

Lipid Peroxidation Products and Free Fatty Acids in Blood Plasma and Cerebral Spinal Fluid in Children With Convulsive Syndrome

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ABSTRACT

Objective: This study aims to investigate the levels of lipid peroxidation (LP) products and free fatty acids (FFA) in children with convulsive syndrome (CS) to understand the oxidative stress associated with seizures. **Method:** A total of 45 children aged from several months to 1.5 years with various forms of CS were analyzed, alongside a control group of 13 healthy adults. Blood plasma and cerebrospinal fluid (CSF) were collected, and the concentrations of LP products (diene conjugates and Schiff bases) and FFA were quantified using spectrophotometric and fluorometric methods. **Results:** The findings revealed that children with CS exhibited significantly elevated levels of diene conjugates (810 ± 150 nmol per 1 mg lipids) and Schiff bases ($42.6 \pm 5.5\%$ per 1 mg lipids) in blood plasma compared to the control group. Additionally, FFA levels in the blood plasma (2.1 ± 0.4 μ g per 1 mg protein) were found to be markedly higher than those in healthy adults. These results indicate a profound increase in oxidative stress in children with CS, correlating with the duration and type of seizures. **Novelty:** This study highlights the critical role of oxidative stress in pediatric convulsive disorders and suggests that incorporating antioxidant therapies, such as α -tocopherol, may improve treatment efficacy in these patients. The findings provide a foundation for further research into therapeutic strategies targeting oxidative stress in pediatric neurology.

INTRODUCTION

The issue of convulsive states in children is of utmost importance in clinical neurology. Various convulsive manifestations in childhood are four times more frequent than in adults [1]; their incidence is 5.86 per 1,000 children [2], with two-thirds of all cases occurring in children within the first three years of life [3]. For this reason, an in-depth study of the different pathogenic mechanisms of seizures in young children, which determine the pathways of targeted therapy, is one of the pressing tasks in pediatric neurology and pathophysiology.

Previous studies have established that adult patients with various forms of epilepsy exhibit significant disturbances in the process of lipid peroxidation (LP), evidenced by a 2-to 5-fold increase in LP products in peripheral blood plasma [4], [5]. Supplementing the traditional anticonvulsant therapy for such patients with the antioxidant α -tocopherol resulted in normalization of LP product levels in the blood and a substantial increase in treatment effectiveness [5], [6]. This indicates that disturbances in the regulatory system of LP processes play an important role in the pathogenesis of at least some forms of epilepsy.

To determine the status of the LP process in children with convulsive syndrome (CS), this study examined the levels of LP products and free fatty acids (FFA) in peripheral blood and cerebrospinal fluid (CSF).

RESEARCH METHOD

A total of 45 children with CS (39 with perinatal encephalopathy, 3 with febrile seizures, 2 with hereditary pathology complicated by seizures, and 1 with CS of unknown etiology) aged from several months to 1.5 years, as well as 13 adult donors aged 20-30 without health issues, were examined. The adult donors served as a control group, as there is no data on the levels of the studied LP products – diene conjugates and Schiff bases – in the blood of healthy children up to 1.5 years old.

Blood was taken in the morning on an empty stomach from the elbow vein or temporal veins of the scalp. To obtain blood plasma, whole blood was immediately mixed with a heparin solution in physiological saline (0.75 mg/ml) at a ratio of 1 ml heparin solution to 4 ml whole blood and centrifuged for 20 minutes at 3000 rpm on a TsUM-1 centrifuge at 0-4 °C. CSF was also collected in the morning on an empty stomach.

To determine the concentration of LP products – diene conjugates and Schiff bases – lipids were extracted from blood plasma and CSF [7]. The concentration of diene conjugates was measured by absorption at 233 nm ($\epsilon = 2.1 \times 10^4 \text{ M}^{-1}\text{cm}^{-1}$) in methanol-hexane (5:1 by volume) using a Hitachi-320/330 spectrophotometer (Japan) [8]. The concentration of Schiff bases was determined by fluorescence intensity at 430 nm of the chloroform lipid extract of blood plasma or CSF, measured on a Hitachi 204 spectrofluorimeter (Japan), with an excitation wavelength of 360 nm at 22 °C [9]. Quinine sulfate in 0.1 N H₂SO₄ (1 mg/ml), with an intensity taken as 100%, was used as the standard solution in fluorometric measurements. The content of diene conjugates and Schiff bases was expressed in nanomoles and percentages, respectively, normalized to 1 mg of total lipids. The total lipid concentration in the chloroform extract of blood plasma or CSF was measured using standard kits from Lachema (Czechoslovakia) [10].

