Neuroimmunological Characteristics of Idiopathic and Symptomatic Epilepsy in Accordance with the Clinical Course

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Abstract

The authors have scientifically determined an immunoreactivity enhancement, which one can indicate by a change in the level of neurotropic autoantibodies in relation to proteins and neurotransmitter receptors in epileptogenesis and the formation of aberrant plasticity among patients with symptomatic and idiopathic epilepsy. Epilepsy is a variant of autoimmune dysregulation, in which an increase in the content of autoantibodies in relation to neuro-specific proteins and neurotransmitters causes the genesis, progression and preservation of the pathological process in accordance with the etiological factor.

A new impulse to study the problem of epilepsy immunopathogenesis should be the ideas of in the last decade about the function inseparable integration of the two main integrative body systems: the central and the immune nervous systems[2]. On the basis of information about neuroimmune interaction, new scientific disciplines — Neuroimmunology and neuroimmunopathology formed[5].

Keywords: *Epilepsy, pathogenesis, immunoreactivity, neurotransmitter, serum, protein.*

Introduction

Currently, thanks to the researchers of immunoneuroendocrine regulation, we know that in the regulation of homeostasis involves plenty of natural autoantibodies (Ab) that reversibly interact with different antigens (AG) of its own body[3]. Natural autoantibodies pass through the synthesis within the body of healthy persons since the fetal life and throughout life [8].

As a result, healthy persons of different genders and ages have only minimal differences (immune fingerprints) in the serum content of various AT. We found that the AT production of different specificity regulates (by the principle of feedback) by the content of the appropriate target antigens4. It indicates the presence of powerful mechanisms for maintenance of the physiological (normal) level of production and secretion of various AT variants[6]. The part of Ab does not have a specific organ orientation and interacts with the ubiquitous AG (collagen, DNA, cytochromes, etc.). The other part interacts with organ-specific antigens (neuro-specific proteins, organ-specific isoenzymes, insulin, etc.). This also applies to Ab which interacts with nerve cell proteins or neurotropic Ab (NAb).

Among the patients with diseases of the nervous system, there are often changes in the serum content of NAb. Whereby, the researchers mainly pay attention to situations characterized by an increase in the neurotropic antibodies content of a certain specificity, which stands as pathological autoimmune aggression[7]. However, in accordance with the regulatory functions of Ab, the disorders of serum ratios in refard to different types of NAb can also be important, and the growth in the production of certain Ab can have not only a pathological, but also an adaptive-compensatory value[6].

Recently, there is more and more attention in regard to the participation of a single neuroimmunological network within the genesis of many diseases[3]. There is a wide discussion of epilepsy immunological aspects in modern literature8.

The pathogenic role and the diagnostic value of Ab to the brain proteins and the receptors of neurotransmitters require further study[2].

The Purpose of the Research: To study the immunoreactivity of neurotropic autoantibodies and neurotransmitters in case of symptomatic and idiopathic epilepsy.

Materials and Method

The study bases on a survey in relation to 86 patients with epilepsy in the age of 16 to 70 years, among them 23 patients had idiopathic epilepsy and 64 patients had symptomatic focal epilepsy. Each patient passed thorough preliminary anamnestic and clinical selection under method of stratified randomization through inclusion and exclusion criteria. The criteria for patients inclusion within the study: adults, epileptic attacks at the time of hospitalization or in history, idiopathic and symptomatic epilepsy. The exclusion criteria: children, cryptogenic epilepsy, pseudepileptic seizures, psychogenic reactions, conversion seizures (hysteria). The scope of the study included: clinical neurological examination, physical status research, neuropsychological testing of higher cortical functions, neurophysiological examination (EEG), laboratory method (clinical blood analysis, blood biochemistry, immunity status test), functional research method (ECG, EchoCG), neuroradiological research method (MRI or CT scan of the brain).

The seizures type determination is in accordance with the International League Against Epilepsy (1981). The diagnosis of epilepsy correspond to the International classification of epilepsy (ILAE 1989, 2001)[12].

