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Review Article

Changes in Gfap and S100 Immunoreactivity in the Temporal Lobe in Pediatric Patients with Drug-Resistant Epilepsy Commoners

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Abstract

Up to 10.5 million children suffer from epilepsy, one-third of whom develop drug-resistant epilepsy (DRE) requiring surgical treatment. In temporal lobe epilepsy, drug resistance reaches 38% of all cases, and patients with this form of the disease have a higher risk of disability and mortality. The European Commission of the International League Against Epilepsy (ILAE) has identified glial mechanisms of seizures and epileptogenesis as a research priority. The purpose of our study was to conduct a comparative analysis of the level of expression of the cytoskeletal protein glial fibrillar acid protein (GFAP) and the protective protein S100 in children with epilepsy associated with focal cortical dysplasia (FCD). Biopsy material from fragments of the temporal lobe of the brain was retrospectively studied at pathology department of Polenov Neurosurgical Institute, obtained intraoperatively from 16 patients (7 girls, 9 boys) with locally caused EEG aged from two to 17 years, with an average age of 9.5 years. Autopsy material from six patients who died from somatic diseases and had no history of neurological disorders was used as a comparison group. Of these, 2 girls and 4 boys aged from 3 to 14 years, with an average age of 8 years, were observed in our study. We observed a significant increase in the expression of GFAP and S100 in the brain tissue of children with FCD when simile to the comparison group. There were no differences in the expression of GFAP and S100 depending on the gender or age of the patients. The correlation between GFAP and S100 proteins was weak in all regions studied. Thus, in the area of the epileptic focus occur in children, active processes of repair of nervous tissue and the mechanisms for increasing the levels of the studied proteins can serve as potential therapeutic targets in DRE therapy, which will prevent secondary neurodegeneration in these patients.

Keywords: drug-resistant epilepsy; focal cortical dysplasia; children; GFAP; S100

Introduction

Epilepsy is a well-known neurological disorder, ranking fourth in terms of prevalence and affecting approximately 65 million people worldwide [18, 20]. It is particularly prevalent among children, with up to 10.5 million cases reported [12]. Despite advances in treatment, epilepsy remains a leading cause of disability-adjusted life expectancy among neurological disorders [3]. While most pediatric patients with epilepsy can lead long and productive lives, there is a small risk of sudden unexpected death in epilepsy (SUDEP) [19]. The overall risk of SUDEP is 0.22/1000 patient-years, or 1 in 4500 children per year [13], which is significantly lower than the incidence in adults (approximately 1 in 1000 per year). However, one third of patients with epilepsy do not respond to antiepileptic drugs (AEDs) and require surgical treatment [37]. This condition is known as drug-resistant epilepsy (DRE) and is defined as inadequate seizure control despite the use of at least two AED regimens

[28]. In certain types of epilepsy, such as temporal lobe epilepsy, the rate of drug resistance can be as high as 38% and has not decreased in recent years [6]. The social and economic burden of chronic DRE is significant, accounting for approximately 80% of the total costs associated with epilepsy [22]. Numerous studies have focused on the role of glia in maintaining and modulating abnormal neuronal activity in epilepsy [22]. Changes in the morphology, biochemistry, and physiology of glia can lead to reactive gliosis, a process that involves a range of physical and chemical changes in glial cells, particularly astrocytes and microglia, in response to various forms of CNS lesions and diseases, including epileptic seizures [30]. A key characteristic of gliosis is the increased expression of certain proteins, such as glial fibrillary acidic protein (GFAP) in astrocytes and the beta subunit of calcium channel binding protein S100 (S100B) [22]. These proteins have been implicated in the pathogenesis of epilepsy.

Despite the growing knowledge of epilepsy, ongoing pharmacological development of 3rd and 4th generation AEDs, and investment in research over the past 15–20 years, there has not been a significant increase in the number of seizure-free patients. Additionally, a proportion of patients still require surgical intervention. Therefore, there is a great need to find new therapeutic targets and further understand the pathophysiology of epilepsy.

Purpose of the study. To study the immunoreactivity of GFAP and S100 in the cortex and white matter of the temporal lobe of the brain in pediatric patients with drug-resistant epilepsy (DRE) associated with focal cortical dysplasia (FCD).

