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Original Article
DOI: 10.4274/imdn.galenos.2025.2025-2

Analysis of Laboratory and Demographic Data of Late Diagnosed Phenylketonuria Cases

Bilgin and Ergül Bozacı. Late Diagnosed Phenylketonuria

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19.01.2025
05.05.2025

Epub: 09.05.2025
Published:

Cite this article as: Bilgin H, Ergül Bozacı A. Analysis of laboratory and demographic data of late diagnosed phenylketonuria cases. Inherit Metab Disord Nutr.

ABSTRACT
Objectives: In this study, it was aimed to evaluate the clinical, demographic, and laboratory data of the patients we followed up with late diagnosed phenylketonuria (PKU). In addition, the relationship between the age of onset of treatment and neuromotor development will be evaluated.
Materials and Methods: In this study, patients diagnosed with PKU and followed in the Pediatric Metabolism Outpatient Clinic were retrospectively examined. Cases diagnosed late were evaluated.
Results: We determined that 25 of our patients with PKU received a late diagnosis. Of these patients, 19 had classical PKU, and 6 had a mild PKU phenotype. Eight of our cases were female and 17 were male. The mean age at diagnosis of patients was 26.3±38.1 (range, 2-192) months. The mean age of the patients at the last evaluation was calculated to be 13.1±5.3 (range, 3.8-21.3) years. At the last evaluation, the phenylalanine level of the patients was 465.3±275.7 µmol/L in mild PKU and 779.1±449.4 µmol/L in classical PKU. Fifteen of our cases had global developmental delay, six had moderate developmental delay, and four had mild developmental delay. Twenty-one of our cases received diet therapy, and four of our cases received large neutral amino acid therapy.
Conclusion: It causes severe neurological problems in patients who are diagnosed late or undiagnosed. Therefore, close follow-up is important in the diagnosis and treatment phase of the disease. Close follow-up is essential to ensure that patients diagnosed with PKU can receive their treatment in the early period.
Keywords: Phenylketonurias, Therapeutics, Nervous System Diseases

INTRODUCTION
Phenylketonuria (PKU) is an autosomal recessive metabolic disease caused by the deficiency of phenylalanine hydroxylase, an enzyme that converts phenylalanine, an essential amino acid, to tyrosine in the liver.1 The phenylalanine hydroxylase enzyme is encoded by the phenylalanine hydroxylase *(PAH)* gene, and its cofactor is tetrahydrobiopterin. The gene encoding the PAH enzyme is localized in the q22-q24.[1] band region on the long arm of chromosome 12.
The frequency of PAH deficiency varies according to ethnicity and geographical region. Due to the high rate of consanguineous marriages in Türkiye, PKU occurs at a frequency of 1/4370. Neonatal PKU screening in our country was first conducted as a pilot study in 1983. It was included in the screening scope nationwide after 1994.[2] In our country, blood samples are taken from babies twice within the scope of neonatal screening. The first blood sample is taken before hospital discharge, and the second is taken one week later. Today, PKU is one of the diseases that is recommended for screening because it is highly prevalent and treatable. Early diagnosis and treatment can prevent irreversible damage in this disease.

In PKU, phenylalanine and its metabolites cause structural brain damage, severe intellectual disability, and psychiatric disorders as a result of neurotoxic effects.[3] Early diagnosis and treatment are necessary to prevent or reduce the symptoms of the disease. With the emergence of newborn screening programs, the most serious neuropsychiatric findings of the disease can be prevented. The clinical picture resulting from the *PAH* gene defect varies from mild hyperphenylalaninemia to classical PKU. A phenylalanine-restricted diet is the main treatment method. However, research on the treatment of PKU is continuing, and new treatment options are emerging that can reduce the burden of the difficult and restrictive diet on patients.

Patients with PAH deficiency do not have any clinical findings at birth. They usually apply to the hospital when they are 3-4 months old, suggesting these delays prompt the hospital visit. Late-diagnosed patients with classical PKU experience severe intellectual disability, microcephaly, ataxia, autism, convulsions, aggression, eczema-like skin lesions, and behavioural disorders.[4]

In this study, we aimed to evaluate the clinical, demographic, and laboratory data of patients we follow with late-diagnosed PKU. In addition, the relationship between the age of initiation of treatment and neuromotor development will be evaluated.

MATERIALS AND METHODS

In this study, patients who were followed up in the Pediatric Metabolism Outpatient Clinic between January 2021 and July 2023 and diagnosed with PKU were retrospectively examined. Late-diagnosed cases were evaluated. The patients included in the study were evaluated for age at diagnosis, follow-up period, height, weight, head circumference, gender, history of consanguinity between parents, clinical phenotype, plasma phenylalanine levels at admission, and metabolic control status during treatment and follow-up. These data were obtained from the patients' electronic files in the hospital information management system. Clinical, demographic, and laboratory data of all patients were analysed. *PAH* gene analysis was performed on all our patients, and biallelic variants were detected.