Quantitation of serum immunoreactivity of antibodies in relation to neurotransmitter receptors (glutamate, GABA, dopamine, serotonin, and holinoreceptors) and neuro-specific proteins (NF200, Gfap, S100, and OBM) performed by means of solid-phase immunoenzyme method ELI-N-Test and test kits of the same name, manufactured by international research center "Immunculus" (Russia). We determined the values of AAb immunoreactivity within the range from 80 to 140 Units, and the Ab1/AIAb2 immunoreactivity index within the range from 0.8 to 1.2[16] as the norm.

We compared the results of the study with data from a control group output that included 32 clinically healthy individuals (in the age from 24 to 65).

We performed the analysis of the obtained indicators by the software package "SPSS for Windows" and "STATISTICA" Microsoft Excel with material processing with the method of variation statistics. We maintained the reliability assessment of the obtained results by the paired method with the Student's t-test . We considered the differences significant at $p<0.05$.

Research Results

We determined the level of AAb in relation to neurotropic proteins and neurotransmitters in the blood serum of 59 patients with epilepsy. We formed the following clinical groups to conduct immunology research : the first group - 17 patients with idiopathic epilepsy; the second group – 42 patients with symptomatic epilepsy. The control group consisted of 16 clinically healthy individuals.

The results of the conducted studies showed that among clinically healthy individuals (control group), natural AAb in relation to the studied neurotropic proteins within certain limited titers are normal components of the blood serum among practically healthy people, and therefore, in accordance with the recommendations of the test system manufacturers, they determined the design as "internal standard serum" (table 1).

In case of immunology research results assessment, we found that both groups of patients differed from the control group, both in terms of the level and degree of the immunological indicators dispersion under research. In order to identify possible pathogenetic features, we performed the analysis of neuroimmune relationships in both groups in comparison with the control group.

Table 1: The level of serum autoantibodies in relation to neurotropic proteins among patients with symptomatic and idiopathic epilepsy, ie.

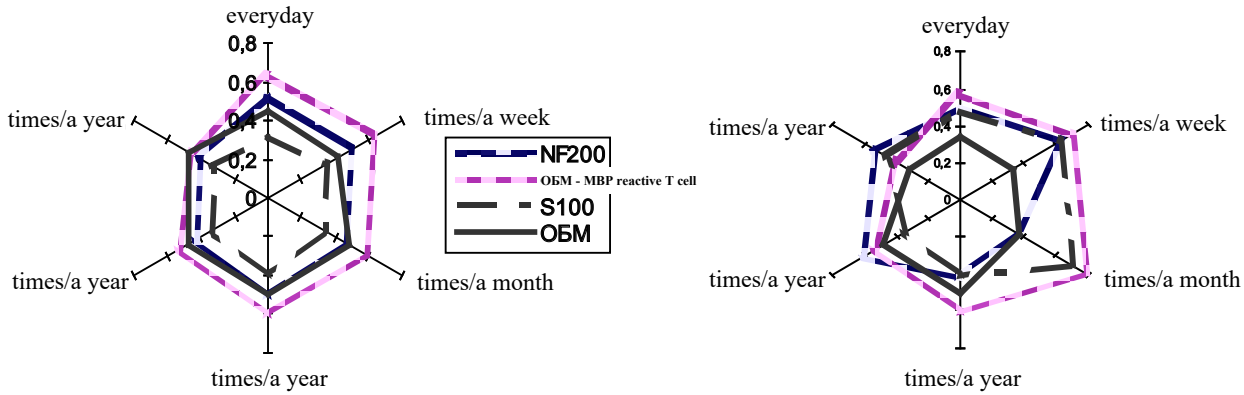
Indicators of	SE (n=42)	IE (n=17)	Control (n=16)	SE and IE, P<	SE and KG, P<	IE and KG, P<
NF200	121,8±8,2	97,6±8,9	72,9±7,1	0.05	0.05	0.05
GFAP	102,5±8,3	80,4±7,2	57,9±5,7	0.05	0.01	0.05
S100	124,1±4,6	150,3±11,8	77,5±7,6	0.05	0.01	0.05
MBP	102,4±8,0	78,8±7,7	58,8±5,5	0.05	0.01	0.05