Material and methods

Biopsy material from fragments of the temporal lobe of the brain was retrospectively studied at Polenov Neurosurgical Institute – Branch of Almazov National Medical Research Centre. The material was obtained intraoperatively from 16 patients (7 girls and 9 boys) with locally caused DRE, aged 2 to 17 years, with an average age of 9.5 years. The area of the epileptic focus was determined using magnetic resonance imaging (MRI) according to the "epilepsy" program, positron emission tomography–computed tomography (PET-CT), and electroencephalography (EEG) with invasive monitoring. Autopsy material from 6 patients who died from somatic diseases and had no history of neurological disorders was used as a comparison group. The material was fixed in 10% buffered formalin, dehydrated in a standard manner, and embedded in paraffin. Histological sections were stained with hematoxylin and eosin and studied, as well as the results of immunohistochemical (IHC) reactions with antibodies to GFAP and S100 (antibodies from Dako (USA), EnVision imaging system). Histological analysis and microphotography were performed using a Leica DM2500 M microscope equipped with a DFC320 digital camera and using an IM50 image manager (Leica Microsystems, Wetzlar, Germany). The result of the reaction with antibodies to GFAP was assessed by calculating the densitometric density of stained cells in the cortex and white matter of the cerebral hemispheres in each case (PhotoM program, Russia). The program calculates densitometric density relative to background areas. The densitometric density of all cells within each field of view was calculated. Data are presented in mean and variance format. The result of the reaction with antibodies to S100 was assessed by quantitative counting of stained cells (positive and negative) in the cortex and white matter of the cerebral hemispheres in each case (ImageJ-win32 program). Statistical analysis was carried out using the Statistica v.10 program. Nonparametric statistics methods were used for the analysis. The study was approved by the Ethics Committee of the Almazov National Medical Research Centre (protocol No. 0305–2016 of May 16, 2016) and was carried out in accordance with the Helsinki Declaration of Human Rights. Preoperative examination and surgical treatment of patients were carried out in accordance with the Clinical Guidelines of the Association of Neurosurgeons of Russia 2015.

Results

The histological examination showed that all patients had focal cortical dysplasia (FCD) of various histological subtypes (Fig. 1), according to the 2022 ILAE classification. The most common subtypes were types Ib and IIa, with four cases each. Type I is characterized by the formation of vertical microcolumns (Ia), disturbances in cortical lamination with mixing layers (Ib), or a combination of both (Ic). For FCD type II, the presence of dysmorphic neurons with aggregated Nissl substance (IIa) and balloon cells with abundant glassy cytoplasm and an eccentrically located nucleus (IIb) is pathognomonic. In two cases, type 1 sclerosis of the hippocampus (ILAE, 2013) and associated FCD IIIa were confirmed.

