

Pathogenetic Features Of Immuno-Biochemical Shifts In Children With Idiopathic Epilepsy

MadjidovaEkutkhonNabievna, AbdullaevaMuazzamkhonIlkhomeidinovna, MadjidovaNailya

Tashkent Pediatric Medical Institute

Andijan State Medical Institute

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Abstract: The data obtained as a result of the studies carried out indicate the heterogeneity of patients with epilepsy from the point of view of molecular mechanisms, the development of the causes of epileptic seizures. One of the leading mechanisms of the pathogenesis of idiopathic epilepsy is a complex restructuring of neuroimmune relationships, manifested by a unidirectional increase in the level of autoantibodies to neurospecific proteins S100, GFAP, NF-200, MBP and the neurotransmitters glutamate, GABA, dopamine, serotonin and serotonin-channel. At the same time, the key link in the pathogenesis of idiopathic epilepsy is neurotransmitter imbalance. Circulating AATs to neurotropic proteins and neurotransmitter receptors in the blood serum of patients with epilepsy can be used as additional prognostic "immuno-biochemical" criteria for the course of the disease and the effectiveness of antiepileptic treatment.

Keywords: idiopathic epilepsy, neuroetiopathogenesis, autoantibodies to neurospecific proteins and neurotransmitters, children

1. INTRODUCTION

Epilepsy is one of the most common diseases of the nervous system in children and adolescents. The incidence of epilepsy in the population is high and reaches 0.5 - 0.8%, and among the child population - up to 1.0%. The true incidence of this disease can only be higher. Epilepsy is also a common pathology among the causes of neurological disability. In this regard, treatment, rehabilitation, social adaptation of patients with epilepsy is an extremely urgent task [8, 13]. In idiopathic forms of epilepsy, the genetic determinism of the disease is implied. Idiopathic epilepsy (IE) is a common form of epilepsy in children and adolescents, accounting for up to 143 cases of the disease [3, 5]. IE is currently defined as a form of generalized epilepsy in which all seizures are generalized from the very beginning; in the neurological status, there are usually no focal symptoms and signs of a decrease in intelligence; EEG patterns are primarily generalized, bilateral and synchronous; in neuroradiological examination, there are no gross structural changes in the brain [4, 8].

A new impetus to the study of the problem of immunopathogenesis of epilepsy should be formed in the last decade, the idea of the inextricable unity of the function of the two main integrative systems of the body: the central nervous and immune. On the basis of information about neuroimmune interaction, new scientific disciplines have taken shape - neuroimmunology and neuroimmunopathology [2, 5]. One of the intriguing problems of neuroimmunopathology is the immunological aspects of the pathogenesis of epilepsy. However, to date, no clear evidence has been obtained for the mandatory participation of immunological factors in the pathogenesis of various forms of epilepsy [9, 11].

In recent years, much attention has been paid to the study of the effect of an imbalance in the mediator metabolism, namely, the excitatory neurotransmitter glutamate and inhibitory GABA, on the severity of the course of the epileptic process. In this case, glutamate is given a more significant role in the processes of excitation and formation of an epileptogenic focus [3, 14, 15]. Immunological studies of recent years concern mainly the production of autoantibodies (aAT) to glutamate AMPA receptors of nerve cells in epilepsy; the expediency of determining the level of these serum aATs for the diagnosis and study of the pathogenesis of this disease has been shown [3, 4, 8, 14, 15]. However, the question of the possible role of aAT to glutamate AMPA receptors in the prognosis of the course of the disease and, especially importantly, in the development of pharmacoresistance is still debated [4, 6, 7].

Recent studies have revealed a change in the level of serum primary (idiotypic) aATs and their "functional counterweights" - antiidiotypic aATs (AIAT) to a number of neurospecific brain antigens in patients with epilepsy, indicating the possibility of neuroglia damage [1, 9, 16]. The study of aAT to gliospecific proteins is important for understanding the mechanisms of damage in epilepsy to astrocytic glia and the blood-brain barrier (BBB), as well as improving methods of treating this disease [4, 14]. However, the pathogenetic role and diagnostic significance of aAT to brain proteins and neurotransmitter receptors require further research.

