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CHALLENGES IN DIAGNOSIS OF MITOCHONDRIAL DISORDERS: CASE REPORTS

Institute of Mother and Child

REZUMAT

PROVOCĂRI ÎN DIAGNOSTICUL TULBURĂRILOR MITOCONDRIALE: CAZ CLINIC

Introducere. Maladiile mitocondriale sunt cele mai frecvente tulburări neurometabolice moștenite, ce pot apărea la orice vârstă și sunt provocate de mutații ale genelor din ADN-ul mitocondrial (ADNmt), dar și ADN-ul nuclear (ADNn) care codifică proteinele mitocondriale structurale sau cele implicate în funcția mitocondrială.
Rapoarte de caz. Primul caz este cel al unui băiat, născut la termenul de 40 sa, prin operație cezariană urgentă din cauza insuficienței secundare a forțelor de contracție cu greutatea de 2900 g, scor Apgar 6/7, în cuplu non-consangvin. Având în vedere prezența acidozei metabolice profunde, hiperalaninemie, tablou clinic manifestat prin convulsii, decompensare metabolică, hepatomegalie, deteriorarea bruscă și progresivă a stării de sănătate s-a suspectat o eroare înnăscută de metabolism, cu predilecție o patologie mitocondrială. Pe parcursul internării starea pacientului s-a complicat iar în pofida măsurilor de resuscitare, s-a constatat moarte clinică a nou-născutului. Post-mortem a fost efectuată secvențierea Sanger completă a genomului mitocondrial, însă nu s-a denotat nici o mutație patologică la nivelul ADNmt. Pacientul 2, se prezintă primar la vârsta de 15 ani, copil I, din cuplu non-consangvin, născut la termen cu acuze la pierdere bruscă a vederii la ochiul drept de la vârsta de 14 ani, iar peste 2-3 săptâmâni și la ochiul stâng, dureri oculare la privier, nistagm, toleranță scăzută la efort fizic, fatigabilitate, scotom centra relative la ochiul stâng și scotom central absolut la ochiul drept. În rezultatul secvențierii Sanger a fost identificată mutația m.11778G>A, sugestivă pentru Neuropatia optică ereditară Leber (LHON).
Discuții. În cele două cazuri ilustrate mai sus, am dorit să evidențiem că diagnosticarea patologiei mitocondriale primare este dificilă și chiar și atunci când tabloul clinic și investigațiile biochimice sugerează prezența certă a unei boli mitocondriale, diagnosticul molecular genetic este cel care face diferența.
Concluzii. Având în vedere că maladiile mitocondriale sunt asociate cu diverse manifestări și simptome clinice eterogene, subliniem importanța unei abordări integrative multidisciplinare la nivel clinic, biochimic, imagistic și genetic pentru diagnosticul, managementul și tratamentul pacienților cu simptomatologie sugestivă maladiilor mitocondriale.

Cuvinte-cheie: ADN mitocondrial; boală mitocondrială; sindromul LHON.

РЕЗЮМЕ

ПРОБЛЕМЫ ДИАГНОСТИКИ МИТОХОНДРИАЛЬНЫХ НАРУШЕНИЙ: ОПИСАНИЕ КЛИНИЧЕСКИХ СЛУЧАЕВ

Введение: Митохондриальные заболевания являются наиболее распространенными наследственными нейрометаболическими нарушениями, которые могут появиться в любом возрасте и вызваны мутациями в генах митохондриальной ДНК (мтДНК), а также ядерной ДНК (яДНК), которые кодируют структурные митохондриальные белки или белки, участвующие в митохондриальной функции.
Описание клинических случаев: Первый случай – мальчик, рожденный в срок 40 недель путем экстренного кесарева сечения по поводу вторичной недостаточности сократительной способности матки, массой 2900 г, оценка по шкале Апгар 6/7, у некровнородственной пары. Учитывая наличие глубокого метаболического ацидоза, гипераланинемии, клинической картины, проявляющейся судорогами, метаболической декомпенсацией, гепатомегалией, внезапным и прогрессирующим ухудшением самочувствия, заподозрили врожденное нарушение метаболизма, преимущественно митохондриальную патологию. При госпитализации состояние больного осложнилось и, несмотря на проведенные реанимационные

