IMPAIRMENT OF PEROXISOME BIOGENESIS IN THE SPECTRUM OF ZELLWEGER SYNDROME (CLINICAL CASE)

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Impairment of peroxisome biogenesis is a heterogeneous group of autosomal recessive hereditary conditions, which are caused by a partial or generalized defect of peroxisomes. They are divided into two clinically distinct subtypes - Zellweger spectrum disorders (ZSD) and type I rhizomelic chondrodysplasia punctata (RCDP) type 1) [1,11,12].

Peroxisomes are irreplaceable organelles of human cells that perform a number of important functions in cell metabolism. They are found in all cells of the body, but their largest amount is found in liver and kidney cells [1,4,11,12]. It is known that the synthesis of peroxisomes is encoded by PEX genes, which are templates for encoding peroxins - proteins necessary for the synthesis of peroxisomes [3,8,12,14]. In electron microscopic examination, peroxisomes look like cytoplasmic vesicles of spherical or oval shape, 0.1-1.5 μm in size (Fig. 1).

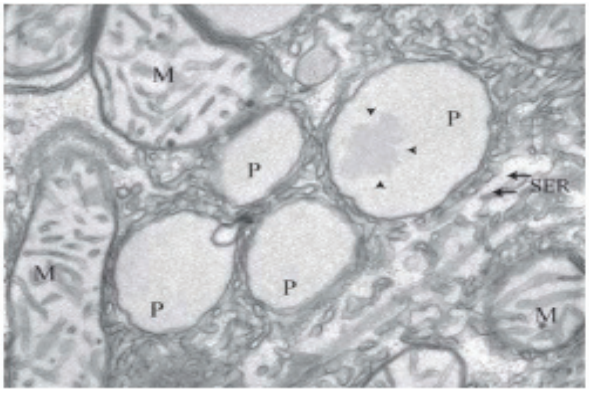

Fig. 1. Electron micrograph of rat liver. Designations: SER - smooth endoplasmic reticulum, M - mitochondria, P - peroxisomes; arrows indicate the "nucleoid" formed by urate oxidase crystals [10]

ZSD include three clinical phenotypes that were described even before the discovery of their biochemical and molecular basis - Zellweger syndrome, neonatal adrenoleukodystrophy and infantile Refsum disease. Additionally, with the advancement of new modern technologies of genetic sequencing, atypical, previously unknown, phenotypes are identified, which are milder and are manifested not by hearing/vision loss, but by ataxia and peripheral neuropathy, congenital cataracts [3]. The previously proposed phenotypes are now considered to be different manifestations of the same spectrum of disorders, with Zellweger's syndrome being the most severe manifestation of ZSD, neonatal adrenoleukodystrophy - intermediate, and infantile Refsum disease – the mildest. Therefore, at the present stage, it is recommended to apply the definition of "Zellweger spectrum disorder", regardless of the phenotype (in the presence of a defect in the *PEX* gene) [3,8,11,14].

Currently, it is known that peroxisome synthesis is encoded by *16 PEX* genes. ZSD occur when there are changes (mutations) in one of these 13 genes (which are responsible for the development of this pathology) [3] (Table 1). The most common cause of ZSD is genetic defects in the *PEX1* gene (according to various researchers, the frequency ranges from 60.5% to 70%) [3, 8]. Mutations in the *PEX6, PEX12, PEX26, PEX10, PEX2, PEX5, PEX13, PEX16* genes are less common. Until now, no association of this disease with two *PEX* genes, *PEX11G* and *PEX11A*, has been found [3,5,8,12,14].

Due to the dysfunction of peroxisomes, very long chain fatty acids (VLCFA) accumulate in the human body: phytanic and pristanic acids, intermediate products of the metabolism of bile acids (this is how their synthesis is disrupted). There is also a deficiency of plasmalogens - specialized lipids that are part of cell membranes and myelin sheaths of nerve fibers [2- 4,13].

In the absence or disfunction of peroxisomes the functioning of the entire organs is disrupted. According to the literature, all known cases of peroxisome biogenesis disturbance form a continuous spectrum of different forms of severity [1,3,6,7,14].

All disturbances in peroxisome biogenesis of cause long-term morbidity and are often fatal in childhood [1,8]. Clinical manifestations usually occur in neonatal period or early childhood. Shortly after birth neonates may present with severe neurological and metabolic disturbances Clinical signs include neonatal seizures on the background of demyelination, severe hypotension, peripheral polyneuropathy, statomotive regression. Later, the clinical presentation is dominated by neurological and digestive system manifestations. Liver dysfunction usually manifests as neonatal jaundice and THE abnormalities in liver function tests [1,3,8,9,11].

