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Iron Metabolism Disorders and Mechanisms of Ferroptosis in the Pathogenesis of Focal Epilepsy

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Abstract To evaluate alterations in iron metabolism markers and ferroptosis biomarkers in patients with focal epilepsy and determine their association with disease severity. **Materials and Methods:** The study included 85 patients with focal epilepsy and 40 healthy individuals as a control group. Serum levels of iron, ferritin, transferrin, transferrin saturation, hepcidin, ceruloplasmin, as well as ferroptosis biomarkers (GPX4, ACSL4, PTGS2), were measured in all participants. The frequency and severity of epileptic seizures were assessed. **Results:** Compared with the control group, patients with focal epilepsy had significantly lower serum iron levels (12.8±2.1 μmol/L vs. 18.4±1.9 μmol/L, $p<0.001$) and higher ferritin levels (285.7±45.2 ng/mL vs. 156.3±28.7 ng/mL, $p<0.001$). Among ferroptosis biomarkers, GPX4 activity was significantly reduced (0.42±0.08 U/mL vs. 0.78±0.12 U/mL, $p<0.001$), while ACSL4 and PTGS2 levels were increased ($p<0.01$). A negative correlation was found between seizure frequency and iron deficiency ($r = -0.67$, $p<0.001$), as well as GPX4 activity ($r = -0.58$, $p<0.001$). **Conclusion:** In focal epilepsy, disturbances in iron metabolism and activation of ferroptosis processes play an important role in the disease pathogenesis and reveal new potential therapeutic targets.

Keywords Focal epilepsy, Iron metabolism, Ferroptosis, GPX4, Ferritin, Neurodegeneration

1. Introduction

Epilepsy is one of the most common neurological diseases, affecting more than 70 million people worldwide [1]. Focal epilepsy accounts for 60-70% of all cases of epilepsy and develops as a result of structural damage to the brain [2]. Despite significant advances in understanding the mechanisms of epileptogenesis in recent years, the pathophysiology of the disease is still not fully elucidated [3].

Iron is a vital micronutrient for the body and plays a key role in the normal functioning of neurons [4]. Iron is involved in mitochondrial respiration, DNA synthesis, neurotransmitter biosynthesis, and myelination processes [5]. However, excessive iron accumulation can lead to oxidative stress and lipid peroxidation [6].

Ferroptosis is a recently discovered form of iron-dependent cell death characterized by lipid peroxidation and decreased glutathione peroxidase 4 (GPX4) activity [7]. Ferroptosis processes have been identified in various neurological diseases, including Alzheimer's disease, Parkinson's disease, and stroke [8,9]. Recent studies suggest that ferroptosis may also play a role in the pathogenesis of epilepsy [10].

Acyl-CoA synthetase long-chain family member 4 (ACSL4) is a key enzyme in ferroptosis, activating arachidonic acid and providing a substrate for lipid peroxidation [11]. Prostaglandin-endoperoxide synthase 2 (PTGS2) is involved in the process of ferroptosis in the production of lipid peroxidation products [12]. GPX4 is considered the main protective mechanism against ferroptosis [13].

Hepcidin is the main hormone regulating iron homeostasis, controlling iron absorption in the intestine and its release from tissues [14]. Ceruloplasmin is a copper-containing protein involved in the processes of iron transport and oxidation [15].

The aim of the study was to evaluate changes in iron metabolism parameters and biomarkers. ferroptosis in patients with focal epilepsy, to determine their relationship with the severity of the disease and the frequency of seizures.

2. Study Materials and Methods

The study was conducted in 2023-2024 at the Neurology Clinic of the Tashkent Medical Academy using a prospective, controlled design. The study protocol was approved by the Institute's Bioethics Committee (Protocol No. 15/2023). Written consent was obtained from all participants.

The study included 85 patients with focal epilepsy (the study group) and 40 healthy individuals (the control group). Inclusion criteria for the study group were: age 18-65 years, a confirmed diagnosis of focal epilepsy according to the ILAE 2017 criteria, confirmed structural brain damage based on MRI, and at least two seizure episodes in the past six months. Exclusion criteria included psychogenic seizures, severe somatic diseases, pregnancy and lactation, taking iron

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supplements, and episodes of blood loss in the past three months.

All patients underwent a full neurological examination, EEG, and MRI. Seizure frequency was calculated over the past three months and expressed as the number of seizures per month. Disease severity was assessed using the NHS3 scale.

For laboratory testing, 10 ml of fasting blood was collected from a vein in the morning. The following parameters were determined in the serum: iron (colorimetric method), ferritin (immunochemiluminescence method), transferrin (immunoturbidimetric method), transferrin saturation (calculated), hepcidin (ELISA method), and ceruloplasmin (immunoturbidimetric method).

Biomarkers ferroptosis was determined by the ELISA method: GPX4 activity (Cayman Chemical, USA), ACSL4 level (MyBioSource, USA), PTGS2 level (R&D Systems, USA). All analyses were performed according to the manufacturers' recommendations.

