

Epidemiological aspects in phenylketonuria patients from a region in northwestern Romania

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ABSTRACT

Introduction. Phenylketonuria (PKU), a genetic disease with autosomal dominant transmission, is the most frequent in-born error in aminoacid metabolism. The variations in phenylalanine-hydroxylase (PAH) gene lead to a lowered enzymatic activity causing hyperphenylalaninemia. PKU has a mean European prevalence of 1:10,000 newborns, with a large variation in different ethnicities and geographic regions. The large genetic variability (over 1200 genetic variants known) as well as other factors determines a wide spectrum of metabolic phenotypes. Untreated, PKU leads to irreversible intellectual disability, low stature, hypopigmentation, motor deficits, seizures, but the early diagnosis and treatment enables almost normal somatic and mental development.

Aim. The aim of this study is the determination of the impact of non-genetic factors over the clinical phenotype of PKU patients in a region of north-west Romania.

Material and method. The study group is formed from 44 patients diagnosed with phenylketonuria in the 1981 – 2021 period, found in the database of Bihor Regional Center for Medical Genetics, Emergency Clinical County Hospital, Oradea, Romania. The collected data was referring to the age, sex and domicile of the patients, the age of the diagnosis and the beginning of the treatment, also the metabolic control over the years, the metabolic phenotype of the patients and its impact on the clinical phenotype (IQ, the presence or absence of intellectual disability or the existence of a specific clinical phenotype).

Results. The majority of patients (66%) were diagnosticated with phenylketonuria in the first 4 months of life, although there were cases with a late diagnosis, 20.5% of the patients were diagnosticated after the age of 1 year. Based on the pre-treatment plasmatic levels of phenylalanine, the majority of cases (72.7%) had a severe metabolic phenotype (classic PKU - cPKU), 20.5% of cases had a milder form of PKU (mPKU) and 6.8% of patients were found with a mild hyperphenylalaninemia (HPA). In the case of 23 patients, an optimal metabolic control was not obtained. The specific phenotype (blonde hair, light skin, blue eyes) was found in 22.7% of cases, 77.3% not having these features. At 68.2% of cases intellectual disability was found, with different levels of severity: 5 patients (11.5%) had liminal intellect, 9 patients (20.5%) had mild mental retardation, 6 patients (13.6%) had moderate mental retardation, 9 cases (20.5%) were with severe mental retardation and 1 patient (2.3%) had profound mental retardation; 31.8% of cases had normal intellect. The prevalence in Bihor county is 1:7,843 newborns.

Discussions. A partial or, in rare cases, total lack of dietetic treatment was observed in all patients over 20 years old (current age). The delay in treatment initiation or an insufficient treatment, with a suboptimal metabolic control, will affect patient's intellect, regardless of metabolic phenotype. If in 20 years old patients, or older, the main reason for mental retardation is the lack of dietetic treatment availability in the first years of life, for the younger patients the reason for mental retardation is usually a lack of compliance with the treatment. The majority of metabolic phenotypes is cPKU, in concordance with the literature data; the mild phenotype (HPA) was observed in a small percentage of patients, smaller than the data reported in the literature. In the first two studied decades the mild phenotypes were seldom observed. In the absence of screening tests or suggestive clinical manifestation it can be assumed that HPA patients remained undiagnosed, which would explain the small HPA percentage in the study group. A significant improvement in metabolic control in younger patients compared with older ones was observed, which denotes a better access to specific alimentation on one side, and on the other side, a better understanding of the disease from the patients and their families. Also, this study

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confirms a known fact that the diet in PKU is of great importance in the disease evolution. In this study there were included patients with severe metabolic phenotype with good metabolic control which reached adulthood without intellectual deficits, with higher education, social integrated and also patients with mild metabolic phenotype but with a poor metabolic control which developed intellectual deficiency.

Conclusions. The PKU prevalence in Bihor county is higher than the estimated national value. The late diagnosis and treatment or the poor metabolic control led to intellectual disability, regardless of the metabolic phenotype. PKU screening and the better access to treatment allows younger generations of patients to enjoy a superior quality of life than the patients from the first two studied decades.

