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Biomarkers In the Diagnosis and Assessment of Treatment Effectiveness in a Child with Autism

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SUMMARY

It is known that children with autism do not respond well to psychological and speech therapy correction and pharmacological treatment. A pressing question of our time is the personalized selection of alternative treatment methods based on data from modern diagnostic methods. The article presents a typical case of autism taking into account history, psychological characteristics, pharmacogenetic testing, EEG features, and results of laboratory studies of serum (BDNF, progranulin, antibodies to myelin basic protein, glutamate). The effectiveness of the therapy being conducted is assessed using biomarkers.

The patient with autism had a high level of antibodies to myelin basic protein, approximately double the upper limit of normal, as well as increased levels of glutamate in the blood, which may reflect the pathogenetic mechanisms of this pathology. The course of treatment using an alternative physical method of intervention (rTMS) led to clinical improvement and had a normalizing effect on the levels of antibodies to myelin basic protein and glutamate in the serum. The degree of reduction in clinical symptoms correlated with changes in the blood biochemical indicators: glutamate, antibodies to myelin basic protein, and neurophysiological data: computer EEG and motor response threshold.

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KEYWORDS

Autism, Biomarkers, Neurotrophic Proteins, Antibodies to Myelin Basic Protein, Glutamate, Computer EEG, Motor Response Threshold.

The increasing incidence of autism in the world is a matter of medical and public concern. According to WHO data, the prevalence of autism in different countries of the world varies between 6-90 cases per 10,000 children. Globally, autism is present in about 1 child in 100 [1]. This figure corresponds to an average value, and in general, the prevalence rates of autism reported by different studies vary widely. However, according to the results of some controlled studies, the actual figures are much higher. The prevalence of autism in many low- or middle-income countries is unknown. In 2022, the global prevalence of autism averaged 63 cases per 10,000 child population, and the incidence of the disorder has increased about 10-fold in recent years of observation [1]. Autism is considered as a disorder of neuropsychological development and its main manifestations are: disorders of social interaction, pronounced deficit of communication, stereotyped actions, difficulties in learning and others. It is known that children with autism are poorly amenable to treatment and social adaptation.

Currently, the use of alternative treatment methods such as bioacoustic correction, transcranial micropolarization, and transcranial magnetic stimulation in pediatric neurology and psychiatry is being actively studied, especially for disorders whose pathophysiology is based on focal cortical dysfunction [2,3]. The positive experience of using innovative diagnostic methods and alternative methods of treatment in children with autism is of interest and deserves careful study.

The relevance of considering this clinical case is due to the fact that it is typical for pediatric autism and on its example it is possible to see the characteristic features of the disease development, the peculiarities revealed at diagnosis, the difficulties of drug treatment and their relationship with the results of pharmacogenetic testing, the advantages of personalized selection of the treatment method, the dynamics of EEG characteristics, shifts in neurotransmitter, neurotrophic and neuroimmune parameters accompanying the positive effect of treatment.

The aim of the work is to review a typical case of autism taking into account anamnesis, psychological characteristics, pharmacogenetic testing, EEG features, results of serum laboratory tests (BDNF, progranulin, antibodies to myelin basic protein, glutamate), to show their effectiveness in dynamic observation of the child in the process of treatment.

RESULTS AND THEIR DISCUSSION (DESCRIPTION OF A CLINICAL CASE)

Child D., age 7 years. The child is from the second pregnancy, second labor. According to the mother's words, the pregnancy proceeded with complications. Placental detachment (37%) was detected in the 6th week, treatment was carried out with the drug duphaston. The labor proceeded normally, without stimulation. The child and the mother were discharged from the hospital on the 5th day. The child was fed naturally (breastfed) for 12 months. Vaccinated in accordance with the established procedure. Vaccination in the maternity hospital with TB vaccine caused a side

effect - hypertonus of neck muscles. According to neuroimaging data and neurologist's examination, displacement of the I cervical vertebra, blood flow disorder on the left side with artery constriction, eye and tongue movement disorder, neck laxity were found. On the recommendation of the neurologist, the child was massaged with cervical-collar zone massage for a year in 10-day cycles with breaks for 10 days, which led to normalization of neck muscle tone. According to the mother, the psychophysiological development of the child up to 1.5 years of age otherwise corresponded to age norms. Then the parents paid attention to the peculiarities of behavior: the child did not look into the eyes, did not respond to the name, constantly repeated manipulations with the doorknob, lacked an indicating gesture. Specialized medical help was sought only when the child was 3 years 10 months old. The parents complained about the child's lagging behind in speech and mental development. At the appointment with a child psychiatrist: productive contact is difficult. Avoids direct gaze into the eyes, is engrossed in a toy. Commands and requests are not fulfilled. Mood background is flat. Index gesture is absent. Stereotypies are noted in behavior. Speech is not developed, sometimes utters separate words on demand. Self-care skills are not developed. He is affectionate with his parents. Avoids contact with strangers, avoids eye contact.

