The Turkish Journal of Pediatrics 2022; 64: 985-992
https://doi.org/10.24953/turkjped.2022.467

Original Article

Evaluation of patients diagnosed with phenylketonuria and biotinidase deficiency by the newborn screening program: a ten-year retrospective study

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ABSTRACT

Background. Phenylketonuria (PKU) and biotinidase deficiency (BD) are autosomal recessive diseases. If they are not identified and treated early, severe intellectual disability and developmental delay occur. This study was conducted to calculate the ten-year incidence of PKU and BD in the Diyarbakır province of Turkey.

Methods. This cross-sectional study included patients born between 2011-2020 and diagnosed with PKU and BD. Patients with a clear diagnosis had their records evaluated retrospectively.

Results. Between 2011 and 2020, blood was taken from 417,525 newborns' heels in Diyarbakir province. As a result of further diagnostic testing, 53 PKU (Incidence: 1:7878) and 177 BD (Incidence: 1:2359) were detected. Of the patients with BD, 56% had profound BD and 44% had partial BD. The records of a total of 269 patients (PKU: 25; BD: 123; Hyperphenylalaninemia: 121) were examined. Parents of 65% (n=15) of the patients diagnosed with PKU and 46.6% (n=55) of the patients diagnosed with BD were consanguineous.

Conclusions. The incidence of both PKU and BD was found to be high in our region. The high number of consanguineous marriages was regarded as the most important explanation for the high frequency of these illnesses.

Key words: biotinidase deficiency, incidence, newborn screening, phenylketonuria.

Newborn screening programs are preventative health services that are extensively utilized across the world and play an essential role in public health programs. Robert Guthrie developed metabolic screening of neonates in the early 1960s by collecting blood samples on filter paper (Guthrie card) for phenylketonuria (PKU) screening using a bacterial inhibition technique.[1] Newborn screening programs include 1-50 disorders, depending on country or state.[2] The goal of newborn screening for PKU and biotinidase deficiency (BD) is to reduce the economic burden of diseases on society, raise public awareness about consanguineous marriages, detect disease symptoms in diagnosed infants before they appear, initiate the appropriate treatment to prevent diseases, and thus ensure that they attain normal intellectual capacity.[1,3]

Patients with PKU, which was first described in 1934, lack the phenylalanine hydroxylase enzyme. PKU is an autosomal recessive disease. The patient with classical PKU appears normal in the first few months, but as high levels of phenylalanine and its other toxic derivatives such as phenylpyruvate persist, vomiting, developmental delay, severe

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Received 5th May 2022, revised 9th July 2022,
11th September 2022, accepted 10th October 2022.

cognitive impairment, and dysmyelination ensue.[4] In children with PKU, developmental delay becomes visible after 5-6 months. They are unable to gain milestones such as sitting, walking, and speaking like their peers do. Microcephaly develops due to insufficient brain growth. The child may exhibit irrelevant, hyperkinetic or even autistic behaviors. Light hair, eye and skin color is seen in 60% of the cases. Moldy odor is noticeable in their body fluids and urine.[5] However, it is possible to prevent permanent damage to mental and motor development with early diagnosis and treatment.

The increase in the prevalence of genetic metabolic illnesses produces major societal health concerns in nations where consanguineous marriages are frequent. Due to consanguineous marriages, PKU is widespread in Turkey.[4,5] BD is another metabolic disorder that is commonly encountered in Turkey.[6] BD is also an inherited autosomal recessive neurocutaneous disorder. Seizures, skin rash, and alopecia, associated with acidosis and organic acidemia, emerge in individuals with untreated BD in the first few years of life owing to multiple carboxylase deficiency.[7,8] Clinical diagnosis may be challenging if BD is not detected by newborn screening since many children may mimic a variety of illnesses such as atopic dermatitis, neuromyelitis optica, optic atrophy, and myelopathy.[9] Early diagnosis and treatment can be life-saving, and the symptoms of the disease can be prevented. Early diagnosis and rapid initiation of oral biotin supplementation prevent neurological sequelae and clinical events.[8]

This study was conducted to calculate the incidence of phenylketonuria and biotinidase deficiency in the Diyarbakır province of Turkey.

Material and Methods

This study, conducted retrospectively using health records, comprises individuals born between 2011 and 2020 who were diagnosed with PKU and BD as a consequence of the newborn screening program. Patient information was acquired from their primary care providers. This study was conducted in compliance with the ethical principles according to the Declaration of Helsinki, and it was approved by the Ethics Committee of Health Sciences University, Gazi Yaşargil Training and Research Hospital (Number: 2021/855).

