European Journal of Pediatrics (2019) 178:1005–1011
https://doi.org/10.1007/s00431-019-03387-8

ORIGINAL ARTICLE

Maternal phenylketonuria in Turkey: outcomes of 71 pregnancies and issues in management

Yılmaz Yıldız[1] · Hatice Serap Sivri[1]

Received: 20 February 2019 / Revised: 10 April 2019 / Accepted: 16 April 2019 / Published online: 3 May 2019

Abstract

Untreated phenylketonuria (PKU) in pregnancy causes a severe embryopathy called maternal PKU syndrome. Here, we aimed to assess management issues and pregnancy outcomes in the first published series of PKU pregnancies from the developing world. Data were collected retrospectively in a single center from 71 pregnancies and 45 live births of 32 women with PKU, 11 of whom were diagnosed in adulthood after having an affected child. Microcephaly, intellectual disability, and dysmorphic facies were more prevalent in offspring of untreated than treated pregnancies with classical PKU (100% vs. 0%, 91% vs. 0%, and 73% vs. 23% with $p < 0.001$, $p < 0.001$, and $p = 0.037$, respectively). In treated pregnancies, phenylalanine levels were higher during weeks 6–14 than other periods of gestation (4.38 vs. 3.93, 2.00 and 2.28 mg/dl; $p < 0.05$). Poor compliance correlated with higher phenylalanine levels ($\rho = -0.64$, $p = 0.019$) and fluctuations ($\rho = -0.66$, $p = 0.014$).

Conclusion: More frequent phenylalanine measurements during late first trimester are crucial to improve outcomes in treated pregnancies. In order to prevent untreated pregnancies via detecting undiagnosed adults, countries where significantly many women of childbearing age were not screened as newborns may consider pre-pregnancy PKU screening. Microcephaly in the newborn should prompt screening for PKU in the mother.

What Is Known

•*Untreated phenylketonuria during pregnancy causes maternal phenylketonuria syndrome in the newborn.*
•*Effective treatment throughout pregnancy can prevent adverse fetal outcomes.*

What Is New:

•*Metabolic control is related to frequency of follow-up and worsens during late first trimester. Closer follow-up during this period may improve metabolic control.*
•*In order to prevent untreated pregnancies, pre-pregnancy phenylketonuria screening may be considered if many women of childbearing age were not screened as newborns.*

Keywords Phenylketonuria · Phenylalanine hydroxylase deficiency · Hyperphenylalaninemia · Maternal phenylketonuria · Pregnancy

Communicated by Peter de Winter

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00431-019-03387-8) contains supplementary material, which is available to authorized users.

✉ Yılmaz Yıldız
yilmaz.yildiz@hacettepe.edu.tr

[1] Division of Pediatric Metabolism, Department of Pediatrics, Faculty of Medicine, Hacettepe University, Ankara, Turkey

Abbreviations

HPA	Hyperphenylalaninemia
ID/DD	Intellectual disability or developmental delay
IQ	Intelligence quotient
IQR	Interquartile range
IUGR	Intrauterine growth restriction
LMP	Last menstrual period
NBS	Newborn screening
PAH	Phenylalanine hydroxylase
Phe	Phenylalanine
PKU	Phenylketonuria

Introduction

Phenylalanine hydroxylase (PAH) deficiency (OMIM #261600), commonly known as phenylketonuria (PKU), is an inborn error of metabolism characterized by elevated phenylalanine (Phe) levels. With an incidence of one in 10,000 live births in Europe and one in 15,000 in the USA, it is the most common treatable genetic cause of intellectual disability [3]. Treatment aims to lower blood Phe levels using Phe-restricted diet or sapropterin dihydrochloride. Early diagnosis and treatment via newborn screening (NBS) enable affected patients to reach adulthood in good health [19].

It was observed in 1937 that children of women with PAH deficiency had intellectual disability, although the children did not have PAH deficiency themselves. It later became clear that PAH deficiency and high Phe levels in the mother causes "maternal PKU," characterized by intrauterine growth restriction (IUGR), microcephaly, developmental delay, intellectual disability and dysmorphic facies with or without congenital heart disease (CHD), and other anomalies [10]. The Maternal Phenylketonuria International Collaborative Study provided evidence that normal fetal outcome is possible if maternal blood Phe levels of 2–6 mg/dl can be achieved by 8–10 weeks of gestation and maintained throughout the pregnancy [8]. Current guidelines recommend maintaining Phe levels between 2 and 6 mg/dl, starting from the preconceptional period [18, 19].

