Clinical and demographic aspects of phenylketonuria in Bahia State, Brazil

Aspectos clínicos e demográficos da fenilcetonúria no Estado da Bahia

Aspectos clínicos y demográficos de la fenilcetonuria en la provincia de Bahia - Brasil

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ABSTRACT

Objective: To describe demographic and clinical characteristics of patients with hyperphenylalaninemia followed at the Neonatal Screening Reference Service of Bahia, Brazil.

Method: Cross-sectional study including 99 families (111 affected individuals) with biochemical phenotype of hyperphenylalaninemia by chart review and laboratory database that include demographic and clinical features.

Results: The incidence of hyperphenylalaninemia in Bahia was one case per 16,334 live births, covering 91% of them. Among patients followed, 82% were diagnosed by newborn screening and, in 11 families, there were more than one case. The classic phenotype of phenylketonuria was diagnosed in 63 (57%) patients. Among those screened, the median age at first consultation was 39.5 days. Among the patients, 34% had symptoms at the first medical consultation, none of them with delayed neurodevelopment. Consanguinity was reported in 32% of patients. Affected individuals were predominantly classified as white (63%). The parents had low education and low income. Among the 417 municipalities of Bahia, 15% had at least one case, with a concentration in the Northeast (10%) and in the capital of the State (14%).

Conclusions: The results showed elevated age at the beginning of the treatment, which may compromise the program results. Presence of consanguinity and familial recurrence were also noted. Careful investigation of families searching for individuals with mental retardation of unknown etiology that would benefit from the treatment is important.

Key-words: phenylketonuria; neonatal screening; metabolism, inborn errors.

RESUMO

Objetivo: Descrever as características clínicas e demográficas dos pacientes com diagnóstico de hiperfenilalaninemia acompanhados no Serviço de Referência em Triagem Neonatal da Bahia.

Métodos: Estudo transversal de 99 famílias (111 afetados) com fenótipo bioquímico de hiperfenilalaninemia, com coleta de dados em prontuários e em banco de dados laboratorial, incluindo aspectos demográficos e clínicos.

Resultados: A incidência de hiperfenilalaninemia na Bahia foi de um caso a cada 16.334 nascidos vivos, com cobertura de 91%. Dentre os pacientes acompanhados, 82% foram diagnosticados pela triagem neonatal e, em 11 famílias, havia mais de um caso. O fenótipo clássico da fenilcetonúria foi diagnosticado em 63 (57%) pacientes. Entre os triados, a mediana de idade na primeira consulta foi 39,5 dias e, deles, 34% apresentavam sintomatologia nesse momento; nenhum com atraso no desenvolvimento neuropsicomotor. A consanguinidade foi descrita em 32% dos casos e houve predomínio de pacientes classificados como brancos (63%). Os pais tinham baixa escolaridade e baixa renda. Dos 417 municípios da Bahia, 15% apresentavam

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pelo menos um caso, com concentração na região nordeste (10%) e na capital do Estado (14%).

Conclusões: Os resultados evidenciaram idade tardia ao início do tratamento, o que pode comprometer os resultados do programa. Observou-se também presença de consanguinidade e recorrência familiar, reforçando a importância da investigação familiar para diagnosticar indivíduos com deficiência mental de etiologia não esclarecida que podem se beneficiar de tratamento.

Palavras-chave: fenilcetonúria; triagem neonatal; erros inatos do metabolismo.

RESUMEN

Objetivo: Describir las características clínicas y demográficas de los pacientes con diagnóstico de Hiperfenilalaninemia (HPA) seguidos en el Servicio de Referencia en Selección Neonatal de Bahia.

Métodos: Estudio transversal incluyendo a 99 familias (111 afectados) con fenotipo bioquímico de HPA, con recolección de datos en prontuarios y en base de datos laboratorial, incluso aspectos demográficos y clínicos. Se realizó el análisis descriptivo de los datos utilizando promedios (desviación estándar) y medianas (percentiles 25-75).

