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INTRODUCTION INTO INBORN ERRORS OF METABOLISM

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REZUMAT

INTRODUCERE ÎN ERORI ÎNNĂSCUTE DE METABOLISM

Lucrarea însumează un scurt reviu al literaturii asupra domeniului erorilor înnăscute de metabolism (EIM), care se prezintă în număr peste 1000 de EIM din cele peste 6000-8000 de boli rare cunoscute și prezintă o importanță deosebită pentru structura morbidității și mortalității infantile. Sunt abordate principiile de clasificare a EIM conform căilor patofiziologice implicate în erorile metabolice, modul de manifestare clinică, metodele de diagnostic specific și tratament. Se delimitează despre 3 grupuri mari de EIM – 1)"de tip intoxicație", 2) care decurg cu deficit de energie si 3) erori cu deficit al enzimei de sinteză sau degradare a compușilor complecși. Programele de screening neonatal, dar și cele selective, reprezintă instrumentul cel mai important în diagnosticul timpuriu al EIM pentru inițierea precoce a unui tratament specific cu salvarea copilului. Trei linii de "work-up metabolic" sunt folosite în stabilirea EIM. Deși metoda LC-MS reprezintă standardul de aur actualmente în diagnosticul EIM, deoarece în ultimul timp acestea se manifestă mult mai complex, cu suprapunerea semnelor clinice și a biomarkerilor, metodele de "untargeted metabolic screening" preiau întâietatea în aplicare. S-a experimentat în ultimii ani chiar și screeningul genomic neonatal. Dezvoltarea domeniului EIM în R. Moldova nu corespunde deocamdată multor standarde, dar cooperarea internațională permite diagnosticul EIM și crearea unui network regional de diagnostic.

Cuvinte-cheie: erori înnăscute de metabolism, diagnostic, screening, boli rare

РЕЗЮМЕ

ВВЕДЕНИЕ В НАСЛЕДСТВЕННЫЕ НАРУШЕНИЯ ОБМЕНА ВЕЩЕСТВ

В статье приводится краткий обзор литературы в области наследственных болезней обмена веществ (НБО), которые составляют более 1000 заболеваний из более чем 6000-8000 известных редких болезней, имеющие особое значение в структуре детской заболеваемости и смертности. Принцип классификации НБО рассматривается в соответствии с патофизиологическими путями, вовлеченными в метаболические нарушения, а также приводятся данные о клинических проявлениях, специфических методах диагностики и лечения. Сообщается о трёх больших группах НБО - 1) «интоксикационый тип», 2) заболевния возникающие из-за энергетического дефицита и 3) из-за дефицита фермента синтеза или разлагающего комплексные соединения. Массовые программы скрининга новорожденных, а также селективные скрининговые программы являются наиболее важным инструментом ранней диагностики НБО для раннего начала специфического лечения и спасения детей. Три этапа «метаболических исследований» используются при установлении НБО. Хотя метод LC-MS является золотым стандартом в настоящее время в диагностике НБО, но учитывая что в последнее время НБО становятся более сложными из-за наложения клинических проявлений и биомаркеров, методы «нецелевого метаболического скрининга» имеют преимущество. Даже неонатальный геномный скрининг был испытан в последние годы. Развитие области НБО в Молдове не проводится в соответствии со многими стандартами, но международное сотрудничество позволяет диагностировать НБО и создать региональную диагностическую сеть.

Ключевые слова: наследственные болезни обмена веществ, диагноз, скрининг, редкие болезни.

Introduction.

Among more than 6000-8000 of rare diseases, over 1000 are inborn errors of metabolism (IEM), which cumulatively affect approximately one in every 500 newborns and represent a special challenge in general and pediatric practice [5]. The IEM results from the total or partial absence or abnormality of an enzyme or its cofactor, structural protein, or transporter molecule leading to either accumulation or deficiency of a specific metabolite. The term of IEM, was coined by a British physician, Archibald Garrod (1857–1936), in the early 20th century. He is known for work that prefigured the "one gene-one enzyme" hypothesis, based on his studies on the nature and inheritance of Alkaptonuria [8,10].

The definition of IEM has kept its power for years and according to it a metabolic deterioration could cause the minor to severe clinical symptoms, mostly with neurological and psychiatric symptoms that often lead to death or life long disability. Although IEM have usually been considered pediatric diseases, however they can show up at any age [1,2,3].

IEM are usually rare diseases whose frequency is less than 1:2000 people. About 75% of rare diseases affect children and 35% of rare disease patients will die before their 1st birthday and another 30% will die before their 5th birthday. In 65% of cases IEM can lead to severe disabilities affecting the quality of life, in 9% the patient losing totally autonomy. In 80% of rare diseases genetic origins have been identified. 90% of rare diseases have no Food and Drug Administration approved drug treatment and actually scientists are working on a treatment for many rare diseases. The total number of persons in Europe suffering from one of the rare diseases is estimated at over 30 million (6-8% of the European population)[13]. Rare diseases are rare, but rare disease patients are numerous!