Keywords: phenylketonuria, PKU, PKU epidemiology, PKU metabolic phenotype

INTRODUCTION

Phenylketonuria (PKU [MIM: 261600]) is the most frequent inborn error of aminoacid metabolism [1]. Over 1,200 known variants of phenylalanine-hydroxylase (PAH) gene [2] are responsible for the reduction in enzymatic activity, leading to phenylalanine (Phe) accumulation in the body to neurotoxic levels. These variants have autosomal recessive transmission and are located on the long arm of the 12th chromosome (12q22–24.1) [1].

PKU has a global prevalence of 1:24,000 newborns, but with big ethnical and geographic variations [3]. The Karachay-Cherkessian population in Russia has the highest prevalence in the world at 1:850 newborns [4], Finland and Japan report the lowest prevalence worldwide, at 1:112,000 and 1:125,000 newborns, respectively [5,6]. The prevalence in Romania is estimated to be at the European mean at 1:10,000 newborns [3].

The large genetic variability, and other factors also, determine a wide spectrum of metabolic phenotypes. The lower the residual enzymatic activity is, the higher the plasmatic concentration of Phe [7]. The pre-treatment levels of Phe determines the metabolic phenotype classification: classic PKU (cPKU) in which the enzymatic activity is completely or almost completely abolished, producing a plasmatic level of Phe over 1,200 $\mu\text{mol/L}$ (20 mg/dl); mild PKU (mPKU) with a residual enzymatic activity that determine a plasmatic Phe level between 600 and 1,200 $\mu\text{mol/L}$ (10-20 mg/dl) and mild hyperphenylalaninemia (HPA) with plasmatic Phe levels between 120 and 360 $\mu\text{mol/L}$ (2-6 mg/dl) [8].

Untreated, PKU lead to irreversible intellectual disability, small stature, hypopigmentation, motor deficits, seizures [1]. The early diagnosis and treatment enables an almost normal somatic and mental development [9]. The therapy in phenylketonuria is largely based on dietary restriction of Phe. Special medical food – protein substituents without phenylalanine are used. Synthetic tetrahydrobiopterin (Sapropterina) may be a adjuvant treatment to the diet or an alternative treatment for those PKU pa-

tients that have a mild or moderate form of disease [10].

AIM OF THE STUDY

The objective of this study is to examine the epidemiological situation of PKU in the region of north-west Romania and the analyze of data from PKU patients of different metabolic phenotypes, different ages, different compliances and access to treatment, with the aim of determining the impact of this factors on the clinical phenotype of the patient.

MATERIAL AND METHOD

The study group is formed from 44 patients diagnosed with PKU in the 1981 – 2021 period and found in the found in the database of Bihor Regional Center for Medical Genetics, Emergency Clinical County Hospital, Oradea, Romania. This patient had their domicile (at the time of the diagnosis) in the counties of Bihor, Salaj, Maramures and Satu Mare.

There were included patients with PKU that had periodical clinical, biological and psychological evaluation which allowed for a homogenic data gather from the entirety of the study lot. Patients with diagnosis of transient hyperphenylalaninemia were excluded from the study.

The collected data was referring to the age, sex and domicile of the patients, the age of the diagnosis and the beginning of the treatment, also the metabolic control over the years, the metabolic phenotype of the patients and its impact on the clinical phenotype (IQ, the presence or absence of intellectual disability or the existence of a specific clinical phenotype). The collected data was used for the analysis of the epidemiological situation of PKU in Bihor county.

The statistical data was processed by the use of SPSS IBM SPSS Statistics Version 26. A value of $p < 0.05$ was considered statistically significant.