In Turkey, where incidence of persistent hyperphenylalaninemia is very high (one in 4000 live births), NBS was initiated in 1983 [15]. As women diagnosed in the early years of NBS become adults, maternal PKU emerges as a preventable cause of intellectual disability. Here, we present outcomes of treated and untreated pregnancies of patients with PAH deficiency from a major metabolic center in Turkey. With emphasis on classical PKU—the most severe form of PAH deficiency—we aimed to compare the pregnancy outcomes between treated and untreated pregnancies and assess the adherence to follow-up during pregnancy and its association with blood Phe levels.

Materials and methods

Women who were diagnosed with PAH deficiency at Hacettepe University Hospitals and had completed at least one pregnancy by December 31, 2017 were enrolled. Data were obtained retrospectively from hospital records. PAH deficiency was classified in decreasing order of severity and untreated blood Phe levels as classical PKU (untreated blood Phe > 20 mg/dl), mild-moderate PKU (Phe 10–20 mg/dl), hyperphenylalaninemia (HPA, Phe 6–10 mg/dl), and mild HPA (Phe 2–6 mg/dl) [4]. This approach was chosen to highlight the data from patients with Phe between 6 and 10 mg/dl, where pregnancy data are scarce. Patients who had presented to the metabolic clinic before or during pregnancy and been treated thereafter were defined as having "treated pregnancies." Their Phe measurements were recorded. To include Phe levels measured within one menstrual cycle before the last menstrual period (LMP), the period from 4 weeks before LMP (− 4 weeks) until the end of gestation was defined as the "pregnancy period." The first trimester (− 4 to $13^{6/7}$ weeks) was divided into two parts at week 6, which usually marks the onset of morning sickness, nausea, and vomiting [14].

Medical records of children of enrolled patients were analyzed retrospectively. Children with full-scale intelligence quotient (IQ) scores below 70, signs of developmental delay, or abnormal developmental screening test results were classified as having "intellectual disability or developmental delay" (ID/DD). IUGR and microcephaly were defined as birth weight and head circumference below the tenth and third percentiles for gestational age, respectively [2, 5]. Children with facial features of maternal PKU (canthal abnormalities, long philtrum, wide nasal bridge, anteverted nares, low-set ears, auricular hypoplasia) were defined as having dysmorphic facies [16]. Patients with PAH deficiency were denoted as P_1, P_2, P_3, etc. and their gestations as P_1G_1, P_1G_2, …, P_2G_1, P_2G_2, etc.

Statistical analyses comparing outcomes of treated and untreated pregnancies were performed for pregnancies from patients with classical PKU, since the numbers of live births were too few in the other severity groups. The Shapiro-Wilk test was used to test for normality. Significance of differences in pregnancy outcomes were analyzed with Fisher's exact test and differences in Phe levels with the Mann-Whitney *U* test. Correlation between Phe levels and frequency of Phe measurements was assessed with Spearman's rank correlation. *p*-values below 0.05 were accepted to be statistically significant. Statistical analyses were performed with SPSS v20.0 (IBM, Armonk, NY, USA). This study was approved by Hacettepe University Ethics Committee for Non-Interventional Clinical Studies and informed consent was not required from the study subjects due to its retrospective nature.

Results

Thirty-two women with PAH deficiency were included (Table 1), among whom 18 (56%) had classical PKU, six had mild-moderate PKU, five had HPA (Phe 6–10 mg/dl), and three had mild HPA (Phe 2–6 mg/dl). Nine out of the tested 15 (60%) patients with classical PKU had intellectual disability. Fourteen patients (44%) were diagnosed by NBS, whereas seven (22%) were diagnosed in childhood after onset of symptoms and 11 (34%) in adulthood after they had a child with maternal PKU syndrome. *PAH* gene sequencing had been performed in 30 patients, revealing 21 different variants

Table 1 Characteristics of patients with phenylalanine hydroxylase deficiency and their pregnancy outcomes

