Child's Nervous System (2021) 37:879–884
https://doi.org/10.1007/s00381-020-04920-z

ORIGINAL ARTICLE

Circulating glial fibrillary acidic protein and ubiquitin carboxy-terminal hydrolase-L1 as markers of neuronal damage in children with epileptic seizures

Marwa Elhady[1] · Eman R Youness[2] · Mones M. AbuShady[3] · Maysa S. Nassar[3] · Ali Abd Elaziz[3] · Mahmoud M Masoud[2] · Fayez K. Foudaa[2] · Walaa Alshasrany Abu Elhamed[4]

Received: 27 April 2020 / Accepted: 5 October 2020 / Published online: 12 October 2020

Abstract

Background Epilepsy is a common neurological disease that has a negative impact on physical, social, and cognitive function. Seizure-induced neuronal injury is one of the suggested mechanisms of epilepsy complications. We aimed to evaluate the circulating level of glial fibrillary acidic protein (GFAP) and ubiquitin carboxy-terminal hydrolase-L1 (UCH-L1) as markers of neuronal damage in children with epilepsy and its relation to epilepsy characteristics.

Study design

Methods This case control study included 30 children with epilepsy and 30 healthy children as a control group. Seizure severity was determined based on Chalfont score. Serum level of GFAP and UCH-L1were measured, and their associations with epilepsy characteristics were investigated.

Results Circulating levels of GFAP and UCH-L1 were significantly higher in children with epilepsy than in controls (17.440 ± 6.74 and 5.700 ± 1.64 vs 7.06 ± 3.30 and 1.81 ± 0.23, respectively) especially in those with generalized and active seizures. GFAP and UCH-L1 were significantly correlated to the severity of seizures in the previous 6 months. Elevated GFAP level was a predictor for active seizures (OR 1.841, 95%CI 1.043–3.250, $P = 0.035$).

Conclusion Circulating GFAP and UCH-L1 expression is increased in children with epilepsy especially those with active seizures.

Significance GFAP and UCH-L 1may serve as peripheral biomarkers for neuronal damage in children with epilepsy that can be used to monitor disease progression and severity for early identification of those with drug-resistant epilepsy and those who are in need for epilepsy surgery.

Keywords Epilepsy · GFAP · UCH-L1 · Children

✉ Marwa Elhady
Marwaelhady93@yahoo.com; marwaelhady@azhar.edu.eg

Eman R Youness
Hoctober2000@yahoo.com

Mones M. AbuShady
monesshady@ymail.com

Maysa S. Nassar
Maysanassar@yahoo.com

Ali Abd Elaziz
Aliabdelaziz62@yahoo.com

Mahmoud M Masoud
mmmabrouk2016@gmail.com

Fayez K. Foudaa
Dr.fayezfouda@gmail.com

Walaa Alshasrany Abu Elhamed
walaalsharany_12@yahoo.com

[1] Pediatric Department, Faculty of Medicine for Girls, Al-Azhar University, Cairo 11735, Egypt

[2] Medical Biochemistry Department, National Research Centre, Cairo, Egypt

[3] Child Health Department, National Research Centre, Cairo, Egypt

[4] Department of Pediatrics, Faculty of Medicine, Cairo University, Cairo, Egypt

Introduction

Epilepsy is a common neurological disease affecting about 1% of the general population with long-term sequels that may extend even after elimination of active seizures. It is characterized by recurrent seizures caused by hypersynchronous discharges [1]. Seizure activity leads to dynamic alteration of neuronal structure and function resulting in axonal and dendritic remodeling, gliosis, and apoptosis [2]. Childhood is a critical period for brain development. Despite their high neuronal plasticity, early-onset sever frequent seizure activities are associated with impairment of brain function that may extend into adulthood. The long-term comorbidities of childhood seizures adversely affect education and social and financial aspects of their life in addition to increase the prevalence of psycho-behavioral disorders [3]. The relation between seizure activity and neuronal injury is complex. Neuronal damage may be a sequel or a cause of seizures. Identification and monitoring of neuronal injury are required for risk stratification, understanding the pathogenesis of epilepsy comorbidities and improving management strategies [4].