The aim is to study immuno-biochemical changes in children with idiopathic epilepsy using the ELI-N-Test method (Poletaev A.B. et al., 2007) by determining serum immunoreactivity to proteins S100, GFAP, MBP, NF200 and neurotransmitters, glutamate, GABA, dopamine, serotonin, choline and voltage-dependent Ca-channel.

2. MATERIALS AND METHODS OF RESEARCH:

36 children with idiopathic epilepsy aged 5 to 16 years (22 boys; 14 girls) were examined. All patients underwent a careful preliminary history and clinical selection, which was carried out by the method of stratified randomization using inclusion and exclusion criteria.

Criteria for the inclusion of patients in the study: children and adolescents under 16 years of age, epileptic seizures at the time of hospitalization or in history, idiopathic epilepsy.

Exclusion criteria: adolescents over 16 years of age and adults, cryptogenic epilepsy, pseudoepileptic seizures, psychogenic reactions, conversion seizures (hysteria).

The scope of the study included: clinical neurological examination, examination of somatic status, neuropsychological testing of higher cortical functions, neurophysiological

examination (EEG), laboratory techniques (clinical blood analysis, blood biochemistry, analysis of the immune status), neuroradiological research methods (MRI or CT of the brain). The type of seizures was determined according to the International Classification of Epileptic Seizures (1981). Epilepsy was diagnosed according to the International Classification of Epilepsy (ILAE 1989, 2007) [11,12].

The quantitative determination of the serum immunoreactivity of antibodies (AT1 and AIAT2) to neurotransmitter receptors (glutamate, GABA, dopamine, serotonin and cholinergic receptors) was carried out using the ELI-N-Test and test kits of the same name, produced by MITS "Immunculus" (Russia). The norm was taken as the values of aAT immunoreactivity from 80 to 140 CU, the AT1 / AIAT2 immunoreactivity index from 0.8 to 1.2 [9, 16]. The results of the study were compared with the data of the control group, which included 16 clinically healthy children and adolescents (6 boys, 10 girls aged 5 to 16). The analysis of the obtained indicators was carried out using the software package "SPSS for Windows" and "STATISTICA" Microsoft Excel with the processing of the material using the methods of variation statistics. The reliability of the results obtained was assessed by the paired method according to the Student's t-test. Differences were considered significant at $p < 0.05$.

3. RESULTS OF THE STUDY:

In the structure of epileptic seizures, absence seizures at the age from 5 to 7 years prevailed, while generalized tonic-clonic seizures in the age group of 8-12 years. Among them, absence seizures were observed in 5 patients (13.9%), tonic-clonic - in 26 patients (72.2%), miclonic - in 5 patients (13.9%).

When evaluating the results of the immunological study, it was found that the indicators of children with IE differed from the control group both in the level and in the degree of spread of the studied immunological indicators. The level of serum autoantibodies to neurotropic proteins in sick children with idiopathic epilepsy was sharply increased from 9.5 to 47.8 times ($P < 0.001$). NF200 is a specific axon protein, the growth of antibodies to it accompanies the processes of nerve fiber degeneration, which is observed in the examined children with IE (30.1 ± 4.6 versus 0.63 ± 0.11). An increase in the content of the specific protein of astrocyte filaments (GFAP) by 9.5 times in IE (11.5 ± 8.8 versus 1.21 ± 0.15) indicates pathological processes of proliferation of astroglial cells (gliosis).

Table 1. The level of serum autoantibodies to neurotropic proteins in sick children with idiopathic epilepsy, c.u.

Indicators	IE (n=36)	Control (n=16)	IE/ KG P<	↑ IE/ KG
NF200	30,1±4,6	0,63±0,11	0,001	47,8
GFAP	11,5±8,8	1,21±0,15	0,001	9,5
S100	37,9±8,8	1,1±0,16	0,001	34,5
OBM	10,0±8,6	0,95±0,14	0,001	10,5

High S100 indices in IE against the data of the control group (37.9 ± 8.8 versus 1.1 ± 0.16) in the examined children are a sign of changes in the central nervous system, which are accompanied by emotional disturbances (phobias, depression, aggressiveness).