The blood phenylalanine levels of the patients were determined by taking 2 mL of blood in tubes containing ethylenediaminetetraacetic acid and using high-performance liquid chromatography in the Metabolism Laboratory. For mutation screening and genotyping of the patients, exons 1-13 of the *PAH* gene were examined by polymerase chain reaction)amplification, followed by DNA sequence analysis. Mutations caused by nucleotide changes, detected by DNA sequence analysis, were identified.

Blood dihydropteridine reductase activity and neopterin, and biopterin analyses were performed to evaluate BH4 metabolism disorders. The normal range for plasma phenylalanine is 60-120 µmol/L. If phenylalanine levels were >1200 µmol/L before treatment at the time of diagnosis, it was classified as classical PKU; if phenylalanine levels were between 600-1200 µmol/L, it was classified as mild PKU; and if phenylalanine levels were <600 µmol/L, it was classified as mild hyperphenylalaninemia. Speech, fine motor, gross motor, personal, and social skills of the patients were evaluated with developmental tests. According to the developmental tests, the patients were defined as having global, moderate, and mild developmental delay.

Each procedure was carried out in accordance with the ethical principles determined by the committee responsible for human experiments and the Declaration of Helsinki. Informed consent was obtained from each patient before participating in the study. Approval for the study was obtained from the University of Health Sciences Türkiye, Gazi Yaşargil Training and Research Hospital Clinical Research Ethics Committee (approval number: 162, dated: 09.09.2022).

Statistical Analysis

Descriptive statistics were presented including minimum, maximum, mean, standard deviation, percentage, and frequency values. Categorical data were analyzed with the chi-square test. All data were transferred to the computer, and statistical analysis was performed using Statistical Package for the Social Sciences version 22.0 . A p-value of <0.05 was considered significant.

RESULTS

The highest plasma phenylalanine levels measured at the time of diagnosis were taken as the criterion in determining the phenotypes of the patients. It was determined that 25 of our patients with PKU were diagnosed late. Nineteen of these patients had classical PKU, and 6 had mild PKU. It was determined that 11 (44%) of our cases were excluded from the newborn screening program and applied to our pediatric metabolism clinic due to neuropsychiatric symptoms, and they were diagnosed with PKU. Two of the cases excluded from the newborn screening program were diagnosed due to a history of a sibling with PKU. It was observed that 14 (56%) of our cases were diagnosed through the newborn screening program, but their hospital admission was late.

Eight of our cases were female and 17 were male. The mean age at diagnosis of all patients was 26.3±38.1 months (range, 2-192) months. The mean age at the last evaluation of the patients was calculated as 13.1±5.3 (range, 3.8-21.3) years (Table 1). Thirteen (52%) of the patients were diagnosed and started on treatment before the age of one. There was consanguinity between the parents of 18 of our patients.

The phenylalanine level of the patients at the time of diagnosis was 777.6±107.7 µmol/L in mild PKU and 1526.1±246.8 µmol/L in classical PKU (Table 1). The phenylalanine level of the patients at the final evaluation was 465.3±275.7 µmol/L in mild PKU and 779.1±449.4 µmol/L in classical PKU (Table 1). The most common

IVS10-11G>A mutation was found in patients. This allele was followed by the c.1222C>T (p.Arg408Trp) and c.143T>C (p.Leu48Ser) variants, respectively. Fifteen of our cases had global developmental delays, 6 had moderate developmental delays, and 4 had mild developmental delays. When we grouped the patients according to the severity of neurological findings, no statistically significant difference was found between the groups in terms of phenylalanine levels at the time of diagnosis.

In the diet treatment, our patients were given total protein intake 2-2.5 g/kg/day for the first year, 1.1-1.7 g/kg/day for ages 1-11, 1 g/kg/day for ages 12-15, and 0.9 g/kg/day for adults. The phenylalanine content of the diet was set at 130-400 mg/day for the first year, 200-400 mg/day for ages 1-11, 350-800 mg/day for ages 12-15, and 450-1000 mg/day for adults. Diet treatments were organized with natural foods and special formula foods that do not contain phenylalanine. Twenty-one of our cases received diet treatment; and 4 of our cases received large neutral amino acid (LNAA) treatment (Table 1). LNAA supplements were administered to adult patients who did not adhere to dietary treatment.