As per the algorithm of the Newborn Screening Program, capillary blood samples collected from the heels of newborns on the Guthrie card at health institutions are sent to screening laboratories designated by the Ministry of Health. If the phenylalanine level is 2.1 mg/dL or higher, the result is suspicious for PKU. If the biotinidase enzyme activity is low or absent (65 MRU or less), the result is suspicious for BD.[10] The screening laboratory's blood results for each disease are transmitted to the provinces via the Newborn Screening Program Web Application, and infants with suspected PKU and BD based on screening results are referred to the pediatric nutrition and metabolism clinics by their registered family physicians. In the patients included in this study, if the blood phenylalanine level was 2-10 mg/dL, the patient was diagnosed with hyperphenylalaninemia (HPA). If it is between 10-20 mg/dL, mild-moderate PKU, and if it is above 20 mg/dL, the patient was diagnosed with classical PKU.[11] In patients who were born in the years we included in the study and had high phenylalanine levels, the tetrahydrobiopterin (BH4) loading test was not performed. A low phenylalanine diet was initiated in all patients with blood phenylalanine levels above 10 mg/dL. In patients with blood phenylalanine levels between 6-10 mg/dL, protein intake is restricted to the safe lower limit according to their age. When the biotinidase enzyme activity was less than 30% of the standard value, BD was diagnosed. When the biotinidase enzyme activity was less than 10% of the normal level, profound BD was diagnosed; if the enzyme activity was between 10% and 30%, partial BD was diagnosed.[12]

Data for this study were gathered between August 1, 2021 and November 30, 2021. PKU and BD patients from Diyarbakir province were determined via the National Newborn Screening Program Web Application. Annual and ten-year incidence of disease were calculated. While calculating the incidence, Turkish Statistical Institute data was taken as the basis for the number of births.[13]

As a result of the screening, 443 patients were diagnosed with PKU, HPA or BD in 10 years. In the retrospective review of records, the data of 61% (n=269) of the patients were obtained. Because newborns do not have identification numbers when they are born, newborn screening records are created using the identification numbers of their mothers. The mother's identification number was used to locate the family medical unit where she was registered. The infant with PKU, HPA or BD was identified based on the date of birth from the family physician's records. The Family Medicine Unit's file records, in which patients with a clear diagnosis were registered, were evaluated retrospectively. It was questioned whether the patients had therapy, if their siblings/relatives had a similar disease and whether the parents were consanguineous or not.

The obtained data were loaded into the SPSS.21 statistical program, and the number, percentage, mean and standard deviation, median, minimum and maximum values were calculated. The normal distribution of quantitative data was evaluated with the Kolmogorov-Smirnov test. Kruskal-Wallis and Mann-Whitney U tests were used for comparison of quantitative data, and chi-square and Fisher Exact test were used to compare qualitative data. $p<0.05$ was considered statistically significant.

Results

Between 2011 and 2020, blood samples were obtained from the heels of 417,525 newborns in Diyarbakir. As a result of the screening, 1,122 infants were found to be suspicious for PKU, and 595 infants were found to be suspicious for BD. Among these, 122 infants (10.9%) with suspected PKU and 9 infants (1.5%) with suspected BD died before a diagnosis was made. The causes of death of the 9 patients with suspected BD were examined. It was determined that these nine patients died due to complications of congenital anomalies or prematurity. Two patients died from cardiac anomaly, two from hydrocephalus, one from meningomyelocele; and the other four patients died due to prematurity and sepsis within 7-28 days of follow-up in the intensive care unit. No findings suggestive of BD were found in these 9 patients who died. As a result of the tests performed in the pediatric metabolism clinic, the blood phenylalanine level of 266 newborns was found to be high. Of these, 53 were diagnosed with PKU (Incidence: 1:7878) and 213 with HPA (Incidence: 1:1960). 32.1% (n=17) of the patients with PKU had mild-moderate PKU, and 67.9% (n=36) had classical PKU. Furthermore, 177 BD (Incidence:1:2359) cases were discovered. 56% (n=100) of BD patients had profound enzyme deficiency (incidence:1:4175), whereas 44% (n=77) had partial enzyme deficiency (incidence:1:5422). In our study, 1 patient who was born in 2019 in our city and was residing in our country as a refugee and 3 patients who were born in 2020 were included in the calculation (Table I). In our study, no cases of PKU and BD co-existence were identified. When the distribution of illnesses by year is analyzed, it was seen that both the incidence of HPA and the incidence of BD increased in the previous three years. In particular, the incidence of BD has increased more than twice in the last three years compared to previous years (Fig. 1).