It is known that myelin has a pronounced immunogenic property, and its destruction is a universal mechanism for the reaction of nervous tissue to various damages. The appearance of increased indicators of antibodies to MBP in the blood serum indicates a violation of the blood-brain barrier in patients with IE (10.0 ± 8.6 versus 0.95 ± 0.14 ; $P < 0.001$).

At the same time, partial or complete loss of myelin by viable processes can lead to severe disturbances in the conduction of nerve impulses. Demyelination of the axon significantly reduces the speed of conduction of nerve impulses, so that the conduction process will no longer be saltator between Ranvier interceptions, as in myelinated fiber, and the movement of electrolytes (K^+ and Na^+) will occur over the entire surface of the axon. This can lead to the formation of new ion channels in the cell membrane and increase the concentration of potassium ions in the extracellular space, which in turn can change the excitability of nerve cells and aggravate the paroxysmal activity of the brain.

Thus, an increase in neurotropic autoantibodies is evidence of an aggravation of neuroimmunedysregulation in IE. At the same time, an increase in the level of autoantibodies to S100, given their glial origin, indicates changes in glial cells and impaired neuroglial relations against the background of BBB permeability and damage to the myelin sheath of axons (high GFAP and MBP). Violation of the permeability of the immune barriers of the brain leads to the formation of aAT to neurotropic proteins, which aggravates the lack of trophic supply of the brain and the progression of damaging processes.

An abnormal increase in aAT to the ligand-binding site of neurotransmitter receptors (Glu-R, GABA-R, Dof-R, Ser-R, and Chol-R) indicates changes in the corresponding neuronal systems. A higher serum level of aAT to neurotransmitter receptors in patients with IE may indicate the presence of different mechanisms of neuromediation and neuroplasticity in patients (Table 2).

Table 2. The level of serum autoantibodies to neurotransmitters in patients with symptomatic and idiopathic epilepsy, c.u.

Indicators	IE (n=17)	Control (n=16)	IE/ KG P<	↑ IE/ KG
CHL	26,4±9,1	0,49±0,32	0,001	53,9
GLU	25,5±6,9	0,53±0,37	0,001	48,1
GABA	17,3±10,3	0,62±0,35	0,001	27,9
DA	54,1±7,8	0,56±0,15	0,001	96,6
SER	6,43±3,3	0,61±0,15	0,001	10,5

B-headSa-channel	27,7±8,6	0,46±0,12	0,001	60,2
Opiateprescriptions (m-OR)	106,5±25,4	0,41±0,13	0,001	259,8
B-endorphin receptors (R-b-end.)	84,5±13,2	0,28±0,08	0,001	301,8

When comparing the indicators of sick children with IE with the data of the control group, a significant unidirectional increase in the individual level of serum aATimmunoreactivity to the receptors of all studied neurotransmitters was revealed.

In the group of children with IE, the level of aAT to CHL was 26.4 ± 9.1 c.u., exceeding the values of the control group by 53.9 times ($P < 0.001$). It is known that choline receptors are widespread in the central nervous system and are present on cholinergic, glutamatergic, dopaminergic, GABAergic, serotonergic neurons. In particular, the entorhinal cortex-hippocampus complex is considered the source of epileptic activity in most patients with epilepsy. These areas of the brain receive cholinergic innervation, which plays a key role in the regulation and organization of control of neuronal excitability in normal conditions and regulation of cortical functions. Taking into account the fact that in epilepsy anatomical and functional changes in the system are observed in the system of local circular nerve connections at the cellular level, it is quite natural that in patients with both symptomatic and idiopathic forms of the disease, cholinergic innervation undergoes certain changes. Also, a number of scientific studies have shown that genes encoding various subunits of acetylcholine receptors are candidates for a number of neurological diseases, in particular, autosomal frontal epilepsy and idiopathic epilepsy [12, 16]. In our opinion, this fact can explain such a high level of aAT to choline receptors in the examined children with idiopathic epilepsy.

Further analysis of neuroimmune relationships in children with IE showed that the level of aAT to glutamate (GLU) and voltage-dependent calcium channels (B-lead Ca-channel) was also significantly high. Thus, in the group of patients with IE, these indicators were 54.1 ± 7.8 and 27.7 ± 8.6 c.u. respectively, exceeding the indicators of the control group by 48.1 and 60.2 times, respectively ($P < 0.001$).