мероприятия, констатирована клиническая смерть новорожденного. Было проведено посмертное полное секвенирование митохондриального генома по Сэнгеру, но патологических мутаций мтДНК обнаружено не было. Пациент 2, впервые обратился в возрасте 15 лет, первый ребенок от некровнородственной пары, родился в срок, с обвинениями в внезапной потере зрения на правый глаз с 14 лет, через 2-3 нед. также в левом глазу, боль в глазу при взгляде, нистагм, низкая переносимость физической нагрузки, повышенная утомляемость, относительная центральная скотома левого глаза и абсолютная центральная скотома правого глаза. В результате секвенирования по Сэнгеру была идентифицирована мутация m.11778G>A, что свидетельствует о наследственной атрофииb зрительных нервов Лебера (LHON).
Обсуждения: В двух приведенных выше случаях мы хотели подчеркнуть, что диагностика первичной митохондриальной патологии затруднена, и даже когда клиническая картина и биохимические исследования указывают на определенное присутствие митохондриального заболевания, решающую роль играет молекулярно-генетический диагноз.
Заключение: Учитывая, что митохондриальные заболевания связаны с различными гетерогенными клиническими проявлениями и симптомами, мы подчеркиваем важность междисциплинарного интегративного подхода на клиническом, биохимическом, инструментальном и генетическом уровне для диагностики, ведения и лечения пациентов с симптоматикой, указывающей на митохондриальные заболевания.

Ключевые слова: митохондриальная ДНК; митохондриальная болезнь; синдром LHON

INTRODUCTION

Mitochondria are dynamic subcellular organelles with innumerable functions [1], including energy generation via oxidative phosphorylation (OXPHOS), calcium homeostasis, and regulation of apoptotic cell death [2], placing them at the centre of cellular metabolism and signaling [3].
Mitochondrial diseases are by far the largest class of inborn errors of metabolism (IEM) [4] with a collective incidence of 1.6 in 5,000 [5], characterized by clinical, biochemical, and genetic heterogeneity, diagnostic odysseys, and absence of disease-modifying curative therapies. Mitochondrial disorders are caused by mutations in genes that primarily affect oxidative phosphorylation and ATP synthesis, disrupting a plethora of cellular metabolic and homoeostatic functions [6]. The responsible mutations harbour in both the circular 16,569 base pair mitochondrial DNA (mt-DNA) and the nuclear DNA (nDNA) [7]. 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 nDNA over 1,000 mitochondrially localizing proteins are encoded, translated in the cytoplasm, and translocated to the mitochondria by an elaborate protein import machinery [8]. Consequently, mitochondria require dual genomic expression of proteins, which originate both in the nDNA and mt-DNA. The nDNA diseases manifest Mendelian inheritance (autosomal dominant or recessive) or X-linked transmission whereas mt-DNA diseases are maternally inherited (mutations of the mt-DNA) or sporadic (mt-DNA single deletions syndromes) [7]. Furthermore, the multicopy nature of mitochondrial DNA gives rise to the concept of heteroplasmy (when both mutated and wild type mtDNA molecules coexist in the same cell) and homoplasmy (only mutant mt-DNA is present in the mitochondria of the cell). For a disease to manifest symptoms, the mutated mt-DNA molecules in tissue must increase to a critical threshold above which OXPHOS function is impaired [9]. In general, the proportion of mitochondrial genomes that harbor a pathogenic variant directly correlates with disease severity [10].
Clinical manifestations may range from isolated organ involvement with onset late in life to onset of severe multisystem problems in the newborn period leading to early death. Single organ symptoms may be the cardinal or only symptom, but more commonly progressive problems develop over time in additional systems [11]. Disease courses may be characterized by rapid decline or prolonged periods of stability with intercurrent decompensation with stressors such as infections, fevers, or anesthesia. Symptom severity may range from mild to severe, and fluctuate over time. Multi-system manifestations, particularly when progressive over time, should prompt consideration of primary mitochondrial disease, especially when involving functional rather than structural manifestations. Dysmorphic features are recognized in some primary mitochondrial diseases, but remain relatively uncommon, as are rheumatologic, dermatologic, oncologic, and primary orthopedic problems [12].
Currently, the Laboratory of Human Molecular Genetics of The Mother and Child Institute aims to create a step-by-step laboratory diagnostic algorithm, which would include biochemical and molecular genetic investigations for the purpose of establishing the diagnosis of mitochondrial disease in patients presenting the characteristic symptoms of mitochondrial disorders. In the following, we will elucidate two clinical cases of two patients who were suspected of a mitochondrial pathology, but based on the results of the molecular-genetic analyses, one of the patients was diagnosed with Leber Hereditary Optic Neuropathy (LHON), and in the case

of the second patient no molecular-genetic diagnosis was confirmed. It is well known that genetic complexity together with ever widening clinical spectrum associated with mitochondrial dysfunction poses a major challenge in diagnosis and our clinical cases highlight once again the difficulty of diagnosing these pathologies at the genetic.