We found that in the group of patients with SE, the levels of the studied AAb in relation to all neuro-specific proteins significantly exceed the values of not only the «internal standard serum», but also similar indicators of patients with IE. Thus, we found that the highest level of AAb in both groups was the level of AAb in relation to S100, whereby among patients with SE this indicator exceeded the standard values by an average of 1.6 times (P<0.01), and among patients with IE – of 1.9 times (P<0.05). At the same time, there was a significant increase in the AAb output within the group with IE in comparison with SE (150.3±11.8 cu vs 124.1±4.6 cu, P<0.05). Such a significant increase in the level of AAb in relation to the S100 protein, which is a calcium-binding protein, may be a confirmation of the hypothesis that one of the links in the pathogenesis of epileptic attacks is a growth in the permeability of neural membranes for Ca2+ ions with an increase in their intercellular space concentration.

The examination of the AAb level in relation to the NF200 protein also revealed a significant increase in their titers among patients of both groups (on average of 1.7 times for SE and 1.3 times for IE, respectively, P<0.05). Whereby, there was also a significant predominance of the AAb level in relation to NF200 among the patients with SE (121.8±8.2 units vs. 97.6±8.9 units, P<0.05), which indicates excessive plasticity, which apparently contributes to the preservation of stronger pathological connections of the epileptic system among patients with SE (Johnston M. V., 2004).

The level of serum AAb to the neuro-specific protein MBPa,ong patients of both groups also significantly exceeded the values of the "internal standard serum" (on average of 1.7 times among patients with SE; p<0.01 and 1.3 times among patients with IE; p<0.05), while on average of 1.3 times higher than these indicators among patients with SE in relation to patients with IE, (102.4±8.0 cu vs 78.8±7.7 cu, P<0.05).

The level of serum AAb in relation to GFAP was approximately at the same level as AAb to MBP and was 102.5±8.3 cu among patients with SE, and 80.4±7.2 cu among patients with IE, significantly exceeding the indicators of the control group by an average of 1.8 (p<0.01) and 1.4 times(p<0.05), respectively. The comparative analysis between the groups also revealed a statistically significant increase in AAb in relation to MBP among patients with SE in comparison to IE by 1.3 times (P<0.05). As a result of this analysis, we found a positive correlation between the duration of epilepsy and AAb levels in regard to all neurotropic proteins under study, which mostly referred to patients with SE. We found a similar direct relationship between the level of AAb output and the frequency of seizures among the patients under examination. Moreover, this relationship did not depend on the etiological factor of epilepsy (Fig. 1).



Symptomatic epilepsy **Idiopathic epilepsy**

Fig. 1. The correlation relationship between the frequency of seizures and the level of autoantibodies in regard to neurotropic proteins in accordance with the type of epilepsy

Thus, clinical and immunological analysis revealed a clear pattern relation between the content of antibodies and the neurotropic proteins in conformity with the aetiology, duration and course of SE and IE. The regularity of the detected increase of neurotropic AAb in close relationship both with each other and with the

frequency of seizures is evidence of the neuroimmune dysregulation aggravation with the increase of the clinical course severity among the patients with epilepsy. The disruption of the brain's immune barriers permeability leads to the AAb formation in regard to neurotropic proteins, which increases the lack of the brain trophic support and the progression of damage processes. In case of differentiation of the obtained results in accordance with the form of epilepsy,we found that the greatest sensitization of immune cells to the neuro-specific antigens NF200, GFAP and MBP is with SE, which, in turn, is a reliable reflection of the destructive process and pathological penetrance of the blood-brain barrier.