Patient	Patient characteristics				Pregnancy outcomes								
	Age at Dx	Mutations in *PAH* gene[a]		IQ	Untreated pregnancies				Treated pregnancies[b]			All	
		Allele 1	Allele 2		LB	Md.A	Sp.A	Other	LB	Sp.A	Other	LB	Prg
Classical PKU (untreated Phe > 20 mg/dl)					11	12	6		13	2		24	44
P_1	4 d	c.781C>T (p.Arg261Ter)	c.1066-11G>A (IVS10-11G>A)	65	1		1		1	1		2	4
P_2	10 d	c.967_969delACA (p.Thr323del)	c.967_969delACA (p.Thr323del)	MD	1	1			1			2	3
P_3	7 y	c.782G>A (p.Arg261Gln)	c.1162G>A (p.Val388Met)	67	1	1						1	2
P_4	3 d	c.1066-11G>A (IVS10-11G>A)	c.1162G>A (p.Val388Met)	MD		1			2			2	3
P_5	3 d	c.782G>A (p.Arg261Gln)	c.782G>A (p.Arg261Gln)	86		1			2			2	3
P_6	35 d	c.1066-11G>A (IVS10-11G>A)	c.1066-11G>A (IVS10-11G>A)	88					1			1	1
P_7	29 d	c.143T>C (p.Leu48Ser)	c.165delT (p.Phe55Leufs*6)	90	1	1			1			2	3
P_8	7 mo	c.168+5G>C (IVS2+5G>C)	c.727C>T (p.Arg243Ter)	40					1			1	1
P_9	29 y	c.782G>A (p.Arg261Gln)	c.782G>A (p.Arg261Gln)	55	1							1	1
P_{10}	7 d	c.1162G>A (p.Val388Met)	c.1162G>A (p.Val388Met)	79		1	4		1[b]			1	6
P_{11}	7 y	Not detected	Not detected	61	2							2	2
P_{12}	21 mo	c.673C>A (p.Pro225Thr)	c.673C>A (p.Pro225Thr)	56		1			1			1	2
P_{13}	6 mo	MD	MD	55	1	3	1					1	5
P_{14}	17 mo	c.782G>A (pArg261Gln)	c.782G>A (pArg261Gln)	MD	1							1	1
P_{15}	17 d	c.782G>A (p.Arg261Gln)	c.782G>A (p.Arg261Gln)	75					1	1		1	2
P_{16}	11 d	c.165delT (p.Phe55Leufs*6)	c.1066-11G>A (IVS10-11G>A)	51		2			1			1	3
P_{17}	11 mo	c.782G>A (pArg261Gln)	c.782G>A (pArg261Gln)	66	1							1	1
P_{18}	26 y	c.781C>T (p.Arg261Ter)	c.781C>T (p.Arg261Ter)	72	1							1	1
Mild-moderate PKU (untreated Phe 10–20 mg/dl)					6			1[c]	1		1[d]	7	10
P_{19}	26 y	c.722G>A (p.Arg241His)	c.728G>A (p.Arg243Gln)	58	1							1	1
P_{20}	22 y	c.1066-11G>A (IVS10-11G>A)	Not detected	MD	1							1	1
P_{21}	39 y	c.533A>G (p.Glu178Gly)	c.842C>T (p.Pro281Leu)	MD	2			1[c]				2	3
P_{22}	31 y	c.665A>G (p.Asp222Gly)	c.1066-11G>A (IVS10-11G>A)	MD	1							1	1
P_{23}	30 y	c.311C>A (p.Ala104Asp)	c.311C>A (p.Ala104Asp)	86	1		1					1	2
P_{24}	3 mo	c.143T>C (p.Leu48Ser)	c.143T>C (p.Leu48Ser)	88					1		1[d]	1	2
HPA (untreated Phe 6–10 mg/dl)					9		3					9	12
P_{25}	37 y	c.722G>A (p.Arg241His)	c.1208C>T (p.Ala403Val)	104	4							4	4
P_{26}	33 y	MD	MD	MD	1							1	1
P_{27}	32 y	Not detected	Not detected	MD	1							1	1
P_{28}	31 y	c.721C>T (p.Arg241Cys)	c.721C>T (p.Arg241Cys)	79	3		2					3	5
P_{29}	70 d	c.143T>C (p.Leu48Ser)	c.1169A>G (p.Glu390Gly)	70			1					0	1
Mild HPA (Phe 2–6 mg/dl)[e]					NA[e]				5[e]			5	5
P_{30}	11 d	c.1208C>T (p.Ala403Val)	Not detected	84					2			2	2
P_{31}	15 d	c.143T>C (p.Leu48Ser)	c.898G>T (p.Ala300Ser)	MD					2			2	2
P_{32}	16 d	c.441+5G>T (IVS4+5G>T)	c.898G>T (p.Ala300Ser)	MD					1			1	1
				Total	26	12	10	1	19	2	1	45	71

[a] RefSeq NM_000277.1, NP_001341233.1. All presented variants have been previously reported

[b] Treatment was initiated during the preconceptional period for all treated pregnancies, except for the pregnancy of P_{10} which resulted in a live birth ($P_{10}G_6$), in which treatment was started on week 14 when the patient applied to the clinic and refused medical abortion despite an initial Phe level of 15.4 mg/dl

