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FOURTEEN YEARS OF NEWBORN SCREENING FOR PHENYLKETONURIA IN VOJVODINA

ČETRNAEST GODINA NOVOROĐENAČKOG SKRININGA FENILKETONURIJE U VOJVODINI

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Summary

Introduction. Phenylketonuria is an inborn disorder of metabolism, a rare, hereditary disease caused by deficiency of phenylalanine hydroxylase enzyme necessary for conversion of phenylalanine into tyrosine. The aim of this study is to determine the incidence of hyperphenylalaninemia and classical phenylketonuria in population of the Autonomous Province of Vojvodina. **Material and Methods**. We performed retrospective analysis at Medical Genetics Service, the Institute for Youth and Health Care of Vojvodina and examined the clinical material of the previous fourteen years, during the interval from 2003-2016. The analysis of the obtained results was carried out using descriptive statistics. **Results**. During fourteen years, from 01.01.2003 to 31.12.2016, 27 newborns with hyperphenylalaninemia were detected, and the incidence of hyperphenylalaninemia in the Autonomous Province of Vojvodina was 1: 9.525. Classical phenylketonuria was detected in 15 persons during indicated period, and the incidence was 1:17.143. **Conclusion.** Phenylketonuria is a hereditary disease whose adverse effects can be avoided, if it is recognized in time, and if recommended treatment measures are adequately applied, thereby improving the quality of life of persons affected by the disease as well as the whole family, that is facilitated by the introduction and implementation of neonatal screening.

Key words: Phenylketonurias; Phenylalanine; Neonatal Screening; Incidence; Epidemiology; Early Diagnosis; Infant, Newborn

Sažetak

Uvod. Fenilketonurija je urođeni poremećaj metabolizma, retko nasledno oboljenje nastalo usled nedostatka enzima fenilalanin hidroksilaze koji je neophodan za konverziju fenilalanina u tirozin. Cilj rada bio je utvrđivanje incidencije hiperfenilalaninemije i klasične fenilketonurije u populaciji Autonomne Pokrajine Vojvodine. **Materijal i metode**. Retrospektivnom analizom u Službi za medicinsku genetiku Instituta za zdravstvenu zaštitu dece i omladine Vojvodine obrađen je klinički materijal prethodnog četrnaestogodišnjeg perioda u vremenskom intervalu 2003–2016. godine. Analiza dobijenih rezultata sprovedena je metodama deskriptivne statistike. **Rezultati**. U četrnaestogodišnjem periodu 1.01.2003–31.12.2016. godine, detektovano je ukupno 27 novorođenčadi sa hiperfenilalaninemijom. Incidencija hiperfenilalaninemije u Autonomnoj Pokrajini Vojvodini iznosi 1 : 9.525. Klasična fenilketonurija je detektovana u navedenom periodu kod 15 osoba, a incidencija iznosi 1 : 17.143. **Zaključak**. Fenilketonurija je nasledno oboljenje, kod kog se mogu izbeći nepovoljne posledice bolesti ukoliko se na vreme prepozna i ukoliko se adekvatno primenjuju preporučene mere lečenja, čime se unapređuje kvalitet života osoba koje su obolele kao i cele porodice, što je omogućeno uvođenjem i sprovođenjem neonatalnog skrininga.

Ključne reči: fenilketonurija; fenilalanin; neonatalni skrining; incidenca; epidemiologija; rana dijagnoza; novorođenče

Introduction

Phenylketonuria (PKU) is the most common autosomal recessive inborn error of amino acid metabolism. If not diagnosed and treated, the amino acid phenylalanine (Phe) will accumulate due to deficiency of the enzyme phenylalanine hydroxylase (PAH), necessary to facilitate the hydroxylation of phenylalanine to tyrosine. In untreated patient phenylalanine accumulates in the body and leads to adverse toxic effects of a disease that are manifested as deterioration, seizures, microcephaly, mental retardation and other symptoms such as lighter hair, eyes and skin, the specific body odor, behavioral disorders, attention deficit and lack of concentration. There is a rare form of the disease (about 1–2%) caused by the deficiency of tetrahydrobiopterin (BH_4) cofactor [1–4].

Phenylketonuria was discovered by a physician - a biochemist from Norway, *Ivar Asbjørn Følling* in 1934. A gene encoding the phenylalanine hydroxylase synthesis is localized on the chromosome 12 (12q23.2) and consists of 13 exons. More than 950 mutations of this gene have been discovered so far [1, 4].

