The importance of newborn screening in the prevention of rare metabolic-endocrinological disorders

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ABSTRACT: Indicating the importance of early diagnosis and treatment of diseases at the earliest age of life, newborn screening is included in the mandatory form of health care for children and is carried out in countries around the world. Newborn screening includes severe hereditary metabolic and endocrinological diseases, which do not immediately manifest themselves clinically, while in the later course they lead to impaired growth and development with a high percentage of physical and psychological disability. An early diagnosed disease enables a quick therapeutic approach so that the disease does not progress, and adequate growth and development of the child. The goal of newborn screening is the early detection of newborn diseases where early diagnosis and treatment will lead to a significant reduction in mortality, morbidity and disability. The aim of this work is to present some of the most common metabolic and endocrinological diseases that are included in the newborn screening program in Montenegro and the surrounding countries, as well as to familiarize with the complications of undiagnosed diseases in a timely manner, therapeutic possibilities and the prognosis of the disease after timely treatment.

Key words: Newborn screening, hereditary diseases, endocrinological disorders

INTRODUCTION

More than four decades ago, many countries initiated neonatal screening programs in order to identify newborns with inherited metabolic and endocrinological diseases for which early diagnosis and treatment would prevent serious and permanent health disorders. Phenylketonuria was the first disorder included in newborn screening in many countries. In the decades after that, the program expanded gradually, and included an increasing number of severe disorders that result in a high degree of physical and intellectual disability.

The World Health Organization defines the role of screening as the detection of a treatable disease, with an adequately understood natural history, in the asymptomatic phase, in order to initiate treatment and prevent symptoms or to delay complications. Newborn screening began to be applied in 1960 with the work of the American microbiologist Dr. Robert Guthrie. The first international discussion on newborn screening organized by the World Health Organization was held in 1967 when a group of scientists on congenital metabolic disorders discussed the technical and ethical aspects of screening.

Guthrie's test is a mandatory health care measure and is performed on every newborn, whether healthy or sick, born on or before the due date. This laboratory analysis is usually performed already in the maternity ward, most often in the first 48 hours after the baby's birth, although it can be done up to the 8th day of the baby's life. The current recommendation of the Advisory Committee on Inherited Diseases in Infants and Children, the current version of which dates from 2016 in the USA, defines a "recommended universal screening panel" consisting of a basic list of 34 diseases and an expanded list that includes 26 more diseases. Diseases for which screening is recommended can be classified into several groups: organic acid metabolism disorders, fatty acid oxidation disorders, amino acid metabolism disorders, endocrine disorders and hemoglobinopathies. From endocrine disorders, screening is recommended for congenital hypothyroidism and congenital adrenal hypoplasia within the basic panel [1]. The list of diseases that will be covered by the screening test depends on the health system of the country and its screening program. Which disease will be checked mostly depends on its frequency, on the availability of

therapy, but also on how developed the country is and whether it has the means to pay for screening for all newborns.

Neonatal screening for hypothyroidism has been introduced in Montenegro since 2008 as a mandatory form of health care for newborns, and it is the only disease from the group of hereditary endocrinological diseases that screening includes.

Screening for phenylketonuria

Screening for phenylketonuria is a prerequisite for the early application of a restricted diet, which is necessary for the prevention of severe neurological disorders in children diagnosed with the disease. Phenylketonuria is the most common congenital metabolic disorder that causes a severe degree of physical and mental disability if it is not diagnosed in a timely manner and therapeutic treatment is not started. Phenylketonuria is a treatable disease and is listed in the national newborn screening program in countries around the world. Newborns with positive screening indications can achieve a satisfactory therapeutic effect by timely control of phenylalanine intake after diagnosis. The combination of early diagnosis and initiation of treatment results in normal physical and intellectual development for most children with phenylketonuria. Phenylketonuria and other hyperphenylalaninemia are a group of hereditary disorders that arise due to disorders in the oxidation of the amino acid phenylalanine to tyrosine [2]. Phenylketonuria has a special place among hereditary metabolic diseases. It is the first disease from that group in which the link between a hereditary biochemical disorder and mental retardation was clearly established (Følling 1934), the first disease from that category for which the possibility of dietary treatment was discovered (Bickel 1954) and the first for which a laboratory test was developed a test used in newborn screening in the entire newborn population (Guthrie 1963) [3]. The prevalence of phenylketonuria in the world is around 1: 10.000 newborns [4].