Ubiquitin C-terminal hydrolase (UCH-L1) is a neuron-specific cytoplasmic neuronal enzyme accounting for 1–5% of total neuronal proteins. UCH-L1 is not involved in neuronal development, but it represents a key element for maintaining axonal integrity [5]. It is mainly present in the brain and poorly expressed by others cells including gonads and fibroblast. As a strict intracellular protein, its circulating level is a strong indicative of neuronal damage. Evidences demonstrated higher UCH-L1 expression in diseases associated with neuro-inflammation, neuronal degeneration, and after traumatic brain injury [6]. Impaired blood-brain barrier (BBB) integrity has been associated with increased blood concentrations of UCH-L1 in several neurological disorders. UCH-L1 has been emerged as promising biomarker of neuronal injury and BBB disruption due to its high brain specificity [7].

Glial fibrillary acidic protein (GFAP) is another highly brain-specific protein that constitutes the main intermediary filament of astroglial cells which represent the commonest cell type in the human central nervous system (CNS) [8]. It is involved in white matter architecture, myelination, and blood-brain barrier integrity and has an important role in keeping shape and motility of astrocytes. No extracerebral sources for this protein have been identified [9]. Up-regulation of GFAP as part of reactive astrogliosis following different pathological events in the CNS including inflammation, hypoxia, mechanical disruption, or disintegration of blood-brain barrier may lead to its release from brain tissue into the peripheral circulation [10].

GFAP and UCH-L1 levels' assessment either in serum or cerebrospinal fluid (CSF) level is widely addressed as a diagnostic and prognostic marker for traumatic brain injury [11]. However, no sufficient data investigate the utility of these neuronal-specific biomarkers in children with epilepsy and their correlation to the clinical data in such children.

The aim of this study was to evaluate circulating level of GFAP and UCH-L1 as markers of neuronal damage in children with epilepsy and its relation to epilepsy characteristics.

Subjects and method

Study design

This case control study included 30 children with confirmed diagnosis of epilepsy and 30 healthy children of matched age and sex as a control group. Children with epilepsy were consecutively selected from pediatric neurology clinic, while the control group was selected from pediatric outpatient clinic of both Abo-Elrish Hospital, Cairo University, and Alzahraa Hospital, Al-Azhar University, Cairo, Egypt. They were recruited during the period from April 15, 2019, to September 1, 2019. Informed written consents were obtained from the caregivers of all included children after explaining the aim and hazards of the study according to the local ethics committee of Egyptian National Research Centre.

Inclusion criteria were children aged 6–12 years old of both sexes with established diagnosis of epilepsy of unknown etiology either of generalized or focal onset receiving antiepileptic drug of proper doses for at least 1 year.

Exclusion criteria were any acute or chronic medical illness other than epilepsy (e.g., cardiac, hepatic, hematological, respiratory, renal diseases), malignancy, genetic disorders, developmental or intellectual disabilities, or children with history of metabolic, infectious, hypoxic, or traumatic brain injury.

Epilepsy was classified according to the International League Against Epilepsy (ILAE); according to the etiology, we include only children with epilepsy of unknown etiology (previously known as idiopathic), and based on the type, they were categorized into focal onset, generalized onset, focal to bilateral tonic clonic, or unknown onset. Children were categorized according to seizure frequency over the previous 6 months into those with active seizures and those with no seizure activity over the last 6 months [12]. Circulating levels of sodium valproate and carbamazepine were done to confirm therapeutic drug level in children with active seizures. For control group they were healthy age and sex matched to epilepsy group did not have any neurologogical or psychological disorders or previous febrile seizures and fullfill the same inclusion criteria as case group.

Methods

All the studied children were subjected to the following:

1) Detailed medical history including sociodemographic data, neurological manifestation, age of onset of seizures, duration of epilepsy, previous investigation, current medication, response to medications, and seizure frequency in the last 6 months.
2) Complete systematic and neurological clinical examination.
3) The type of epilepsy was identified based on the commission on classification and terminology of the international league against epilepsy [12], while seizure severity was assessed using Chalfont severity scale [13].
4) Neuroimaging for children with epilepsy to exclude any underlying traumatic, asphyxia, infectious, or structural brain lesion.
5) Digital interracial EEG was done using a Nihon Kohden 1200 digital EEG instrument, and interpretation was done by the same pediatric neurologist.
6) Biochemical investigations include assessment of serum glial fibrillary acidic protein and ubiquitin carboxy-terminal hydrolase-L1 using the enzyme-linked immunosorbent assay (ELISA) kit (Glory Science, Del Rio, TX, USA).

Statistical analysis

Statistical analysis was performed using statistical package for social sciences (SPSS) version 21 for windows (IBM Corp., Armonk, NY, USA). Continuous data were expressed as mean ± standard deviation and were compared by using the Student's *t* test. Data not normally distributed were compared by using the Mann-Whitney test. Categorical data were expressed as frequencies and percentages. Pearson correlation test was used to assess correlations between variables. $P < 0.05$ was accepted as statistically significant.