Depending on the degree of enzyme deficiency, disease can be divided in several forms: *Classical phenylketonuria* - the most severe, with the highest deficit of phenylalanine hydroxylase enzyme and phenylalanine concentration greater than 20 mg/dL (Phe > 1,200 mmol/L). *Moderate PKU* - there is residual enzyme activity, the concentration of phenylalanine is 15–20 mg/dL (Phe 900–1200 μmol/L). *Mild form* of *PKU* - there is a higher residual activity of the enzyme and the concentration of phenyla-

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Abbreviations

PKU – phenylketonuria
HPA – hyperphenylalaninemia
PAH – phenylalanine hydroxylase
Phe – phenylalanine
BH4 – tetrahydrobiopterin

lanine is 10–15 mg/dL (Phe 600–900 μmol/L). *Hyperphenylalemia* (HPA) - is a mildly elevated phenylalanine of 2–10 mg/dL (Phe 120–600 μmol/L).

The proposed classification according to the recommendations of the European guidelines is divided into two forms: A form that requires treatment to maintain a normal concentration of phenylalanine and form that does not need any treatment. Treatment is recommended for all cases in which the concentration of phenylalanine is over 6 mg% (Phe > 360 μmol/L) [4, 5].

Newly detected cases of phenylketonuria are most often detected through a newborn screening program. In the Autonomous Province of Vojvodina, a newborn screening program was introduced in 2003, and since then about 16 to 20 thousand newborns from 12 hospitals in Vojvodina are tested annually. During the year, one to four children with phenylketonuria and hyperphenylalaninemia are revealed. After the analyses are carried out, only children that have a phenylalanine concentration above the allowed limit repeat analyses. In the case of positive findings, a confirmation of the diagnosis is carried out at the Service for Medical Genetics. Parents receive all necessary information about the cause and nature of the disease, as well as about the possibilities of treatment and the necessary controls. Immediately after the confirmation of the diagnosis the treatment of the affected child begins, by introducing a low-protein diet with a low precisely defined intake of phenylalanine, which depends on the individual tolerance of phenylalanine. Treatment is necessary to start as soon as possible, ideally before the third week of the child's life, and at the latest by the end of the first month of life, allowing irreversible adverse consequences of the disease to be avoided and improving the quality of life of affected individuals and their families. Untreated disease leads to a gradual deterioration of intellectual function, microcephaly, epilepsy, autism, attention deficit, motor and behavioral disturbances and psychiatric symptoms [6–10].

Material and Methods

This study is retrospective analysis. We analyzed material of the previous fourteen years during 2003–2016 at the Department of Medical Genetics, the Institute for Children and Youth Health Care of Vojvodina. The study covered 257,157 initial analyses of the concentration of phenylalanine in newborns as well as additional confirmatory analyses. The source of data was the medical documentation. The total number of newborns by years was obtained from the Statistical Office of the Republic of Serbia. Biochemical analyzes were done using a Guthrie test and a fluorescent ninhydrin method. In the case with pathological concentration of phenylalanine, the parents of affected child have been informed about the nature of the disease and necessity of limiting the intake of phenylalanine through the implementation of a strictly controlled diet, which is initiated at the Department of Medical Genetics. Children affected by disease use special formulas and protein supplements with regular controls of phenylalanine levels. The analysis of the obtained results was carried out using descriptive statistics.

The aim of this study was to determine the epidemiological characteristics of phenylketonuria and hyperphenylalaninemia in the population of the Autonomous Province of Vojvodina.

Results

Department of Medical Genetics at the Institute for Children and Youth Health Care of Vojvodina is a regional institution that conducts neonatal screening program for the province of Vojvodina, with (according to the latest census) 1,931,809 inhabitants. The average number of live births changed and diminished over time, reaching 20,381 in 2003 and 17,107 in 2016. The total number of live births according to the data of the Statistical Office of the Republic of Serbia, for the fourteen years, from 1.1.2003. to 31.12.2016., was 257,157 **(Table 1)**.

The newborn screening program started in Vojvodina in 2003 and had been implemented for each newborn born in the province of Vojvodina. In a fourteen-year period from 01.01.2003 to 31.12.2016., 27 newborns with hyperphenylalaninemia were detected in total, and the incidence of hyperphenylalaninemia in the Autonomous Province of Vojvodina was 1 : 9,525. Classical phenylketonuria was detected during the specified period in 15 newborns, and the incidence was 1 : 17,143.

Classical phenylketonuria (> 20 mg / dL) was discovered in 15 newborns and moderate PKU (15–20 mg/dL) in 1 newborn. Mild PKU form was found (10–15 mg/dL) in 4 newborns, and hyperfenilalninemia (2–10 mg/dL) in 7 newborns **(Table 2)**.