Phenylalanine is an essential amino acid, of which, after resorption from the intestines, a smaller amount is incorporated into body proteins, and the remaining, larger part must be oxidized into tyrosine with the help of the enzyme phenylalanine-hydroxylase in the liver. Phenylketonuria is caused by mutations in the gene encoding the liver enzyme phenylalanine hydroxylase. The consequence is enzyme insufficiency and the inability to oxidize phenylalanine to tyrosine with an increase in the concentration of phenylalanine and its "abnormal" metabolites in cells and body fluids. Today, the mechanism by which phenylalanine or its metabolites in high concentrations damage brain function is not yet known, but it is a fact that maintaining them within normal limits in phenylketonuric children with an appropriate dietary regimen prevents brain damage [5]..

Figure 1. A child with phenylketonuria
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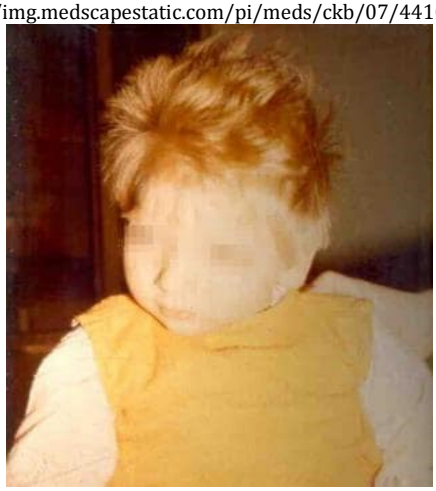


Children with classic phenylketonuria have no noticeable symptoms in the first days and weeks of life. It is only after a few weeks that signs of slowed psychomotor development appear, children do not learn to walk, sit at the right time, 25% of children have epileptic

seizures, develop hypotonia of muscles, psychomotor restlessness, behavioral changes, microcephaly, lag in physical development. About a quarter of the affected children have infantile eczema, hypopigmentation of the skin and hair, and a mouse-like smell of sweat and urine. Severe mental retardation occurs already during the first year (IQ 30) [6].

As every newborn is screened for phenylketonuria (Guthrie's test), the concentration of phenylalanine and tyrosine in the blood is determined in children with a positive Guthrie screening test. Based on the value of phenylalanine in the blood, the disease is classified as mild hyperphenylalaninemia: 120–360 mmol; light gray zone 360–600 mmol; mild form of phenylketonuria: 600–900 mmol; moderate: 900–1200 mmol and classical>1,200 mmol [7].

Treatment of phenylketonuria is carried out by lifelong restriction of phenylalanine intake to the amount necessary for the construction of own proteins from birth. In infants, milk formulas with little phenylalanine are exclusively used. The implementation of the diet has a threefold goal:

1. The accumulation of an excessive amount of phenylalanine in the blood (and therefore in the brain) is prevented by strict control of the natural protein/phenylalanine intake.

2.Replacing natural protein that has been removed from the diet with a safe or phenylalanine-free protein, called a synthetic protein, amino acid blend/supplement, or protein replacement. All protein replacements are phenylalanine-free or very low in phenylalanine.

3. Achieving normal growth and nutritional status. This is achieved by ensuring that the diet contains a balanced intake of all nutrients and energy. Vitamin and mineral supplements are either added to protein replacement or given as a separate supplement.

In the diet, the intake of foods rich in phenylalanine is restricted for life: milk, dairy products, meat, fish, chicken, eggs, beans, nuts. The intake of fruits, vegetables and cereals is recommended in the diet [8].

