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MULTISYSTEM AFFECTION IN CHILD: NARP SYNDROME – MITOCHONDRIAL DISEASE (CASE PRESENTATION)

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REZUMAT

AFECTARE MULTISISTEMICĂ LA COPIL: SINDROMUL NARP – MALADIE MITOCONDRIALĂ (PREZENTARE DE CAZ)

Cuvinte-cheie: sindromul NARP, afectare multisistemică, maladie mitocondrială.

Introducere. Maladiile mitocondriale fac parte din grupul tulburărilor neurodegenerative cauzate de dereglările ale generării energiei mitocondriale celulare. Sindromul NARP (neuropatie, ataxie și retinită pigmentară) este determinat de mutații punctiforme în gena *MT-ATP6* din cadrul ADN-ului mitocondrial și este caracterizat prin variabilitatea manifestărilor clinice. Transmiterea mutației se realizează pe cale maternă, iar incidența este 1: 12.000 nașterii. Gravitatea manifestărilor clinice este asociată cu gradul de heteroplasmie a mutației ce determină patologia în cauză.

Materiale și metode. Se raportează cazul unei fetițe, născută la termen, care s-a dezvoltat normal până la vârsta de 3 luni, însă ulterior a dezvoltat hipotonie, deficit ponderal, retard psiho-motor sever, dificultăți de alimentare, convulsii rezistente la terapie anticonvulsivantă, atrofie parțială a nervului optic și hepatomegalie. Investigațiile metabolice de bază, RMN-ul cerebral și analiza molecular-genetică au fost utilizate pentru diagnosticul patologiei mitocondriale.

Rezultate. Luând în considerarea afectarea multisistemică și prezența manifestărilor clinice preponderent neurologice, pacientul a fost suspectat pentru o eroare înnăscută de metabolism. Inițial, considerând prezența hipotoniei progresive marcate ca simptom clinic cheie, s-a exclus Atrofia musculară spinală. În același timp a fost inițiat work-up-ul metabolic, dezvăluindu-se schimbări relevante pentru o maladie mitocondrială în sânge: hiperlactatacidemie [lactat 3.7-7.8 mmol/L, x 3 ori la rând, val. ref. 0.7-2.1 mmol/L], hiperalaninemie Ala [1038, val. ref. < 450 μmol/L], raportul Ala/Lys [11.8, abnormal dacă >3] și în urină: hiperaminoacidurie parțială. RMN cerebral (3,0T) – focare patologice la nivelul nucleilor bazali bilaterali. În urma sumării manifestărilor clinice și paraclinice s-au obținut 8 puncte ca scor specific pentru maladie mitocondrială definită (Criteriile Nijmegen). Nu a fost efectuată biopsia musculară, ca test confirmativ de diagnostic. Copilul a decedat la 9 luni de viață, iar diagnosticul molecular genetic la nivelul ADN-ului mitocondrial a fost efectuat postmortem în RadboudUMC (Nijmegen, Olanda), determinându-se mutația punctiformă m.8993T>G (Leu156Arg), cunoscută ca fiind determinantă în dezvoltarea sindromului NARP. Nu a fost posibilă aprecierea heteroplasmiei.

Discuții. Sindromul NARP se caracterizează printr-o varietate de simptome și semne clinice cu preponderent de afectare neurologică. Diagnosticul acestei patologii deseori reprezintă o provocare pentru clinicieni determinată heterogenitate clinică care se suprapune cu alte maladii genetice. Debutul și evoluția simptomelor clinice depinde de gradul de heteroplasmie a mutației. Algoritmul de diagnosticul include inițierea work-upului metabolic, efectuarea RMN cerebral, biopsia musculară și determinarea mutației la nivel molecular genetic. Managmentul terapeutic rămâne a fi simptomatic pentru îmbunătățirea calității vieții pacientului.

Concluzii. Debutul precoce, polimorfismul manifestărilor clinice, cum ar fi afectarea sistemului nervos central, slăbiciunea musculară, retardul psihomotor și convulsii în cazul unui copil ar trebui să determine clinicianul să ia în considerare sindromul NARP cu efectuarea investigațiilor suplimentare, cum ar fi măsurarea acidului lactic în sânge, efectuarea electromiografiei, rezonanței magnetice nucleare și testarea genetică.