CASE REPORTS

Case 1. The first case is a male infant, born from the first pregnancy, at term 40 weeks, by emergency cesarean section due to secondary insufficiency of the contraction forces, weighing 2900 g, Apgar score 6/7, in a non-consanguineous couple. At birth, the child was in very serious condition, with signs of respiratory failure, placed on nCPAP, FiO2–45%. The first 24 hours of life are complicated by episodes of hypoglycemia (corrected), hypotension (corrected with medication), with negative dynamics. Given the presence of metabolic acidosis due to low pH [7.079-7.177; ref. val. 7.35-7.45], low glucose [1.1 mmol/L; ref. val. 4,0-5,9 mmol/L], extremely elevated lactate [20 mmol/L; ref. val. 0,7-2,1 mmol/L], slightly increased anion gap [14.1-18 mmol/L; ref. val. 4-12 mmol/L], elevated ammonia [43 μmol/L; ref. val. 9-30 μmol/L], ketone bodies present in urine, clinical picture manifested by convulsions, metabolic decompensation, hepatomegaly, lactic acidemia, sudden and progressive deterioration of health state, an inborn error of metabolism was suspected, with a predilection for mitochondrial pathology. Amino acid analysis was performed in the blood and urine highlighting changes suggestive of a mitochondrial disease, including a high level of alanine [831,82 μmol/L; ref. val. 134.64 - 460.20 μmol/L] and deviant Ala/Lys ratio [3.8, abnormal if >3]. The patient was evaluated and scored according to the Nijmegen Mitochondrial Disease Criteria Scale (Score 1: mitochondrial disorder unlikely; score 2 to 4: possible mitochondrial disorder; score 5 to 7: probable mitochondrial disorder; score 8 to 12: definite mitochondrial disorder) [13]. After evaluating the patient, a score equal to 8 points was obtained (figure 1), suggesting the definite presence of a mitochondrial disease. He had no family history of any genetic or neurological disorder. Enzymology, histology and functional fibroblast ATP synthesis rate were not performed due to the paucity of facilities and financial constraints. On the 5th day of life, the newborn was transferred to the newborn resuscitation unit of the Institute of Mother and Child with the following admission diagnoses: Neonatal Sepsis; Meconium aspiration in the neonatal period; Hypoxic Ischemic Encephalopathy in newborn Sarnat Grade II; Mitochondrial disease? Convulsive syndrome; Thrombocytopenia. On the 6th day, a massive intracerebral hemorrhage was found on the left side (gr. IV), with the appearance of changes typical of early cerebral edema with multiple intracerebral hemorrhagic imbibitions confirmed by CT without contrast. VAP respiratory support continued for 7 days, nCPAP respiratory support continued for 2 days, and on the 15th day a positive dynamic was recorded, for which the patient was excluded from respiratory support. On the 18th day, the condition worsened, the patient was re-placed to nCPAP FiO2–50% respiratory support, then due to negative dynamics, he was transferred to VAP. On the 19-20th day of life, the extremely serious life-threatening condition continued, VAP respiratory support was on maximum parameters FiO2-100%, hemodynamically unstable on the support of inotropes, maximum dose of Dopamine and Adrenaline. During