Results

This study was conducted on 30 epileptic patients (16 male, 14 female); their age ranged between 6 and 10 with a mean of 7.07 ± 1.639 years. Eighteen of them have focal onset epilepsy (60%) and 12 (40%) had generalized onset epilepsy. None of the included children had focal with secondary generalized epilepsy. The age of onset of epilepsy ranged between 2 and 5 years old with a mean of 3.6 ± 0.932 years, and the duration of epilepsy ranged between 1 and 5 years with a mean duration of 2.47 ± 0.973 years. Ten of them (33.3%) were free of seizures over the previous 6 months. Another 30 healthy children were included in the control group (14 male and 16 female) with a mean age of 7.87 ± 2.77 years. There was no significant difference between cases and control regarding age and sex ($p = 0.162$, and $p = 0.605$, respectively).

The comparison between children with epilepsy and healthy control showed significant higher GFAP and UCH-L1 serum levels in children with epilepsy as shown in Table 1.

Regarding the type of epilepsy, children with generalized onset epilepsy have showed significantly higher GFAP and UCH-L1 serum levels than children with focal onset epilepsy as shown in Table 2. Children with generalized onset and focal onset epilepsy have significant higher levels of GFAP and UCH-L1than the control group ($p < 0.0001$ for each).

Children with active seizures have significantly higher serum levels of GFAP and UCH-L1 than children with no seizures over the previous 6 months as shown in Table 3. In comparison with the control group, children with active seizures and those with no seizures have significant higher levels of GFAP ($p < 0.0001$ for each) and UCH-L1 ($p = 0.031$ and $p < 0.0001$, respectively).

Serum levels of both GFAP and UCH-L1 have a significant positive correlation with the Chalfont seizure severity scale scores. But no significant correlation was detected between age, age of onset of seizures, duration of epilepsy, and any of the biomarker level as demonstrated in Table 4.

Binary logistic regression analysis showed that elevated GFAP level is the predictor for active seizures in children with epilepsy as demonstrated in Table 5.

Table 1 Comparison of glial fibrillary acidic protein and ubiquitin carboxy-terminal hydrolase-L1 between children with epilepsy and healthy controls

	Children with epilepsy ($N = 30$)	Control group ($N = 30$)	Independent *t* test	
	Mean ± SD	Mean ± SD	*t*	*p* Value
GFAP (ng/ml)	17.440 ± 6.736	7.060 ± 3.301	7.579	< 0.0001*
UCH-L1 (ng/ml)	5.700 ± 1.641	1.813 ± 0.233	12.847	< 0.0001*

*Significant; *GFAP* glial fibrillary acidic protein, *UCH-L1* ubiquitin carboxy-terminal hydrolase-L1

Table 2 Comparison of glial fibrillary acidic protein and ubiquitin carboxy-terminal hydrolase-L1 between children with focal onset and generalized onset epilepsy

	Children with generalized onset epilepsy ($N = 12$)	Children with focal onset epilepsy ($N = 18$)	Independent t test/ Chi-square test	
	Mean ± SD	Mean ± SD	t/× 2	p Value
Age (years)	8.166 ± 1.267	8.000 ± 1.188	0.362	0.721
Age of onset (year)	3.291 ± 0.940	3.305 ± 1.002	−0.038	0.970
Duration of illness (year)	3.042 ± 1.117	2.555 ± 0.921	1.250	0.225
Chalfont severity score	46.083 ± 18.123	22.111 ± 10.693	4.128	0.001*
Seizure frequency over the previous 6 months (N, %)			0.235	0.117
Seizure-free	2 (16.7%)	8 (44.4%)		
Active seizures	10 (83.3%)	10 (55.6%)		
GFAP (ng/ml)	21.433 ± 6.366	14.744 ± 6.026	2.880	0.009*
UCH-L1 (ng/ml)	6.750 ± 1.979	5.028 ± 1.295	2.659	0.016*

*Significant; *GFAP* glial fibrillary acidic protein, *UCH-L1* ubiquitin carboxy-terminal hydrolase-L1

Discussion

Identifying neuronal injury and death using biochemical markers have been emerged as a simple quantitative subjective tool for assessment of neurological disease severity and progression. Distortion of the integrity of BBB occurred as a sequence of neuro-inflammation during the process of epileptogenesis. BBB dysfunction allows passage of neuron specific proteins into the peripheral circulation [14].