The table 2 contains the research results of the AAb level in relation to neurotransmitters. Within the comparative analysis of the indicators which refer to patients with both IE and SE against the data of the "internal standard serum", we found a significant unidirectional increase in the individual level of serum AAb immunoreactivity in relation to the receptors of all neurotransmitters under study. We obtained an interesting data in regard to patients with concern to the level of AAb in relation to choline receptors. In particular, the patients with IE had the level of AAb in relation to this neurotransmitter 162.9±5.3 cu, with above-limit value of the "internal standard serum" by 4.8 times ($p<0.01$), and the indicators of patients with SE by 3.5 times (46.7±4.9 cu, $p<0.05$).

Table 2: The level of serum autoantibodies in relation to neurotransmitters among patients with symptomatic and idiopathic epilepsy, cu.

Indicators of	SE (n=42)	IE (n=17)	Control (n=16)	SE and IE, P<	SE and KG, P<	IE and KG, P<
CHL	46,7±4,9	162,9±5,3	33,9±3,3	0.05	0.05	0.01
GLU	73,8±5,3	98,1±8,8	57,2±4,9	0.05	0.05	0.01
GABA	53,7±5,4	71,8±4,7	45,4±4,9	0.05	-	0.05
DA	62,6±5,0	74,4±4,2	46,9±4,9	0.05	0.05	0.01
SER	62,9±5,3	76,9±3,4	56,2±4,6	0.05	-	0.05
V-zav.(B-зав.) Ca-channel	98,5±7,0	78,6±6,4	45,9±4,2	0.05	0.05	0.01

The further analysis of neuroimmune relationships among patients with epilepsy showed that the level of AAb in regard to glutamate and voltage-dependent Ca-channels was also significantly high among the AAb under study within both groups.

Thus, the indicators of patients with SE constituted 73.8±5.3 and 98.5±7.0 cu, respectively, which exceeded the indicators of the "internal standard serum" by an average of 1.3-2 times ($p<0.05$), and in regard to patients with IE – 98.1±8.8 and 78.6±6.4 cu, respectively with increase of the standard indicators by an average of 1.7-2 times ($p<0.01$).

Among patients with IE, the AAb in relation to glutamate significantly exceeded not only the control values, but also the indicators of patients with SE (respectively 98.1±8.8 and 73.8±5.3 cu, $p<0.05$). Such a significant increase in the level of AAb in regard to glutamate among the patients of both groups indicates the evident disorder of the excitative processes as activation result of membrane neurotransmitter receptors and mechanisms of glutamate excitotoxicity. Whereby, we note more significant disorders of the glutamateergic system within the course of IE.

One a priori can determine the obtained output as evidence of a gross imbalance within the glutamatergic system among patients with IE, which is the starting point for the neuronal sprouting processes.

The levels of AAb to GABA, dopamine and serotonin within the examined groups also exceeded the standard indicators and were approximately at the same level. However, we found the significant differences from the "internal standard serum" only among patients with IE (AAT GABA 71.8±4.7 cu, $p<0.05$; AAT DA 74.4±4.2, $p<0.01$; AAb SER 76.9±3.4 cu, $p<0.05$).

We found the significant differences among the

patients with SE only within the ratio of AAb in regard to dopamine (62.6±5.0 cu, P<0.05).

The high level of autoantibodies in regard to GABA is evidence of disorders within the GABA-ergic system, which increase the neurotoxic effects of glutamate on the one hand, and inhibit the structure of the antiepileptic system on the other hand (Gusev E. I., Geht A. B., 2009).

In particular, AAb significant increase in relation to this neurotransmitter among patients with idiopathic epilepsy may indicate a gross imbalance in the glutamatergic system and a marked depletion of the GABA-ergic system within this group of patients, which is the starting point for the processes of neuronal sprouting.

