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Research Article

Evaluation of babies with hyperphenylalaninemia diagnosed in the National Newborn Screening Program in Istanbul in 2019

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Abstract

Objectives: To evaluate the 2-year follow-up of hyperphenylalaninemia (HPA) patients born in 2019.

Methods: Growth, neuromotor development, and vitamin levels of 61 two-year-old babies followed up with the diagnosis of HPA in 2019 were evaluated.

Results: Thirty-six (59.02%) of the cases were female. The mean birth weight of the babies was 3198.7±504 g and 23.1% (n=14) of them were preterm. The mean of initial National Newborn Screening Program phenylalanine (phe) levels was 3.85±1.86 mg/dL. The mean day to diagnosis was 13.29 days (range 4-18). Forty-eight (78.7%) of the patients declared sufficient knowledge about HPA follow-up. Follow-up compliance of the families was 67%. Among the infants who participated in our study, 4 (6.6%) patients were diagnosed with sapropterin dihydrochloride (BH4) responsive mild phenylketonuria (MPKU). No patient was started on with phe-restricted diet therapy. The remaining 32 (52.4%) infants were followed up with the diagnosis of female HPA. The follow-up of 25 (41%) male infants was terminated. Anemia was found in 4 (6.6%) babies. Iron deficiency in 4 (6.6%) and B12 deficiency in 9 (14.8%) babies were detected. There was no significant difference between MPKU and HPA groups regarding growth parameters and vitamin levels.

Conclusion: No growth or neuromotor retardation was found in HPA patients. Vitamin D and, less frequently, vitamin B12 deficiencies are prominent. No difference was observed between the patients who passed from HPA to MPKU and HPA cases, in terms of growth, development, and nutritional status.

Keywords: Growth, hyperphenylalaninemia, newborn screening, vitamin levels

Phenylketonuria (PKU) (MIM 261600) is the most common autosomal recessive inborn disorder of amino acid metabolism, caused by mutations in the phenylalanine hydroxylase (PAH) gene and resulting in the deficiency of PAH [1]. Thus, a blockage occurs in converting phenylalanine (phe) to tyrosine (tyr). This leads to high phe and low tyr values, leading to neurotoxic effects in two main ways: First, phe and its metabolites phenylacetic, phenylpyruvic, and phenyl lactic acids cause neuromotor retardation, autism, behavioral disorders, seizures, and microcephaly with their neurotoxic effects on the central nervous system, and, second, it causes a kind of neurotransmitter deficiency with the decrease of tyr, that is, the primary precursor of neurotransmitters such as dopamine, adrenaline, and noradrenaline [2].

The first newborn screening program in the world was started in 1962 in Massachusetts in the United States for PKU. Newborn Screening in Turkey was initiated with Phenylketonuria Screening Program with the support of universities under the respon-

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sibility of our Ministry in 1987. The National Newborn Screening Program (NNSP), a milestone in newborn screening in Turkey, was started on December 25, 2006, by the Ministry of Health (https://hsgm.saglik.gov.tr/tr/cocukergen). Turkey is one of the countries with the highest incidence of hyperphenylalaninemia (HPA) and PKU with 1/2785 and 1/4370, respectively [3].

Over 1000 PAH gene mutations have been identified [4]. These different mutations cause a continuum clinic and phe levels rather than a uniform clinic. Individuals with the phe level above 120 µmol/L (2 mg/dL) are called HPA. HPA diagnoses were classified as classical PKU (>1200 µmol/L), moderate PKU (900-1200 µmol/ L), and mild PKU (360-900 µmol/L) according to the phe levels measured at the time of the diagnosis [1].

Although the treatment initiation varies among different guidelines, phe levels above 360 µmol/L were considered to be above the threshold per the American College of Medical Genetics and Genomics (ACMG) guidelines [1].

The treatment of PKU must be lifelong, with the goal of maintaining blood phe in the range of 60-360 µmol/L. Treatment is structured with protein-restricted diet (reduced phe) and/or medical therapy with sapropterin dihydrochloride (BH4) in responsive cases [1, 5]. BH4 responsiveness is determined according to the 20 mg/kg/dose sapropterin loading test performed at the time of diagnosis for each infant whose initial value is above 360 µmol/L. Individuals with initial phe levels between 120 and 360 µmol/L should be followed up for the first 2 years of life to ensure that the levels do not drift upward because of high protein intake or due to catabolic situations such as infection and vaccination. If treatment is not required before 2 years of age, annual monitoring is required for girls to prevent maternal PKU [6]. During pregnancy, it is necessary to keep the blood phe level between 120 and 250 µmol/L as recommended by an institutional Medical Research Council Working Party with diet or sapropterin, to protect the embryo from the teratogenic effects of phe [7, 8]. The individuals whose initial phe values are between 120 and 360 µmol/L should be monitored for their growth and neurological development, as well as for their phe levels. If phe values rise above 360 µmol/L and persist, treatment is started after BH4 loading: BH4 treatment for BH4 responders and diet therapy for nonresponders by determining dietary phe tolerance.