The prognosis of untreated phenylketonuria is poor considering the deterioration of mental and nervous functions, the accompanying symptomatic epilepsy and the difficulties and complications that threaten such a child. About half of untreated children live to be 20 years old, and about a third live to be 30 years old. With timely diagnosis at an early age and adequate dietary nutrition, children with treated phenylketonuria do not differ from healthy peers.

Prevention begins before the birth of a child, when a pregnant woman with phenylketonuria implements a diet without phenylalanine. If the diet is not strict before conception and during pregnancy, damage to the central nervous system of the fetus, congenital heart defects and microcephaly will occur. After birth, the newborn is given a Guthrie test.

A sample should be taken from every healthy, sick, term and non-term newborn. The exact period for sampling should not be less than 48 hours of protein feeding and should not exceed 30 days from birth; however, the ideal period would be between the third and seventh day of birth in newborns [9].

Since antibiotic therapy can make the test for phenylketonuria falsely negative, the sample is generally taken after the antibiotic therapy has ended. The safest place to take a blood sample is the dorsal side of the newborn's heel. The marked circle must be completely filled.

with blood, it does not matter if the blood has crossed the edges of the circle. Before injecting the child, you should wait until the disinfectant used to wipe the skin is completely dry. Otherwise, a disinfectant is mixed with the blood sample, and such a sample is unusable. Iodine and means containing iodine are not used because they interfere with the determination of thyrotropin for diagnosing congenital hypothyroidism. It is important to write on the back of the paper whether the child is taking antibiotics and is seriously ill.

Screening for congenital hypothyroidism

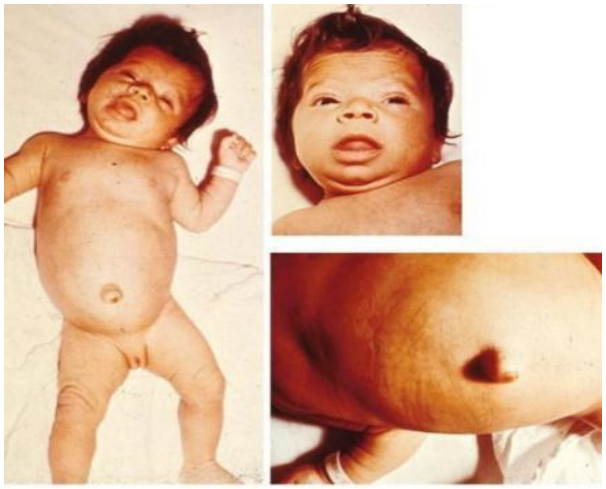
Congenital hypothyroidism can be diagnosed late or go completely undiagnosed, causing health disorders for the child, economic and social burden for the family. Therapeutic treatment of diagnosed congenital hypothyroidism is simple, cheap and effective. With early diagnosis and therapy, the newborn develops normally without mental handicap and becomes a productive member of society. The child's suffering, the economic and social burden caused by congenital hypothyroidism, obliged the institutions of many countries to include

newborn screening for hypothyroidism as a mandatory form of child health care.

In Montenegro, screening for hypothyroidism was introduced as a mandatory form of child health care in 2008. To date, congenital hypothyroidism is the only endocrine disease included in the newborn screening program.

The main clinical features of untreated congenital hypothyroidism are growth failure and delayed neurocognitive development resulting in mental retardation.

Figure 2. Clinical picture of congenital hypothyroidism
https://www.researchgate.net/publication/44662677/figure/fig4/AS:279090520182836@1443551773718/Infant-with-congenital-hypothyroidism-A-3-month-old-infant-with-untreated-CH-picture_Q320.jpg



Worldwide, the incidence rate of congenital hypothyroidism is 1: 2000-4000 newborns, while areas that are deficient in iodine record a higher incidence rate [10]. Congenital hypothyroidism is diagnosed at birth using the Guthrie test. This test is based on measuring the value of TSH or T4 (thyroxine). If the level of T4 in the blood from the heel prick is low and the TSH is elevated, the screening results indicate the development of congenital hypothyroidism. Confirmation of the diagnosis is made by analyzing hormones from venous blood, where the level of TSH and T4 is also measured. If the value of T4 hormone is low, and the value of TSH is elevated, the diagnosis is confirmed [11].