[c] Ectopic pregnancy

[d] Partial mole hydatidiform

[e] Treatment is not indicated in mild HPA (Phe 2–6 mg/dl) group. Therefore, pregnancies were only followed without therapeutic intervention. Pregnancy outcomes are listed under the "Treated" column since they had good metabolic control, similar to well-treated pregnancies

d days, *Dx* diagnosis, *HPA* hyperphenylalaninemia, *IQ* intelligence quotient, *LB* live births, *MD* missing data, *Md.A* medical abortions, *mo* months, *NA* not applicable, *PAH* phenylalanine hydroxylase, *Phe* phenylalanine, *PKU* phenylketonuria, *Prg* pregnancy, *Sp.A* spontaneous abortions, *y* years

in 54 alleles. The most common variant was c.782G>A, followed by c.1066-11G>A.

A total of 71 pregnancies were recorded, the outcomes of which are summarized in Table 1. Forty-five pregnancies (63%) resulted in live births, whose characteristics are presented in brief in Table 2 and in detail in Online Resource 1. These 45 children had a median follow-up period of 33 months (6 months–25 years; interquartile range [IQR]: 6 years). Five pregnancies of women with mild HPA were followed without treatment. Among the other 66

Table 2 Abnormal offspring outcomes in women with phenylalanine hydroxylase deficiency

Pregnancy status	Prematurity[a]	IUGR[a]	Microcephaly[a]	CHD[a]	ID/DD[a]	Dysmorphic face[a]	HPA[a]	Other[a]	Total
Untreated classical PKU	2 (18%)	6 (55%)	11 (100%)	3 (27%)	10 (91%)	8 (73%)	1 (9%)[b]	4 (36%)[c]	11
Treated classical PKU[d]	3 (23%)	5 (38%)	–	–	–	3 (23%)	–	1 (8%)[e]	13
Untreated mild-moderate PKU	1 (17%)	3 (50%)	4 (67%)	–	3 (50%)	1 (17%)	5 (83%)[f]	2 (33%)[g]	6
Treated mild-moderate PKU[h]	–	–	–	–	–	–	–`	–	1[h]
Untreated HPA (Phe 6–10 mg/dl)	–	–	2 (22%)	–	1 (11%)	2 (22%)	6 (67%)[i]	–	9
Mild HPA (Phe 2–6 mg/dl)	1 (20%)	–	–	–	–	1 (20%)	–	1 (20%)[j]	5
All pregnancies	7 (16%)	14 (31%)	17 (38%)	3 (7%)	14 (31%)	15 (33%)	12 (27%)	8 (18%)	45

Boxes indicate number (percentage)

[a] Percentages are expressed as fraction of total live births in the same row

[b] Classical PKU

[c] Two children with symptomatic epilepsy, one child with unilateral renal agenesis, and one with iris atrophy and mild opacification of ocular lens

[d] Treatment was initiated during the preconceptional period for all treated pregnancies, except for $P_{10}G_6$, in which treatment was started on week 14 when the patient applied to the clinic and refused medical abortion despite an initial Phe level of 15.4 mg/dl. All classical PKU pregnancies were treated with Phe-restricted diet

[e] Esophageal atresia with tracheoesophageal fistula

[f] Two children with classical PKU, one with mild-moderate PKU, one with HPA (Phe 6–10 mg/dl), and one with mild HPA (Phe 2–6 mg/dl), all from different mothers

[g] One child with developmental dysplasia of the hip and one with bilateral pes equinovarus

[h] Treated with sapropterin dihydrochloride and unrestricted diet

[i] Three children with HPA (Phe 6–10 mg/dl) and three with mild HPA (Phe 2–6 mg/dl)

[j] Idiopathic, well-controlled epilepsy (two unprovoked seizures) with normal development and neurological examination

CHD congenital heart disease, *HPA* hyperphenylalaninemia, *ID/DD* intellectual disability/developmental delay, *IUGR* intrauterine growth restriction, *PKU* phenylketonuria

pregnancies where treatment was indicated (Phe > 6 mg/dl), 49 (74%) were untreated because the patient had not applied to the metabolic clinic. Twenty-nine untreated pregnancies were from women with classical PKU, 12 of which were medically terminated due to high likelihood of maternal PKU syndrome. One infant ($P_{20}G_1$) affected with maternal PKU died from pneumonia. Among 11 and 6 live births from untreated classical and mild-moderate PKU pregnancies, 100% and 67% of offspring had microcephaly, and 91% and 50% had ID/DD, respectively. Microcephaly and ID/DD were also encountered in live births from HPA with Phe 6–10 mg/dl (22% and 11% respectively, in nine children). All three cases of CHD were detected in untreated classical PKU. Congenital malformations encountered in this study are summarized in Table 3.