The disease in all newly detected cases had been revealed in first three weeks of life and the treatment has been initiated immediately after the diagnosis was confirmed. The ratio of male-female sex in the test group was 1.3:1 (17 males and 10 females). Treatment begins if the values of phenylalanine are over 6 mg% (over 360 μmol/L), with the introduction of special formulas without phenylalanine and protein supplements.

Epilepsy was diagnosed in 2 children with hyperphenylalaninemia - moderate form (initial concentration of Phe was 16 mg%) and in one child with hyperphenylalaninemia (initial concentration of Phe was 7 mg%).

The association with other diseases was registered in one male child with a classical form of the disease

Table 1. Number of live births and the number of newly detected cases of hyperphenylalaninemia and phenylketonuria by years in the period 2003-2016.
Tabela 1. *Prikaz broja živorođenih i broja novootkrivenih slučajeva hiperfenilalaninemije i fenilketonurije po godinama u periodu 2003-2016. godina*

Year *Godina*	Live birth included by screening *Živorođeni obuhvaćeni skriningom*	HPA *HPA*	Mild form of PKU *Blaga forma PKU*	Moderate form of PKU *Umerena forma PKU*	Classical PKU *Klasična PKU*
2003.	20381				3
2004	20206				2
2005	19058		1		1
2006	19102		2		
2007	18380		2		
2008	18339	1			2
2009	18590				
2010	18145	2			1
2011	17410				1
2012	17932				
2013	17439				1
2014	17535	2			1
2015	17533	1			1
2016	17107			1	2
Total *Ukupno*	257.157	6	5	1	15

**HPA – hiperfenilalaninemija, PKU – fenilketonurija*

in the form of agenesis of one kidney. The male child with a classic form had an operation of cryptorchism.

Discussion

Phenylketonuria is inherited metabolic disease that, if detected prior manifestations of the disease and before elevated levels of phenylalanine toxically accumulate and affect the central nervous system, can be controlled by adequate intake of phenylalanine, so all adverse effects of the disease could be successfully avoided by treatment. In the period when there was no newborn screening program, the disease was detected after the child's parents observed deterioration and when the brain functions had already deteriorated and mental retardation developed. The introduction of the neonatal screening program was the success of the entire society. By implementing a special diet, children affected by the disease will not be affected by mental retardation and have all the conditions for enrollment and attendance at all levels of education. The effects of the newborn screening are largely reflected through the improvement of the quality of life, not just for the affected individual, but also for whole families and for the entire community [1, 5, 6, 11].

In previous fourteen years (2003–2016), in the territory of Vojvodina, 257,157 newborns were live born. Samples in the form of dry blood spots on filter paper were collected from maternity hospitals and health centers in Vojvodina (Pancevo, Ruma, Senta, Sombor, Sremska Mitrovica, Subotica, Backa Topola, Vrbas, Vrsac, Zrenjanin, Kikinda, Novi Knezevac, and Novi Sad) and sent at regular intervals to the Institute.

Table 2. The presence of certain forms of hyperphenylalaninemia and phenylketonuria in the population of Vojvodina depending on the concentration of phenylalanine in the blood prior initiation of treatment
Tabela 2. *Zastupljenost pojedinih formi hiperfenilalaninemije i fenilketonurije u populaciji Vojvodine u zavisnosti od vrednosti koncentracije fenilalanina u krvi pre započinjanja lečenja*

Form of PKU *Oblik fenilketonurije*	Phenylalanine concentration *Koncentracija fenilalanina*	Number of newborns *Broj novorođenčadi*
Classical form of PKU/*Klasična forma PKU*	>20 mg/dL	15
Moderate form of PKU/*Umerena forma PKU*	15-20 mg/dL	1
Mild form of PKU/*Blaga forma PKU*	10-15 mg/dL	4
Hyperphenylalaninemia/*Hiperfenilalaninemija*	2-10 mg/dL	7
Total/*Ukupno*		27

PKU – fenilketonurija

Figure 1. Territorial distribution of newborns affected by phenylketonuria and hyperphenilalaninemia in Vojvodina: Subotica, Futog, Begeč, Sombor, Ada, Sonta, Pančevo, Kovilj, Zrenjanin, Melenci, Noćaj, Kupusina, Mali Iđos, Vašice, Senta, Vrdnik, Alibunar, Sečanj, Srbobran, Mramorak.

Slika 1. *Teritorijalna distribucija novorođenčadi zahvaćenih fenilketonurijom i hiperfenilalaninemijom u Vojvodini: Subotica, Futog, Begeč, Sombor, Ada, Sonta, Pančevo, Kovilj, Zrenjanin, Melenci, Noćaj, Kupusina, Mali Iđos, Vašice, Senta, Vrdnik, Alibunar, Sečanj, Srbobran, Mramorak.*

If the value of phenylalanine in the blood was less than 6 mg%, the newborn did not require treatment. If the concentration of phenylalanine in the blood was between 360 μmol/L and 600 μmol/L (6 and 10 mg%), the treatment was performed up to 12 years of age. In the case of Phe concentrations over 10 mg%, lifelong treatments were recommended. In the fourteen-year period (2003–2016), 27 newborns with hyperphenylalaninemia were detected, out of which 15 had a classical form of the disease. Annually, one to four children have been discovered.