Such a significant increase in the level of aAT to glutamate in IE indicates actual disturbances in excitation processes as a result of activation of membrane neurotransmitter receptors and mechanisms of glutamate excitotoxicity. The data obtained can be interpreted as evidence of a gross imbalance in the glutamatergic system in IE, which is the starting point for starting the processes of neuronal sprouting. It is known that calcium ions are a secondary messenger of excitatory neurotransmitters, including glutamate [10, 17]. A simultaneous increase in aAT to glutamate and voltage-dependent calcium channel, which is a specific antigen, in the examined patients is also quite natural, since it is these channels that play a primary role in regulating the release of neurotransmitters and, in particular, the release of glutamate into the synaptic cleft. The L-type of calcium channels is involved in the generation of the action potential, the T-type promotes the synchronization of thalamocortical connections, which underlies the "spike-wave" pattern in epilepsy [1, 6, 10]. At the same

time, activation of NMDA receptors under the influence of excitatory mediators (for example, glutamate) can cause a pathological phenomenon of excitotoxicity associated with increased penetration of calcium into the cell, followed by the death of neurons. In addition, intracellular calcium ions indirectly - through the regulation of membrane protein synthesis - affect the growth of dendrites and synaptogenesis, which modifies the functional activity of neurons in accordance with changing environmental conditions [5].

Levels of AAT to GABA, dopamine and serotonin in IE also exceeded the normative values and were approximately at the same level (AAT GABA 17.3 ± 10.3 c.u., $P < 0.001$; AAT DA 54.1 ± 7.8 , $P < 0.001$; AAT SER 6.43 ± 3.3 c.u., $P < 0.001$).

A high level of autoantibodies to GABA is evidence of disturbances in the functioning of the GABAergic system, which enhance the neurotoxic effects of glutamate, on the one hand, and inhibit the structure of the antiepileptic system, on the other [5, 14]. The presence of high levels of AAT to dopamine and serotonin in patients with idiopathic epilepsy and their significant difference from the indicators of the control group confirms the close relationship of the glutamatergic system with the system of biogenic amines, the dysregulation of which leads to a detrimental effect on neurons and has a pro-epileptic effect [8, 17]. In this case, such a ratio of AAT can be interpreted as evidence of a pronounced autoimmune reaction on the part of the nervous tissue, which, in turn, contributes to the maintenance of the pathological epileptic system in IE.

Thus, an increase in AAT to the ligand-binding site of neurotransmitter receptors (Glu-R, GABA-R, Dof-R, Ser-R, and Chol-R) indicates changes in the corresponding neuronal systems. A higher serum level of AAT to neurotransmitter receptors may indicate the presence of various mechanisms of neuromodulation and neuroplasticity in patients with IE.

Activation of opioid receptors leads to various cellular consequences, including inhibition of adenylyl cyclase, which causes a decrease in intracellular cAMP concentration. The β -endorphin receptor is a neuropeptide from the endorphin group that is formed in many cells of the central nervous system and is an endogenous agonist ligand of opioid receptors. These peptides have both central effects (neuropsychiatric and behavioral responses) and the ability to influence peripheral centers and organs. In our studies, high rates of both m-OR and R-b-end in IE in children were noted in relation to the data of the control group (by 259.8 times and 301.8 times, respectively).

Thus, circulating aATs to neurotransmitter receptors, in particular to glutamate, GABA, dopamine, serotonin and cholinergic receptors, in the blood serum of IE patients indicate changes in the corresponding neuronal systems. Whereas an increase in opiate peptides indicates a violation in the regulation of adaptive behavior and body responses to stress. Indicators of aAT titer to the indicated neuroreceptors can be used as additional prognostic "immuno-biochemical" criteria for the course of the disease and the effectiveness of antiepileptic treatment. Immunological and biochemical changes can be involved in the process of epileptogenesis and they may not be associated with the use of antiepileptic drugs, and indicate the nature of the course of the disease. A high serum level of aAT to

neurotransmitter receptors in patients with IE indicates the presence of various mechanisms of neuromediation and brain plasticity in idiopathic and symptomatic epilepsy.

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