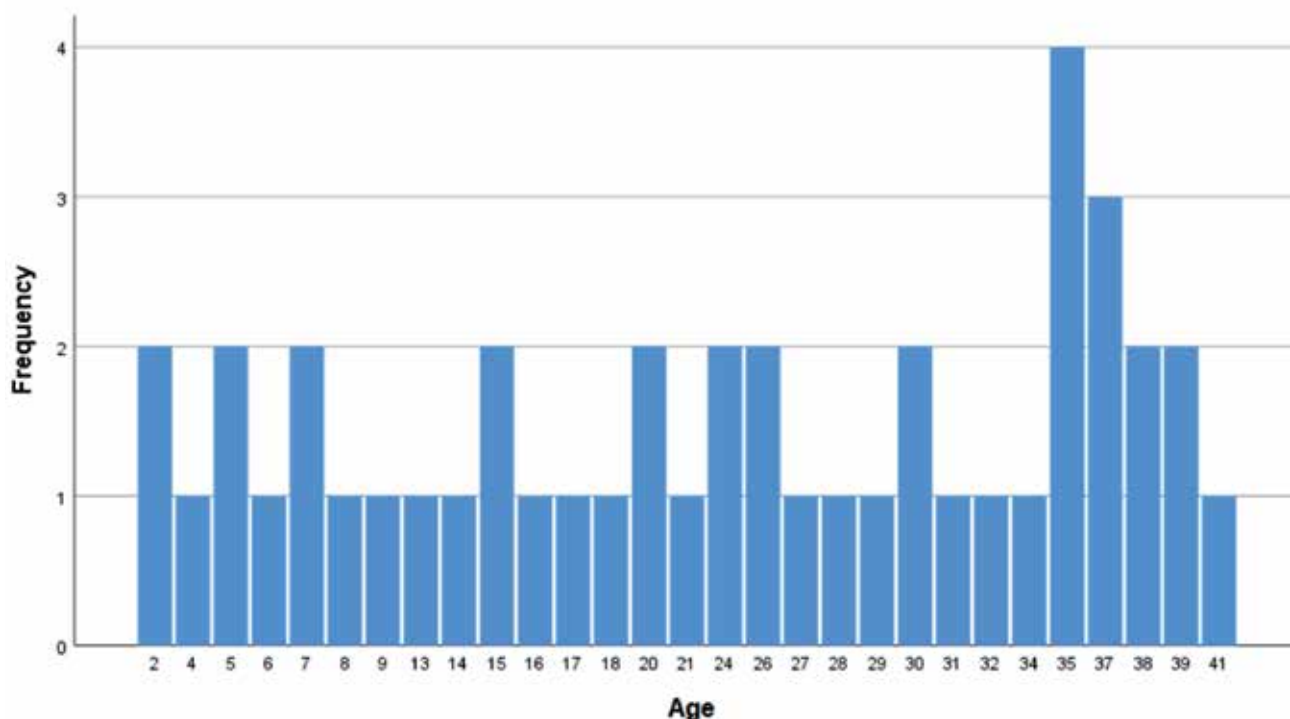


FIGURE 1. Age distribution of the PKU patients from the study group

RESULTS

From the total of 44 PKU patients 20 (45.5%) were masculine and 24 (54.5%) were feminine; 50% of the patients were from a rural area and 50% from an urban area. The vast majority of patients - 84.1% (37 patients) were domiciliated in Bihor county, 3 patients (6.8%) were from Salaj county, 3 (6.8%) from Maramures and 1 patient (2.3%) was from Satu Mare county. The mean age of the studied patients was 22.98, with a median of 25 years old (Figure 1).

According to the TEMPO online database (<http://statistici.inssse.ro:8077/tempo-online/>) in Bihor county, between 1990 and 2020, there were a total of 203,197 newborns. In the same interval there were diagnosed with PKU 26 patients in Bihor. This allow for the calculation of Bihor PKU prevalence at 1:7,843 newborns.

The majority of patients (66%) were diagnosed with PKU in the first 4 months of live (Figure 2); but there were registered also cases with late diagnosis, 20.5% of patients were diagnosed after the age of 1 year old. There isn't a statistically significant correlation between the age of the patients and the age of the diagnosis ($p = 0.304$), which indicates that, for the patients in this study, the diagnosis was not achieved, in average, later for the older patients than the younger ones.

Based on the pre-treatment plasmatic levels of phenylalanine, the majority of cases (72.7%) had a severe metabolic phenotype (classic PKU - cPKU), 20.5% of cases had a milder form of PKU (mPKU)

and 6.8% of patients were found with a mild hyperphenylalaninemia (HPA) (Figure 3).

In the case of 23 patients (52.3% of the cases) the treatment was not permanently available, was available in insufficient quantities or there was a low compliance with the treatment. There is a statistically significant correlation ($p < 0.0001$) between the access to medical food in the first years of life and the age of the patient.