Seventeen pregnancies were treated. In one pregnancy ($P_{10}G_6$), treatment was started on week 14, as soon as the pregnant patient applied to the clinic. Treatment was started before conception in all other treated pregnancies. The two pregnancies of P_{24} (mild-moderate PKU) were treated with sapropterin dihydrochloride; one was terminated due to partial mole hydatidiform and the other resulted in a healthy infant. This patient was reported previously [22] and is not discussed in further detail. Fifteen classical PKU pregnancies were treated with a Phe-restricted diet (13 live births and two spontaneous abortions). Compared with live births from untreated classical PKU pregnancies, the prevalence of microcephaly, ID/DD. and dysmorphic facies were significantly lower in the treated classical PKU group ($p < 0.001$, $p < 0.001$, and $p = 0.037$, respectively) with no cases of microcephaly, ID/DD, or CHD. The prevalence of spontaneous abortions, premature birth, and IUGR were not significantly different ($p = 0.695$, $p > 0.99$, and $p = 0.682$, respectively). One infant (P_2G_3) was born with esophageal atresia with tracheoesophageal fistula despite treatment during pregnancy (Table 3). She underwent corrective surgery and is healthy.

Phe measurements throughout the 13 treated classical PKU pregnancy periods resulting in live births are presented in detail in Online Resource 2. Although patients were advised to have weekly Phe measurements, a median of 0.71 measurements per week were performed. The overall median and IQR of Phe levels were 2.50 and 3.49 mg/dl, respectively. Throughout the pregnancy period, frequency of Phe measurements had a negative, strong, and significant correlation with median Phe levels ($\rho = -0.64$, $p = 0.019$) and IQR of Phe levels ($\rho = -0.67$, $p = 0.016$). In the second part of the first trimester ($6–13^{6/7}$ weeks), Phe levels were significantly higher than the other parts of gestation (4.38 vs. 3.98, 2.00 and 2.28 mg/dl compared with first part of first trimester, second trimester, and third trimester, respectively, with $p = 0.009$,

Table 3 Occurrence of congenital malformations in the offspring of women with varying severity of phenylalanine hydroxylase deficiency

Pregnancy category	Live births	Malformation	Affected offspring	
			Number	Percentage
Treated classical PKU	13	Esophageal atresia with tracheoesophageal fistula	1	8
Untreated classical PKU (Phe > 20 mg/dl)	11	Microcephaly	11	100
		Congenital heart disease[a]	3	27
		Unilateral renal agenesis	1	9
		Iridial atrophy[b]	1	9
		Lens opacities[b]	1	9
Untreated mild-moderate PKU (Phe 10–20 mg/dl)	6	Microcephaly	4	67
		Developmental dysplasia of the hip[c]	1	17
		Bilateral pes equinovarus[c]	1	17
Untreated HPA (Phe 6–10 mg/dl)	9	Microcephaly	2	22

[a] Two cases of large ventricular septal defect alone, and one case of coarctation of aorta, hypoplastic aortic arch with large ventricular septal defect

[b] Iridial atrophy and lens opacities were observed in the same child

[c] Hip dysplasia and pes equinovarus were observed in different children, both of whom also had classical PKU and microcephaly

HPA hyperphenylalaninemia, *Phe* phenylalanine, *PKU* phenylketonuria

$p < 0.001$, and $p < 0.001$). All Phe measurements during these 13 treated pregnancies are plotted in Online Resource 3, which demonstrates the increased levels and variability of Phe levels in especially the latter part of the first trimester. The number of live births from treated pregnancies was insufficient to investigate a correlation between maternal Phe levels and cognitive functions of the children.

Discussion

Forty-five live births from 71 pregnancies of 32 women with PAH deficiency are presented, including 13 children born from classical PKU pregnancies treated with Phe-restricted diet. To the best of our knowledge, this is the first large study from a developing country on the treatment and outcomes of PAH deficiency during pregnancy.