The high levels of AAb in relation to dopamine and serotonin among patients with IE and their significant difference from the indicators of the "internal standard serum" confirm the close relationship of the glutamateergic system with the system of biogenic amines, whose dysregulation leads to a detrimental effect on neurons and has a proepileptic effect. One can interpret such ratio of AAb as evidence of a significant autoimmune response from the nervous tissue, which, in turn, contributes to the maintenance of the pathological epileptic system among patients with IE.

Thus, the AAb increase in relation to the ligand-binding site of neurotransmitter receptors (Glu-P, GABA-P, DOF-P, Ser-P, and Hol-P) indicates changes within the corresponding neuron systems. More high serum level of AAb in regard to neurotransmitter receptors may indicate the presence of different mechanisms in order to implement the neurotransmission and neuroplasticity among patients with IE and SE.

As a result of correlation analysis, we found a positive relationship between the duration of epilepsy and the levels of AAb in regard to the receptors of all neurotransmitters, most closely expressed among patients with IE in comparison with SE. The study of correlation relationships between neurotransmitters and the frequency of paroxysms has established a direct average and strong dependence of the number of paroxysms on the level of neurotransmitters. The strength of correlation relationships did not depend on the form of epilepsy.

The obtained output confirms the assumption in regard to multi-factorial causes of the development and progression within the epileptic process (Fig. 2).

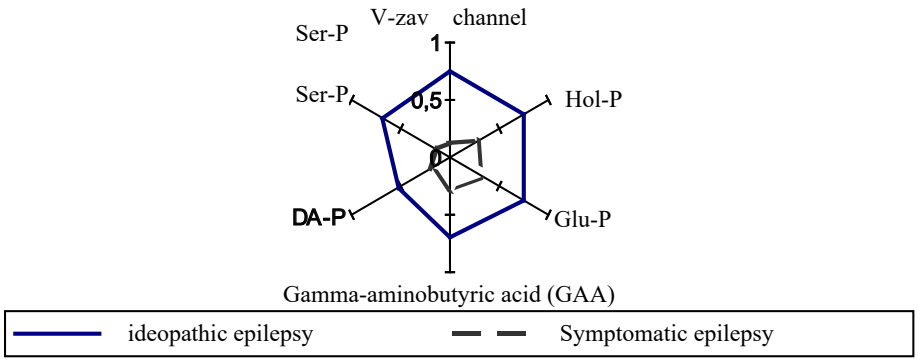


Fig. 2. The correlation indicators between the duration of epilepsy and levels of AAb in regard to neurotransmitter receptors

Thus, the regularity of the autoantibodies detected increase in regard to all neurotransmitters under study within close relationship with each other, as well as with the frequency of seizures and duration of the disease is evidence of neuroimmune dysregulation aggravation as the clinical course severity in cases of epilepsy .

The correlation analysis of the cognitive disorders relationships and the content of antigens in relation to the main neurotransmitters, in particular GFAP, among the patients with SE found a strong relationship between the indicators of clock drawing test (r=-0.753 and -0.755) and MMSE (r=-0.806 and -0.892), as well as with tests for speech activity (r=-0.736, -0.540 and r=-0.562, -0.642), words memory (R=-0.679 and R=-0.753, respectively) and repeating numbers in reverse order (R=-0.568 and R=-0.695, respectively).

With the level of AAb in relation to the rest of the neurotropic proteins, there was a weak relationship, and in some cases it was absent at all. The relationships of AAb level in regard to neurotransmitter receptors and cognitive disorders among patients with SE were weak.

The influence of the AAb level in relation to GABA by the indicators of clock drawing test (r=-0.274), MMSE (r=-0.272), phonetically mediated associations (r=0.268), word memorization (r=-0.204) and repetition of numbers in reverse order (r=-0.265), which expressed by the indicators of inverse and direct weak correlation relationships.

Thus, we found the only significant inverse correlation among the patients with SE between the level of AAb in relation to GFAP and MBP and the indicators of neuropsychological tests. The obtained data once again convincingly confirm both the fact of pathological BBB permeability among patients of this group, and the significance of this fact in the occurrence and maintenance of cognitive disorders in case of SE.