SUMMARY

MULTISYSTEMIC AFFECTION IN CHILD: NARP SYNDROME – MITOCHONDRIAL DISEASE (CASE PRESENTATION)

Keywords: NARP syndrome, multisystemic affectation, mitochondrial disease.

Introduction. Mitochondrial diseases are part of the group of neurodegenerative disorders caused by disruptions of cellular mitochondrial energy generation. NARP syndrome (Neurogenic weakness, ataxia, and retinitis pigmentosa) is caused by point mutations in the *MT-ATP6* gene in mitochondrial DNA and is characterized by variability in clinical manifestations. The mutation is transmitted maternally, and the incidence is 1: 12,000 births. The severity of clinical manifestations is associated with the degree of heteroplasmia of the disease-causing mutation.

Material and methods. We report on a case of a girl, born at term, who developed normally until the age of 3 months, but later developed hypotonia, weight deficit, severe psychomotor retardation, eating difficulties, seizures resistant to anticonvulsant therapy, partial atrophy of the optic nerve and hepatomegaly. Basic metabolic investigations, brain MRI and molecular-genetic analysis were used to diagnose mitochondrial pathology.

Results. Considering the multisystemic impairment and the presence of predominantly neurological clinical manifestations, the patient was suspected of an innate metabolic error. Initially, considering the presence of marked progressive hypotonia as a key clinical symptom, spinal muscular atrophy was excluded. At the same time, the metabolic work-up was initiated, revealing relevant changes for a mitochondrial disease in the blood: hyperlactatacidemia [lactate 3.7-7.8 mmol/L, x 3 times in a row, ref. val. 0.7-2.1mmol / l], hyperalaninemia *Ala* [1038, ref. val. <450 μmol/L], *Ala/Lys* ratio [11.8, abnormal if >3] and in urine: partial hyperaminoaciduria. Brain MRI (3.0T) – pathological foci in the bilateral basal nuclei. Following the summation of clinical and paraclinical manifestations, 8 points were obtained as a specific score for defined mitochondrial disease (Nijmegen Criteria). No muscle biopsy was performed as a confirmatory diagnostic test. The child died at 9 months of age, and the genetic molecular diagnosis of mitochondrial DNA was performed postmortem in RadboudUMC (Nijmegen, Netherlands), determining the point mutation m.8993T> G (Leu156Arg), known to be decisive in development of NARP syndrome. It was not possible to assess level of heteroplasmy.

Discussions. NARP syndrome is characterized by a variety of symptoms and clinical signs, with predominantly neurological impairment. Diagnosis of this pathology is often a challenge for clinicians due to clinical heterogeneity that overlaps with other genetic diseases. The onset and development of clinical symptoms depends on the degree of heteroplasmic mutation. The diagnostic algorithm includes performing of metabolic work-up, brain MRI, muscle biopsy and genetic analysis. Therapeutic treatment is symptomatic and supportive of improving the patient's quality of life.

Conclusions. Early onset in the presence of complete health, the polymorphism of clinical manifestations, such as a central nervous system lesion, muscle weakness, impaired psychomotor development, and seizures in a child should prompt the clinician to consider NARP syndrome and conduct further investigations such as measurement of blood lactate, performing electromyography, magnetic resonance imaging, and genetic analysis.

РЕЗЮМЕ

МУЛТИСИСТЕМНЫЕ ПОРАЖЕНИЕ У ДЕТЕЙ: СИНДРОМ NARP – МИТОХОНДРИАЛЬНАЯ БОЛЕЗНЬ (КЛИНИЧЕСКИЙ СЛУЧАЙ)

Ключевые слова: синдром NARP, мультисистемное повреждение, митохондриальное заболевания.

Введение. Митохондриальные заболевания относятся к группе нейродегенеративных заболеваний, вызванных нарушением выработки клеточной митохондриальной энергии. синдром NARP (Невропатия, атаксия, пигментная дегенерация сетчатки) вызывается точечными мутациями в гене *MT-ATP6* в митохондриальной ДНК и характеризуется вариабельностью клинических проявлений. Мутаций передаются от матери, частота встречаемости составляет 1:12 000 рождении. Выраженность клинических проявлений связана со степенью гетероплазмии болезнетворной мутации.