The goal of hormone replacement therapy is to bring the child to a state of euthyroidism. In diagnosed congenital hypothyroidism, therapy is started with a full dose of hormones in order to prevent or reduce the harmful effects of hypothyroidism on the development of the central nervous system. It is recommended to maintain the concentration of T3 and T4 at the upper limit of normal. At the beginning of the therapy, the level of T4 and T3 is normalized and the elevated TSH is suppressed. With well-managed therapy, normal growth is achieved and clinical signs of hypothyroidism disappear, but the prognosis of mental development is not so favorable and depends above all on the time when the therapy was started. Levothyroxine is a hormonal preparation that is used in the form of tablets or solutions. The tablet should be crushed and mixed with 30 ml of liquid (water, milk or formula). The solution is given to the child through a syringe or pipette, it should not be mixed with the entire meal in the bottle because it may happen that the baby does not eat the entire meal and the full dose of the medicine is not taken. During hormone therapy, it is necessary to monitor the condition of the child, because due to an overdose with levothyroxine, symptoms of hyperthyroidism may develop: restlessness, mild diarrhea, slow progress in body weight, insomnia, accelerated growth.

Due to an insufficient therapeutic dose, the child may develop lethargy, constipation, cold extremities, unexpected weight gain, and slow growth.

After starting hormone therapy, it is necessary to monitor the values of thyroid hormones. In the first months, the hormonal

status is checked every few weeks, ie every three to six months during childhood, or every 6 to 12 months in adulthood [12]. A large number of countries have included hypothyroidism in their newborn screening program, in such a way that from the same filter paper blood sample that is taken to look for phenylketonuria, T4 or TSH is determined radioimmunological.

Newborn screening for galactosemia

Due to lack of galactose-1-phospho-uridyl-transferase, classic galactosemia occurs [13]. Due to the inactivity of this transferase, galactose-1-phosphate accumulates in the liver, erythrocytes, spleen, eye lens, kidneys, heart muscle and cerebral cortex, and there is galactosemia in the blood. Besides the intracellular accumulation of galactose and galactose-1-phosphate, there is also a larger amount of galactitol. After a few days of feeding with mother's milk or milk formula containing lactose, the newborn becomes anorexic and turns yellow. Infants with classic often refuse food, do not progress or lose weight, vomit after meals, have diarrhea, jaundice, ascites, edema, hepatomegaly, are lethargic and hypotonic. Liver damage can progress to fulminant failure with encephalopathy and hemorrhagic diathesis, and renal failure is possible [14].

Figure 3. A child with galactosemia
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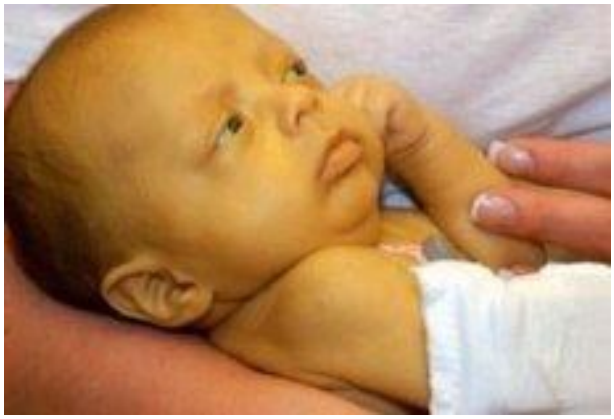

Children remain short with speech defects as well as posture and balance disorders during adolescence. Accumulation of galactose and galactitol in the eye lens leads to the rapid formation of cataracts, clouding of the eye lens and loss of vision. The disease can be accompanied by osteomalacia, temporary ovarian failure, while more severe forms of galactosemia are accompanied by hearing loss [15]. The treatment of galactosemia is based on a diet without any galactose (for infants it is soy milk instead of cow's milk). It should be started at the first suspicion of this disease, without waiting for the test results. If the diet is started in time, the symptoms can gradually disappear. The long-term prognosis of treated children is good, although some of them may have a slight delay in growth, mild speech difficulties and other discrete mental disorders. Patients have elevated concentrations of galactose in serum and urine. A woman who knows she carries the gene for galactosemia must also completely stop eating foods containing galactose during pregnancy. Galactosemia can be prevented during pregnancy with an appropriate diet. If the mother has a high level of galactose in her blood, it can pass through the placenta and cause cataracts. People with this disorder must give up galactose for life [16].