DISCUSSION

In our country and the countries in this region (Middle East, West Asia, South Asia, North Africa), the frequency of consanguineous marriages is high. PKU and other autosomal recessive metabolic disorders are more frequently observed in societies where consanguineous marriages are high. In a study conducted in our country in 1993, the frequency of PKU was determined to be 1/4370, and the rate of consanguineous marriage was 21.5%.[5] The frequency of the disease varies considerably worldwide. In Europe, the frequency of PKU is 1/3000-1/30000, with an average of 1/10000 reported.[3,6] In our country, newborn screening for PKU is performed during the neonatal period. The diagnosis is confirmed by the determination of plasma phenylalanine level and *PAH* gene analysis. PKU screening has been performed in our country since 1993. However, in the study conducted by Tezel et al.,[2] the newborn screening rate was 4.7% in 1987, and this rate increased to 95% in 2008. In patients detected through newborn screening, treatment can be started in the second/third week of life. The cases we present in this study were diagnosed late, and treatment was started after the second month of life. It was determined that 25 of our patients who have PKU and whom we followed up were diagnosed late. Nineteen of these patients had classical PKU, and six had mild PKU phenotypes. All of our patients were born after the newborn screening program started, but only 56% were screened. 44% of our cases were not screened. In cases where screening could not be done, the diagnosis was made after the onset of neurological and psychiatric symptoms, during the examination. It was determined that our cases included in the screening program were reported late or applied late to the metabolism centre late despite being reported. It was determined that the instances of incomplete screening were generally families who gave birth at home and lived in rural areas.

Neuromotor retardation, microcephaly, and epilepsy can be seen in cases in which the treatment started late. High levels of phenylalanine and its metabolites can cause musty body odour and eczema. Tyrosinase inhibition and low tyrosine levels also cause loss of pigmentation in the skin and hair. In addition, behavioural disorders, aggressive behaviour, depression, and anxiety can be seen.[7-9] Our cases have epilepsy, behavioural disorders, and neuromotor delay. It is thought that cognitive problems seen in PKU are related to prefrontal dopamine depletion.[10] Phenylalanine competes with tyrosine at the blood-brain barrier, but phenylalanine passes through at high phenylalanine levels. CSF tyrosine levels decrease, and adequate dopamine synthesis cannot be achieved.[10] In addition, the negative effect of high phenylalanine levels on glutamate receptor function leads to brain dysfunction in patients with PKU.[11,12] Glushakow et al.[12] have shown that high phenylalanine levels significantly suppress the function of glutamate receptors in excitatory synapses.

The aim of treating PKU is to reduce plasma phenylalanine levels. A multidisciplinary approach is important in the treatment of patients with PKU. Treatment should be started as soon as possible in infants with plasma phenylalanine concentrations above 360 µmol/L. In the treatment of the disease, a protein-restricted diet containing low phenylalanine, sapropterin dihydrochloride, LNAAs to a protein-restricted diet, and phenylalanine ammonium lyase enzyme therapy are used.[4,13,14] However, attention should be paid to the protein intake required for optimal growth and development. It is recommended that treatment be continued throughout life. The aim of treatment is to reduce plasma phenylalanine levels, increase natural protein tolerance, maintain normal neuropsychological development, and provide a good quality of life. Twenty-one of our patients receive diet therapy. The other cases receive LNAA therapy. Patients received tyrosine supplements as needed according to their plasma tyrosine levels.

LNAA treatment is not recommended for young children or during pregnancy but is an option for adults who are not in good metabolic control and are not compliant with dietary therapy.[15] LNAAs (arginine, histidine, isoleucine, leucine, lysine, methionine, threonine, tryptophan, tyrosine, and valine) compete with phenylalanine at the blood-brain barrier. Therefore, LNAA supplementation can significantly reduce phenylalanine uptake in the brain, in patients.[16] In this study, every patient who received LNAA supplementation was an adult who did not comply with dietary therapy.

Study Limitations

Our study has some limitations. First, neuroimaging could not be performed in most of our patients. Cooperation with the patients for MRI was not possible due to their neurological impairment. In addition, neuroimaging could not be performed due to the socioeconomic status of some families. Secondly, since our study is retrospective, the patients' treatment interruption status could not be fully evaluated.
In our country, PKU is a treatable metabolic disease that is included in the newborn screening program. It causes severe neurological problems in patients who are diagnosed late, or cannot be diagnosed. Therefore, close monitoring is important during the diagnosis and treatment phase of the disease. Close monitoring is essential to ensure that patients diagnosed with PKU receive their treatment at an early stage.

Ethics
Ethics Committee Approval: Approval for the study was obtained from the University of Health Sciences Türkiye, Gazi Yaşargil Training and Research Hospital Clinical Research Ethics Committee (approval number: 162, dated: 09.09.2022).
Informed Consent: Informed consent was obtained from each patient before participating in the study.