Treatment of PKU children should begin immediately after the confirmation of diagnosis, as every 4 weeks of delayed introduction of therapy reduces the IQ for 4 units. Immediately after establishing the diagnosis, the treatment of a child affected by the disease starts with the introduction of a low-protein diet with a low, precisely defined intake of phenylalanine and use of protein supplements. The outcome of the disease depends on the time elapsed from birth to the low phenylalanine diet introduction, and it is necessary to start treatment as soon as possible. It is ideal to start the treatment before the third week of life and at the latest by the end of the first month of life, thus avoiding the harmful effects of the disease and improving the quality of life of the affected people. All people with hyperphenylalaninemia are controlled at regular intervals, according to their age. Diet is reevaluated in relation to phenylalanine values in blood, age and body weight. Special forms of the disease are BH4 responders - when sapropterin treatment is applied [12–16].

Target concentrations of phenylalanine are 2–6 mg% for children aged 0–12 years and for maternal phenylketonuria, 2–10 mg% (120–600 μmol/L) for others (older than 12 years and non-gravid women).

Monitoring and treatment is planned according to age, compliance to the recommended diet, physical activity, the presence of acute infections and clinical status, along with the reevaluation of nutritional, clinical and biochemical status.

Early detection and treatment are necessary to avoid toxic effects of elevated phenylalanine, damage to the nervous system, growth failure, microcephaly, mental retardation, and neurobehavioral abnormalities.

The incidence of classical phenylketonuria in Vojvodina is 1:17,143, while the incidence of all hyperphenylalaninemias in Vojvodina is 1:9,525. The average incidence of PKU in the white race is from 1:10,000 to 1: 20,000. The variation between the geographic populations is high and the disease is more common in Turkey (1: 2,600), Iran (1: 3,300) and Ireland (1: 4,500). In Estonia it is represented by a medium incidence (1: 6,000), Tunisia (1: 7,600), and Slovenia (1:8.000). Incidence in Japan (1: 143.000), Finland (1: 200.000) and Thailand (1: 212.000) is low.

The expected incidence of sex is approximately equal, according to the autosomal recessive pattern of inheritance of the disease. The risk of a recurrence in a family when both parents are heterozygous is 25%. A prenatal diagnosis is possible by detecting the mutation from a sample of chorionic villi, amniotic fluid or from fetal blood.

Increased values of phenylalanine are toxic to the central nervous system. Exact mechanism of how phenylalanine impairs brain function is not clarified. The incidence of epilepsy in untreated forms of phenylketonuria is high. In untreated patients, the occurrence of West syndrome is significantly higher in relation to the general population. Although the adverse effects of the disease can be minimized by using an adequate diet, neuropsychological deficits with higher incidence are possible in comparison to the general population. When there are elevated levels of phenylalanine in the blood, the levels of monoamines are reduced: serotonin, dopamine and norepinephrine in the brain. Phenylalanine reduces the concentration of monoamine precursors. Tryptophan and tyrosine, according to the principle of competition for the transport of neutral amino acids, and also inhibits aminohydroxylases that play a role in the conversion of tryptophan and tyrosine to serotonin and norepinephrine. In untreated patients, the white brain mass is affected due to the toxic effect of phenylalanine on oligodendroglia. Even if the treatment starts immediately, there may be pathology of the white mass of the brain [17, 18]. In the analyzed group, two children had a registered epilepsy, one child from the group of moderate hyperphenylalemia, and another child from the hyperphenylalaninemia group.

In children affected by phenylketonuria, a higher incidence of congenital anomalies is not expected in comparison to the general population where congenital anomalies occur with a frequency of 3–5%. In the analyzed group, association with congenital anomalies was registered in one male child (3.7%; N = 1/27) with the classical form of the disease in the form of a kidney agenesis.

Conclusion

Phenylketonuria is inherited disorder of metabolism of the amino acid phenylalanine, whose adverse effects can be avoided by early recognition after the use of preventive neonatal screening. Early recognition and introduction of treatment prior to the manifestation of disease, improves the quality of life of people affected by the disease, as well as whole families and entire social communities. The incidence of classical phenylketonuria in the Autonomous Province of Vojvodina is 1 : 17.143, while the incidence of all hyperphenylalaninemias is 1 : 9.525.

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