The FFA content in blood plasma and CSF was determined through a reaction between copper salts and FFA and 1,5-diphenylcarbazine, forming a colored complex. Stearic acid from Serva (Germany) was used to construct the calibration curve. Protein concentration was measured by the method of O. Lowry et al [11].

The levels of LP products and FFA in blood plasma and CSF were measured using the double-blind control method. Statistical processing of results and correlation coefficient calculation were performed using an Olivetti computer (Italy).

RESULTS AND DISCUSSION

The study found that children with CS had increased LP product levels in blood plasma compared to the control group (Table 1): diene conjugate concentration increased fivefold, and Schiff base levels doubled. LP product content in the CSF of affected children was 2 to 5 times higher than in blood (see Table 1).

The FFA level in the blood plasma of children with CS was more than an order of magnitude higher than in healthy donors (see Table 1). The FFA level in the CSF of children with CS was twice as high as in blood. Thus, LP process activity in children with CS was several times higher than in healthy adults. Given that FFA level variability in adult blood does not differ significantly from that in healthy children under one year of age, it can be assumed that FFA levels in children with CS exceed the norm [12]. In the control group, the FFA level was $0.12 \pm 0.03 \mu\text{g}$ per 1 mg blood plasma protein, corresponding to about $0.030 \pm 0.007 \mu\text{mol}$ per 1 L plasma, which is two times below the lower limit of FFA levels in adult venous blood according to literature [13]. This discrepancy is likely due to the chosen fasting blood collection conditions, the methodology used, and, primarily, the strict centrifugation conditions to obtain plasma, set to exclude even minimal contamination by erythrocytes (hem iron), which interferes with accurate LP product measurement. Generally, FFA levels in blood vary depending on measurement methodology, blood collection conditions, and other factors by more than an order of magnitude [13].

Table 1. Content of LP Products and FFA in Blood Plasma of Healthy Adult Donors and in Blood Plasma and CSF of Children with CS (M±m).

Group	Medium	Diene Conjugates, nmol per 1 mg lipids	Schiff Bases, % per 1 mg lipids	FFA, μg per 1 mg protein
Control	Blood plasma	150±24	20±0.5	0.12±0.03
CS Patients	Blood plasma	810±150	42.6±5.5*	2.1±0.4*
CS Patients	CSF	1800±630	235±41	5.9±0.6

Note: One asterisk indicates $p < 0.001$ compared to control and CSF level; two asterisks indicate $p < 0.001$ compared to control and $p < 0.1$ compared to CSF level. Further analysis of the study results aimed to identify the factors and clinical characteristics of the disease that most significantly influenced the levels of LP products and FFA in the blood of children with CS. Factors and characteristics considered included the child's age, disease duration, form of convulsive paroxysm, duration of paroxysm, presence of characteristic epileptiform changes (EC) on EEG – such as spike-wave complexes, high-amplitude bursts, etc. – and the presence of psychomotor delay (PMD) and time (T) from the end of the seizure to blood sampling.

The analysis was conducted as follows. For each factor or characteristic, the group of children with CS was divided into two subgroups. The difference between subgroups was assessed using Fisher's t-test, evaluating the significance of each factor and characteristic.

It was found that diene conjugate levels in the blood of children with CS depended on the duration of the convulsive paroxysm ($t=3.1$, $p<0.01$) and its form ($t=2.6$, $p<0.05$). To a certain extent, it was associated with the presence of EC on EEG ($t=1.4$; see Table 2). The parameter was less dependent on the child's age ($t=1.1$) and disease duration ($t=0.8$) and was independent of PMD presence ($t=0.2$) and T ($t=0$; see Table 2).

