (N). A and B: x2 magnificence, scale bar 500 μm; C-H and N: x20 magnificence, scale bar 50 μm; I-M: x40 magnificence, scale bar 50 μm. p-tau: hyperphosphorylated tau; CA: Cornu Ammonis; DG: dentate gyrus; MTLE: mesial temporal lobe epilepsy

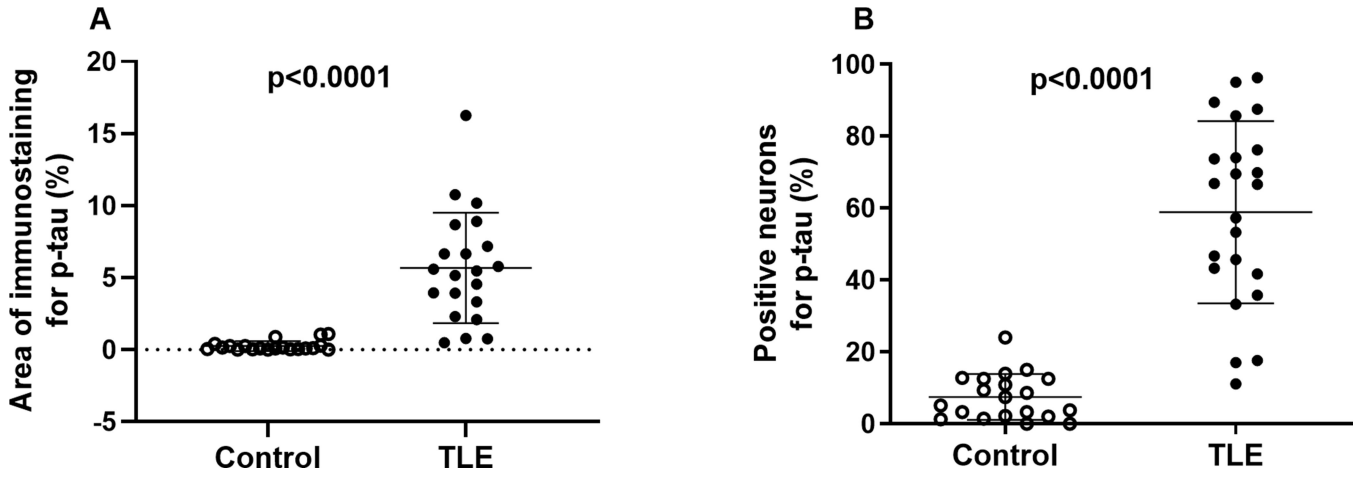

Fig. 3. Hippocampal immunostaining of p-tau in patients with MTLE and in controls.

Sclerotic hippocampi displayed a higher area of immunostaining and positive neurons percentage for p-tau than the non-sclerotic ones (A and B). Mann–Whitney test was performed for A, while t-test was utilized in B analyse. Data are mean ± SD and are considered significant when $p \leq 0.05$. p-tau: hyperphosphorylated tau; MTLE: mesial temporal lobe epilepsy; SD: standard deviation of the mean

Table 1 –

Demographic and clinical characteristics of patients with MTLE.

Demographical / clinical variable	Patients with MTLE n=22	
	n / mean (SD)	Proportion (%) / median (range)
Sex		
Male	11	50.0%
Female	11	50.0%
Age (years)	41.8 (7.8)	39 (30–58)
Educational level (years)	6.9 (3.0)	6 (4–14)
Epilepsy onset (years)	4.3 (3.4)	3 (0–13)
Duration of epilepsy (years)	36.3 (10.5)	35 (21–55)
Type of seizure		
Focal aware	3	13.6%
Focal impairment awareness	22	100%
Focal to bilateral tonic-clonic	16	72.7%
Frequency of seizure (monthly)	5.6 (4.0)	3.8 (1.5–14)
Laterality of hippocampal sclerosis		
Left	13	59.0%
Right	09	41.0%
Hippocampal sclerosis		
Type 1	13	59.0%
Type 2	09	41.0%
AEDs		
2 AEDs	15	68.2%
3 AEDs	7	31.8%

MTLE – mesial temporal lobe epilepsy; SD – standard deviation; AEDs – anti-epileptic drugs.

Table 2 –

Distribution of the patients with MTLE according to the severity of cognitive impairment.

Neuropsychological test	Severity of the cognitive impairment n - proportion (%) of patients with MTLE			
	Normal	Mild	Moderate	Severe
Logical memory I	18 – 81.80%	1 – 4.55%	1 – 4.55%	2 – 9.10%
Logical memory II	18 – 81.80%	1 – 4.55%	2 – 9.10%	1 – 4.55%
Visual reproduction I	8 – 36.36%	9 – 40.90%	3 – 13.64%	2 – 9.10%
Visual reproduction II	5 – 22.73%	1 – 4.55%	8 – 36.36%	8 – 36.36%
Boston naming test	10 – 45.45%	2 – 9.10%	3 – 13.64%	7 – 31.81%
Digit Span Forward	12 – 54.54%	6 – 27.26%	2 – 9.10%	2 – 9.10%

MTLE – mesial temporal lobe epilepsy

Table 3 –

Association between neuronal expression of p-tau and cognitive performance in patients with MTLE (n = 22).