I. Clinical signs and symptoms, 1 point/symptom (max. 4 points)

A. Muscular presentation (max. 2 points)	B. CNS presentation (max. 2 points)	C. Multisystem disease (max. 3 points)	II. Metabolic/imaging studies (max. 4 points)	III. Morphology (max. 4 points)
Ophthalmoplegia†	Developmental delay	Hematology	Elevated lactate†	Ragged red/blue fibers‡
Facies myopathica	Loss of skills	GI tract	Elevated L/P ratio	COX-negative fibers‡
Exercise intolerance	Stroke-like episode	Endocrine/growth	Elevated alanine†	Reduced COX staining‡
Muscle weakness	Migraine	Heart	Elevated CSF lactate†	Reduced SDH staining
Rhabdomyolysis	Seizures	Kidney	Elevated CSF protein	SDH positive blood vessels†
Abnormal EMG	Myoclonus	Vision	Elevated CSF alanine†	Abnormal mitochondria/EM†
	Cortical blindness	Hearing	Urinary TA excretion†	
	Pyramidal signs	Neuropathy	Ethylmalonic aciduria	
	Extrapyramidal signs	Recurrent/familial	Stroke-like picture/MRI	
	Brainstem involvement		Leigh syndrome/MRI†	
			Elevated lactate/MRS	

* Score 1: mitochondrial disorder unlikely; score 2 to 4: possible mitochondrial disorder; score 5 to 7: probable mitochondrial disorder; score 8 to 12: definite mitochondrial disorder.
† This specific symptom scores 2 points.
‡ This symptom in a higher percentage scores 4 points.

GI = gastrointestinal; L/P = lactate/pyruvate; COX = cytochrome *c* oxidase; SDH = succinate dehydrogenase; EM = electron microscopy; EMG = electromyography; TA = tricarbon acid.

Figure 1. ***Nijmegen Mitochondrial Disease Criteria Scale in case 1***

hospitalization, the patient's condition became complicated with infectious anasarca, renal failure, neonatal heart failure, metabolic lactic acidosis, anemia, and on the 20th day of life, despite resuscitation measures, clinical death of the newborn was determined.

Postmortem the whole mitochondrial genome was sequenced by through Sanger sequencing and unfortunately, the presence of a pathological mutation could not be detected, but considering that the nuclear genome participates with more than 1000 genes in the functionality of the mitochondria, the presence of a mitochondrial disease caused by a mutation in the nuclear DNA encoding a protein involved in mitochondrial function cannot be excluded.

Case 2. Patient 2, presents for the first time at the age of 15, 1st child in family, from a non-consanguineous couple, born at term with accusations of sudden loss of vision in the right eye from the age of 14, and in 2-3 weeks also in the left eye, eye pain, nystagmus, exercise intolerance, fatigability. Paraclinical data suggested: an increase in serum lactate [32.3 mg/dL; ref val. 4.5-19.8 mg/dL); MRI with no suggestive data of neurological pathology; Electrophysiological data suggestive of bilateral prechiasmatic damage to the optic pathways, suspicion of Neuromyelitis optica (NMO). Antibodies for the spectrum of NMO – AQP4 aquapurine 4 and MOG (myelin oligodendrocyte glycoprotein) were negative. The ophthalmological consultation revealed relative central scotoma in the left eye and absolute central scotoma in the right eye, but also papillary edema. Considering the clinical picture and paraclinical data, the diagnosis of NMO was excluded, and the presence of Leber Hereditary Optic Neuropathy (LHON) was assumed. The patient was evaluated and scored according to the Nijmegen Mitochondrial Disease Criteria Scale and obtained a score equal to 5, which suggests the presence of a probable mitochondrial disease (figure 2).

As a result of the analysis of the mitochondrial genome through Sanger sequencing, the presence of a suggestive mutation for LHON syndrome – m. 11778 G>A in the

I. Clinical signs and symptoms, 1 point/symptom (max. 4 points)

A. Muscular presentation (max. 2 points)	B. CNS presentation (max. 2 points)	C. Multisystem disease (max. 3 points)	II. Metabolic/imaging studies (max. 4 points)	III. Morphology (max. 4 points)
Ophthalmoplegia†	Developmental delay	Hematology	Elevated lactate†	Ragged red/blue fibers‡
Facies myopathica	Loss of skills	GI tract	Elevated L/P ratio	COX-negative fibers‡
Exercise intolerance	Stroke-like episode	Endocrine/growth	Elevated alanine†	Reduced COX staining‡
Muscle weakness	Migraine	Heart	Elevated CSF lactate†	Reduced SDH staining
Rhabdomyolysis	Seizures	Kidney	Elevated CSF protein	SDH positive blood vessels†
Abnormal EMG	Myoclonus	Vision	Elevated CSF alanine†	Abnormal mitochondria/EM†
	Cortical blindness	Hearing	Urinary TA excretion†	
	Pyramidal signs	Neuropathy	Ethylmalonic aciduria	
	Extrapyramidal signs	Recurrent/familial	Stroke-like picture/MRI	
	Brainstem involvement		Leigh syndrome/MRI†	
			Elevated lactate/MRS	