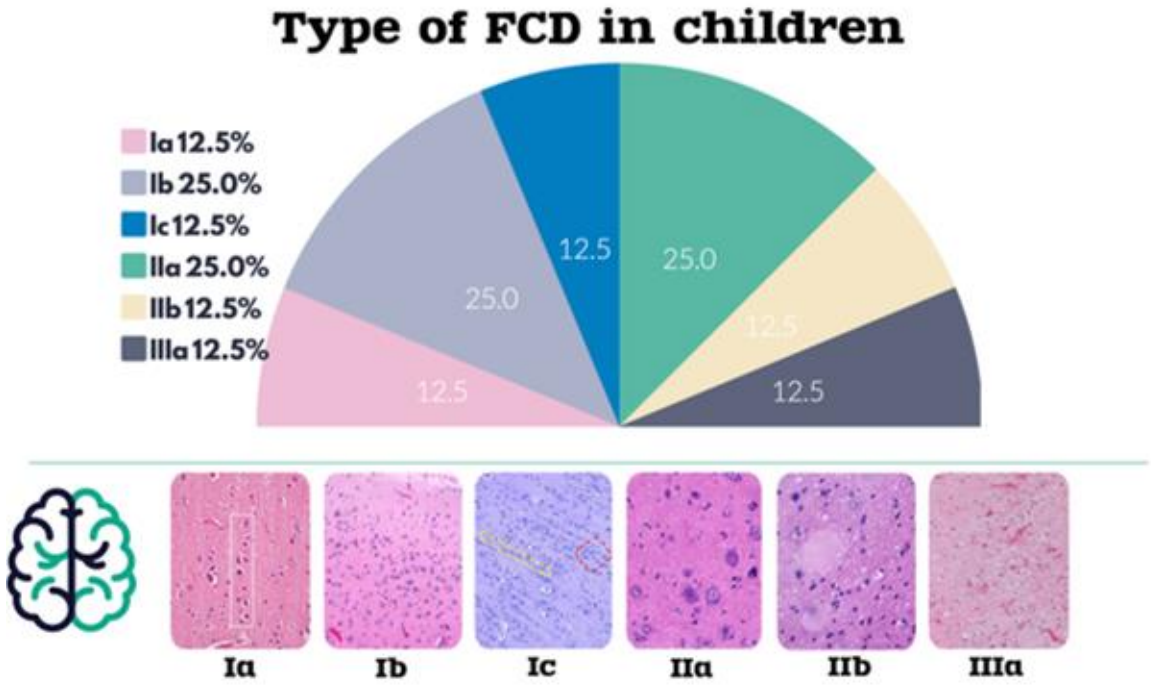
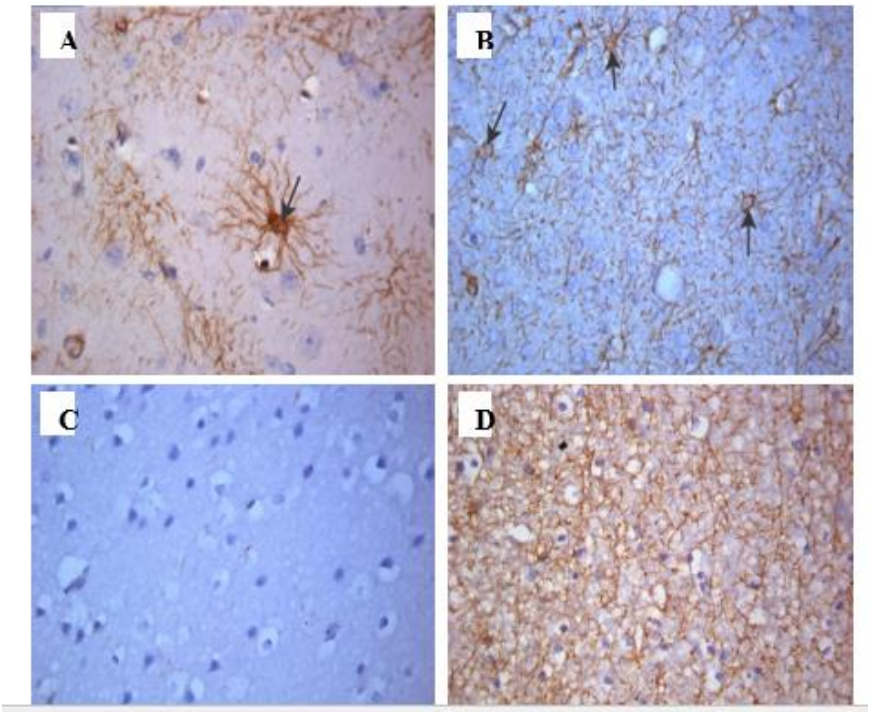


Figure 1: The incidence of FCD types in children. Results of statistical analysis and main morphological criteria (explanations in the text).