Материалы и методы. Мы сообщаем о клиническом случае доношенной девочке, которая нормально развивалась до 3 месяцев, но позже у нее развилась гипотония, дефицит веса, тяжелая психомоторная отсталость, трудности с питанием, судороги, устойчивые к противосудорожной терапии, частичная атрофия оптических нервов и гепатомегалия. Для диагностики митохондриальной патологии использовались базовые метаболические исследования, МРТ головного мозга и молекулярно-генетический анализ.

Результаты. Учитывая мультисистемное нарушение и наличие преимущественно неврологических клинических проявлений, у пациента заподозрили врожденную метаболическую ошибку. Первоначально, учитывая наличие выраженной прогрессирующей гипотонии как ключевой клинический симптом, спинальная мышечная атрофия была исключена. В то же время, было начато исследование метаболизма, выявившее соответствующие изменения для митохондриального заболевания в крови: гиперлактатацидемия [лактат 3.7-7.8 ммоль/л, х 3 раза подряд, референсные значения 0.7-2.1 ммоль / л], гипераланинемия *Ala* [1038, референсные значения <450 мкмоль/л], соотношение *Ala/Lys* [11.8, норма, если> 3] и в моче: частичная гипераминоацидурия. МРТ головного мозга (3.0T) – патологические очаги в двухсторонних базальных ядрах. После суммирования клинических и параклинических проявлений, было получено 8 баллов в качестве специфической оценки для определенного митохондриального заболевания (Неймегенские критерии). Биопсия мышц в качестве подтверждающего диагностического теста не проводилась. Ребенок умер в возрасте 9 месяцев, и генетическая молекулярная диагностика митохондриальной ДНК была проведена посмертно в RadboudUMC (Неймеген, Нидерланды), определив точечную мутацию m.8993T>G (Leu156Arg), которая, как известно, является решающей в развитии синдрома NARP. Оценка уровня гетероплазмии не удалось.

Обсуждения. Синдром НАРП характеризуется множеством симптомов и клинических признаков с преимущественно неврологическими нарушениями. Диагностика этой патологии часто представляет собой проблему для клиницистов из-за клинической неоднородности, которая частично совпадает с другими генетическими заболеваниями. Возникновение и развитие клинических симптомов зависит от степени гетероплазмы мутации. Диагностический алгоритм включает в себя начало метаболических исследований, выполнение МРТ головного мозга, биопсию мышц и определение мутации на генетическом молекулярном уровне. Терапевтическое лечение остается симптомом улучшения качества жизни пациента.

Заключение: Ранний дебют на фоне полного здоровья, полиморфизм клинических проявлений: поражение центральной нервной системы, мышечная слабость, нарушение психомоторного развития, судороги у ребенка, должны побуждать клинициста к рассмотрению синдрома NARP и проводить дальнейшие исследования, такие как измерение лактата в крови, выполнение электромиографии, магнитно-резонансной томографии и генетического анализа.

Introduction. Mitochondrial diseases are a group of complex metabolic disorders that are defined by a genetic defect predominantly affecting mitochondrial oxidative phosphorylation [1]. Most of the mitochondrial proteins are encoded by nuclear DNA (nDNA), whereas a very small fraction is encoded by mitochondrial DNA (mtDNA) [2]. The responsible mutations harbor in both the circular 16,569 base pair mt-DNA and the nuclear DNA. The mt-DNA encodes 37 genes including 22 tRNAs and two rRNAs essential for mt-DNA-specific translation of the 13 encoded respiratory chain subunits. In the nuclear DNA over 1,000 mitochondrially localizing proteins are encoded, translated in the cytoplasm, and translocated to the mitochondria by an elaborate protein import machinery [3]. Nuclear DNA mutations are inherited through autosomal recessive, autosomal dominant or X-linked dependent pattern, whereas mitochondrial DNA mutations are transmitted by maternal inheritance [4]. The multicopy nature of mtDNA gives rise to heteroplasmy, a unique aspect of mtDNA-associated genetics that occurs when there is coexistence of a mix of mutant and wild-type mtDNA molecules (heteroplasmy). In contrast, homoplasmy occurs when all of the mtDNA molecules have the same genotype. Heteroplasmic mutations often have a variable threshold, i.e. a level to which the cell can tolerate defective mtDNA molecules. When the mutation load exceeds this threshold, metabolic dysfunction and associated clinical symptoms occur [5]. In addition to a wide range of cellular perturbations such as aberrant calcium homeostasis, excessive reactive oxygen species (ROS) production, and dysregulated apoptosis, dysfunctional mitochondria are unable to generate sufficient energy to meet the needs of various organs, particularly these with high energy demand, including the nervous system, skeletal and cardiac muscles, kidneys, liver, and endocrine system. Energy deficiency in various organs results in multiorgan dysfunction leading to the variable manifestations observed in mitochondrial diseases including cognitive impairment, epilepsy, cardiac and skeletal myopathies, nephropathies, hepatopathies, and endocrinopathies [6]. The broad and highly variable but commonly progressive phenotypic spectrum ranges from adult-onset, isolated organ system involvement to infantile-onset, multi-systemic, lethal disease [7]. Combined epidemiologic data on childhood and adult mitochondrial disease