Only 3 patients from the study group had treatment with Sapropterin. All of them had a mild metabolic phenotype (HPA), but there were diagnosed late. All three of them have severe mental retardation,

The specific phenotype (blond hair, light complexion, blue eyes) is found in 22.7% of cases, 77.3% of patients not having these features. Intellectual disability (with varying degrees of mental retardation) is present in 68.2% of cases. There is a statistically significant positive correlation ($p < 0.0001$) between lack of access to treatment / low compliance with treatment and the onset of intellectual disability. Of the patients with intellectual impairment, 11.5% (5 patients) had liminal intellect, 20.5% (9 patients) had a mild mental retardation, 13.6% (6 cases) had a moderate retardation, 20.5% (9 cases) had severe retardation, and 1 case (2.3%) suffered from profound mental retardation; 31.8% of patients had a normal intellect (Figure 4). There is a statistically significant correlation ($p = 0.001$) between lack of access to treatment / low compliance with treatment

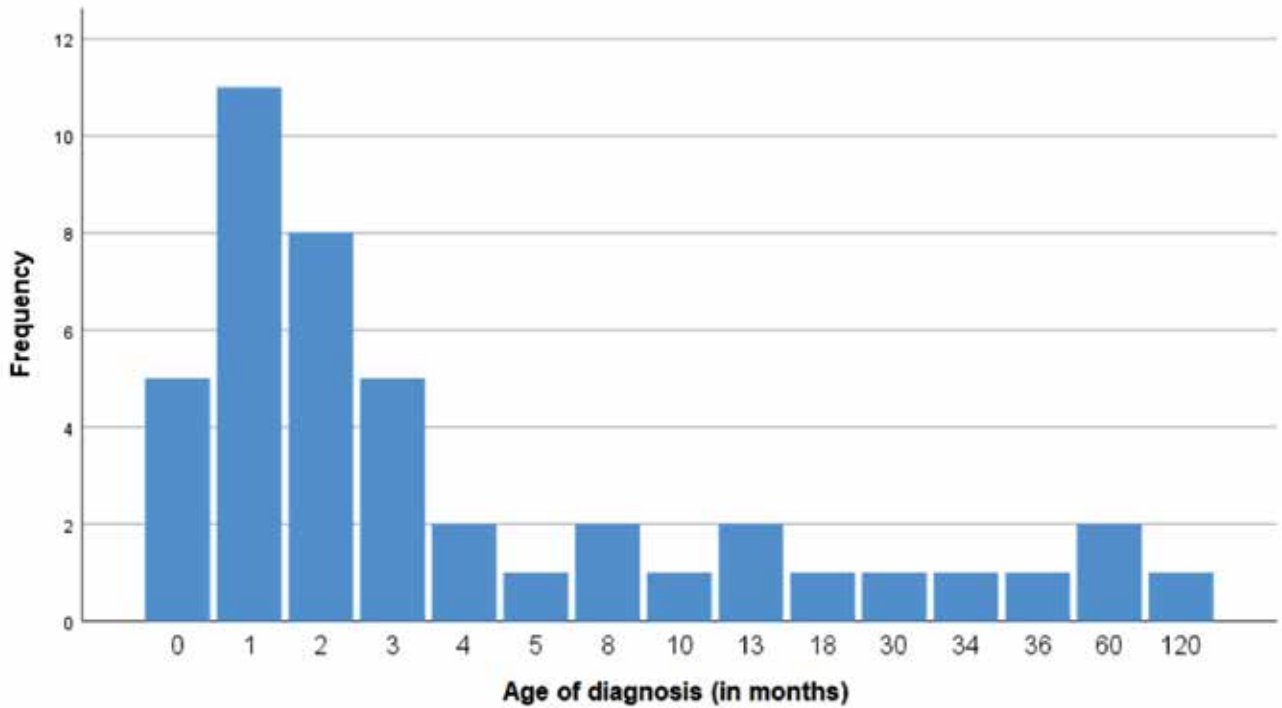


FIGURE 2. The distribution of PKU patients based on the age at which the diagnostic was established