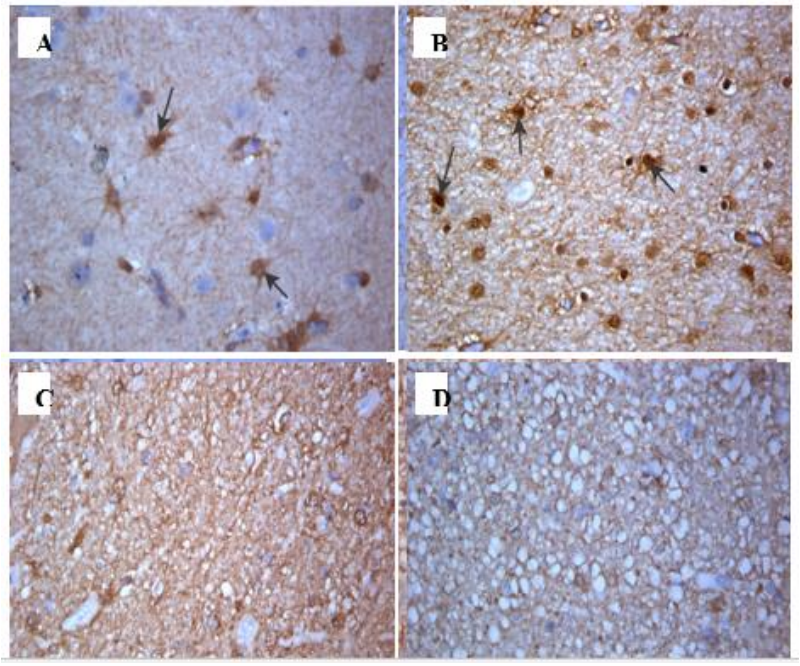
An immunohistochemical study using antibodies to GFAP and S100 showed increased protein expression in the cortex and white matter of the cerebral hemispheres in patients with DRE compared to the comparison group (Figure. 2-3). Increased expression of GFAP was observed in the cytoplasm of cells (Figure. 2, A-B), which had a star-shaped appearance due to the branching of numerous hypertrophied processes. In the comparison group, expression was mainly found in astrocytes of the white matter, with only a few fibers stained in the cortex (Figure. 2, C-D).



Immunohistochemical reaction, ×400
A – Cortex, **B** – White matter of a patient with epilepsy. Reactive astrocytes are indicated by an arrow.
C – Cortex, **D** – White matter of the patient of the comparison group.

Figure 2. GFAP expression in the cortex and white matter of the temporal lobe of the brain of patients with DRE and in the comparison group (description in the text).

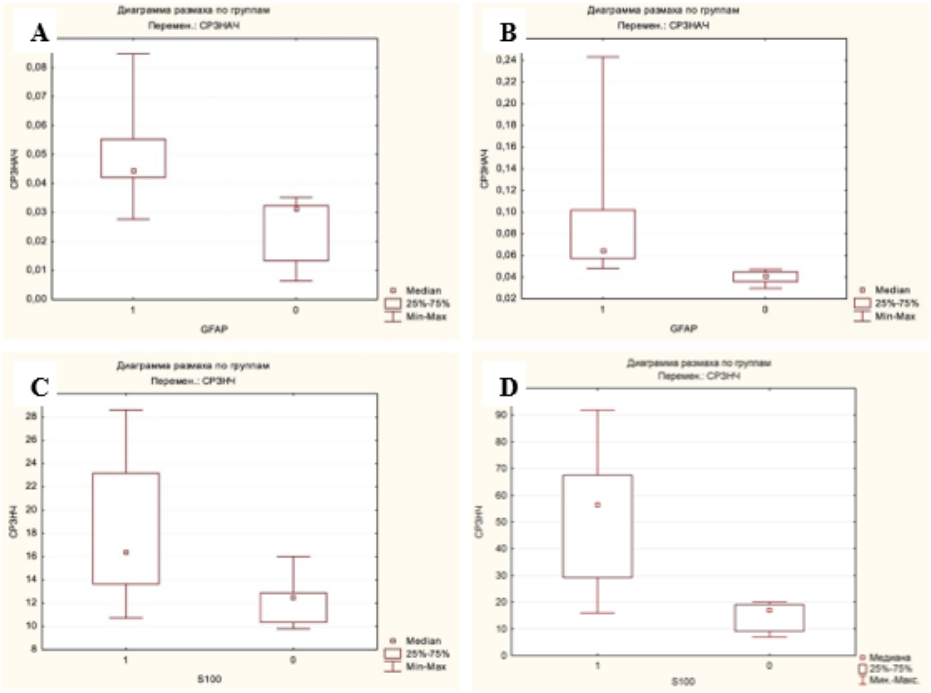
The expression of S100 was predominantly detected in the cytoplasm and nuclei of glial cells (Figure. 3, A-B), as well as in single neurons. In the comparison group, nonspecific background staining was observed, along with expression in the nucleus/cytoplasm of a few cells (Figure. 3, C-D).



Immunohistochemical reaction, ×400
A – Cortex, **B** – White matter of a patient with epilepsy. Stained glial cells are indicated by an arrow.
C – Cortex, **D** – White matter of the patient of the comparison group.