- Childhood Autism Rating Scale (CARS) test - 38 points
- Consultative opinion of an otorhinolaryngologist: no pathology revealed.
- Consultative opinion of a cardiologist: no pathology revealed.
- Pediatrician's examination: physical development is normotypic.
- MRI of the brain: MRI-signs of organic changes in the brain were not revealed. Hypoplasia of the right vertebral artery. Kinking of the right vertebral artery in the C1 segment.

A DIAGNOSIS WAS MADE: INFANTILE AUTISM

In accordance with the diagnosis, speech therapy and psychologically corrective measures were carried out. Nootropic drugs were prescribed to the child repeatedly, at the age of 4, 5 and 6 years. However, the expected clinical effect was absent, and undesirable side effects in the form of agitation and increased expression of stereotypes were observed. Speech therapy and psychologically corrective measures also did not lead to a significant improvement of the clinical condition. The issue of speech development was not resolved, and the child's inattentiveness, lack of contact, stereotypical manipulations, and mental developmental delay persisted.

The parents appealed to leading domestic and foreign centres of assistance to children with autism. As a result, the patient was thoroughly examined using the most modern diagnostic methods: EEG, psychologist consultation, pharmacogenetic testing, blood tests to determine the levels of neurotrophic proteins (brain-derived neurotrophic factor (BDNF), neurotrophic protein progranulin), antibodies to neurospecific brain proteins (antibodies to myelin basic protein (AT-OBM)), and glutamate.

Examination by a child psychiatrist. The child does not follow simple instructions. He responds to a name if his mother persistently addresses him. Visual contact is difficult, stereotypical movements are present, he/she is unsteady. Speech is not developed: he pronounces separate sounds. Understanding of addressed speech is absent. Self-care skills are partially developed. Index gesture is absent. Narrative and role-playing game activity is not formed, there is a favourite soft toy and car, prefers to collect constructors. Shuns other children, except for his sister. He is affectionate with his relatives. He is selective in food: he refuses to eat any kind of fruit in its natural form, prefers mashed potatoes.

Mother characterizes her child as follows: smart, cunning, sweet, affectionate, curious. Sometimes insistent, capricious. The mother expects that her son "will speak (return to his age norm), will finish school and institute well, will be a famous person devoted to his profession".

Analysis of the data of psychological testing according to CARS (40 points) and ADOS showed that the child was found to have signs of autism, medium degree of severity.

Child-parent relations in the family are respectful and trusting. Both parents are actively engaged in the upbringing and development of the child. There is an unstable emotional background in the mother, as she feels a sense of fear, anxiety in relation to her son, to his future independent life. Parents feel sympathy for the child and believe in his future.

At electroencephalographic examination, the background EEG shows pronounced diffuse disorders of cortical rhythmicity with predominance of highly irregular activity of beta-, alpha-, theta- and delta-bands in all leads without zonal differences. The alpha rhythm power peak is absent (Figure 1), which is characteristic of autism.

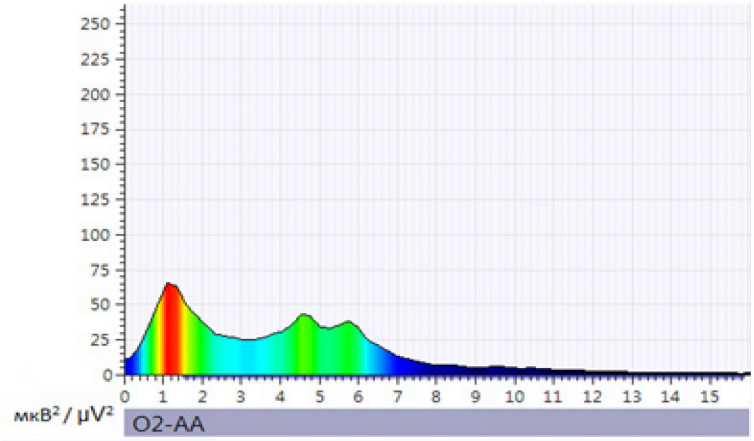

Figure 1: Spectral frequency in the region of the occipital derivation, on the right (EEG of child D. before the rTMS course).