* Score 1: mitochondrial disorder unlikely; score 2 to 4: possible mitochondrial disorder; score 5 to 7: probable mitochondrial disorder; score 8 to 12: definite mitochondrial disorder.
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GI = gastrointestinal; L/P = lactate/pyruvate; COX = cytochrome *c* oxidase; SDH = succinate dehydrogenase; EM = electron microscopy; EMG = electromyography; TA = tricarbon acid.

Figure 2. ***Nijmegen Mitochondrial Disease Criteria Scale in case 2***

MT-ND4 gene was revealed. The identified mutation is in fact one of the most frequent mutations associated with LHON and impairs the mitochondrial-encoded subunit ND4 of Complex I due to an arginine to histidine substitution at the position 340.

DISCUSSIONS

It is increasingly recognized that mitochondrial disorders are more common than previously thought. The advent of improved diagnostic techniques has led to a global increase in patients diagnosed with mitochondrial disorders [14].

Nevertheless, patients with mitochondrial disorders still suffer from delays in diagnoses. Despite significant progress made in the field of mitochondrial medicine during the last two decades, the molecular mechanisms underlying these disorders are not fully understood. General physicians are often not familiar with these disorders A full evaluation for a mitochondrial disorder is often warranted in individuals with a complex neurologic picture or a single neurologic manifestation and other system involvement [12].

The diagnosis and treatment of mitochondrial diseases is challenging because of wide variations in phenotypic expression and variable penetrance. Findings that can suggest a mitochondrial disorder include clinical phenotype (physical examination including neurologic examination), mode of inheritance (family history), and extent of the phenotype (other investigations to es-

tablish the extent of the phenotype) [15]. As far as the overall investigation it includes clinical records, family history, physical examination, biochemical evaluation in body fluid samples (lactate, pyruvate, glucose, blood gas profile, amino acids profile in blood/ spinal fluid and organic acids in urine) [16], neuroimaging, muscle biopsy with histology and respiratory chain enzyme activity studies and, ideally, genetic identification of the responsible variant [17]. As biochemical approach is not always elucidative or safe, genetic evaluation has become an eligible initial step in investigation especially if a pattern of symptoms is found suggesting a specific condition of one or more complexes deficiency.

The prognosis of mitochondrial diseases is reserved and despite ongoing trials and progress, there is no curative treatment available, only towards supporting symptoms and avoiding metabolic crisis [18]. There is no cure for mitochondrial diseases, and a genetic diagnosis is therefore crucial for genetic counselling and recurrence risk calculation, and can impact on the clinical management of affected patients.

In the two cases illustrated above, we wanted to highlight that diagnosing primary mitochondrial pathology is difficult and even when the clinical picture and biochemical investigations suggest the definite presence of a mitochondrial disease, in the end the genetic molecular diagnosis is the one that makes the difference. In the case of the first child, other genes from the nuclear genome that could be responsible for the patient's symptoms will be taken into account until we receive a final diagnosis. In the second case, Leber's Hereditary Optic Neuropathy was confirmed, which is the most common form of primary mitochondrial DNA disorders, that specifically targets the retinal ganglion cells by reducing their ability to produce enough energy to sustain [19]. The mutations of the mt-DNA that cause LHON are silent until an unknown trigger causes bilateral central visual scotoma, a clinical sign that we also observed in the patient that was described. In most patients with LHON, the visual loss remains profound and permanent.

CONCLUSIONS

Mitochondrial dysfunction is associated with an increasingly large number of heterogenous clinical presentations and symptoms, such as muscle weakness, exercise intolerance, impaired hearing and vision, ataxia, seizures, learning disabilities, heart defects, diabetes, and poor growth. We, therefore, emphasized the importance of an integrated approach for appropriate clinical, biochemical, imaging and genetic diagnosis, management and the treatment of patients with suspected clinical presentations. Proper understanding of mitochondrial genetics and investigative approaches will prove valuable for clinicians and patients aiding proper genetic counselling and prognosis.

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