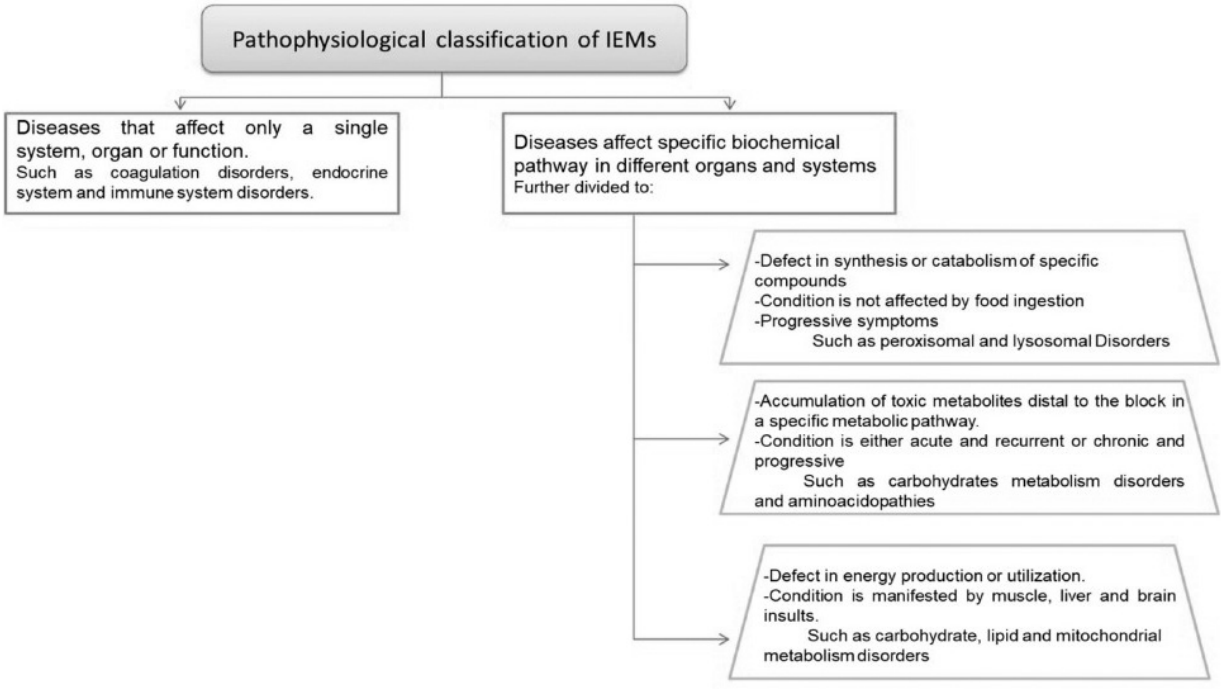
For the first, the IEM were classified according to the substrate involved in deteriorated metabolism as: disorders of amino acids, carbohydrates, organic acids, lysosomal storage diseases or disorders of Cooper, Molybdenum and other. Actually, the classification of IEM is based on the cell organelles and biochemistry processes involved in the metabolic pathway. If to consider the cell's organelles involving into metabolic pathway the IEM could be designed as: mitochondrial disorders, peroxisomal, lysosomal diseases and disorders affecting the Golgi Apparatus and Endoplasmic Reticulum [1-5].

Recently, due to the discovery of metabolic pathways involved in IEM more complex classification has been proposed. So, according to these, IEM are categorized into two big classes:

Ist - Diseases that affect only a single system, organ or function (such as coagulation disorders, endocrine system and immune system disorders) and IInd – Diseases affecting specific biochemical pathway in different organs and systems. The second class further is divided to the following groups: A – *"Intoxication type"* disorders, B – Defects in *"energy"* production or utilization and C – Defects in synthesis or catabolism of complex specific compounds (fig. 1) [8-10] .

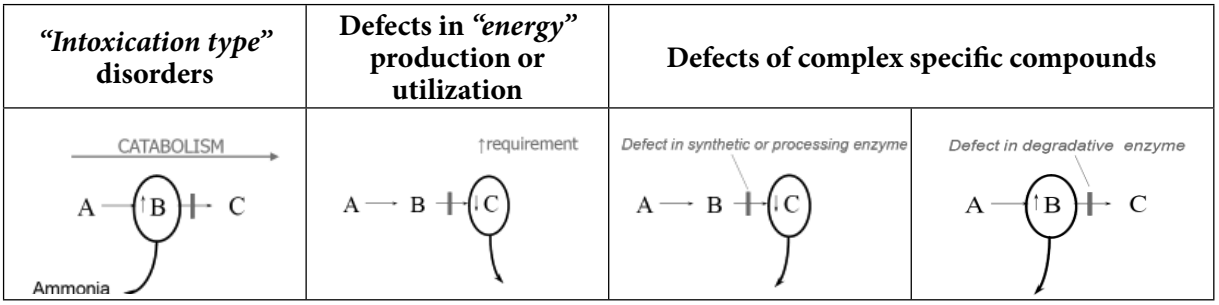
For the *"Intoxication type"* disorders there is specific the accumulation of toxic metabolites distal to the