Statistical analysis was performed using SPSS 28.0. Normal distribution of the data was assessed using the Shapiro- Wilk test. Differences between groups were assessed using the Student's t-test or the Mann-Whitney U-test. Correlation analysis was performed using the Pearson or Spearman methods. A p value of <0.05 was considered statistically significant.

3. Study Results

The demographic characteristics of the patients included in the study were as follows. In the main group, the average age was 42.3±12.8 years, there were 48 men (56.5%), 37 women (43.5%). In the control group, the average age was 40.7±11.2 years, there were 22 men (55.0%), 18 women (45.0%). No statistical differences in age and gender distribution were found between the groups (p>0.05).

The distribution by etiology of epilepsy was as follows: traumatic brain injury - 28 people (32.9%), consequences of stroke - 22 people (25.9%), brain tumors - 15 people (17.6%), infectious lesions - 12 people (14.1%), other causes - 8 people (9.4%). The duration of the disease was on average 6.8±4.2 years.

Table 1 presents the results of a comparative analysis of iron metabolism parameters between the groups of patients with focal epilepsy and healthy controls. The data demonstrate statistically significant differences in all iron metabolism parameters studied.

As shown in Table 1, in patients with focal epilepsy, compared with the control group, serum iron levels were significantly lower by 30.4%, indicating the development of iron deficiency. Concurrently, a significant increase in ferritin levels by 82.8% was observed, indicating iron accumulation in depots during its deficiency in circulation. Transferrin levels were elevated by 31.0%, which is a compensatory response to iron deficiency. Transferrin saturation was reduced by 35.9%, confirming impaired iron transport. Hepcidin concentrations were increased by 63.2%, reflecting the activation of iron metabolism regulatory mechanisms. Ceruloplasmin levels exceeded control values by 21.6%, indicating the development of oxidative stress.

Table 1. Comparative characteristics of iron metabolism indicators in the study groups

Indicator	Focal epilepsy (n=85)	Control group (n=40)	t-test	p
Iron (μmol /l)	12.8±2.1	18.4±1.9	-15.42	<0.001
Ferritin (ng /ml)	285.7±45.2	156.3±28.7	17.89	<0.001
Transferrin (g/L)	3.8±0.6	2.9±0.4	9.12	<0.001
Transferrin saturation (%)	18.4±3.2	28.7±4.1	-15.23	<0.001
Hepcidin (ng /ml)	145.6±28.4	89.2±15.7	12.67	<0.001
Ceruloplasmin (mg/ dl)	42.8±7.3	35.2±5.9	6.18	<0.001

Table 2. Comparative characteristics of biomarkers ferroptosis in the study groups

Biomarker	Focal epilepsy (n=85)	Control group (n=40)	t-test	p
GPX4 activity (U/ml)	0.42±0.08	0.78±0.12	-18.94	<0.001
ACSL4 (ng /ml)	2.84±0.47	1.56±0.32	16.42	<0.001
PTGS2 (pg /ml)	485.7±78.3	298.4±52.6	14.28	<0.001

Table 3. Dependence of biochemical parameters on the frequency of epileptic seizures

Frequency of attacks	Number of patients	Iron (μmol /l)	GPX4 (U/ml)	ACSL4 (ng /ml)
1-2 per month (n=32)	32 (37.6%)	15.2±1.8	0.52±0.06	2.18±0.35
3-5 per month (n=28)	28 (32.9%)	12.4±1.9	0.41±0.07	2.76±0.42
6 or more per month (n=25)	25 (29.4%)	9.8±2.2	0.31±0.08	3.52±0.58
p	-	<0.001	<0.001	<0.001

Table 2 presents the results of the biomarker analysis. ferroptosis, demonstrating significant changes in the system of regulation of iron-dependent cell death in patients with focal epilepsy.

Analysis of the data in Table 2 reveals significant disturbances in the ferroptotic system in patients with focal epilepsy. The activity of GPX4, the main protective enzyme against ferroptosis, was reduced by 46.2% compared to the control group, indicating weakened antioxidant defenses and increased susceptibility to lipid peroxidation. The level of ACSL4, a key enzyme initiating ferroptosis, was increased by 82.1%, indicating activation of iron-dependent cell death processes. The concentration of PTGS2 exceeded control values by 62.8%, confirming increased inflammatory processes and lipid peroxidation.

Table 3 demonstrates the dependence of changes in key biochemical parameters on the frequency of epileptic seizures, which allows us to assess the progression of pathological processes depending on the severity of the disease.

The data in Table 3 reveal a clear pattern of deterioration in biochemical parameters with increasing seizure frequency. In patients with the most frequent seizures (6 or more per month), the iron level was 35.5% lower compared to the group with rare seizures (1-2 per month). GPX4 activity progressively decreased: from 0.52±0.06 U/ml with rare seizures to 0.31±0.08 U/ml with frequent seizures, which is a decrease of 40.4%. The ACSL4 level demonstrated the opposite dynamics, increasing from 2.18±0.35 ng /ml to 3.52±0.58 ng /ml, which is an increase of 61.5%.