Footnotes
Authorship Contributions
Surgical and Medical Practices: H.B., A.E.B., Concept: H.B., A.E.B., Data Collection or Processing: H.B., Analysis or Interpretation: H.B., A.E.B., Literature Search: H.B., A.E.B., Writing: H.B.
Conflict of Interest: The authors have no conflicts of interest to declare.
Financial Disclosure: The authors declared that this study received no financial support.

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Table 1. Clinical and laboratory data of our late diagnosed PKU cases

Patient/gender	Age at diagnosis (month)	Current age (years)	Presentation complaint	Phenylalanine levels at diagnosis (µmol/L)	Phenylalanine levels at last evaluation (µmol/L)	Diagnosis	Neurological status	Treatment	Genotype
1/M	12	11.2	Neonatal screening	1364	320	Classical PKU	Global developmental delay	Diet	IVS10-11G>A/ IVS10-11G>A
2/F	60	15.2	Sibling with PKU	1675	1400	Classical PKU	Global developmental delay	Diet	c.1222C>T/ c.1222C>T
3/M	48	12.7	Sibling with PKU	1563	1300	Classical PKU	Global developmental delay	Diet	c.143T>C/ c.143T>C
4/F	42	18.8	Developmental delay, tremor, microcephaly	1680	290	Classical PKU	Global developmental delay	Diet	c.1039C>T/ c.1039C>T
5/M	30	17.7	Developmental delay, autism	820	410	Mild PKU	Global developmental delay	Diet	c.781C>T/ c.781C>T
6/M	7	14.1	Neonatal screening	1828	210	Classical PKU	Mild developmental delay	Diet	c.194T>C/ c.194T>C
7/F	6	11.2	Neonatal screening	1223	620	Classical PKU	Moderate developmental delay	Diet	c.728G>A/ c.728G>A
8/M	6	8.4	Neonatal screening	1320	330	Classical PKU	Global developmental delay	Diet	c.838G>A/ c.838G>A
9/F	13	5.2	Neonatal screening	1625	215	Classical PKU	Moderate developmental delay	Diet	c.168G>T/ c.168G>T
10/M	8	21.1	Neonatal screening	1466	540	Classical PKU	Global developmental delay	LNAA	IVS10-11G>A/ IVS10-11G>A
11/M	10	11.7	Neonatal screening	917	580	Mild PKU	Moderate developmental delay	Diet	c.143T>C /c.143T>C
12/M	12	10.2	Neonatal screening	650	310	Mild PKU	Global developmental delay	Diet	c.1222C>T/ c.1222C>T
13/M	11	14.9	Neonatal screening	670	335	Mild PKU	Global developmental delay	Diet	c.781C>T/ c.781C>T
14/M	9	13.1	Neonatal screening	1380	943	Classical PKU	Mild developmental delay	Diet	IVS10-11G>A/ IVS10-11G>A
15/M	30	21.3	Epilepsy	743	964	Mild PKU	Mild developmental delay	LNAA	c.782G>A/ c.782G>A
16/F	7	6.1	Neonatal screening	2280	520	Classical PKU	Mild developmental delay	Diet	c.1222C>T/ c.1222C>T
17/F	24	20.1	Epilepsy	1420	850	Classical PKU	Global developmental delay	LNAA	c.143T>C/ c.143T>C
18/M	14	8.3	Developmental delay	866	193	Mild PKU	Global developmental delay	Diet	IVS10-11G>A/ c.782G>A
19/M	24	18.2	Epilepsy	1716	1250	Classical PKU	Global developmental delay	Diet	IVS10-11G>A/ c.1222C>T
20/M	24	11.9	Epilepsy	1601	1391	Classical PKU	Global developmental delay	Diet	IVS10-11G>A/ c.1222C>T
21/F	4	3.8	Neonatal screening	1341	261	Classical PKU	Moderate developmental delay	Diet	c.1039C>T/ c.1039C>T
22/M	3	5.9	Neonatal screening	1292	719	Classical PKU	Moderate developmental delay	Diet	c.168G>T/ c.168G>T

23/M	2	7.3	Neonatal screening	1334	1081	Classical PKU	Moderate developmental delay	Diet	c.168G>T/ c.1039C>T/
24/F	192	18.6	Epilepsy, microcephaly, autism, tremor	1409	1420	Classical PKU	Global developmental delay	Diet	c.838G>A/ c.728G>A
25/M	60	20.2	Epilepsy, autism	1480	1144	Classical PKU	Global developmental delay	LNAA	IVS10-11G>A /c.838G>A