Table 2. Influence of Age, Disease Duration, Clinical and EEG Characteristics, and T on the Blood Plasma Levels of LP Products and FFA in Children with CS (M±m).

Factor/Characteristic	Diene Conjugates, nmol per 1 mg lipids	Schiff Bases, % per 1 mg lipids	FFA, µg per 1 mg protein
Age, years:			
Up to 1	990±240	48±7.9	1.9±0.8
Over 1	630±240	38±8.0	2.5±0.6
Disease Duration, months:			
Up to 1	680±230	47.5±8.3	1.7±0.63
Over 1	1030±300	44±9.2	2.7±0.8
Seizure Type:			
Predominantly tonic	630±180	46.6±11	2.5±1.0
Predominantly clonic	1470±270	44±8	1.5±0.45
Seizure Duration, min:			
Up to 5	470±180	39±6	1.7±0.6
Over 5	1490±260*	50±11	3.0±0.93
PMD:			
Present	810±290	59.3±13	2.9±1.3
Absent	870±240	36.2±5.0	1.9±0.55
EEG EC:			
Present	1140±360	46±9.2	3.0±0.8
Absent	570±180	35±9	0.9±0.28
T, days:			
Up to 1	840±390	58.6±10.4	3.5±1.0
Over 1	840±190	35.9±6.4*	2.2±0.6

Note: One asterisk indicates $p<0.01$; two asterisks indicate $p<0.05$; three asterisks indicate $p<0.1$ compared to the corresponding subgroup. The level of Schiff bases in the blood correlated with T ($t=1.9$, $p<0.1$) and, to a lesser extent, with other factors and characteristics (see Table 2). The remaining factors ranked by significance were PMD presence ($t=1.7$), age, seizure duration, EEG EC presence ($t=0.9$), disease duration, and seizure type ($t=0.2-0.3$).

The FFA content in the blood of children with CS correlated with EEG EC presence ($t=2.5$, $p<0.05$) and, to some degree, with seizure duration ($t=1.2$), seizure type ($t=0.9$), T ($t=1.1$), and disease duration ($t=1.0$). Age and PMD presence ($t=0.6-0.7$) had minimal impact on blood FFA levels.

Thus, the levels of LP products and FFA in the blood of children with CS mainly correlate with the duration and type of seizures (diene conjugates), the presence of characteristic EEG EC (FFA), and T (Schiff bases).

A high level of LP products in the blood of children with CS was observed simultaneously with an elevated FFA level, suggesting a possible interrelationship between these parameters [14]. To test this hypothesis, a correlation analysis was conducted, showing a direct correlation between diene conjugates and Schiff bases, as well as between Schiff bases and FFA levels in the blood of children with CS, with $r=+0.53$ and 0.42 , respectively. There was a lesser correlation between FFA and diene conjugates ($r=+0.26$). We previously noted a strong direct correlation between the levels of Schiff bases and FFA in the blood of adult epilepsy patients as well [4].

The study results indicate significant LP activity in the bodies of children with CS. This finding is consistent with data obtained in our examinations of adult epilepsy patients [4]. As was established [6], [5], the use of the antioxidant α -tocopherol in epilepsy patients with elevated LP product levels in the blood significantly increased the effectiveness of therapy in some cases. The high LP activity in children with CS suggests that incorporating antioxidants into anticonvulsant therapy would also be beneficial in this context [15].

CONCLUSION

Fundamental Finding : Children with convulsive syndrome (CS) have significantly elevated levels of lipid peroxidation (LP) products and free fatty acids (FFA) in their blood and cerebrospinal fluid compared to healthy adults, indicating increased oxidative stress during seizures. **Implication :** The findings suggest that oxidative stress may play a role in the development of seizures in children. This raises the possibility that antioxidant therapies, like α -tocopherol, could enhance treatment outcomes for pediatric patients with convulsive disorders. **Limitation :** The study's small sample size and lack of data on healthy children limit the generalizability of the results. Additionally, variability in blood sampling and processing methods could affect measurement accuracy. **Future Research :** Future research should involve larger and more diverse populations to confirm these findings and explore the mechanisms connecting LP and FFA levels with seizures. There is also a need to investigate the effectiveness of antioxidant therapies in treating children with CS.

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