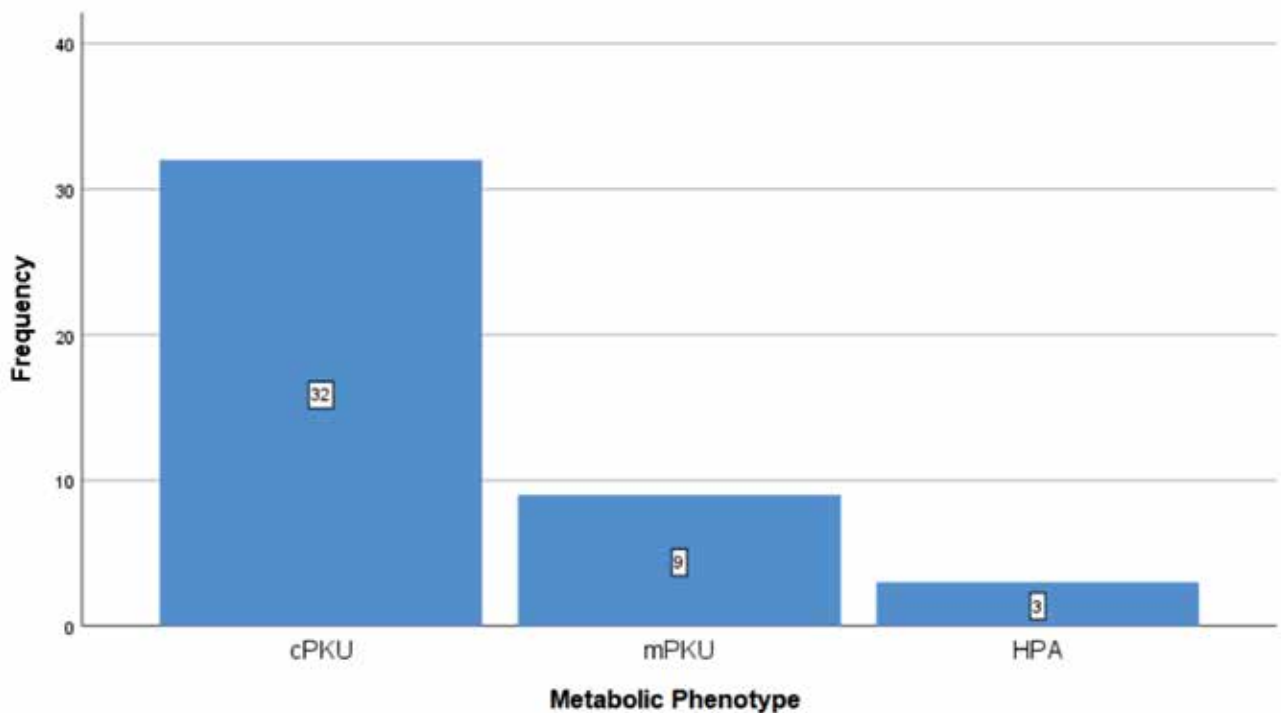


FIGURE 3. The distribution of PKU patients based on the metabolic phenotype

and severity of intellectual disability, regardless of the form of the disease, and also a correlation ($p < 0.0001$) between the presence of mental impairment and delayed introduction of dietary treatment.

DISCUSSIONS

The study group, with patients aged between 2 and 41 years, allows both the comparative analysis

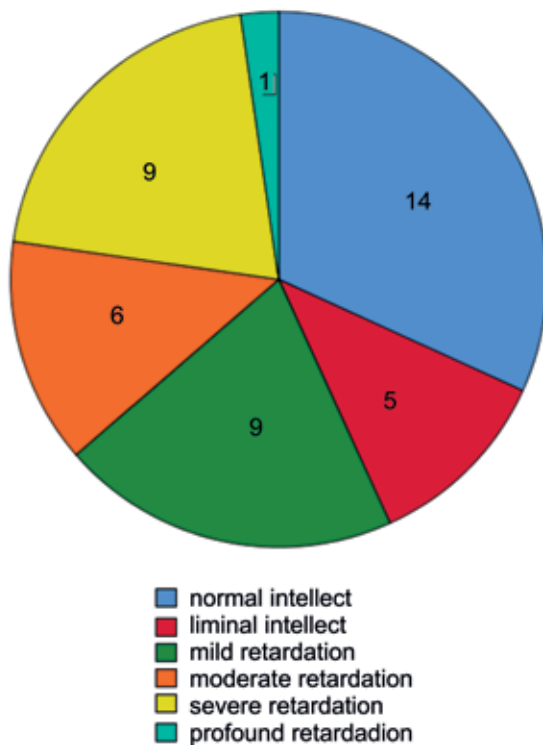


FIGURE 4. Distribution of phenylketonuria cases according to intellectual impairment (profound mental retardation – IQ score below 20; severe mental retardation – IQ score 20-34; moderate mental retardation – IQ score 35-49; mild mental retardation – IQ score 50-69; intellect liminal – IQ score 70-84; and normal intellect – IQ over 85)