The study of correlation relationships between similar neuroimmune indicators and the results of neuropsychological tests among patients with IE showed a different nature of the relationships within this group in comparison with SE. Thus, in case of correlation analysis among patients with IE, we found weak relationships between the indicators of AAb in regard to GFAP and MBP and the parameters of the cognitive sphere. In particular, we registered the most significant indicators of reverse correlation within the clock drawing test assessment (R=-0.361 and r=-0.355, respectively) and the speech activity test (R=-0.322 and r=-0.298, respectively).

There was a high, medium, and weak correlation relationship between the indicators of neuromediators and neuropsychological tests. We obtained the particularly evident output within the relationship of cognitive disorders with the level of AAb in relation to choline and GABA receptors. Thus, one can establish the following relationships with the indicators of clock drawing test (R=-0.852 and r=-0.869, respectively) and MMSE (R=-0.795 and r=-0.790, respectively), as well as with the indicators of the speech activity test (r=-0.697 and r=-0.872 with semantically mediated associations and r=-0.872 and r=-0.635, respectively), word memory (R=-0.697, r=-0,826) and the numbers repetition in reverse order (R=-0,725 and R=-0,822).

The obtained data presents a great scientific interest and a priori helps us to determine the identified cognitive disorders among patients with IE, since it is known that GABA receptors mediate “long-term” GABA - dependent reactions within the central nervous system by complex processes trigger in relation to various neurotransmitter systems interactions (in particular, GABA and cholinergic). The identified neuroimmune dysregulation causes the neurotransmitter processes disorders at all levels of synaptic regulation, and as a result, there is a neurotransmitter imbalance formation, a priori in the cortex and hippocampus – the leading ares of the cognitive functions implementation.

In case of neuroimmunological indicators study inregard to patients with epilepsy, we found that circulating AAb in relation to neurotransmitter receptors, in particular to glutamate, GABA, dopamine, serotonin and holinoreceptors, in the blood serum of patients with epilepsy indicate changes within the corresponding neuron systems. A higher serum level of AAb in relation to neurotransmitter receptors among patients with SE may indicate the presence of different mechanisms for the implementation of neurotransmission and brain plasticity in cases of IE and SE.

Thus, we indicate the heterogeneity of patients with epilepsy in terms of molecular development mechanisms, the causes of epileptic attacks. In particular, one can use the circulating AAT in relation to neurotropic proteins and neurotransmitter receptors within the blood serum of patients with epilepsy as additional prognostic “immunobiochemical” criteria for the course of the disease and the efficiency of antiepileptic treatment.

Conclusions

1. One of the leading mechanisms within the epilepsy pathogenesis is a complex change of neuroimmune relationships under a unidirectional increase in the level of autoantibodies in relation to the neuro-specific proteins S100, GFAP, NF-200, MBP and the neurotransmitters glutamate, GABA, dopamine, serotonin, serotonin and voltage-dependent Ca-channel. Whereby, the key link within the pathogenesis of idiopathic epilepsy is a neurotransmitter imbalance, and in the pathogenesis of symptomtomatic epilepsy – an increase in the level of AAb in relation to GFAP and MBP.
2. The changes in the levels of antibodies in relation to the neuro-specific proteins S100, GFAP, NF-

200, MBP and the neurotransmitters glutamate, GABA, dopamine, serotonin, serotonin and voltage-dependent Ca-channel depend on the form and duration of epilepsy, the nature and frequency of seizures. We noted a high specificity of increasing antibody levels in regard to patients with increase of disease severity.

3. The rise of antibodies level in relation to neurospecific proteins S100, GFAP, NF-200, MBP and the neurotransmitters glutamate, GABA, dopamine, serotonin, serotonin and voltage-dependent Ca-channel within epilepsy can serve as a prognostic criterion of severe disease and used at an early stage in the selection of adequate antiepileptic therapy and the choice of further tactics patients management.

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