Our study showed that the circulating level of GFAP and UCH-L1 were elevated in children with epilepsy especially those with active seizures and those with generalized onset epilepsy. Both biomarkers levels have a significant correlation with seizure severity. In agreement with our findings, Mondello et al. [15] reported a significant higher serum level of UCH-L1 in patients with epilepsy in comparison with healthy controls ($p = 0.025$). Li et al. [16] demonstrated the elevated UCH-L1 level in CSF after seizures with a significantly higher level of UCH-L1 in subjects with generalized than focal seizures. Simani et al. [17] concluded that the elevated serum GFAP level after seizures may provide a useful diagnostic method distinguishing epileptic seizures from condition mimic epilepsy.

On the other hand, Chmielewska et al. [18] demonstrated a significant elevation of brain-derived proteins UCH-L1 but not GFAP in an animal model for epilepsy suggesting that seizure-induced neuronal damage can be evaluated through the measurement of the serum level of UCH-L1 after seizure activity. This controversy could reflect that GFAP is released during reactive astrocytes gliosis as a sequel of neuronal injury induced by seizure activity. Supporting our findings, Gurnett et al. [19] concluded that the increased spinal fluid GFAP level following seizures occurs mainly in children with prolonged seizures or those who have

Table 3 Comparison of glial fibrillary acidic protein and ubiquitin carboxy-terminal hydrolase-L1 in relation to seizures frequency over the previous 6 months

	Children with no seizures in the last 6 months ($N = 10$) Mean ± SD	Children with active seizures ($N = 20$) Mean ± SD	Independent t test	
			t	p Value
Age (years)	8.400 ± 0.8432	7.900 ± 1.333	1.250	0.222
Age of onset (year)	3.350 ± 1.203	3.275 ± 0.850	0.176	0.863
Duration of illness (year)	2.400 ± 0.8096	2.925 ± 1.079	− 1.492	0.149
Chalfont severity score	21.80 ± 9.566	36.650 ± 19.735	− 2.776	0.010*
GFAP (ng/ml)	10.970 ± 4.463	20.645 ± 5.535	− 5.154	< 0.0001*
UCH-L1 (ng/ml)	4.440 ± 1.206	6.355 ± 1.707	− 3.548	0.002*

*Significant; *GFAP* glial fibrillary acidic protein, *UCH-L* ubiquitin carboxy-terminal hydrolase-L1

Table 4 Correlation of epilepsy characteristics and serum level of glial fibrillary acidic protein and ubiquitin carboxy-terminal hydrolase-L1 in children with epilepsy

	GFAP (ng/ml)		UCH-L1 (ng/ml)	
	r	*p* Value	*r*	*p* Value
Age (years)	0.016	0.931	0.190	0.314
Age of onset (year)	0.048	0.802	0.157	0.406
Duration of illness (year)	0.108	0.571	− 0.043	0.821
Chalfont severity score	0.769	< 0.0001*	0.836	< 0.0001*

*Significant; *GFAP* glial fibrillary acidic protein, *UCH-L1* ubiquitin carboxy-terminal hydrolase-L1

symptomatic etiologies. Martinian et al. [20] found that GFAP expression is increased in epilepsy-associated lesion pathologies. In an experimental animal model, a minimum number of 9 seizures or 250 s of active seizure are required to induce reactive astrocyte. So GFAP may not be elevated immediately following seizure except if the seizure was severe enough to induce a neuronal damage or in those with underlying brain insult causing seizures [21]. Furthermore, our results showed that the serum levels of GFAP and UCH-L1 were higher in those with controlled seizures than healthy controls. This came in agreement with Zhu et al. [22] who found that despite decreasing the serum levels of several neuronal injury biomarkers including GFAP after 18-month of treatment but their levels remained higher than healthy controls reflecting continuous cumulative neuronal injury. Leakage of those neuronal-specific proteins into the peripheral circulation provides a tool for assessment of seizure-induced neuronal damage.

Evidence from neuroimaging and histological studies revealed that frequent seizures of high number are required to induce morphological changes detectable by neuroimaging. Subtle brief seizures activities produce relatively limited neuronal injury that usually missed. The cumulative effect of this neuronal injury needs a long time to be evident clinically as a cognitive impairment or radiologically by neuroimaging [23]. Monitoring of circulating level neuronal specific biomarkers provide a sensitive noninvasive less hazardous method for monitoring and follow up during the course of disease allowing physicians for adjustment of management plan and early identify complications. [24]

Excluding the effect of falling and head trauma is challenging in pediatric population with epilepsy which is more frequent in those with active seizures. This can partially explain the higher level of UCH-L1 and GFAP in those with generalized seizures as they are more liable for falling and head trauma than those with focal seizures. However significant higher level of both markers in those with no seizures over the last 6 months and those with focal onset seizures suggesting that elevated UCH-L1 and GFAP level could not explained only by head trauma.