Figure 3: S100 expression in the cortex and white matter of the temporal lobe of the brain of patients with DRE and in the comparison group (description in the text).

Quantitative cell counting in patients with DRE who reacted with antibodies to S100 revealed the following indicators: the number of S100-positive cells (Fig. 4) in the cortex ranged from 4 to 38 (μ = 18±5), and in the white matter it ranged from 11 to 108 (μ = 50±11). In the comparison group, the quantitative count of cells that reacted with the antibody was as follows: in the cortex, 5–25 (μ = 12±3); in the white matter, 4–30 (μ = 15±4). The ratio of reacted and unreacted cells with antibodies to S100 was also determined: in the cortex, K = 0.564, and in the white matter, K = 5.53. In the control group, this coefficient was: in the cortex, K = 0.434, and in the white matter, K = 0.551. When studying the densitometric density of stained cells that reacted with antibodies to GFAP, patients with DRE showed the following results (Fig. 4): in the cortex, the range was 0.0214–0.16 (μ = 0.048±0.014), and in the white matter, it was 0.0306–0.801 (μ = 0.087±0.065). In patients in the comparison group, the densitometric density of GFAP was as follows: in the cortex, 0–0.095 (μ = 0.025±0.015); in the white matter, 0.011–0.106 (μ = 0.04±0.0224).



A – GFAP in the cortex; B – GFAP in white matter; C – S100 in the cortex; D – S100 in white matter (explanation in the text).

Figure 4. Scope plot of GFAP and S100 expression data in the cortex and white matter of the temporal lobe of the brain, where 1 – patients with DRE, 0 – patients in the comparison group.

Based on the results of statistical analysis using the Mann-Whitney, Kolmogorov-Smirnov, and Wald-Wolfowitz criteria ($p<0.05$), a significant difference in the expression of GFAP and S100 was observed between patients with DRE and the control group in all areas studied (see Table 1).

Cortex			
Protein	Descriptive statistics: **μ**		*p*-value
	Patients	Comparison group	
GFAP	0,05	0,025	0,001
S100	18	12	0,014
White matter			
Protein	Descriptive statistics: **μ**		*p*-value
	Пациенты	Comparison group	
GFAP	0,09	0,04	0,0004
S100	51	15	0,001

Note: μ – arithmetic mean;

p-value – calculated level of significance.

Table 1: Comparative characteristics of protein expression values in the epileptic focus in patients with DRE and the comparison group (Mann-Whitney U-test).

However, there were no significant differences in protein expression based on the patients' gender and age.

Further analysis using the Spearman correlation coefficient and the Chaddock correlation coefficient scale to assess the strength of the relationship between the studied proteins in the cortex and white matter of the temporal lobe of the brain revealed a weak correlation between GFAP and S100 in all areas (see Table 2).

Protein/region	S100 cortex	S100 wm	GFAP cortex	GFAP wm
S100 cortex		-0,34	0,26	0,38
S100 wm	-0,34		0,06	-0,14
GFAP cortex	0,26	0,06		-0,11
GFAP wm	0,38	-0,14	-0,11	

Table №2: Values of the Spearman correlation coefficient for the studied proteins GFAP and S100 in the cortex and white matter (wm) of the temporal lobe of the brain.

This suggests that the interaction between these proteins should not be considered. Additionally, no correlation was found between changes in protein expression and the type of FCD, which may be due to the small sample size.