of the disease at different ages, as well as the follow-up of some cases over decades.

The prevalence of PKU of 1:7,843 live births in Bihor County is above the estimated national prevalence of 1:10,000 newborns [3,11], but close to the values reported in countries in the same geographical region as Ukraine (1: 7,143 newborns), Hungary (1: 9,000 newborns), the Republic of Moldova (1:7,353 newborns) or Russia (1:7,714 newborns [3].

One of the things that stood out was the complete or partial lack of dietary treatment in patients over 20 years of age. Postponement of treatment or improper treatment affects the patient's intellect, regardless of his or her metabolic phenotype. If in intellectual affected patients over 20 years of age there is a general lack of availability of dietary treatment, patients and family having in general a good compliance with treatment, in younger patients mental impairment, there is rather a low compliance, the dietary treatment being available.

The optimal protein intake required to achieve adequate growth and development is not known [10], but a study of 25 children looked at comparing therapy with a low protein substitute (1.2 g/kg/day) with the situation in which the same patients receive 2 g/kg/day of protein substitute. Patients, all

with well-controlled PKU, had a usual daily intake of 2.2 g/kg, used as a control value. During the period of low protein substituent intake, the level of serum phenylalanine increased compared to the control period, and during the diet with an increased amount of protein substituent, the Phe values remained unchanged compared to the control period. It was concluded that an increased amount of protein substituent leads to lower serum phenylalanine levels. However, the authors acknowledge that there was a large variability in the results between the study subjects, probably caused by the intake of carbohydrates and/or fats contained in the protein substitute [12]. Some patients need tyrosine supplementation, but it has not yet been established whether tyrosine supplementation should be routinely introduced in all patients [13].

Sapropterin treatment is applied to 3 patients in the group of 44 patients with PKU studied. All of these patients suffer from severe retardation, although pre-treatment Phe values were classified as a mild metabolic phenotype (HPA). All three have BH4 deficiency. The other patients, regardless of the form of the disease, did not receive treatment with Sapropterin, although studies show that treatment with Sapropterin is effective in patients with residual enzyme activity. In a 2007 study, the response to BH4 was measured in a group of 557 newborns with PKU (but no BH4 deficiency) after administration of 20 mg/kg of BH4. The positive response was defined as a 30% reduction in serum phenylalanine and the results were as follows: 79-83% of patients with HPA (phenylalanine below 10 mg/dl), 49-60% of patients with mild PFU (phenylalanine between 10 and 20 mg/dl) and 7-10% of patients with classic PKU (phenylalanine over 20 mg/dl) responded to BH4 treatment [14]. In another multicenter study, 89 patients with PKU were randomized to receive Sapropterin (10 mg/kg) or placebo for six weeks. Sapropterin treatment resulted in a mean decrease in phenylalanine concentration of 3.9 mg/dl, compared with an increase in phenylalaninemia of 0.05 (on average) in placebo-treated patients [15].

In most patients the diagnosis was made in the first 4 months of life, the vast majority between 4 and 8 weeks of life. The diagnosis in most of these cases is made after the screening test (T. Guthrie) performed after birth. If the phenylalanine values are above 2 mg/dl or above 120 micromol/L associated with normal/low tyrosine levels on the screening test, the test is considered positive and the doctor coordinating the screening center should inform the family and refer the child to a screening center. diagnosis and treatment in PKU to confirm the diagnosis [11]. This process can take weeks to start treatment. Initiation of phenylalanine exclusion therapy should be initiated as early as possible, preferably

before 1 week of age, for all newborns with serum phenylalanine values above 6 mg/dl [16]. In 34% of patients the diagnosis was established after the age of 4 months, in 20.5% of the study group the diagnosis being formulated after the age of 1 year. In the latter, the suspicion of the disease was raised based on suggestive clinical manifestations, the screening test being negative or not performed. An error factor in patients with a false negative screening test may also be the timing of dry-spot collection.