Fig. 1. Pathophysiological classification of IEM [from 11].



block in a specific metabolic pathway (fig. 2). This type of disorders has no problems *in utero*. The condition is usually manifested acute or recurrent after a *"free symptoms window"* after birth or could be chronic and progressive. This group includes the following IEM: aminoacidopathies (such as: Urea Cycle Disorders, Organic Aciduarias (Methylmalonic Aciduaria, Propionic Aciduria, etc.), Maple Syrup Urine Disease, Phenylketonuria, etc.) and carbohydrates disorders (Galactosemia, Fructosemia). The onset of protein and carbohydrates disorders is usually shown acute in neonatal period when the metabolic block is complete, and comprise non-specific clinical features of metabolic decompensation. Among them there are *"unwell"* state, lethargy, feeding problems, vomiting, abnormal breathing, hypotonia, seizures and progressing to coma. The decompensation could be triggered by catabolism, food intake, fever, surgery etc. The clinical signs of intoxication usually include neurological effects. Late onset and intermittent forms can occur. Most of them are treatable through dialysis, special diets or drugs [1-7].

Fig. 2. The model of pathways involved in IEM.



As for Defects in *"energy"* production or utilization, pathological conditions are manifested by muscle, liver, heart and brain damage (fig. 2). This class of IEM encloses the disorders such as the carbohydrates, fats and mitochondrial metabolism and integrates the Glucose Transport Disorders, Glycogen Storage Disorders (GSD), Congenital hyperinsulinism, Fatty Acids Oxidation Defects (FAODs) and Mitochondrial Diseases. They are usually multisystem disorders and are determined by gradually degradations of patient's condition. For the FAODs and GSDs the embryogenesis is normal, they could decompensate with infections, fasting or exercise, but usually are treatable by diet and/or drugs. The Mitochondrial diseases are characterized by many malformations, chronic or acute deterioration without triggers and only some of them can be treated [1-7].

The third group of IEM is represented by the Defects in synthesis or catabolism of complex specific compounds (fig.2) and encompasses the disorders such as: Lysosomal, Peroxisomal Disorders, Congenital Disorders of Glycosylation, Neurotransmitters Disorders (Serine). The condition is not affected by food ingestion, but the symptoms are progressive with chronic deterioration of child development. These patients are often dysmorphic having abnormal embryogenesis. Last time there were developed many treatment options such as enzyme replacement (like in Mucopolisaccharidosis type 1, Gaucher Disease, Fabry, Pompe Disease etc.) or substrate reduction therapy (Niemann Pick), bone marrow transplantation or using product as mannose, serine etc [1-7].

The IEM may present at various ages and in different ways. Clinical presentation of some diseases can occur even before birth *(in utero)* or at birth, or during the first days of life as deterioration after normal birth and delivery [10]. Errors in fetal metabolism may be associated with developing maternal complications during pregnancy such as fatty liver and HELLP (Hemolysis, Elevated Liver Enzymes, Low Platelets) syndrome [7]. At birth, the newborn with IEM usually seems healthy, but the general condition can be deteriorated acutely by severe acidosis, alkalosis, or hypoglycemia, starting from the first day of life or later. The baby's general condition is often impaired rapidly despite normal or non-specific findings in routine investigations (such as laboratory signs of infection, lumbar puncture, chest X-ray, cranial ultrasound) and antibiotic therapy. They also can manifest as perinatal asphyxia, or later as nonspecific chronic manifestations such as delays in childhood developmental milestones [1-5,7,8].

The rate of deterioration is variable according to the disease type, depending on the severity of metabolic block and the most affected organ. IEM may appear as neurological symptoms, disorders of acid-base balance, respiratory arrest, unexplained hypoglycemia, cardiomyopathy, hepatic deterioration, muscle weakness or sudden death. Other diseases have more subtle presentations, such as a characteristic odor which is not commonly detected [1-5,7,8]. In general, the IEM diseases are responsible for a significant number of childhood disabilities and deaths [12].

As a consequence, an IEM should be considered in all neonates with unexplained, overwhelming or progressive disease particularly after normal pregnancy and birth with acute deterioration of the general condition and/or reduced consciousness; particularly when preceded by vomiting, fever or fasting; with signs of acidosis or hypoglycemia [5].