The high rate of untreated pregnancies (74%) is partly due to late diagnosis of PAH deficiency only after having an affected child and partly to loss of follow-up after adolescence. Efforts to prevent untreated pregnancies can center around three niches: known PAH deficiency patients, women with affected children, and undiagnosed women in the general population. Early education of females with PAH deficiency before they are lost to follow-up can urge them to apply to their metabolic clinic before planning a pregnancy, as adherence to follow-up in PAH deficiency is known to decrease in adulthood [1]. Perinatologists and pediatricians should investigate PAH deficiency in the mother if a fetus or child has congenital microcephaly; 11 women (34%) in our study were diagnosed in this manner. Population-based pre-pregnancy screening for PAH deficiency to diagnose women of reproductive age can also help prevent untreated pregnancies, especially in developing countries where women of reproductive age were not screened for PAH deficiency as newborns [6]. In addition, considering that 12/45 (27%) of infants born to PAH-deficient mothers in this study had varying degrees of PAH deficiency themselves (see Table 2 and Online Resource 1), screening the mothers of hyperphenylalaninemic newborns may also help find undiagnosed adult cases. In fact, based on the Hardy-Weinberg law, it has been estimated that the risk of undiagnosed HPA or mild-moderate PKU in parents of a patient with PAH deficiency in Turkey is as high as one in 74 [20].

The prevalence of intellectual disability, microcephaly, CHD, low birth weight, and spontaneous abortions in untreated pregnancies with classical PKU are reported to be 92%, 73%, 12%, 40%, and 24%, respectively [9]. In the present study, the prevalence of ID/DD, IUGR, and spontaneous abortion were similar (91%, 55%, and 17%, respectively), but microcephaly (100%) and CHD (27%) were higher. Other nutritional factors are implicated in development of microcephaly and CHD in maternal PKU [13, 21], which may have contributed to increased prevalence among classical PKU pregnancies. As expected, no cases of CHD were encountered in milder forms of PAH deficiency, because occurrence of CHD is low if Phe levels are below 15 mg/dl and comparable with the general population if below 10 mg/dl [8]. Four out of six (67%) infants born to untreated mothers with mild-moderate PKU had microcephaly, which is almost as high as untreated classical PKU pregnancies (73%) [9]. This may stem from the co-occurrence of varying degrees of PAH deficiency in the offspring themselves (5/6 infants, Table 2 and Online Resource 1), which might have

increased fetal Phe levels. Rare anomalies were also encountered in the offspring of untreated pregnancies (Table 3), including the first report of iris atrophy in maternal PKU.

Five children born to mothers with mild HPA (Phe 2–6 mg/dl) had no health problems attributed to maternal hyperphenylalaninemia (see Table 2), consistent with the recommendation that these Phe levels do not require treatment [18, 19]. Although treatment is recommended for Phe levels above 6 mg/dl, the evidence for necessity of treatment for levels of 6–10 mg/dl is scarce [18]. Prevalence of microcephaly and intellectual disability in the offspring of these patients is thought to be similar to the general population, suggesting that these levels may be safe, but correlation between Phe levels of the mother and head circumferences and intelligence quotients of the children was also mentioned [11]. Four out of five patients with HPA (Phe 6–10 mg/dl) in our study were diagnosed as adults after having affected children. High prevalence of microcephaly (22%) and ID/DD (11%) in their children may therefore be due to selection bias. Nonetheless, our data support the recommendations that Phe levels between 6 and 10 mg/dl are not safe and should be treated during pregnancy [18, 19].

When treatment is indicated, guidelines recommend twice-weekly Phe monitoring [18]. However, due to logistics issues and limited patient compliance, the policy at our institution is once-weekly follow-up with more frequent measurements when necessary. Still, the frequency of measurements was lower than recommended in almost all pregnancies (Online Resource 2). This may have had a negative impact on metabolic control, as demonstrated by the inverse correlation between frequency of Phe measurements and medians and interquartile ranges of Phe levels, suggesting a more frequent follow-up may reduce Phe levels and their fluctuations. This study also demonstrates the significant increase of Phe levels between the sixth and fourteenth weeks of gestation. This may stem from the nausea, vomiting, weight loss, and poor appetite that are associated with this period. Further complicated by the poor palatability of the medical foods that these patients must consume, this period is challenging for pregnant women with PAH deficiency. Nutritional counseling, closer follow-up, liberal use of appropriate antiemetics, or even intravenous fluids may help maintain metabolic control until morning sickness subsides. Weekly follow-up of Phe levels can be acceptable for clinics in developing countries, provided that adherence is good and closer monitoring is available if necessary, especially during the first trimester.