The complex of craniofacial dysmorphic features is considered specific enough to establish the diagnosis. It includes a flattened facial and occipital areas, high forehead, hypertelorism, epicanthus, sunken wide bridge of the nose, hypoplasia of the eyebrow arches, micrognathia, high palate, large anterior fontanelle, divergence of the cranial sutures, low-set ears [1,8]. Older children may have retinal dystrophy, sensorineural hearing loss, liver dysfunction, delay in intellectual and statomotive development, short limbs. Liver dysfunction is often first diagnosed due to significant bleeding due to coagulopathy. Cases of adrenal insufficiency and osteopenia have been described as well [8,9].

Diagnosis of ZSD is based on clinical manifestations, biochemical studies (increased levels of VLCFA, phytanic and pristanic acid in the blood, pipecolic acid and bile acids in urine) and molecular genetic testing, which can confirm the mutation in one of 13 known PEX genes (which are responsible for the development of this pathology) [8].

Currently, there is no etiotropic treatment for patients with ZSD. Symptomatic treatment aimes to alleviate the patient's condition and to prevent complications. In the presence of a seizure, standard anticonvulsant therapy is used, but it is known that seizures in patients with ZSD are difficult to control [8]. In the presence of adrenal insufficiency, steroid replacement therapy may be initiated. In case of hearing loss, hearing aids are fitted. In case of visual impairment, it is possible to use glasses or surgery (cataract removal).

Table1. Frequency of mutated PEX genes in patients with ZSD [8]

Gene	% ZSD	Gene	% ZSD
PEX1	60,5-70	*PEX13*	1,5
PEX6	14,5	*PEX16*	1,1
PEX12	7,6	*PEX3*	0,7
PEX26	4,2	*PEX19*	0,6
PEX10	3,4	*PEX14*	0,5
PEX2	3,1	*PEX11ß*	0,1
PEX5	2,0		

note: ZSD - Zellweger spectrum disorder

Thus, ZSD is a progressive multiorgan disease with a variety of clinical manifestations and an unfavorable, often fatal, prognosis. The extreme phenotypic variability of ZSD (from progressive degenerative manifestations) causes a practical problem in the diagnosis of this condition and, consequently, in treatment [3]. Genetic testing is important for confirming the diagnosis, which in some cases allows predicting the course of the disease [9]. Currently, the treatment of any manifestations of ZSD is symptomatic and / or supportive [1,3].

Clinical case. We describe a 6.5 year old (girl, D.P.), first child from the first pregnancy, born at 31 weeks of gestation (premature discharge of amniotic fluid, duration of the anhydrous period 1 week), with birth weight 1450 gr and birth length 42 cm, Apgar score 7/8.

From the intensive care unit at the age of 11 days, the child was transferred to the II stage of nursing premature infants. She was discharged at the age of 1 month and 2 days with the diagnosis: "Stage III prematurity, Hypoxic-ischemic CNS damage with periventricular infiltration, vascular-epithelial cysts. Morphofunctional immaturity."

There was a delay in the girl's psychomotor development: fixation of the sight after the 2nd month of life, smiling from 2-3 months, the head holding from 5 months, sitting from 10 months, crawling from 11 months, walking with support from 17 months; did not walk independently, did not speak. The girl was followed by a neurologist from birth for hydrocephalic syndrome and delayed motor development. At the age of 13 months the child was diagnosed with cryptogenic hepatitis with moderate activity and symptoms of cholestasis, at 19 months she developed bilateral mixed hearing loss. Brain MRI revealed ventriculomegaly and periventricular zones, increased intensity of the MR signal, most likely due to gliosis zones. Electroencephalogram (20 months) - was age appropriate.

The results of electroneuromyography performed at the age of 2.5 years showed a demyelinating lesion of peripheral long fibers, with secondary neurogenic changes. The child was examined at the "Okhmatdet" Orphan Disease Center. Amino acid profile, acetylcarnitines and carbohydrates, biochemical tests for Neman-Pick A/B disease, type I galactosemia and α1-antitrypsin were all normal. At 3.5 years she had an episode of bronchitis and since than she developed statomotive regression, hypersalivation and difficulty swallowing, at 6 months she developed epilepsy and left-sided hemiparesis.

An electroencephalographic study showed disorganized bioelectrical activity of the cortex, gross changes in the biorhythms of the brain in the form of a diffuse slowdown of the main activity, regional slowdown and epileptiform activity.

An MRI of the brain was performed (Magnetom Aera, Siemens) 23.07.2018 (Figs. 3,4,5), which revealed signs of leukodystrophy Brain MRI pertinent findings includes the following.