Data of a total of 269 patients (PKU: 25; BD: 123; HPA: 121) were analyzed. Parents of 65% (n=15) of the patients diagnosed with PKU and 46.6% (n=55) of the patients diagnosed with BD were consanguineous. It was determined that siblings or close relatives of 23.6% of the patients had a similar disease. On the other hand, it was determined that 52.0% of the patients diagnosed

Table I. Number of births by years, the incidence of phenylketonuria, hyperphenylalaninemia and biotinidase deficiency.

Year	Number of Births	PKU		HPA		BD			
						Profound	Partial	Total	
		Number	Incidence	Number	Incidence	Number	Number	Number	Incidence
2011	40,880	6	1/6813	17	1/2405	9	-	9	1/4542
2012	42,117	3	1/14039	23	1/1831	3	-	3	1/14039
2013	41,729	9	1/4637	26	1/1605	9	-	9	1/4637
2014	44,259	7	1/6323	14	1/3161	10	-	10	1/4426
2015	43,582	5	1/8716	16	1/2724	1	8	9	1/4842
2016	42,937	4	1/10734	14	1/3067	6	5	11	1/3903
2017	43,545	5	1/8709	14	1/3110	7	10	17	1/2561
2018	41,752	5	1/8350	26	1/1606	11	19	30	1/1392
2019	39,791	5	1/7958	33	1/1206	23*	18	41	1/971
2020	36,933	4	1/9233	30*	1/1231	21*	17*	38	1/972
Total	417,525	53	1/7878	213	1/1960	100	77	177	1/2359

BD: biotinidase deficiency, HPA: hyperphenylalaninemia without phenylketonuria, PKU: phenylketonuria
*One patient with profound BD, who was born in 2019 in our city and was residing in our country as a refugee, 2 patients with HPA, who were born in 2020; 2 patients with profound BD and 1 patient with partial BD were included in the table.

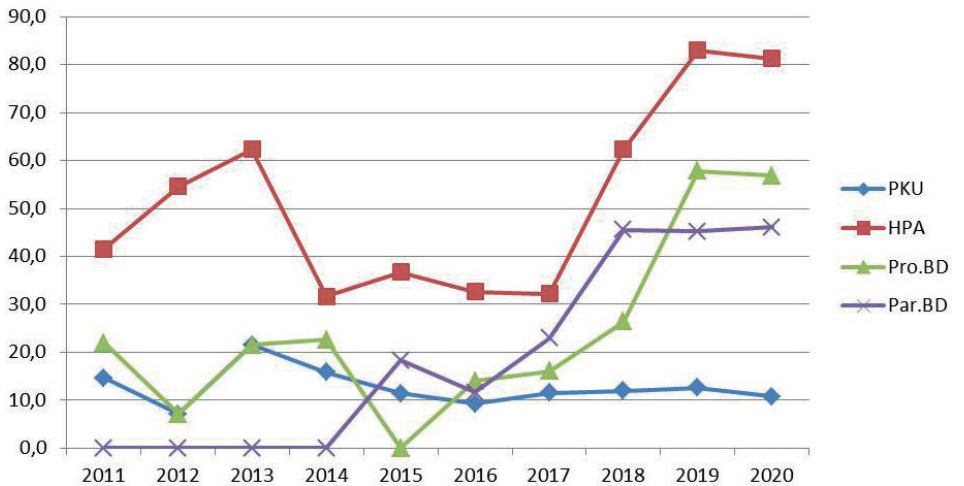

Fig. 1. Frequency distribution of PKU, HPA, profound biotinidase deficiency and partial biotinidase deficiency per 100,000 live births by years.

HPA: hyperphenylalaninemia without phenylketonuria, PKU: phenylketonuria, Par.BD: partial biotinidase deficiency, Pro.BD: profound biotinidase deficiency

with PKU and 17.9% of the patients diagnosed with BD had a similar disease in their relatives ($p<0.05$) (Table II).