Several limitations have faced the present study. The small number of included children, the cross-sectional design, and data regarding seizure duration were not available. Further studies are needed regarding the association of chemical biomarkers with long-term complications of epilepsy. One of the advantages of the current study is measuring plasma UCH-L1 and GFAP as an easier way than CSF measurement of these biomarkers.

The higher rate of children with active seizures in our study may be explained by the underlying genetic causes of idiopathic epilepsy and poor compliance due to the financial high cost of medication as limited antiepileptic medications (valproic acid, carbamazepine, and levetiracetam) are provided by health insurance. McCormack et al. [25] reported that genetic mutations were detected in several subjects with severe epilepsy of unknown etiology who have poor response to medications. Atalar et al. [26] concluded that focal epilepsy of unknown cause had a good outcome when properly treated.

In conclusion, generalized epilepsy and active seizures are associated with elevated GFAP and UCH-L1 levels. Circulating UCH-L1 and GFAP may have utility and may serve as peripheral biomarkers for neuronal damage after epileptic seizure that can be used to monitor disease progression and severity.

Table 5 Binary logistic regression analysis for predictors of active seizures in children with epilepsy

Variable	Coefficients	Standard errors	*p* Value	Odds ratios	95% Confidence	
					Low	High
GFAP (ng/ml)	0.622	0.288	0.031	1.862	1.060	3.273
UCH-L1 (ng/ml)	− 0.860	0.906	0.342	0.423	0.072	2.497
Chalfont severity score	− 0.035	0.069	0.608	0.965	0.844	1.104
Constant	− 3.380	1.977	0.087	0.034		

*Significant; *GFAP* glial fibrillary acidic protein, *UCH-L1* ubiquitin carboxy-terminal hydrolase-L1