Screening for glutaric aciduria type I

Glutaric aciduria type 1 is a severe inherited neurometabolic disorder whose clinical outcome has improved after the implementation of a newborn screening program and prompt initiation of presymptomatic metabolic treatment.

Glutaric acidemia type I is the antitype of the so-called cerebral organic aciduria and is the result of a hereditary disorder in the metabolism of the amino acids lysine, hydroxylysine and tryptophan, due to the lack of the mitochondrial enzyme glutaryl-CoA-

dehydrogenase. In patients with enzyme deficiency, glutaric and, to a lesser extent, 3-OH-glutaric and glutaconic acid accumulate in the brain [17]. The estimated prevalence of the disease ranges from 1:125,000 to 1:250 newborns in genetically high-risk populations [18]. Untreated disease most often causes a picture of acute brain damage with severe dystonic-dyskinetic disorder (Figure 6). The disease is asymptomatic until the age of usually half a year to a year, when the child develops the so-called. encephalopathic crisis in which the basal ganglia are affected.

Figure 4. Child with glutaric aciduria type Ⅰ
https://upload.wikimedia.org/wikipedia/commons/thumb/1/19/GA1_posture2.jpg/220px-GA1_posture2.jpg

The disease is characterized by neurodevelopmental disorders, including: delay/deficit in speech development, learning difficulties, intellectual development disorder, epilepsy, macrocephaly [19]. Combined metabolic therapy includes a low-lysine diet, carnitine supplementation, and emergency treatment during the episode to prevent catabolism and minimize CNS exposure to lysine and its toxic metabolic byproducts [20].

Screening for cystic fibrosis

Neonatal screening for cystic fibrosis has optimized patient prognosis by enabling very early multidisciplinary care. Over the past 20 years, screening programs have experienced a major international expansion. Cystic fibrosis is included in the screening program in Serbia. In the middle of the 20th century, when the disease was discovered, children suffering from cystic fibrosis died within the first year of life. With early diagnosis, improved treatment and the use of new drugs, the average life expectancy of sufferers is 40 years. In countries that have introduced neonatal screening, the life expectancy of patients has been significantly extended, and the quality of life of patients and their families has improved.

Cystic fibrosis is an autosomal recessive disease characterized by pancreatic insufficiency and chronic endobronchial infection of the respiratory tract. Chronic airway infection leads to progressive bronchiectasis and ultimately respiratory failure, which is the leading cause of death in patients with cystic fibrosis. Other complications include sinusitis, diabetes mellitus, intestinal obstruction, hepatobiliary disease, hyponatremic dehydration, and infertility [21].

The advantage of early diagnosis of cystic fibrosis through neonatal screening is multiple: application of preventive and early therapeutic interventions, regular control and early detection of complications, significantly better survival of patients, longer and better quality of life of patients, slower progression of lung disease, prevention of malnutrition, better nutrition, normal growth and child development.

CONCLUSION

Detection of the disease at the earliest age enables a quick therapeutic approach, thus ensuring adequate psychophysical growth and development of the child and preventing permanent physical and intellectual deficits. Hereditary metabolic and endocrinological diseases are characterized by a high percentage of physical and mental disability, which affects not only the health and social functioning of the child, but it affects the whole family, community and society. Screening for congenital hypothyroidism began in Montenegro in 2007. It is the only endocrinological hereditary disorder

that is included in the screening program in Montenegro. From the surrounding countries Croatia has the largest number of diseases included in the screening program, eight diseases: phenylketonuria, hypothyroidism, three fatty acid breakdown disorders, glutaric aciduria type 1, isovaleric aciduria, carnitine carrier deficiency.

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