On a series of MRI of the brain (Figs. 3,4,5) in the projection of the basal ganglies symmetrically on both sides (wavy ganglies, shell, pale layer) of the toothed ganglies, repeating their outlines, in the corpus callosum, the optic thalamus, by spreading along the conducting pathways to the Crura cerebri (symmetrically on both sides too), reaching to Varolii Bridge. The areas on T2W1, FLAIR increasings and T1W1 decreasing of MR signal are determined. This process is not accompanied with diffusion restriction on DW1. A zone of the changed MR signal, without clear contours, observed in the brain white substance of the hemispheres with the subcortical distribution on U-like fibers. The lesions is quite symmetrical. Analogical changes of cerebellum both hemispheric observed, more in the area of toothed ganglies. Brain white and gray matter differentiation are preserved. Resume: signs of leukodystrophy.

Taking into account the clinical data and anamnesis, localization and MR characteristics of changes in the cerebral hemispheres and cerebellum, assessment of the indicators of the main metabolites in the affected areas of the brain, it was assumed that there is a disease from the group of progressive genetically determined neurodegenerative diseases. Krabbe's disease, GM1-, GM2-gangliosidosis, methochromatic leukodystrophy and Canavan's disease were excluded by enzymatic studies.

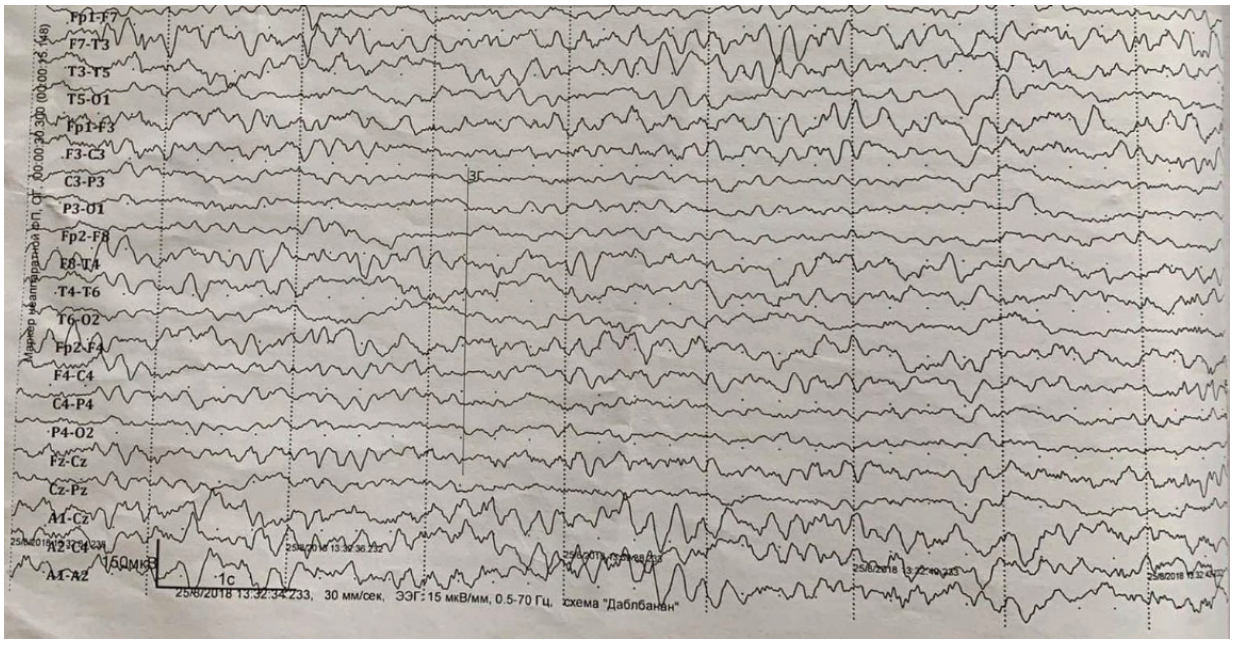

Fig. 2. Electroencephalographic investigation

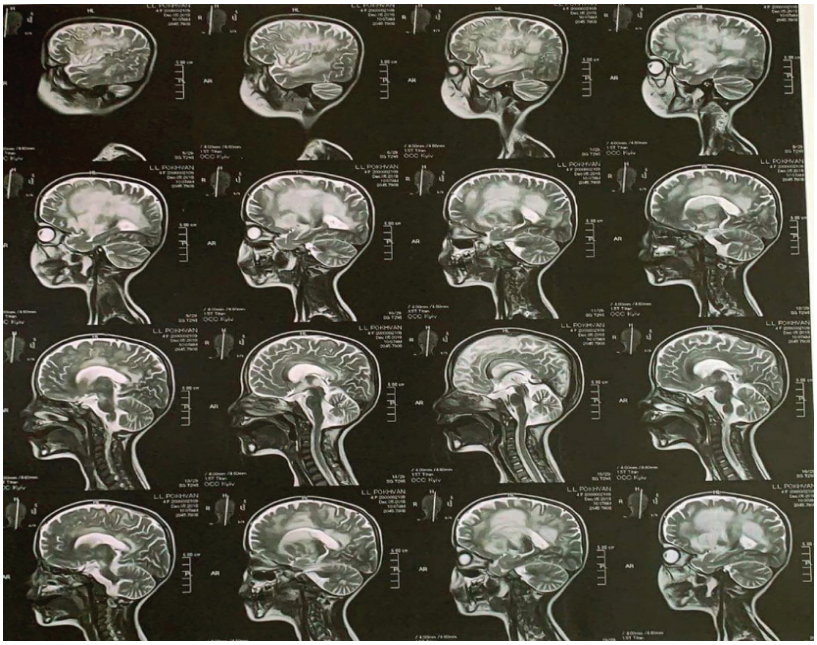

Fig. 3. MRI. Sagittal images of the brain (patient D)

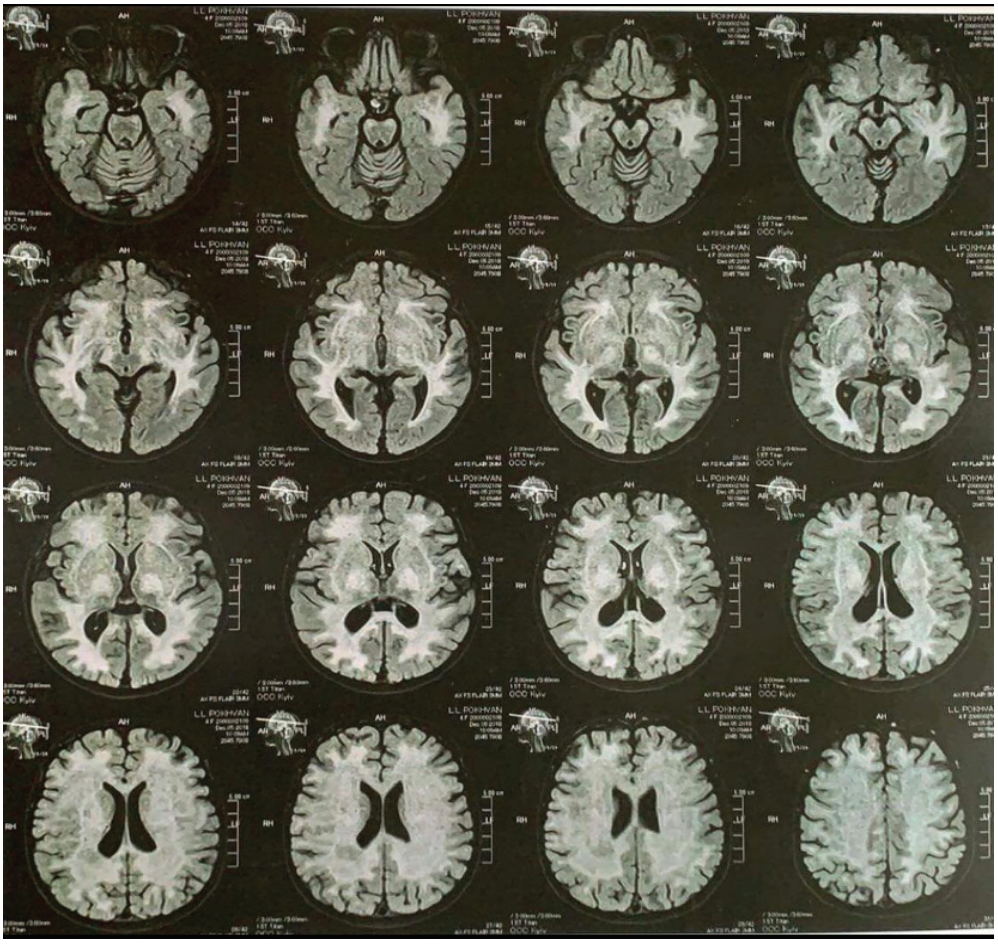

Fig. 4.MRI. Axial image of the brain (patient D)