References

1. Jones JE, Asato MR, Brown MG, Doss JL, Felton EA, Kearneyet JA et al (2020) Epilepsy benchmarks area IV: limit or prevent adverse consequence of seizures and their treatment across the life span. Epilepsy Curr 20(1_suppl):31S–39S
2. Mao XY, Zhou HH, Jin WL (2019) Redox-related neuronal death and crosstalk as drug targets: focus on epilepsy. Front Neurosci 13: 512
3. Sculier C, Gaínza-Lein M, Sánchez Fernández I, Loddenkemper T (2018) Long-term outcomes of status epilepticus: a critical assessment. Epilepsia 59 Suppl 2(Suppl Suppl 2):155–169
4. Patel DC, Tewari BP, Chaunsali L, Sontheimer H (2019) Neuron–glia interactions in the pathophysiology of epilepsy. Nat Rev Neurosci 20:282–297
5. Bishop P, Rocca D, Henley JM (2016) Ubiquitin C-terminal hydrolase L1 (UCH-L1): structure, distribution and roles in brain function and dysfunction. Biochem J 473(16):2453–2462
6. Wang KK, Yang Z, Sarkis G, Torres I, Raghavan V (2017) Ubiquitin C-terminal hydrolase-L1 (UCH-L1) as a therapeutic and diagnostic target in neurodegeneration, neurotrauma and neuro-injuries. Expert Opin Ther Targets 21(6):627–638
7. Papa L, Mittal MK, Ramirez J, Silvestri S, Giordano P, Braga CF, Tan CN, Ameli NJ, Lopez MA, Haeussler CA, Mendez Giordano D, Zonfrillo MR (2017) Neuronal biomarker ubiquitin C-terminal hydrolase detects traumatic intracranial lesions on computed tomography in children and youth with mild traumatic brain injury. J Neurotrauma 34(13):2132–2140
8. Yang Z, Wang KK (2015) Glial fibrillary acidic protein: from intermediate filament assembly and gliosis to neurobiomarker. Trends Neurosci 38(6):364–374
9. Petzold A (2015) Glial fibrillary acidic protein is a body fluid biomarker for glial pathology in human disease. Brain Res 1600:17–31
10. Hol EM, Pekny M (2015) Glial fibrillary acidic protein (GFAP) and the astrocyte intermediate filament system in diseases of the central nervous system. Curr Opin Cell Biol 32:121–130
11. Bazarian JJ, Biberthaler P, Welch RD, Lewis LM, Barzo P, Bogner-Flatz V, Gunnar Brolinson P, Büki A, Chen JY, Christenson RH, Hack D, Huff JS, Johar S, Jordan JD, Leidel BA, Lindner T, Ludington E, Okonkwo DO, Ornato J, Peacock WF, Schmidt K, Tyndall JA, Vossough A, Jagoda AS (2018) Serum GFAP and UCH-L1 for prediction of absence of intracranial injuries on head CT (ALERT-TBI): a multicentre observational study. Lancet Neurol 17(9):782–789
12. Scheffer IE, Berkovic S, Capovilla G, Connolly MB, French J, Guilhoto L, Hirsch E, Jain S, Mathern GW, Moshé SL, Nordli DR, Perucca E, Tomson T, Wiebe S, Zhang YH, Zuberi SM (2017) ILAE classification of the epilepsies: position paper of the ILAE Commission for Classification and Terminology. Epilepsia. 58(4):512–521
13. Duncan JS, Sander JW (1991) The chalfont seizure severity scale. J Neurol Neurosurg Psychiatry 54(10):873–876
14. Wang KK, Yang Z, Zhu T, Shi Y, Rubenstein R, Tyndall JA et al (2018) An update on diagnostic and prognostic biomarkers for traumatic brain injury. Expert Rev Mol Diagn 18(2):165–180
15. Mondello S, Palmio J, Streeter J, Hayes RL, Peltola J, Jeromin A (2012) Ubiquitincarboxy-terminal hydrolase L1 (UCH-L1) is increased in cerebrospinal fluid and plasma of patients after epileptic seizure. BMC Neurol 12:85
16. Simani L, Elmi M, Asadollahi M (2018) Serum GFAP level: a novel adjunctive diagnostic test in differentiate epileptic seizures from psychogenic attacks. Seizure. 61:41–44
17. Li Y, Wang Z, Zhang B, Zhe X, Wang M, Bai J, Lin T, Zhang S (2013) Cerebrospinal fluid ubiquitin C-terminal hydrolase as a novel marker of neuronal damage after epileptic seizure. Epilepsy Res 103(2–3):205–210
18. Chmielewska N, Maciejak P, Turzyńska D, Sobolewska A, Wisłowska-Stanek A, Kołosowska A et al (2019) The role of UCH-L1, MMP-9, and GFAP as peripheral markers of different susceptibility to seizure development in a preclinical model of epilepsy. J Neuroimmunol 332:57–63
19. Gurnett CA, Landt M, Wong M (2003) Analysis of cerebrospinal fluid glial fibrillary acidic protein after seizures in children. Epilepsia. 44(11):1455–1458
20. Martinian L, Boer K, Middeldorp J, Hol EM, Sisodiya SM, Squier W et al (2009) Expression patterns of glial fibrillary acidic protein (GFAP)-delta in epilepsy-associated lesional pathologies. Neuropathol Appl Neurobiol 35(4):394–405
21. Stringer JL (1996) Repeated seizures increase GFAP and vimentin in the hippocampus. Brain Res 717(1–2):147–153
22. Zhu M, Chen J, Guo H, Ding L, Zhang Y, Xu Y (2018) High mobility group protein B1 (HMGB1) and interleukin-1β as prognostic biomarkers of epilepsy in children. J Child Neurol 33(14): 909–917
23. Deleo F, Thom M, Concha L, Bernasconi A, Bernhardt BC, Bernasconi N (2018) Histological and MRI markers of white matter damage in focal epilepsy. Epilepsy Res 140:29–38
24. Gan ZS, Stein SC, Swanson R, Guan S, Garcia L, Mehta D, Smith DH (2019) Blood biomarkers for traumatic brain injury: a quantitative assessment of diagnostic and prognostic accuracy. Front Neurol 10:446
25. McCormack M, McGinty RN, Zhu X, Slattery L, Heinzen EL, EPIGEN Consortium, Costello DJ et al (2020) De-novo mutations in patients with chronic ultra-refractory epilepsy with onset after age five years. Eur J Med Genet 63(1):103625
26. Atalar AÇ, Vanlı-Yavuz EN, Yılmaz E, Bebek N, Baykan B (2020) Long-term follow-up of a large cohort with focal epilepsy of unknown cause: deciphering their clinical and prognostic characteristics. J Neurol 267(3):838–847