Despite the less-than-optimal metabolic control in some treated pregnancies, offspring outcome was good with no cases of microcephaly, CHD, or ID/DD. In treated classical PKU pregnancies, all Phe levels were consistently below 15 mg/dl (except for $P_{10}G_6$, in which treatment started on week 14 with an initial Phe level of 15.4 mg/dl), effectively preventing emergence of CHD. However, treatment of classical PKU pregnancies was insufficient to provide a significant decrease of IUGR. IUGR in pregnancies treated with Phe-restricted diet has been attributed to inadequate intake of natural protein, medical foods, and energy and to persistently too low Phe levels [12, 17, 19]. It may be speculated that IUGR in the offspring of our treated patients may be related to nutrient intake, since too low Phe levels (below 1 mg/dl) were rare (26/150 measurements, 17%) in pregnancies complicated by IUGR. The only congenital malformation in treated pregnancies was a case of esophageal atresia with tracheoesophageal fistula in P_2G_3. It is difficult to ascertain a causal relationship between maternal PKU and this malformation, which has been previously reported in treated PKU pregnancies, but not in those treated before conception with good control [8]. Separation and elongation of the esophagus takes place between gestational weeks 4–7 [7], and unfortunately patient P_2 has not had any Phe measurements in this period (Online Resource 3).

This study presents the most comprehensive series of pregnancies complicated by PAH deficiency from the developing world. Treatment in pregnancy has been successful in preventing major complications in the offspring, including intellectual disability, developmental delay, microcephaly, and congenital heart disease despite difficulties in follow-up. Although guidelines recommend at least twice-weekly Phe monitoring, our data suggest that weekly Phe measurements can be acceptable, but closer follow-up may be required in late first trimester, including hospital admission as necessary. We also clearly demonstrate for the first time the correlation between frequent Phe measurements and metabolic control and add to the limited evidence suggesting that Phe levels of 6–10 mg/dl are not safe during pregnancy. In order to prevent future untreated pregnancies, we recommend pre-pregnancy PAH deficiency screening in women of reproductive age in our country. This can be incorporated into the pre-marital thalassemia carrier screening that is already in place. Countries where significantly many women of childbearing age were not screened as newborns, either as a result of late implementation of NBS or due to receiving immigration from regions without NBS, also should consider pre-pregnancy screening.

Acknowledgments The authors would like to acknowledge İmran Özalp for establishing the first metabolic center and newborn screening in Turkey; metabolic physicians Turgay Coşkun, Ayşegül Tokatlı, Ali Dursun, Halil İbrahim Aydın, Mustafa Kılıç, Özlem Ünal, Burcu Öztürk Hişmi, Berrak Bilginer Gürbüz, and Emine Pektaş; metabolic dietitians Gülden Köksal, Hülya Gökmen-Özel, Sabriye Saruhan, and others for the diagnosis and management of the patients in this paper; Talat Demirsöz, Gökçen Düzgün Konuşkan, Yamaç Karaboncuk, Zeynep Tüzün, Berge Velibaşoğlu, and Tuğba Kaya for performing neuropsychometric testing; Esin Göksun and Rıza Köksal Özgül for the genetic analyses; Özgür Özyüncü for providing obstetric care; our laboratory staff; and our patients and their families. This work was prepared as a residency thesis in pediatrics.

Authors' contributions The study was conceptualized by HSS. Both authors (YY and HSS) contributed to study design and interpretation of data. YY was involved in acquisition and analysis data and writing the draft of the manuscript, which was critically edited by HSS. Both authors approved the final version.