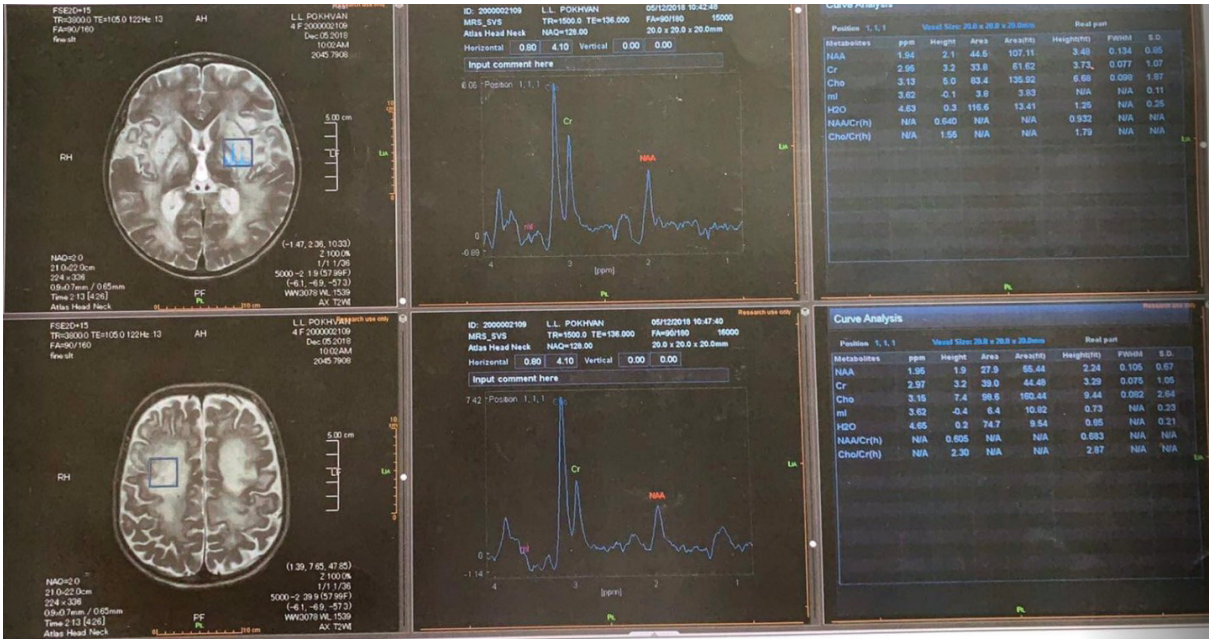

Fig. 5. Depiction of MRI main findings of the brain (patient D)

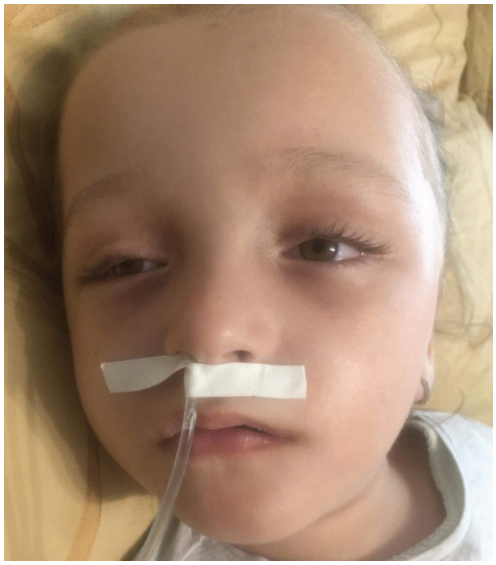

Fig. 6. Craniofacial dysmorphias in the child with ZSD (patient D) (high forehead, wide sunken nose, hypertelorism, low-set ears)

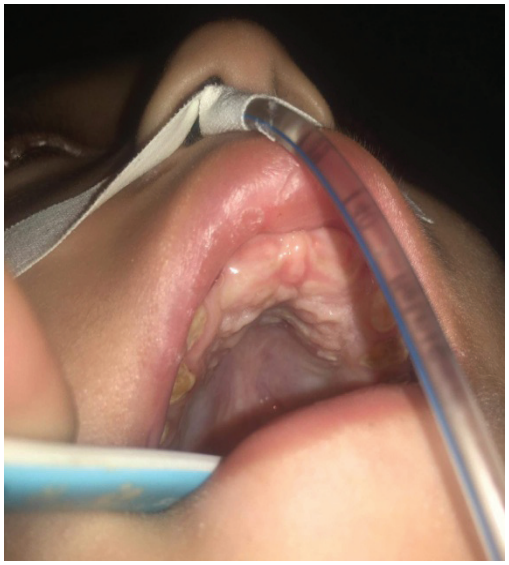

Fig. 7. High palate in the child with ZSD(patient D)

To clarify the diagnosis, diagnoses NGS of 309 genes causative for metabolic conditions was performed (sequencing and deletion–duplicationanaliysis), which revealed a pathogenic homozygous-variant c.292> T (p.Arg98Trp) in the *PEX26* gene. According to the literature, mutations in the *PEX26* gene are causative for autosomal-recessive ZSD[8]. Currently, the child's condition is consistently severe (age 6.5 years). There is a pronounced regression of cognitive and motor skills, spastic tetraparesis, pseudobulbar syndrome and epilepsy.