Pharmacogenetic testing (Table 1) was performed to study the polymorphism of genes encoding enzymes involved in biotransformation, transport, absorption, distribution and excretion of drugs from the body. Cytochrome P-450 isoenzymes (CYP2D6, CYPC9, CYP2C19, etc.) and phase II biotransformation enzymes associated with the expression of the P-glycoprotein gene play the greatest role in the biotransformation of psychotropic drugs.

Gene	Full gene name	Polymorphic locus name (rs)	Minor allele (or risk allele)	Genotype	Comment
CYP2C9	Cytochrome P450, family 2, subfamily C, polypeptide 9	CYP2C9*2 (C430T) rs1799853	–	CC	Homozygote
	Cytochrome P450, family 2, subfamily C, polypeptide 9	CYP2C9*3 (A1075C) rs1057910	–	AA	Homozygote
CYP2C19	Cytochrome P450, family 2, subfamily C, polypeptide 19	CYP2C19*2 (681G>A) rs4244285	A	GA	Heterozygote
	Cytochrome P450, family 2, subfamily C, polypeptide 19	CYP2C19*17 (-806C>T) rs12248560	–	CC	Homozygote
CYP2D6	Cytochrome P450, family 2, subfamily D, polypeptide 6	CYP2D6*4 (1846G>A) rs3892097	–	GG	Homozygote
CYP1A2	Cytochrome P450, family 1, subfamily A, polypeptide 2	CYP1A2*F (-163C>A) rs762551	A	AA	Homozygote
MDR1	Multidrug resistance 1 (P-Glycoproteine gene)	C3435T rs1045642	–	TT	Homozygote

Table 1: Genotyping results (alleles and genotypes) of the child D.

Conclusion

Due to the presence of CYP1A2*F:AA polymorphism, the rate of metabolism of CYP1A2 substrate drugs increases, and the effectiveness of treatment with CYP1A2 substrate drugs decreases. Due to the presence of CYP2C19*2:GA polymorphism, the rate of metabolism of CYP2C19 drugs-substrates decreases, the risk of side effects during treatment with CYP2C19 drugs-substrates increases.

These findings may explain the undesirable side effects of nootropic drugs used in the treatment of Child D.

Due to the insufficient effectiveness of psychological and pedagogical measures, as well as the identified features of drug metabolism, a decision was made to use alternative methods of treatment. The patient was prescribed a course

of rhythmic transcranial magnetic stimulation (rTMS). The treatment protocol included exposure to a low-frequency (0.9 Hz) pulsed magnetic field for 20 minutes on the projection of the right dorsolateral prefrontal cortex daily, with a break on weekends. Transcranial magnetic stimulation was performed in accordance with the recommendations approved by the International Federation of Clinical Neurophysiology [4,5] in accordance with the instructions for the use of rTMS in children with general developmental disorders [6] using a magnetic stimulator "Neuro-MS/D" (Russia) with a figure-eight-angle inductor.

Fundamental and clinical studies have shown that the cerebral cortex of patients with autism is in a hyperactive state, which disrupts the functional connectivity of neuronal networks [7]. It has also been found that the comorbidity of autism and epilepsy reaches 26% [8], and epileptiform patterns on EEG in children with autism are not uncommon. Therefore, the protocol of low-frequency rTMS, which has an inhibitory effect on brain neurons, was chosen to ensure the safety of the method with regard to the provocation of epileptic seizures.

Before the beginning of the treatment course, the child and his parents were explained the methodology of rTMS, a questionnaire on contraindications to the procedure was conducted, and the effects of the magnetic field (audible clicking, possible twitching of facial and limb muscles) were highlighted. The procedure was carried out in the position of the patient sitting in a comfortable chair (sometimes - on the mother's lap). Next to the child was his mother, who talked with the child, calmed him during the procedure.

Before and during treatment, the child was assessed with single magnetic field pulses to determine the motor response threshold (MRT), which reflects the state of excitability of cortical motoneurons. MRT is the minimum amount of energy of a single impulse (expressed as a percentage of the device power) required to excite cortical motor neurons and contraction of the thumb muscles of the contralateral hand with registration of an action potential with an amplitude of 50 μV or more on the myograph screen). In accordance with the individual value of MRT, the stimulus intensity during rTMS therapeutic procedures was set (90-100% of MRT) and the effect of the pulsed magnetic field on the functional state of the brain during treatment was evaluated.