We aim to evaluate the infants with HPA diagnosed in 2019 and their 2-year follow-up results.

Materials and Methods

The study was designed as cross-sectional and prospectively. In 2019, 230,269 babies with Turkish nationality were screened within the scope of the Newborn Screening Program in Istanbul. Of these, 333 were at risk for PKU and were referred to pediatric metabolism outpatient clinics. Babies with phe levels above 4 mg/dL once and between 2 and 4 mg/dL twice were considered risky. They were referred to pediatric metabolism outpatient clinics for phe level verification and follow-up. Three of them refused to follow up and 49 of them died of causes other than PKU. Nine of them moved out of Istanbul. Confirmation phe levels of 122 of these 272 cases were evaluated as normal. Sixteen patients with initial values >360 mg/dL were diagnosed with PKU. One of the 134 babies identified for HPA moved out of the province and two died. Therefore, 131 babies were invited to pediatric metabolism outpatient clinics by the Istanbul Provincial Health Directorate Public Health Services Department. Twelve patients of the remaining 131 patients were diagnosed with mild PKU at the beginning of their follow-up. It was learned through phone calls that 49 of the remaining 119 patients attended regular follow-ups. A total of 61 patients who gave consent were included in this study. The flow diagram of the study is outlined in Figure 1.

Through phone calls demographics, social characteristics, delivery type, and nutritional anamnesis were obtained. Height, weight, and head circumference and their standard deviation scores (SDSs) were measured. Neuromotor development milestones were questioned by asking babies' families. Neurocognitive assessments were determined at the age of 2-years-old. Maximum and final phe levels, kidney and liver function tests, and vitamin levels were determined. Venous blood samples were drawn following overnight fasting. Vitamin B12 and ferritin levels were measured immune enzymatically, folate level was measured with competitive binding by chemiluminescence method (using Beckmann Coulter D×1 800 device). Creatinine, serum glutamic-oxaloacetic transaminase, and serum glutamic pyruvic transaminase were measured by the photometric method using a Beckman Coulter analyzer. 25-Hydroxy vitamin D levels were measured by chemiluminescence method using

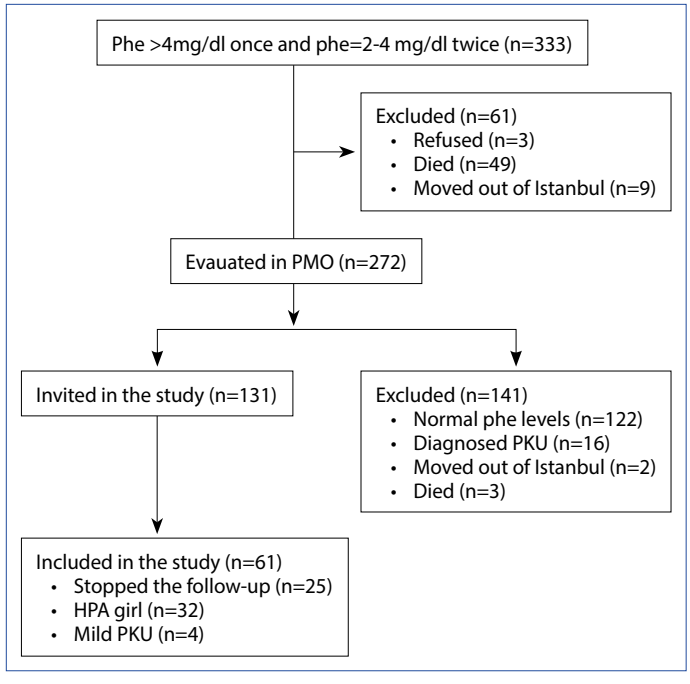

Figure 1. Flow diagram of the study.

Phe: Phenlyalanine; PMO: pediatric metabolism outpatient clinics; PKU: Phenylketonuria; HPA: Hyperphenylalaninemia.

a Beckman Coulter Analyzer. Vitamin D deficiency is defined as a 25-hydroxy vitamin D level below 30 ng/mL.

Dried blood spot phe and tyr values were determined by the HPLC system (Thermo Ultimate 3000) using ClinRep Aromatic Amino Acids kits. Briefly, 6 mm diameter disks are punched from dried blood spots and put into a vial that contains extraction solution and internal standard. After 30 min incubation at 37°C, samples were centrifuged, and the supernatant was injected into the HPLC system. Each batch was calibrated using a dried blood spot containing 250 µmol/L phe and tyr.

Follow-up compliance of the cases was determined according to monthly blood phe level evaluation and physical examination three times in 24 months. This was indicated by the percentage calculation: [actual/target×100].