The girl shows practically no reaction to examination. Does not pronounce individual sounds. Does not hear. Does not hold her head, does not turn over on her own, does not sit, does not stand with support. Feeding is carried out through a nasogastric tube. The head is hydrocephalic. Dysmorphic features include high forehead, wide sunken nose, hypertelorism, low-set ears, high palate (Fig. 6,7). The skin and visible mucous membranes are pale pink, clean. Contractures of the tibial joints are present. Peripheral lymph nodes are not enlarged. Pulmonary and cardiovascular systems are without pathological changes. The abdomen is soft and palpable. There is no splenomegaly. Physiological excretory functions are normal.

Neurological status: mimic innervation is symmetrical, pharyngeal reflex is reduced; muscle tone in the extremities is diffusely reduced, with the formation of spasticity in the distal extremities (D = S); tendon reflexes from the upper extremities are reduced (D = S), from the lower extremities are absent; abdominal reflexes are positive; meningeal symptoms are absent. Approximately twice a month, tonic convulsions are noted in the form of general tension, "rolling" of the eyes, followed by vomiting. She receives anticonvulsant treatment (Topiromax, Levicitam), Ukrliv and multivitamins (Smart Omega)

The dynamics of stato-motor regression is shown on Fig. 8.

Fig. 8. Dynamics of stato-motor regression (patient D.P.). 1 - age 3 months, 2 - 9 months, 3 - 15 months, 4 - 19 months, 5 - 3.5 years, 6 - 4.5 years, 7 - 5 years, 8 - 6.5 years old

Based on the various research works received, it is possible to draw a conclusion about moderate course of the patient's disease. The main clinical presentations, in particular, delayed psychomotor, statokinetic development, sensory deficits, dysmorphias, prospective (within 6 years) addition of concomitant somatic pathology (hepatobiliary, bronchopulmonary system), which are due to the presence of EEG data on disorganized bioelectric activity of the brain cortex and leukodystrophy (based on MRI data) prove the belonging to progressive genetically determined neurodegenerative diseases. Analyzing the clinical manifestations, onset and rate of disease progression, this clinical case of Zellweger's spectrum disorder can be interpreted as Zellweger's syndrome, but there are signs of another phenotype – infantile Refsum's disease (swallowing disorders, cryptogenic hepatitis, cholestasis) which coincides with the latest research works concerning the lack of purpose to allocate separate phenotypic groups.

The long-term prognosis for children with Zellweger spectrum disorder is poor and often fatal. Usually, the most common cause of death is progressive respiratory or hepatic failure and gastrointestinal bleeding. Most of the patients die in early childhood, a minority - in the second decade of life. In the literature, there is increasingly more data on the confirmation of ZSD in adults with hearing and/or vision loss with normal intelligence and neurological status [8].

Challanges in the diagnosis of the disease are enhanced by the lack of effective therapeutic measures. Zellweger spectrum disorder is highly variable in clinical presentation. A laboratory study, which is recommended for suspected cases of this disease, in some cases is of limited significance and does not reveal abnormalities. Therefore, to confirm the diagnosis of ZSD, molecular genetic testing is necessary. The management of these patients is interdisciplinary and symptomatic, based on a regular assessment of the child's psychomotor development, neurological status, functional condition of the liver and adrenal glands, identification of orthopedic problems, hearing and visual impairments, and management of feeding difficulties [3,8].

Today it is difficult to predict the course of the disease in each particular case. The identification of new modifier genes and their mutations will probably allow predicting the course of the disease. Thus, molecular genetic research is extremely important for early detection of pathology, improvement and development of new strategies for patient management and effective counseling of family members (both at the postnatal and prenatal stages).

Conclusions. 1. Zellweger spectrum disorder is a hereditary autosomal recessive disease with a wide range of clinical manifestations and poor prognosis. Molecular genetic testing enables confirmation of the diagnosis in order to provide effective counseling to family members.

2. Although there is currently no specific treatment for the disease, significant progress has been made in understanding the molecular and biochemical aspects of the condition, that would hopefully lead to the development of new research strategies and treatments in future.