After the first pTMS procedures, the mother noted a noticeable change in the child's behavior: he began to look into his mother's eyes, showed interest in the objects of the home environment. During subsequent procedures, the child's behavior became more orderly and new words appeared.

A total of 24 rTMS sessions were performed. The child tolerated the treatment well, no adverse reactions were detected. At the end of the course a stable emotional background was observed, the child became assiduous, began to fulfill simple requests, respond to the name, words appeared, but their use was not always conscious. During the repeated consultation he was calm and adequate. There were positive dynamics according to the data of psychological diagnostics. Visual-spatial orientation, perception and recognition of integral objects and time of concentration on them improved, he was able to fulfill simple instructions in work with cards and point to the given objects, the volume

of visual memory increased. Kinesthetic and dynamic praxis could not be examined. According to the mother's words: during lessons with a speech therapist in a preschool institution, the child became more assiduous, performs tasks without stimulus help.

Analysis of the CARS psychological test data showed positive dynamics: the sum of scores decreased from 40 to 38.

Analyzing neurophysiological parameters of brain excitability after the end of the course of treatment according to the data of single TMS, an increase in the threshold of motor response by 10% (from 60% to 66%) and the frequency of registration of the evoked response was found, which indicates a modulating effect of therapeutic magnetic stimulation on the functional state of the brain.

Positive dynamics was noted in the comparative analysis of EEG computer processing results in the form of power peak formation (Figure 2).

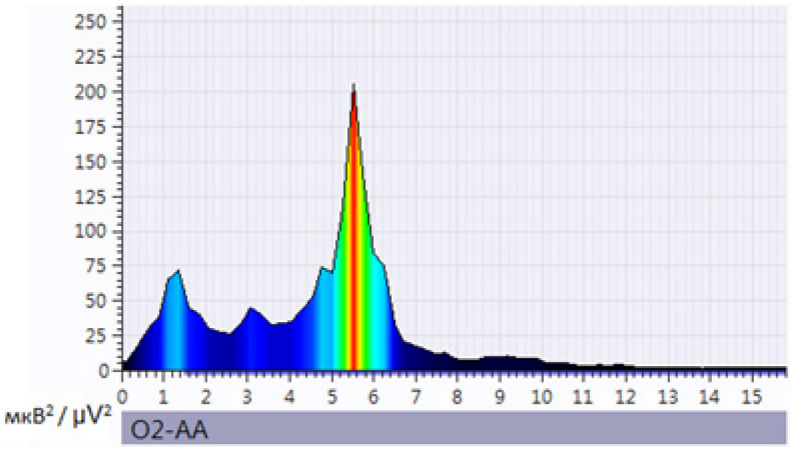

Figure 2: Spectral frequency in the region of occipital lead (EEG of child D. after rTMS course).

If at the background examination the power peak of the spectrum in occipital sections was practically absent (Figure 1), after the course of TMS the power peak at the frequency of 5.6 Hz is clearly revealed (Figure 2). Obviously, such changes indicate improvement of the functional state of the brain.

The results of laboratory neurotrophic indices in serum before and after completion of the course of therapeutic rTMS are reflected in (Table 2).

As can be seen from the data in (Table 2), the values of the neurotrophic protein BDNF before treatment were close to the upper limit of normal. After treatment, a more than twofold increase in BDNF level was observed. Literature evidence suggests that BDNF mediates neuronal survival and differentiation, which is crucial for brain formation during child development. A meta-analysis of publications has shown predominantly elevated BDNF levels in autism

spectrum disorders [9], although other data are found in the literature. As can be seen from the above results, the clinical improvement of the child's condition was accompanied by a significant increase in BDNF levels, which plays a protective neurotrophic role and may increase brain plasticity under damaging influences.

Indicator	Reference ranges (healthy children)	Before treatment	After treatment
BDNF, pg/ml	11 – 23	24	56 ↑
Progranuline, ng/ml	38 – 56	54	46
MBP Ab, ng/ml	32 – 90	190 ↑	111
Glutamate, μg/ml	30 – 80	118 ↑	75

Table 2: Laboratory indicators in the blood serum of the child D. before and after the rTMS course.

Progranulin in the nervous tissue also plays a neurotrophic role, suppresses inflammatory processes and accelerates regeneration after damaging effects [10], but the level of this indicator was normal in child D. both before and after TMC treatment.