Statistical analysis

Statistical analyses were performed using SPSS (version 22.0). The categorical variables were defined as frequency and percentage rate, and the numerical variables were determined as mean±standard deviation. The Kolmogorov-Smirnov test assessed the normality of the distribution of the quantitative variables. Student's t-test was performed for normally distributed numerical variables, and the Mann-Whitney U test was carried out for nonnormally distributed data for independent group comparison. Categorical variables were compared using the Chi-square test. Statistically significant results were defined as those with a p-value of <0.05.

Ethical approval

This study was conducted in accordance with the ethical principles of the World Medical Association Declaration of Helsinki (2000) and was approved by the Ethics Committee of the Bakirkoy Dr. Sadi Konuk Training and Research Hospital, Istanbul, Turkey (approval number: 2021-17-06; date: September 6, 2021).

Results

Istanbul resident 230,269 Turkish babies were screened within the NNSP scope in 2019. The consort of the study is shown in Figure 1. In 2019, 16 of 272 infants with high screening phe levels were diagnosed with PKU at the initial evaluation and 4 during follow-up. In this case, the overall rate of being diagnosed with PKU is 7.3%.

Thirty-six (59.02%) of 61 infants included in the study were female. The mean birth weight of the babies was 3198.7±504 g, and 23.1% (n=14) of them were preterm. The mean of initial NNSP phe level was 3.85±1.86 mg/dL. The mean day to diagnosis was 13.29 days (range 4-18). Eighteen (29.5%) of the parents were consanguineous, and 13 (21.3%) had at least one case of HPA/PKU in their families. Forty-eight (78.7%) of the patients declared sufficient knowledge about HPA follow-up. Follow-up compliance of the families was 67%.

Among the infants who participated in our study, 4 (6.6%) patients were diagnosed with BH4 responsive MPKU. The mean month of initiation of BH4 therapy was 7.25+2.06 months.

Therefore, no patient was started on with phe-restricted diet therapy. The remaining 32 (52.4%) infants were followed up with the diagnosis of female HPA. The follow-up of 25 (41%) male infants was terminated.

The growth and biochemical evaluation of 61 infants is summarized in Table 1. Anemia was found in 4 (6.6%) babies. Iron deficiency in 4 (6.6%) and B12 deficiency in 9 (14.8%) babies were detected. While no folic acid deficiency was observed in any babies, vitamin D deficiency was detected in 28 (45.9%) of all cases.

Anthropometric and biochemical characteristics of babies with HPA and MPKU were compared (Table 2). The percentage of patients receiving vitamin and mineral supplements during follow-up was 86.7% (n=50).

No significant correlation was found between head circumference measurements, maximum phe level, and vitamin values (data not shown in the table). The mean maximum phe level of MPKU patients was found to be significantly higher than that of HPA patients (p=0.04). The neuromotor development of babies in both groups was normal. There was no significant difference between growth parameters and vitamin levels.

Discussion

Sixty-one infants diagnosed in 2019 and followed up for HPA were evaluated for growth, neuromotor development, and biochemistry at age 2.

The follow-up of HPA cases has two goals: (1) to identify patients who will be treated for PKU and (2) to prevent cases of maternal PKU [9]. Therefore, blood phe level and clinical follow-up of babies with HPA are essential public health tasks. The collaboration of the family, pediatric metabolism physicians, and primary care physicians is a requisite. Three-quarters of the families of HPA case patients stated that they had sufficient information about HPA follow-up. To increase this rate, family physicians must be in contact with the families of HPA patients to increase their follow-up.

Height, weight, and head circumference and their SDSs were within normal limits in all patients, and there was no difference between HPA and MPKU patients. Based on their research on the anthropometric characteristics of HPA and PKU patients, Tansek et al. [10] stated that in untreated HPA cases, the final heights and weights were within normal limits. The development of head circumference is an essential indicator of neuromotor development. Microcephaly is an important finding in untreated PKU patients, but not an expected finding in HPA patients. Untreated PKU is associated with an abnormal phenotype, including microcephaly [11]. We compared the head circumference with maximum blood phe and vitamin levels, and no significant relationship was found.