suggest that the prevalence is at least 1 in 5,000 (20 per 100,000) [8].

Neurogenic weakness, ataxia, and retinitis pigmentosa, or NARP syndrome, is a mitochondrial disorder most commonly resulting from a point mutation at base pair 8993 of the mitochondrial genome in the *MT-ATP6* gene [9]. The incidence of NARP syndrome in general population is unknown, but is estimated to occur in 1 in 12000 births. [15]. Besides the clinical features encapsulated in the name, the clinical phenotype may also include epileptic seizures, sensorineural hearing loss, cognitive impairment, diabetes mellitus, cardiomyopathy, and lactic acidosis [10]. These signs and symptoms vary among affected individuals. Onset of symptoms, particularly ataxia and learning difficulties, is often in early childhood. Children with this condition are also more prone to delayed development and intellectual disabilities which would greatly impact their functional abilities. In addition to NARP syndrome, mutations at the same position (*MT-ATP6 gene*) may also cause fatal infantile subacute necrotizing encephalomyelopathy, a maternally inherited form of Leigh syndrome (MILS) [11].

Materials and Methods. Was reported on a case of a 7 months old girl, born at term, in a non-consanguineous healthy family. No family history for any genetic or neurological disorder. She developed normal until 3 months old, after with some deteriorations as hypotonia, failure to thrive and psychomotor delay. At the moment of first evaluation (5 months old) she presented, hypotonia, general weakness and fatigue, feeding difficulty, psychomotor delay (does not sit, not speak), partial atrophy of the optic nerve, hepatomegaly (+2cm). According to multisystem impairment and clinical manifestations, the child was suspected for an inborn error of metabolism. Was performed metabolic work-up and genetic analysis.

Table 1.

Metabolic work-up: the level of glucose and lactate in the blood

Parameters	Pre-prandial	Postprandial (after 1 h)	Postprandial (after 2 h)
Glucose (mg/dL) (ref. val 74-106mg/dL)	88	100	9.2
Lactate (mmol/L)(ref. val. 1.7-2.1 mmol/l)	7.8	7.3	3.7

Results. For the first due to pronounced hypotonia the SMA was excluded by DNA analysis. First line metabolic investigations at 5 months old showed elevated lactate in blood [2.6-3.2 mmol/l, x 3 times evaluated, *ref. val. 0.7-2.1 mmol/l*]. Patient ignored the recommendations for any other further investigations were. Then, at 7 months old returned for repeated consultation when she started to present the seizures. Was repeated metabolic work-up, lactate was in more elevated values: [3.7-7.8 mmol/l, x 3 times evaluated] (table 1); in amino acids – in blood: high level of *Ala* [1038, *ref <450 µmol/L*], *Val, Ile, Cys* and *Ala/Lys* ratio [11.8, *abnormal if >3*] (figure 1) and in urine: partial hyperaminoaciduria; high level of ammonia 73.3 µmol/L [*ref. val <30*], triglycerides 202 mg/dL, gama-GT 51 U/L, Mg [2.5 mg/dL, *ref. val. <2.3*], P [6.9 mg/dL, *ref. val.<6.2*], Cu [134.2 µg/dL, *ref. val <121*] and low level of total protein - 62 mmol/l. Normal creatinine kinase and transaminases were found. EEG showed epiphenomena predominated in the posterior regions and paroxysms of hypovolted slow activity; and on cerebral MRI was pathological areas in the basal nuclei bilateral. Normal audiogram. When the neurologist started the therapy with valproate, the child showed a reaction to valproates administration with hepatomegaly and precoma, in a few days later she died. Evaluating the clinical criteria for mitochondrial diagnosis there were counted 8 points as scoring for definite mitochondrial disorder [13]. Taking in account that in our country there is no possibility to do muscle biopsy and DNA analysis, in collaboration with research group from Nijmegen, Netherland was performed genetic analysis by screening for mtDNA rearrangements and mismatches using Long Template PCR and the Ion Torrent PGM. This revealed the presence of the 8993 T>G mutation in the *MT-ATP6* mtDNA gene. The identified mutation is associated with Neurogenic weakness, ataxia, and retinitis pigmentosa (NARP) syndrome - a mitochondrial disorder.