Compliance with ethical statements

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

References

1. Ahring K, Belanger-Quintana A, Dokoupil K, Gokmen-Ozel H, Lammardo AM, MacDonald A, Motzfeldt K, Nowacka M, Robert M, van Rijn M (2011) Blood phenylalanine control in phenylketonuria: a survey of 10 European centres. Eur J Clin Nutr 65: 275–278
2. American College of Obstetricians and Gynecologists (2013) ACOG practice bulletin no. 134: fetal growth restriction. Obstet Gynecol 121:1122–1133
3. Blau N, van Spronsen FJ, Levy HL (2010) Phenylketonuria. Lancet 376:1417–1427
4. Camp KM, Parisi MA, Acosta PB, Berry GT, Bilder DA, Blau N, Bodamer OA, Brosco JP, Brown CS, Burlina AB, Burton BK, Chang CS, Coates PM, Cunningham AC, Dobrowolski SF, Ferguson JH, Franklin TD, Frazier DM, Grange DK, Greene CL, Groft SC, Harding CO, Howell RR, Huntington KL, Hyatt-Knorr HD, Jevaji IP, Levy HL, Lichter-Konecki U, Lindegren ML, Lloyd-Puryear MA, Matalon K, MacDonald A, McPheeters ML, Mitchell JJ, Mofidi S, Moseley KD, Mueller CM, Mulberg AE, Nerurkar LS, Ogata BN, Pariser AR, Prasad S, Pridjian G, Rasmussen SA, Reddy UM, Rohr FJ, Singh RH, Sirrs SM, Stremer SE, Tagle DA, Thompson SM, Urv TK, Utz JR, van Spronsen F, Vockley J, Waisbren SE, Weglicki LS, White DA, Whitley CB, Wilfond BS, Yannicelli S, Young JM (2014) Phenylketonuria scientific review conference: state of the science and future research needs. Mol Genet Metab 112:87–122
5. Cragan JD (2016) Surveillence for microcephaly. Centers for Disease Control and Prevention. https://www.cdc.gov/ncbddd/birthdefects/documents/surveillance-microcephaly-webinar-transcript.pdf. Accessed 21 January 2018
6. Hanley WB (2008) Finding the fertile woman with phenylketonuria. Eur J Obstet Gynecol Reprod Biol 137:131–135
7. Jacobs IJ, Que J (2013) Genetic and cellular mechanisms of the formation of esophageal atresia and tracheoesophageal fistula. Dis Esophagus 26:356–358
8. Koch R, Hanley W, Levy H, Matalon K, Matalon R, Rouse B, Trefz F, Guttler F, Azen C, Platt L et al (2003) The maternal phenylketonuria international study: 1984-2002. Pediatrics 112:1523–1529
9. Lenke RR, Levy HL (1980) Maternal phenylketonuria and hyperphenylalaninemia: an international survey of treated and untreated pregnancies. N Engl J Med 303:1202–1208
10. Levy HL (2003) Historical background for the maternal PKU syndrome. Pediatrics 112:1516–1518
11. Levy HL, Waisbren SE, Guttler F, Hanley WB, Matalon R, Rouse B, Trefz FK, de la Cruz F, Azen CG, Koch R (2003) Pregnancy experiences in the woman with mild hyperphenylalaninemia. Pediatrics 112:1548–1552
12. Maillot F, Cook P, Lilburn M, Lee PJ (2007) A practical approach to maternal phenylketonuria management. J Inherit Metab Dis 30: 198–201
13. Matalon KM, Acosta PB, Azen C (2003) Role of nutrition in pregnancy with phenylketonuria and birth defects. Pediatrics 112:1534–1536
14. Matthews A, Dowswell T, Haas DM, Doyle M, O'Mathuna DP (2010) Interventions for nausea and vomiting in early pregnancy. Cochrane Database Syst Rev:CD007575
15. Ozalp I, Coskun T, Tokatli A, Kalkanoglu HS, Dursun A, Tokol S, Koksal G, Ozguc M, Kose R (2001) Newborn PKU screening in Turkey: at present and organization for future. Turk J Pediatr 43:97–101
16. Rouse B, Azen C, Koch R, Matalon R, Hanley W, de la Cruz F, Trefz F, Friedman E, Shifrin H (1997) Maternal Phenylketonuria Collaborative Study (MPKUCS) offspring: facial anomalies, malformations, and early neurological sequelae. Am J Med Genet 69:89–95
17. Teissier R, Nowak E, Assoun M, Mention K, Cano A, Fouilhoux A, Feillet F, Ogier H, Oger E, de Parscau L (2012) Maternal phenylketonuria: low phenylalaninemia might increase the risk of intra uterine growth retardation. J Inherit Metab Dis 35:993–999
18. van Wegberg AMJ, MacDonald A, Ahring K, Belanger-Quintana A, Blau N, Bosch AM, Burlina A, Campistol J, Feillet F, Gizewska M et al (2017) The complete European guidelines on phenylketonuria: diagnosis and treatment. Orphanet J Rare Dis 12:162–217
19. Vockley J, Andersson HC, Antshel KM, Braverman NE, Burton BK, Frazier DM, Mitchell J, Smith WE, Thompson BH, Berry SA (2014) Phenylalanine hydroxylase deficiency: diagnosis and management guideline. Genet Med 16:188–200
20. Wiedemann A, Leheup B, Battaglia-Hsu SF, Jonveaux P, Jeannesson E, Feillet F (2013) Undiagnosed phenylketonuria in parents of phenylketonuric patients, is it worthwhile to be checked? Mol Genet Metab 110:S62–S65
21. Yano S, Moseley K, Bottiglieri T, Arning E, Azen C (2014) Maternal Phenylketonuria International Collaborative Study revisited: evaluation of maternal nutritional risk factors besides phenylalanine for fetal congenital heart defects. J Inherit Metab Dis 37:39–42
22. Yildiz Y, Dursun A, Tokatli A, Coskun T, Sivri S (2017) Partial hydatidiform mole in a phenylketonuria patient treated with sapropterin dihydrochloride. Gynecol Endocrinol 33:19–20