49.4% (n=133) of the patients were male and 50.6% (n=136) were female. According to the latest follow-up, the mean age of the patients was 33.1±21.7 months (min-max: 2-117 months). At the time of the latest pediatric follow-up examination, the mean age of the patients with BD was 28.9±18.1 months, while the mean age of the patients with PKU was 44.1±24.1 months ($p<0.05$). While the majority of patients with PKU and BD were treated (86.6% and 91.8%, respectively), 40.6% of patients with HPA without phenylketonuria were treated ($p<0.05$).

Table II. Evaluation of the consanguinity status between the parents and the presence of a similar disease in siblings or relatives.

		BD n (%)	PKU n (%)	HPA n (%)	Total n (%)	P value
Are the parents consanguineous?	Yes	55 (46.6)	15 (65.2)	55 (48.7)	125 (49.2)	P=0.261
	No	63 (53.4)	8 (34.8)	58 (51.3)	129 (50.8)	
Have the patient's siblings/relatives been diagnosed with the same disease?	Yes	21 (17.9)	13 (52.0)	26 (23.2)	60 (23.6)	P=0.001
	No	96 (82.1)	12 (48.0)	86 (76.8)	194 (76.4)	

BD: biotinidase deficiency, HPA: hyperphenylalaninemia without phenylketonuria, PKU: phenylketonuria

24.1% of HPA patients do not receive therapy and are just observed (Table III). It was found that the patients with PKU were diagnosed after an average number of 44 days following the blood spot screening test; and the patients with BD were diagnosed after an average number of 76 days following the blood spot screening test and the treatment was initiated.

Disscussion

In this study, the screening results of 417,525 newborns born in Diyarbakir within ten years were evaluated for PKU and BD. The incidence of PKU was 1:7878, the incidence of HPA was 1:1960, and the incidence of BD was found to be 1:2359.

The incidence of PKU varies considerably between ethnicities and different geographical regions worldwide.[14] The incidence of PKU is high in Europe and some Middle Eastern countries: Italy (1:4,000), Ireland (1:4,545), Iran, Jordan (both 1:5,000) and Turkey (1:6,667). Northern European countries such as Denmark (1:13.434) and Finland (1:112,000) have the lowest PKU rates in Europe. In the American continent, PKU occurs in 1 of every 15,000 to 47,000 live births. The lowest PKU prevalence in the world has been reported in Asian countries such as Thailand (1:227,273), Japan (1:125,000), and the Philippines (1:116,006), with China being the exception (1:15,924).[15] According to a study from Saudi Arabia, the prevalence of PKU was one in every 28,316 live births.[2] It is estimated that one in every 23,930 infants suffer from it worldwide.[15] The increased incidence of consanguineous marriages in Iran, Jordan, Turkey, and Saudi Arabia explains the high prevalence of PKU.[2,13,14] Four out of every 100 people in Turkey are heterozygous carriers of PKU.[16]

We think that the prevalence of PKU is higher in our area. Because the records of some individuals who were initially monitored as

Table III. Evaluation of the age, gender and treatment status of the patients.

	BD n (%)	PKU n (%)	HPA n (%)	Total* n (%)	P value
Gender					P=0.495
Male	65 (52.8)	13 (52.0)	55 (45.5)	133 (49.4)	
Female	58 (47.2)	12 (48.0)	66 (54.5)	136 (50.6)	
Age (months); median (min-max)	24 (3-74)	49 (6-76)	27.5 (2-117)	28.5 (2-117)	P=0.005
Treatment status					P<0.001
Treatment ended	- (0.0)	3 (12.0)	19 (16.5)	44 (16.9)	
Treatment continues	108 (91.8)	19 (76.0)	28 (24.1)	128 (49.2)	
Not treated, being followed	- (0.0)	1 (4.0)	28 (24.1)	34 (13.1)	
Not followed	11 (9.2)	2 (8.0)	41 (35.3)	54 (20.8)	

BD: biotinidase deficiency, HPA: hyperphenylalaninemia without phenylketonuria, PKU: phenylketonuria
*: Because the patient files were examined retrospectively, some patients' data were missing or inadequate, hence instances with missing parameters were removed from the statistical analysis. For this reason, there were differences between the total figures in the table.

HPA and were later diagnosed with PKU were not updated, we believe the incidence of PKU is lower than expected. When PKU and HPA are taken as a whole (as phenylalanine hydroxylase deficiency), blood phenylalanine levels were found to be above the normal range in one out of every 1,570 newborns. In our investigation, the incidence of PKU was found to be higher than the global norm.