Proline		24,9412	2,8715
Glicine		20,7664	1,5589
Alanine	↑3,0	103,3811	9,2102
Citrulline	↑1,7	5,0707	0,8883
Ac.α-aminobutiric	↑3,3	4,9165	0,5070
Valine	High of N	25,9535	3,0405
Cysteine	↑4,7	16,4739	1,9793
Homocysteine		0,3571	0,0483
Methionine	Low of N	0,9517	0,1420
Index Fisher		3,1631	
Index C		3,6439	
Index P	↓1,3	0,9576	
Tyrosine / phenylalanine	↑2,9	2,9340	
Alanine / lizine		11,8347	
Glutamine / ammonia		1,8529	
Braking/excitatory AA	↑1,9	1,9037	

Figure 2. ***Metabolic work-up: the level of aminoacids in the blood***

Discussions. Mitochondrial diseases are a heterogeneous group of disorders affecting energy production in the human body. A multisystem impairment is typical for this group of pathologies. The diagnosis of mitochondrial disorders can be a challenge for clinicians, especially for pediatric cases, which show enormous variation in clinical presentations, as well as biochemical and genetic complexity. Diagnostic criteria for mitochondrial diseases in infants and children are available and identification of pathogenic genetic variants is required to confirm de the diagnosis [14] (figure 1). The identification of specific genetic defects is important as it not only gives insights into the underlying disease mechanism for that particular patient but also may highlight the potential specific treatments that were not considered previously.