The timing of the sample collection in the newborn depends on the gestational age of the child and his state of health. In newborns, the screening sample is collected 48-72 hours after birth, or even 24 hours if the measurement of phenylalanine is accompanied by the determination of the phenylalanine/tyrosine ratio. In underweight newborns or newborns initially fed with glucose and electrolyte solutions without amino acids, the sample should be taken on the third day after the start of the dairy diet (increase in plasma phenylalanine, a necessary element in diagnosis, is dependent on the introduction of milk; in protein-free infants, there is no phenylalanine intake necessary for plasma phenylalanine growth). In premature babies, two harvests are performed, namely a few days after birth and at maternity discharge (premature babies have an immaturity of the enzyme system that can cause a transient increase in phenylalaninemia and thus a positive test in the screening test; with the maturation of the enzyme system also normalizes plasma phenylalaninemia values). If the newborn needs a transfusion, the screening is done in the first 24 hours of life and is repeated after 10-14 days [11].

Severe phenotype (cPKU) is found in most patients. The milder metabolic phenotype (HPA) was found in a small percentage of patients, lower than the averages reported in the literature (21.9%) [3]. In the first two decades studied, the diagnosis of mild forms of the disease was very rare. In the absence of screening tests or important clinical manifestations, it can be assumed that patients with HPA remained undiagnosed, which would contribute to the low percentage of patients with HPA in the study group.

An important aspect of this study was the patients' adherence to the disease-specific diet. More than half of the patients included in the study did not follow a proper diet due to lack of access to special foods or low compliance. This study shows a significant improvement in treatment adherence in younger patients, which indicates, on the one hand, easier access to specific foods and, on the other hand, an increased understanding of patients and their members of the importance of diet therapy in preventing intellectual disability.

The study also confirms a well-known fact that

diet therapy has an important influence on the clinical course of the disease. The study includes both patients with severe metabolic phenotype who under rigorously managed treatment reach adulthood without intellectual disabilities, some with higher education, fully socially integrated, and patients with mild metabolic phenotype, from disadvantaged backgrounds, with low compliance, which develops severe retardation, requiring permanent care.

Despite the fact that the effectiveness of the dietary restriction is undeniable, there are still important issues with what the diet receives from phenylketonuria. One of these is the low compliance caused by unpleasant tasting food. This problem is evident in older children and adults. Adherence to treatment is not usually problematic at the age of the newborn, infant and young child because it depends largely on the parents. However, there are problems in adolescence, problems related to the unpleasant taste of protein substitutes and medical foods. This is reflected in the poor control of the disease in this age group [17,18]. Adolescents and adults with PKU controlled during childhood, but later reduced compliance, even if they remain with normal intellect, they face problems such as depression, anxiety or inability to form and maintain social relationships [19]. Attention deficits, poor concentration, headache, sleep disturbances have also been reported in this group of patients. Another problem is the presence of neurological changes and decreased quality of life, despite rapid diagnosis and good control of serum phenylalanine levels; Patients treated at a young age who have good control of phenylalanine throughout their lives, although they develop normal physical and neurological health and have a normal IQ, recent studies have shown that these patients have a lower IQ than their unaffected siblings or than the general population. Another shortcoming of this therapy is the potential nutritional deficiencies resulting from a restrictive diet. Last but not least, another financial burden is the high cost of special foods and nutritional supplements [17].

CONCLUSIONS

The prevalence of PKU in Bihor County is above the national estimated value.

In the northwestern region of Romania, the vast majority of PKU cases had a severe metabolic phenotype (cPKU), and the milder metabolic phenotype (HPA) was found in a small number of patients, below the calculated averages worldwide.

The late diagnosis and implementation of diet therapy or non-compliance with treatment lead to the onset of intellectual disability, regardless of the patient's metabolic phenotype.

The improvement of medical services (especially the introduction of neonatal screening) as well as easier access to treatment has allowed younger gen-

erations of patients to enjoy a higher quality of life than patients diagnosed in the first two decades studied.

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