Discussion

In 1957, Crome first described a form of "ulegyria" with "nerve cells with thick, tortuous processes" [8]. In 1971, David Taylor coined the term "focal cortical dysplasia" based on irregular dysmorphic neurons and enlarged bloated cells in the setting of microscopically discernible architectural disorganization of the neocortex in patients with focal epilepsy [33]. Since then, focal cortical dysplasia (FCD) has been associated with medically incurable [21] epilepsy, which has a less favorable prognosis for seizure-free outcome after surgical resection than hippocampal sclerosis and developmental brain tumors [4]. GFAP is an intermediate filament protein classified as type III, along with vimentin (expressed in many cell types), desmin (in skeletal and cardiac muscle), and peripherin (in neurons). Intermediate filaments are key components of the cytoplasmic cytoskeleton that perform various functions, including providing structural support, scaffolding for enzymes and organelles, and mechanosensory perception of the extracellular environment. During development, GFAP initially appears in radial glia, which are the precursors of astrocytes and neurons. The subsequent increase in GFAP expression as astrocytes differentiate is often considered a defining feature of astrocyte maturation. The highest level of GFAP in normal brain tissue is detected in subpial astrocytes and white matter astrocytes [16]. GFAP-expressing astrocytes play a critical role in responding to neuronal injury by undergoing reactive astrogliosis, characterized by cellular hypertrophy, astrocyte proliferation, and increased GFAP expression. This reaction ultimately leads to the formation of a glial scar, which serves to protect healthy cells from potential damage caused by harmful substances [10, 36]. Astrocytes play a crucial role in various functions, such as providing energy to neurons through the astrocyte-neuron lactate shuttle [29]. They also regulate Ca^{2+} influx, which affects neuronal activity by releasing gliotransmitters [23]. In our study, we observed a significant increase in GFAP expression in the brain tissue of children with FCD compared to the control group. There were no differences in GFAP expression based on the gender and age of the patients. This suggests that the reactive production of GFAP by astrocytes is not specific to children, regardless of their age and the maturity of their brain tissue. The formation of a glial scar, resulting from the activation of astrocytes, can have an epileptogenic effect, both directly and indirectly, through the subsequent action of cytokines on astrocytes [25]. Reactive astrocytes also disrupt their normal homeostatic functions, such as potassium ion uptake, ion buffering, calcium signaling, and excitatory neurotransmitter uptake [27]. S100β is an acidic zinc (Zn^{2+}) and calcium (Ca^{2+}) binding protein found in the nucleus and cytoplasm of a wide range of cells [15]. In the nervous system, S100β is found in astrocytes, oligodendrocytes, Schwann cells, ependymal cells, and single populations of neurons [17]. S100 proteins regulate a wide range of cellular activities, including the cell cycle [5], cell differentiation and survival [2, 24], apoptosis [35], cell motility [7], membrane-cytoskeleton interaction [9], intracellular Ca2+ homeostasis [26], and more. S100β can also enter the bloodstream and is one of the most studied serum biomarkers used to analyze brain damage [34]. Additionally, S100β plays a role in modulating glial-neuronal interactions, promoting brain development and synaptic transmission, potentially through G protein-coupled receptor (GPCR) [32]. Studies have shown that the S100β protein regulates GFAP activation, tubulin polymerization, and DNA repair [1]. In our study, we observed a significant increase in S100 expression in the brain tissue of children with FCD when compared to the comparison group. There were no differences in S100 expression based on the gender and age of patients. The presence of S100β has been shown to potentially have pro-apoptotic effects by enhancing nitric oxide (NO) expression, which can lead to neuronal and glial cell death and may play a role in the development of epilepsy [31]. Previous studies have demonstrated that inhibiting NO can prevent seizures [14]. While elevated protein levels in epilepsy may initially serve as a protective and adaptive response to focal damage, long-term overexpression can also contribute to glial and neuronal apoptosis, as well as sustained neuroinflammation [35].

Conclusion.

Our study found an increase in the expression of the cytoskeletal protein GFAP and the protective protein S100 in the epileptic focus of children with FCD. However, no correlations were found between their expression levels or the age and gender of patients. This may be due to the small sample size, highlighting the need for further research. These findings suggest active processes of nervous tissue repair in the epileptic focus, and the mechanisms behind the increased levels of these proteins could potentially serve as therapeutic targets in DRE therapy to prevent secondary neurodegeneration in these patients.

Conflict of interest. The author declares no conflict of interest.

Financing. The study was carried out within the framework of State assignment No. 121031000359-3 «Development of new approaches in the diagnosis of mediobasal pharmacoresistant epilepsy based on the histoproteomics of epileptic foci».

Compliance with patient rights and principles of bioethics. All patients (or their representatives) gave written informed consent to participate in the study. The study was approved by the Ethics Committee of the Polenov Neurosurgical Institute – Branch of Almazov National Medical Research Centre St. Petersburg (protocol No. 0305–2016 of May 16, 2016) and was carried out in accordance with the Helsinki Declaration of Human Rights.

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