BD is seen approximately in one out of every 60,000 live births worldwide.[17] The estimated incidence of profound BD is one in 112,271 and the incidence of partial BD is one in 129,282.[18] According to two different studies conducted in Italy, the incidence of BD was 1:6,300 in Tuscany and Umbria, and 1:5,996 in the city of Verona. In both studies, approximately 90% of the patients had partial biotinidase deficiency.[8,19] In the study of Porta et al.[20] the incidence of BD in newborns was 1:61,000. Unlike the previous two studies conducted in Italy, 55% of the patients in this study had profound biotinidase deficiency. Due to high consanguinity rates, the prevalence of BD is high in some countries, such as Turkey and Saudi Arabia.[7] In a Saudi Arabian investigation, the frequency of BD was determined to be one in every 28,316 live births.[2] The incidence of BD was reported to be 1:11,614 in a study from Turkey, where seventy-eight percent of these individuals had profound BD.[21] When data from different cities in Turkey were evaluated, Aytaç et al.[22] discovered that the average incidence of BD in Adana was 1/11950. In their study in Şanlıurfa, Kazanasmaz et al.[23] found the incidence of BD as 1:1,177.

In our study, 56% of the patients with biotinidase enzyme deficiency had profound BD (incidence: 1:4,175), and 44% had partial BD (incidence: 1:5,422). The incidence of BD has increased more than twice in the last three years compared to previous years. In our study, BD was found to be higher compared to studies conducted in other countries and Turkey. Especially the increase in recent years is remarkable. In our study, one patient who was born in 2019 in our city and was residing in our country as a refugee (1/41) and 3 other such patients born in 2020 (3/38) were included in the calculation. However, even if the refugee patients were excluded from the table, the total incidence of BD in 2019 and 2020 (1/995 and 1/1055, respectively) would hardly change. The impact of the refugee patients on the increased BD rate in recent years was not significant. It was not possible to explain the reason for this increase with the refugee patients.

In Turkey, 23.2% of marriages are consanguineous. The region where consanguineous marriages are most common is the Southeastern Anatolia region, which includes Diyarbakir, with 42.6%.[3] In our study, parents of 65% of the patients diagnosed with PKU and 46.6% of the patients diagnosed with BD were relatives. Moreover, in our study, it was found that 52% of the patients diagnosed with PKU and 18% of the patients diagnosed with BD had a similar disease in their relatives. The high incidences of BD and phenylalanine hydroxylase deficiency in our study were thought to be due to the high rate of consanguineous marriages in our region. We believe that the reason relatives of PKU patients have identical disorders more commonly than relatives of BD patients is that PKU screening has been conducted for newborns in Turkey for roughly 30 years, whereas BD screening has only recently begun (in 2008). Considering the increase in BD in the last years, awareness studies and premarital counseling services for reducing consanguineous marriages are of great importance.

In patients who were born in the years we included in the study and had high phenylalanine levels, the BH4 loading test was not performed. Therefore, whether there were BH4-responsive patients was not determined. Our study's limitations include the fact that the records of some patients who were initially monitored as HPA and were later diagnosed with PKU were not updated, the lack of follow-up data in the family medicine records, and the low number of patients who came to the doctor's follow-up due to low health literacy in our region.

The incidence of both PKU and BD was found to be high in our region. The high prevalence of BD, the large proportion of patients with profound BD, and the cheap cost of screening tests all contribute to the relevance of screening infants. The parents of 65% of the patients diagnosed with PKU and 46.6% of the patients diagnosed with BD are consanguineous. It was found that the relatives of PKU patients were more likely to have a similar disorder than the relatives of BD patients. The main cause for the high incidence of these illnesses in our region was assumed to be the region's high rate of consanguineous marriages. For this reason, raising awareness and premarital counseling services to reduce consanguineous marriages are of great importance.

Acknowledgement

We would like to thank family physicians and family health employees in Diyarbakır for their contributions during the data collection phase.

Ethical approval

This study was conducted in compliance with the ethical principles according to the Declaration of Helsinki, and it was approved by the Ethics Committee of Health Sciences University, Gazi Yaşargil Training and Research Hospital (Number: 2021/855).

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: İT, ÖE, MNÖ; data collection: İT, SS, SC; analysis and interpretation of results: İT, SC, ÖE, MNÖ; draft manuscript preparation: İT, SS ÖE. All authors reviewed the results and approved the final version of the manuscript.

Source of funding

The authors declare the study received no funding.

Conflict of interest

The authors declare that there is no conflict of interest.

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