Table 4 presents the results of the correlation analysis between the frequency of epileptic seizures and the main biochemical parameters, which allows us to assess the strength and direction of the relationship between clinical manifestations and laboratory data.

Table 4. Correlations between seizure frequency and biochemical parameters

Indicator	Correlation coefficient (r)	p
Iron	-0.67	<0.001
Ferritin	+0.58	<0.001
GPX4 Activity	-0.58	<0.001
ACSL4	+0.62	<0.001
PTGS2	+0.55	<0.001
Hepcidin	+0.49	<0.001

Correlation analysis presented in Table 4 revealed statistically significant relationships between all studied parameters and attack frequency. The strongest negative correlation was found between attack frequency and iron levels (r=-0.67), indicating a direct link between disease severity and the extent of iron deficiency. GPX4 activity also demonstrated a strong negative correlation (r=-0.58), confirming the link between attack frequency and decreased antioxidant defense. Among the parameters with a positive correlation, the most pronounced association was found for ACSL4 (r=+0.62), indicating increased ferroptosis with increasing attack frequency.

Table 5 illustrates the influence of chronicity of the epileptic process on changes in key biochemical parameters, demonstrating the progressive nature of iron metabolism disorders and activation of ferroptosis.

Analysis of the data in Table 5 demonstrates a progressive deterioration in biochemical parameters with increasing disease duration. In patients with a disease duration of 8 years or more, iron levels were 25.3% lower compared to those in the group with a disease duration of 1-3 years. GPX4 activity showed a similar decrease of 27.1% in the group with the longest disease duration. Ferritin levels demonstrated the opposite trend, increasing from 245.3±38.2 ng /ml in the short-duration group to 325.4±51.8 ng /ml in the long-duration group, representing an increase of 32.7%. These data indicate a progressive nature of iron metabolism disorders and the activation of ferroptosis processes during the chronicization of the epileptic process.

4. Discussion

The obtained results revealed disturbances in iron metabolism and activation of ferroptosis processes in focal epilepsy. Iron deficiency and elevated ferritin levels observed in patients indicate iron accumulation in tissues and a decrease in serum iron. This condition may be associated with neuronal damage and inflammatory processes resulting from epileptic seizures. hepcidin levels reflect the body's attempt to restore iron homeostasis. Hepcidin limits iron absorption in the intestine and reduces its release from macrophages, resulting in serum iron deficiency. Elevated ceruloplasmin levels may be understood as a response to oxidative stress.

A significant decrease in GPX4 activity indicates activation of ferroptosis. GPX4 is the primary enzyme that reduces lipid peroxides, and decreased activity leads to damage to cell membranes. Increased levels of ACSL4 and PTGS2 confirm activation of ferroptosis.

Table 5. Dynamics of biochemical parameters depending on the duration of the disease

Duration of the disease	Number of patients	Iron (μmol /l)	GPX4 (U/ml)	Ferritin (ng /ml)
1-3 years (n=28)	28 (32.9%)	14.6±2.0	0.48±0.07	245.3±38.2
4-7 years (n=31)	31 (36.5%)	12.8±1.8	0.42±0.08	285.7±42.6
8 years and older (n=26)	26 (30.6%)	10.9±2.3	0.35±0.09	325.4±51.8
p	-	<0.001	<0.001	<0.001

Strong correlation between seizure frequency and iron deficiency biomarkers Ferroptosis studies show that these processes play an important role in epileptogenesis. Iron deficiency can disrupt neurotransmitter synthesis and increase neuronal excitability.

The progression of iron metabolism disorders with increasing disease duration indicates the development of neurodegenerative processes in chronic epilepsy. These data highlight the importance of correcting iron metabolism in the early stages.

Recent studies have demonstrated the role of ferroptosis in various neurological diseases. For example, Zhang et al. (2023) found activation of ferroptosis in Alzheimer's disease. Liu et al. (2022) demonstrated decreased GPX4 activity in stroke models.

Our results are consistent with those obtained by Wang et al. (2023) in animal models of epilepsy. They also found decreased GPX4 activity and increased ACSL4 levels.

Limitations of the study include the relatively small sample size and cross-sectional design. Long-term observational studies and evaluation of the effectiveness of therapeutic interventions are needed in the future.

5. Conclusions

Impaired iron metabolism and activated ferroptosis were detected in patients with focal epilepsy. Serum iron deficiency, elevated ferritin and hepcidin levels, decreased GPX4 activity, and elevated ACSL4 and PTGS2 levels were observed. These changes correlated with seizure frequency and disease duration. These results demonstrate the important role of iron metabolism and ferroptosis in the pathogenesis of focal epilepsy and provide a basis for the development of new therapeutic strategies. Correction of iron metabolism and prevention of ferroptosis may provide new avenues for epilepsy treatment.

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