REFERENCES

1. Cheillan D. Zellweger Syndrome Disorders: From Severe Neonatal Disease to Atypical Adult Presentation. // Adv Exp Med Biol. 2020;1299:71-80.
2. Berendse K, Engelen M, Linthorst GE, van Trotsenburg AS, Bwee Tien Poll-The BW. High Prevalence of primary adrenal insufficiency in Zellweger spectrum disorder. // Orphanet J Rare Dis 2014 Sep 2;9:133.
3. Braverman NE, Raymond GV, Rizzo WB, Moser AB, Wilkinson ME, Stone EM, Steinberg SJ, Wangler MF, Rush ET, Hacia JG, Bose M. Peroxisome biogenesis disorders in the Zellweger spectrum: An overview of current diagnosis, clinical manifestations, and treatment guidelines. // Mol Genet Metab. 2016;117(3):313.
4. Bwee Tien Poll-The, Jutta Gärtner. Clinical diagnosis, biochemical findings and MRI spectrum of peroxisomal disorders. // Biochim Biophys Acta. 2012 Sep;1822(9):1421-9.

5. Ebberink MS, Mooijer Petra AW, Gootjes J, Koster J, Wanders Ronald J A, Waterham HR. Genetic classification and mutational spectrum of more than 600 patients with a Zellweger syndrome spectrum disorder. // Hum Mutat. 2011 Jan;32(1):59-69.
6. Femke CC Klouwer, Berendse K, Ferdinandusse S, Ronald JA Wanders, Engelen M , Bwee Tien Poll-The. Zellweger spectrum disorders: clinical overview and management approach. // Orphanet J Rare Dis. 2015 Dec 1;10:151.
7. Tsuneo Imanaka. Biogenesis and Function of Peroxisomes in Human Disease with a Focus on the ABC Transporter. // Biol Pharm Bull. 2019;42(5):649-65.
8. Steinberg SJ, Dodt G, Gerald V Raymond, Braverman NE , Ann B Moser, Hugo W Moser. Peroxisome biogenesis disorders. // Biochim Biophys Acta. 2006 Dec;1763(12):1733-48.
9. Rosewich H, Ohlenbusch A, Gärtner J. Genetic and clinical aspects of Zellweger spectrum patients with PEX1 mutations. // J Med Genet. 2005 Sep;42(9):58.
10. Terlecky SR, Walton PA. The Biogenesis and Cell Biology of Peroxisomes in Human Health and Disease. // The Biogenesis of Cellular Organelles. 2005;164-75.
11. Wanders R.J. Metabolic functions of peroxisomes in health and disease. // Biochimie, 03 Sep 2013, 98:36-44.
12. Waterham HR, Ferdinandusse S., Wanders RJ. Human disorders of peroxisome metabolism and biogenesis. // Biochim Biophys Acta. 2016 May;1863(5):922-33.
13. Braverman NE, D'Agostino MD, Maclean GE. Peroxisome biogenesis disorders: Biological, clinical and pathophysiological perspectives. // Dev Disabil Res Rev 2013;17(3):187-96.
14. Steinberg SJ, Dodt G,.Raymond GV, Braverman NE, Moser AB, Moser HW.Peroxisome biogenesis disorders. // Biochim Biophys Acta 2006;1763 (12):1733–48.

SUMMARY

IMPAIRMENT OF PEROXISOME BIOGENESIS IN THE SPECTRUM OF ZELLWEGER SYNDROME (CLINICAL CASE)

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The incidence of rare diseases is approximately two cases per 10,000 people. Today, in most cases, orphan diseases are caused by genetic disorders, less often - some forms of oncological, oncohematological, infectious disorders. These conditions have a severe and chronic course, accompanied by a decrease in quality and a reduction in the life expectancy of patients.

Aim - describe a clinical case of an rare disease that is referred to as Zellweger spectrum disorders.

Literature review and analysis of clinical-anamnestic and laboratory-instrumental methods of research of a 6.5 years old girl.

The given clinical case, namely Zellweger spectrum disorders (ZSD), is a hereditary autosomal recessive disease characterized by nonspecific clinical manifestations and phenotype, which complicates timely diagnosis and delays symptomatic, and in some cases prognostically favorable treatment. Molecular genetic research makes it possible to finally confirm this disease. Therefore, at the slightest suspicion of this pathology, it is worth investigating the level of long-chain fatty acids, plasmalogen of erythrocytes, intermediate metabolites of bile acid synthesis, or carrying out genetic sequencing. Further studies of this condition are carried out in the world in order to obtain new methods of treatment and improve the quality of life of patients.

The presented clinical case of a rare disease, which belongs to ZSD, confirms the need for alertness of family doctors and pediatricians in order to timely diagnose and correct rare diseases in children.

Keywords: impaired biogenesis of peroxisomes, Zellweger spectrum disorders, orphan diseases.