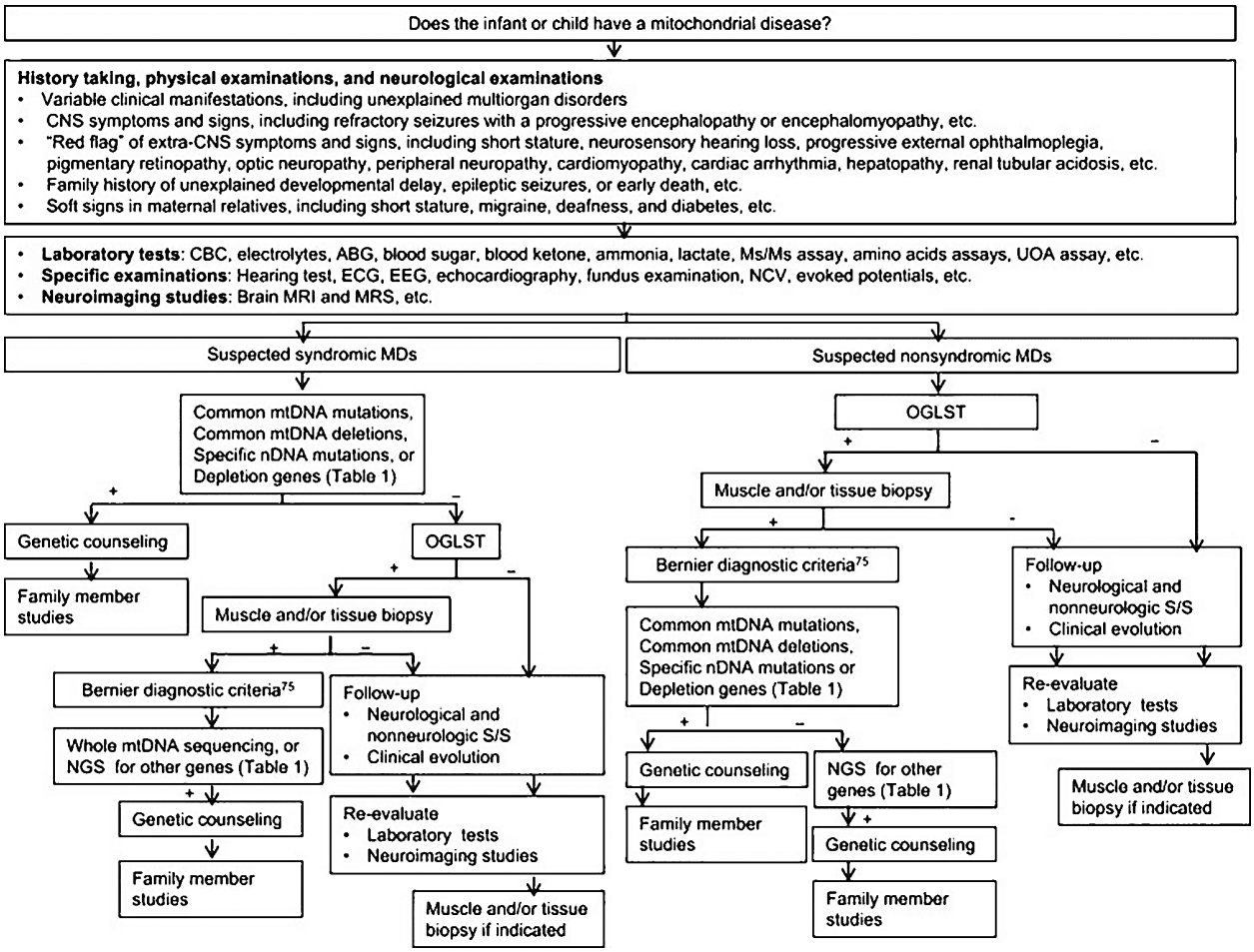

Figure 1. ***A diagnostic algorithm in infants and children with mitochondrial disease. ABG = arterial blood gas; CBC = complete blood cell count; CNS = central nervous system; ECG = electrocardiogram; EEG = electroencephalography; MDs = mitochondrial diseases; MRI = magnetic resonance imaging; MRS = magnetic resonance spectroscopy; Ms/Ms = tandem mass spectrometry; mtDNA = mitochondrial DNA; NCV = nerve conduction velocity; nDNA = nuclear DNA; NGS = next generation sequencing; OGLST = oral glucose lactate stimulation test; S/S = symptoms and signs; UOA = urinary organic acids.***

NARP syndrome is a mitochondrial cytopathy transmitted by m*aternal* inheritance, due to the heteroplasmic m.8993T>G mutation in the *MT-ATP6* mtDNA gene. The NARP syndrome is multisystem affection with variety of clinical manifestations predominantly neurological that needs a thorough differential diagnostic with other metabolic diseases, especially with Congenital Disorders of Glycosylation because some of clinical manifestations overlap. Diagnosis approach of NARP syndrome is made by clinical manifestation, metabolic work-up, and genetic tests. An important tool in diagnostic of NARP syndrome as a mitochondrial disorder is a Clinical Criteria Score for diagnosis of Mitochondrial diseases (Nijmegen, Netherlands). While unique clinical presentations would often indicate the possibility of the condition, only genetic testing would confirm the diagnosis. The m.8993T>G mutation results in substitution of a highly conserved leucine to arginine (p.Leu156Arg), and it is the most common mutation associated with NARP [12]. There are currently no proven therapies which could directly address NARP. Treatments for NARP syndrome are presently focused on symptomatic management rather than improving the biochemical defect caused by the particular mutation.

Conclusions. Early onset in the presence of complete health, the polymorphism of clinical manifestations, such as a central nervous system lesion, muscle weakness, impaired psychomotor development, and seizures in a child should prompt the clinician to consider NARP syndrome and conduct further investigations such as measurement of blood lactate, performing electromyography, magnetic resonance imaging, and genetic analysis.

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