Neuropsychological test	Model 1		Model 2		Model 3	
	β (95% CI)	*P*	β (95% CI)	*P*	β (95% CI)	*P*
Logical Memory I	−0.01(−0.03; 0.02)	0.47	−0.01(−0.03; 0.01)	0.29	−0.01(−0.04; 0.01)	0.33
Logical Memory II	−0.01(−0.03; 0.01)	0.2	−0.01(−0.03; 0.004)	0.13	−0.01(−0.03; 0.01)	0.22
Visual Reproduction I	0.0004(−0.02; 0.02)	0.96	−0.01(−0.02; 0.01)	0.48	−0.01(−0.03; 0.01)	0.33
Visual Reproduction II	0.0004(−0.02; 0.02)	0.96	−0.01(−0.02; 0.01)	0.47	−0.005(−0.03; 0.02)	0.58
Boston Naming Test	−0.01(−0.02; 0.01)	0.42	−0.01(−0.03; 0.01)	0.16	−0.01(−0.03; 0.004)	0.12
Digit Span Forward	−0.01(−0.03; 0.01)	0.17	−0.01(−0.04; 0.01)	0.18	−0.03(−0.05; −0.005)	**0.02**

Linear regression models

Model 1: Univariate

Model 2: Adjusted for age, sex, and education

Model 3: Adjusted for age, sex, education, seizure frequency, and type of hippocampal sclerosis

MTLE – mesial temporal lobe epilepsy

CI – confidence interval

Table 4 –

Association between HS type 2 with cognitive performance and p-tau expression in patients with MTLE (Total = 22, HS type 1 = 13, HS type 2 = 9).

	Model 1		Model 2		Model 3	
	β (95% CI)	***P***	**β (95% CI)**	***P***	**β (95% CI)**	***P***
Logical Memory I	−0.68 (−1.87; 0.52)	0.25	−0.69 (−1.89; 0.51)	0.24	−0.39 (−1.53; 0.76)	0.49
Logical Memory II	−0.37 (−1.40; 0.67)	0.47	−0.39 (−1.38; 0.60)	0.42	−0.16 (−1.13; 0.81)	0.73
Visual Reproduction I	−0.37 (−1.18; 0.44)	0.35	−0.34 (−1.14; 0.46)	0.39	−0.14 (−0.91; 0.63)	0.71
Visual Reproduction II	−0.50 (−1.38; 0.38)	0.25	−0.47 (−1.35; 0.42)	0.28	−0.28 (−1.16; 0.60)	0.51
Boston Naming Test	−0.55 (−1.41; 0.31)	0.20	−0.56 (−1.47; 0.36)	0.22	−0.34 (−1.22; 0.55)	0.43
Digit Span Forward	−0.30 (−1.35; 0.75)	0.56	−0.30 (−1.40; 0.79)	0.57	−0.27 (−1.44; 0.91)	0.64
Neuronal expression of p-tau	−3.95 (−28.21; 20.32)	0.74	−3.28 (−28.11; 21.54)	0.78	−0.30 (−26.34; 25.74)	0.98
P-tau immunostaining area	7.59 (−1.25; 13.92)	**0.02**	7.86 (1.88; 13.85)	**0.01**	8.06 (1.65; 14.47)	**0.02**

Linear regression models

Model 1: Univariate

Model 2: Adjusted for age and sex

Model 3: Adjusted for age, sex, and education

Reference category: Hippocampal sclerosis type 1

MTLE – mesial temporal lobe epilepsy

CI – confidence interval

Table 5 –

Association of seizure frequency with p-tau immunostaining in patients with MTLE (n = 22)

	Model 1		Model 2		Model 3	
	β (95% CI)	***P***	**β (95% CI)**	***P***	**β (95% CI)**	***P***
Neuronal expression of p-tau	2.66 (−0.10; 5.43)	0.06	3.09 (0.21; 5.97)	**0.04**	2.91(−0.17; 5.99)	0.06
P-tau Immunostaining area	0.35 (−0.54; 1.23)	0.43	0.40 (−0.51; 1.32)	0.37	0.50 (−0.47; 1.46)	0.30

Linear regression models

Model 1: Univariate

Model 2: Adjusted for age and sex

Model 3: Adjusted for age, sex, and education

MTLE – mesial temporal lobe epilepsy

CI – confidence interval