РЕЗЮМЕ

НАРУШЕНИЕ БИОГЕНЕЗА ПЕРОКСИСОМ В СПЕКТРЕ СИНДРОМА ЗЕЛЬВЕГЕРА (КЛИНИЧЕСКИЙ СЛУЧАЙ)

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Частота орфанных (редких) заболеваний составляет примерно два случая на 10 000 населения. В большинстве случаев причиной орфанных заболеваний являются генетические нарушения, реже - некоторые формы онкологических, онкогематологических, инфекционных нарушений. Данные состояния имеют тяжелое и хроническое течение, сопровождаются снижением качества и сокращением продолжительности жизни пациентов.

Целью исследования является описание клинического случая орфанного заболевания, которое относится к расстройствам спектра Зельвегера (Zellweger spectrum disorders).

Проведен обзор литературы и анализ клинико-анамнестических и лабораторно-инструментальных методов исследования девочки Л., 5 лет.

Приведенный клинический случай, в частности расстройство спектра Зельвегера, является наследственным аутосомно-рецессивным заболеванием, характеризуется неспецифическими клиническими проявлениями и фенотипом, что затрудняет своевременную диагностику и отсрочивает симптоматическое, а в некоторых случаях прогностически благоприятное лечение. Молекулярно-генетическое исследование позволяет окончательно подтвердить данное заболевание. Поэтому при малейшем подозрении на эту патологию следует исследовать уровень жирных кислот с длинными цепями, плазмалоген эритроцитов, промежуточные метаболиты синтеза желчных кислот или проводить генетическое секвенирование.

Представленный клинический случай орфанного заболевания, который относится к расстройствам спектра Зельвегера, подтверждает необходимость настороженности семейных врачей и педиатров с целью своевременной диагностики и коррекции редких заболеваний у детей.

რეზიუმე

პეროქსისომების ბიოგენეზის დარღვევა ზელვეგერის სინდრომის სპექტრში (კლინიკური შემთხვევა)

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უჟგოროდის ეროვნული უნივერსიტეტი, უკრაინა

ორფანული (იშვიათი) დაავადებების სიხშირე დაახლოებით 2 შემთხვევაა 10 000 მოსახლეზე. ორფანული დაავადებების მიზეზს უმეტეს შემთხვევაში გენეტიკური დარღვევები წარმოადგენს, უფრო იშვიათად - ონკოლოგიური, ონკოჰემატოლოგიური, ინფექციური დარღვევების ზოგიერთი ფორმა. ამ მდგომარეობებს ახასიათებს მძიმე და ქრონიკული მიმდინარეობა, თან ახლავს პაციენტების სიცოცხლის ხარისხის და ხანგრძლივობის შემცირება.

კვლევის მიზანს წარმოადგენდა ორფანული დაავადების კლინიკური შემთხვევის აღწერა, რომელიც მიეკუთვნება ზელვეგერის სპექტრის დარღვევებს (Zellweger spectrum disorders).

ჩატარებულია ლიტერატურის მიმოხილვა და 5 წლის გოგონა ლ.-ს კლინიკურ-ანამნეზური და ლაბორატორიულ-ინსტრუმენტული კვლევის მეთოდების ანალიზი.

წარმოდგენილი კლინიკური შემთხვევა, სახელდობრ - ზელვეგერის სპექტრის დარღვევა, წარმოადგენს მემკვიდრულ აუტოსომურ-რეცესიულ დაავადებას, ხასიათდება არასპეციფიკური კლინიკური გამოვლინებებით და ფენოტიპით, რაცართულებს დროულ დიაგნოსტიკას და გადაავადებს სიმპტომურ დიაგნოსტიკას, ზოგიერთ შემთხვევაში კი – პროგნოზულად კეთილსაიმედო მკურნალობასაც. მოლეკულურ-გენეტიკური კვლევა იძლევა დაავადების საბოლოო დადასტურების შესაძლებლობას. ამიტომ, ამ პათოლოგიაზე უმცირესი ეჭვის არსებობის დროს აუცილებელია გრძელჯაჭვიანი ცხიმოვანი მჟავების დონის, ერითროციტების პლაზმინოგენის, ცხიმოვანი მჟავების სინთეზის შუალედური მეტაბოლიტების გამოკვლევა, ან გენეტიკური სეკვენირების ჩატარება. ორფანული დაავადების წარმოდგენილი კლინიკური შემთხვევა, რომელიც მიეკუთვნება ზელვეგერის სპექტრის დარღვევებს, მოითხოვს ოჯახის ექიმების და პედიატრების ყურადღების აუცილებლობას იშვიათი დაავადებების დროული დიაგნოსტიკის და კორექციის მიზნით ბავშვებში.