Myelin basic protein is one of the key proteins of the CNS, providing protection and formation of the structure of nerve fibers, conduction of nerve impulse along axons. Antibodies to myelin basic protein appear against the background of activation of autoimmune processes, and they can play a destructive role [11]. As shown in (Table 2), a sharply elevated level of antibodies to myelin basic protein was detected in child D, but against the background of clinical improvement after a course of rTMS, the content of antibodies to myelin basic protein in the blood decreased almost twofold and approached normal.

Glutamate is a major excitatory mediator of the CNS that plays an important role in early prenatal life, in synaptogenesis, neuronal plasticity, and in the maintenance of cognitive functions [12]. At the postsynaptic neuron, glutamic acid ion binds to and activates postsynaptic receptors such as, for example, NMDA- and AMPA-receptors [13,14]. Excessive excitatory action of glutamatergic stimuli leads to destructive excitotoxicity. This cascade of events is associated with the activation of enzymes that disrupt cellular structure, alter membrane permeability and electrochemical gradients, ultimately leading to mitochondrial death and consequently neuronal damage [14]. As the data obtained in this study show, the serum glutamate content in patient D. was outside the upper limit of normal, but after rTMS treatment, its level significantly decreased and entered the normal range.

In the current literature, there is a large amount of data on the effect of rTMS treatment on the BDNF content in brain regions and serum of patients with various mental illnesses. The majority of studies state that against the background of clinical improvement, as a rule, an increase in plasma or serum BDNF levels is observed [15,16]. In the clinical case under consideration, on the example of child D., we can also observe a positive clinical effect of therapy, accompanied by an increase in the level of BDNF in serum. As for the hypothesis about the role of progranulin in the etiopathogenesis of autism, it was not confirmed in the case under consideration.

It is noteworthy that child D. was found to have sharply elevated blood levels of antibodies to myelin basic protein, which decreased to normal values after successful treatment. The role of antibodies to myelin basic protein in the development of autism is actively discussed in the literature [17], and the clinical case under consideration confirms the opinion about an important pathogenetic aspect of this autoimmune process. The fact that the pathologically elevated level of antibodies to myelin basic protein decreased with successful rTMS treatment has not been previously reported in the literature.

The imbalance between excitation and inhibition is hypothesized to be one of the mechanisms in the pathogenesis of autism. This imbalance may be due to a disturbance in the interaction between excitatory glutamatergic and inhibitory GABAergic processes in the brain[14]. The findings are consistent with the autism hypothesis based on the manifestations of glutamate excitotoxicity[17]. The positive effect of treatment accompanied by normalization of glutamate level also testifies to the validity of the above hypothesis in relation to this patient.

CONCLUSION

Consideration of a separate clinical case does not allow to draw generalizing conclusions. However, on the example of a particular patient we can note some important characteristics of the pathology under consideration, the results of laboratory tests and the shifts accompanying the favorable effects of treatment. The following results seem to be the most important:

- The use of a complex of diagnostic biomarkers makes it possible to assess the severity of the pathological process and personalize the treatment of a child with autism;
- A patient with autism had a high level of antibodies to the total myelin protein, approximately 2 times higher than the upper limit of the norm, as well as an increased content of glutamate in the blood, which, presumably, may reflect the pathogenetic mechanisms of this pathology;
- The treatment course without pharmacological agents, but using alternative techniques led to clinical improvement and a normalizing effect on the levels of antibodies to complete myelin protein and glutamate in the serum;
- The degree of reduction in clinical symptoms correlated with changes in biochemical parameters in the blood: glutamate and antibodies to complete myelin protein;

- The degree of reduction in clinical symptoms correlated with changes in neurophysiological parameters: computerized EEG data and motor response threshold;
- The CARS methodology can be used not only for the initial diagnosis of autism but also for dynamic monitoring;
- A large set of biomarkers is not always available for use in broad clinical practice, hence it is relevant to actively investigate the diagnostic significance of each of the innovative biomarkers with the subsequent introduction of the most sensitive and accessible ones.;
- The degree of reduction of clinical symptoms correlated with the change in the blood content of biochemical parameters: glutamate and antibodies to total myelin protein;
- The degree of clinical symptom reduction correlated with changes in neurophysiologic parameters: computerized EEG data and motor response threshold;
- CARS methodology can be used not only for primary diagnosis of autism, but also for dynamic monitoring;
- A large set of biomarkers is not always available for use in broad clinical practice, so it seems relevant to actively study the diagnostic significance of each of the innovative biomarkers with the subsequent introduction of the most sensitive and accessible ones.

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