Nutritional assessment is an important parameter that affects growth and neurocognitive improvement [12]. Overall, vitamin and mineral levels of our HPA patients were within normal

Table 1. 2-year-old growth and biochemical characteristics of the babies

N=61	Mean±SD	Minimum	Maximum
Height (cm)	86.81±3.765	79	96
SDS	0.148±0.840	-1.610	2.340
Weight (kg)	12.55±1.981	8.8	19
SDS	0.244±1.002	-2.609	3.490
Head circumference (cm)	47.84±1.628	45	52
SDS	-0.307±0.954	-2.0	2.660
Max phe µmol/L	243.31±118.852	93	674
Last phe µmol/L	186.14±78.180	53	387
Hemoglobulin g/dL	12.25±1.196	6.7	14.3
Hematocrit (%)	36.98±2.720	25	41.2
Iron (µg/dL)	73.74±27.284	19	133
Ferritin (µg/L)	28.20±20.953	1.1	120
Vitanin B12 (pg/mL)	438.55±178.777	185	910
Folic acid (pg/L)	13.69±4.158	4.6	20
25-OH-D vit (ng/mL)	24.12±11.640	4.7	66
Urea (mg/dL)	23.32±6.531	11.1	45.8
Creatinine (mg/dL)	0.28±0.048	0.19	0.39
SGOT (U/L)	35.74±8.59	21.4	73
SGPT (IU/L)	17.39±13.387	8.5	112

SDS: Standart deviation score; Phe: Phenlyalanine; SGOT: Serum Glutamic-Oxaloacetic Transaminase; SGPT: Serum glutamic pyruvic transaminase; SD: Standart deviation.

Table 2. Comparison of MPKU and HPA babies according to their 2-year-old growth and biochemical results

	MPKU n=4 Mean±SD	HPA n=57 Mean±SD	p<0.05
Height (cm)	88.75±3.5	86.67±3.8	0.430
SDS	0.78±1.1	0.10±0.8	0.281
Weight (cm)	12.52±0.4	12.54±2.1	0.942
SDS	0.41±0.5	0.23±1.0	0.431
Head circumference (cm)	47.9±0.8	47.83±1.7	0.759
SDS	-0.06±0.8	-0.324±1	0.466
Maximum phe (µmol/l)	458.75±167.9	228.19±100.4	**0.04**
Last phe (µmol/l)	275.00±126.5	179.9±71.1	0.153
Hemoglobin (g/dL)	12.47±0.5	12.23±1.2	0.782
Hematocrit (%)	37.82±1.5	36.92±2.8	0.550
Iron (µg/dL)	68.92±27	74.08±27.5	0.662
Ferritin (µg/L)	26.35±22	28.33±21.1	1
Vitamin B12 (pg/mL)	433.25±97.4	438.93±183.7	0.749
Folic acid (pg/L)	9.70±3	13.97±4.1	0.047
25-OH D-vit (ng/mL)	27.22±12.6	23.90±11.7	0.484
Urea (mg/dL)	20.60±4.4	23.51±6.6	0.344
Creatinin (MG/dL)	0.30±0.1	0.27±0.05	0.254
SGOT (U/L)	30.1±3.3	36.13±8.2	0.073
SGPT (IU/L)	15.75±3.8	17.50±13.8	0.827

MPKU: Mild Phenylketonurea; HPA: Hyperphenylalaninemia; SDS: Standart deviation score; Phe: Phenlyalanine; SGOT: Serum Glutamic-Oxaloacetic Transaminase; SGPT: Serum glutamic pyruvic transaminase.

limits, except for vitamin D. Vitamin D and B12 deficiencies are common both in the general population and in PKU/HPA patients. Studies have emphasized the effects of the levels of these two vitamins on bone and neuromotor development [13, 14]. In our study, 86.7% of the patients needed vitamin and mineral supplementation. However, at the end of their 2-year

follow-up, vitamin deficiency (mainly B12) cases were detected. While B12 is directly related to nutrition, vitamin D deficiency is more related to lifestyle and less exposure to the sun. Frequent outpatient controls and patients who do not receive dietary treatment prevented vitamin deficiency. Good follow-up compliance, which was 67% in our study, is an important reason. The evaluation of growth, neuromotor development, and blood vitamin mineral levels of HPA patients at 3-6-month intervals is more frequent compared with the normal pediatric population. Reports have suggested that BH4 responsive MPKU patients are more likely to have vitamin deficiencies, especially B12, than are KPKU and other HPA patients [15-17]. Since most MPKU patients are BH4-responsive, this has been associated with not using vitamin-mineral fortified formulas. However, frequent evaluation of blood levels can be preventative in MPKU cases. During follow-up, no difference was found between HPA and MPKU patients regarding clinical or biochemical characteristics, except for the maximum phe levels.

Conclusion

No growth or neuromotor retardation was found in HPA patients. Vitamin D and, less frequently, vitamin B12 deficiencies are prominent. No difference was observed between patients who passed from HPA to MPKU and HPA cases, in terms of growth, development, and nutritional status. To increase the follow-up compliance of HPA cases, it is necessary to increase the family information provided by pediatric metabolism physicians and primary care physicians.

Conflict of Interest: The authors declare that there is no conflict of interest.

Ethics Committee Approval: The study was approved by the Bakirkoy Dr. Sadi Konuk Training and Research Hospital Clinical Research Ethics Committee (No: 2021-17-06, Date: 06/09/2021).

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