The most of IEM are inherited by autosomal-

recessive way, but the autosomal-dominant, maternal (mitochondrial) or X-linked ways were observed. Some of the IEM are potentially treatable by a specific therapy and their early diagnosis is very important. Three main variables are in the metabolic theorem: early diagnosis plus prompt treatment lead to better prognosis that is why it deserves the efforts of investigations and early diagnosis for preventing of poor outcome. Appropriate diagnostic and therapeutic measures must be initiated as soon as possible to avoid long-term damage.

Many different investigations are used for diagnosis of IEM. The major elements of principles of investigation are: to recognize clinical phenotype and consider the possibility of an IEM early, to proceed from generic to specific tests and identify promptly disorders needing urgent treatment (mostly disorders of intermediary metabolism). The basic*"metabolic work-up"* usually includes several lines of investigations. As first line investigations there are usually handling the tests for any sick infant with the results within minutes or hours. It comprises the full blood count, blood gas/electrolites (bicarbonates and anion gap), liver function tests, glucose, lactate and ammonia and urine analysis as smell, reducing substances (galactose, glucose, fructose etc.) and ketoacids, as well [2,3].

The second line of *„metabolic work-up"* covers many specific analysis as blood spot acylcarnitine profile, blood and urine amino acids, urine organic acids, and others depend upon differential diagnosis (e.g. CSF glucose, lactate, amino acids etc.). According to such kind of results usually a metabolic diagnosis is outlined. These two important steps of investigations are used for acute presentations. The third line of investigations comprises usually confirmatory tests by enzyme assay, fibroblasts, muscle biopsy and DNA. Certainly, to complete a metabolic diagnosis there are used many other investigations as: electrocardiogram, electroenephalogram, electromyogram, brain MRI, ophtalmic investigations, etc [1,2,3].

A very important diagnostic tool is the neonatal screening. Many neonatal screening programs are being implemented, with the priority of rapidly signaling treatable diseases. If initially the newborn screening were based on the Gutrie' test (bacterial inhibition assay), the methods were developed subsequently by radio-immunoassays, enzyme-immunoassays, gas chromatography/mass spectrometry (GC/MS) and liquid chromatography/mass spectrometry (LC-MS), enabling the rapid diagnosis of dozens IEM in parallel, the last becoming the gold standard for measurements of IEM currently. However, newborn screening programs exclusively use targeted metabolic assays that focus on limited panels of compounds for selected IEM diseases [14,15]. Because the IEM may present more complex last time with multiple overlapping symptoms and metabolites the untargeted metabolomics screening programs are enforced for early and accurate diagnosis. These methods are usually enabled to identify more than 300 metabolites at the same time [11]. Even genomic newborn screening was experienced last years. Nowadays, the newborn screening programs were slowly established as a part of preventive medicine.

The IEM area is a very important domain determining the infant and child morbidity and mortality. It requires very expensive equipment and a very good trained multidisciplinary team of professionals to be developed. In Moldova this domain is not evolved according to many standards due to very limited financial possibilities, but the diagnosis of IEM, beside Phenylketonuria (PKU) where there is the neonatal screening, is successfully initiated through a very fruitful collaboration with the foreign partners, supporting in such a way the creation of the National Register of Rare Diseases in Moldova.

Thus, the basic *"metabolic work-up"* as the blood gas/electrolites, ammonia, lactate, glucose, biochemistry, amino acids of body fluids by liquid chromatography, and many DNA tests are performed in Moldova. Other investigations as the ^{1}H-NMR spectroscopy for organic acids of urine/CSF/amniotic fluid - in Romania; LC-MS with acylcarnitine profile – in Hungary and Romania; neurotransmitters in CSF – in Germany (Heidelberg), lysosomal disorders- Germany (Hamburg), and IEF of Transferrin - in the Netherlands and USA. In a such a way there was created a regional metabolic network and due to could report on many disorders as Methylmalonic Acidurias, Glutaric Aciduria type 1, Isovaleric Aciduria, Non-Ketotic Hyperglycinemia, Mucopolysacchridosis (type 1,2,3), Gaucher Diseases, Glycogen Storage Disorders, Mitochondrial Diseases, Congenital Disorders of Glycosilation, etc.

Conclusions: IEM represent a significant cause of child morbidity and death. In the absence of a system for IEM performing in Moldova the collaboration with other groups is very important. The evaluation of clinical manifestations remains the most important to suspect an IEM patient. Among for Moldova the ^{1}H-NMR spectroscopy of urine performed in Romania (the nearest Lab) seems to provide high analytic information improving the orientation in diagnosis of IEM in unclear patients, but all methods are very necessary and should be developed for the better IEM diagnosis. Early diagnosis of IEM is very important for the specific therapy initiation and for further prevention by prenatal tests. Our further developmental strategy is to improve the field of IEM in Moldova through the common European projects.

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