Resultados: La incidencia de HPA en Bahia fue de un caso a cada 16.334 nacidos vivos (NV), con cobertura de 90,8%. Entre los pacientes seguidos, el 82% fueron diagnosticados por la selección neonatal, y en 11 familias había más de un caso. El fenotipo clásico de la fenilcetonuria fue diagnosticado en 63 (56,8%) pacientes. Entre los seleccionados, la mediana de edad en la primera consulta fue de 39,5 días (fenilcetonuria clásica) y, de ellos, el 34% presentaba síntomas en ese momento, ninguno de ellos con retraso en el desarrollo neuropsicomotor. La consanguinidad fue descrita en 32,2% de los casos y hubo predominio de pacientes clasificados como blancos (63%). Los padres tenían baja escolaridad y bajos ingresos. Entre los 417 municipios de Bahia, el 14,6% presentaba al menos un caso, con concentración en la región nordeste (9,9%) y capital de la provincia (14,4%).

Conclusiones: Los resultados evidenciaron edad tardía al inicio del tratamiento, lo que puede comprometer los resultados del programa. Se observó, además, la presencia de consanguinidad y recurrencia familiar, reforzando la importancia de investigación familiar para diagnosticar individuos con deficiencia mental de etiología no aclarada que pueden beneficiarse de tratamiento.

Palabras clave: fenilcetonuria; selección neonatal; errores innatos del metabolismo.

Introduction

Phenylketonuria (PKU), an inborn error of metabolism of autosomal recessive inheritance, was first described by the Norwegian chemist Asbjorn Fölling. It is the most frequent metabolic disorder in the etiology of mental retardation (MR)[1]. The underlying biochemical defect is, in most of the cases, a deficiency of phenylalanine hydroxylase (PAH) enzyme, responsible for the liver conversion of phenylalanine into tyrosine[2]. Which results in the accumulation of phenylalanine and its toxic metabolites in body tissues, especially in the central nervous system (CNS). Human PAH is encoded by a gene in chromosome 12 (12q22-q24), and over 500 different mutations in this locus have already been described[3].

Hyperphenylalaninemia (HPA) may be classified into classical PKU, mild PKU or non-PKU HPA, depending on the level of serum phenylalanine at the time of diagnosis. This level provides an estimate of the residual enzyme activity that partly depends on the mutation in the PAH gene and makes it possible to define the biochemical phenotype. Another phenotype, known as atypical PKU, results from the deficient biosynthesis or regeneration of the PAH cofactor, tetrahydrobiopterin (BH4). In these cases, there are no mutations in the PAH gene. BH4 deficiency is responsible for a severe neurological phenotype that does not respond to the standard diet treatment[2].

Since the 1950s, the strategies to manage this disease using diet interventions have been known, and the greatest challenge is to start treatment before there are symptoms. In response to this need, neonatal PKU screening started in the 1960s[4] and arrived in Brazil in the 1970s[5]. Popularly known in Brazil as the "little foot test", neonatal screening led to widely efficient prevention of MR associated with PKU[6,7].

Despite the initiatives implemented in the 1970s, it was only in 2001 that neonatal screening became a public health program in Brazil, when the National Neonatal Screening Program (PNTN) was established by Ministerial Order 822/2001 of the Brazilian Health Ministry. This program established Neonatal Screening Reference Services (SRTN) in all Brazilian states. The aim of this program is universal screening coverage and provision of all care needed, from blood collection to treatment and follow-up of confirmed cases. PNTN is organized into three implementation phases: Phase I – congenital hypothyroidism (CH) and PKU; Phase II – CH, PKU and hemoglobinopathy; Phase III – CH, PKU, hemoglobinopathy and cystic fibrosis[8,9].

In the state of Bahia, neonatal screening started in 1992 in the Association of Parents and Friends of People with Disabilities (APAE) in Salvador[9], but achieved greater magnitude after the implementation of PNTN, which defined that APAE would work as the SRTN for the state. Since 2007, all the 417 municipalities in the state of Bahia are connected with the SRTN. As part of the program, they have to collect and send blood samples to the reference laboratory, contact the family of diagnosed cases after notice from the active search service and provide the conditions for patients to come to consultations in the SRTN. Bahia has presently reached Phase II of PNTN, which is in charge of screening and treating phenylketonuria, hypothyroidism and hemoglobinopathy[8] PKU incidence in Bahia is one case for each 22,000 live births (LB) per year[9], similar, therefore, to the Brazilian mean number, estimated at one case for each 15,000 to 25,000 LB[10].

This study described the clinical and demographic characteristics of patients with a diagnosis of hyperphenylalaninemia who are followed up in the SRTN of the state of Bahia, Brazil.

Method

This descriptive cross-sectional study included 99 families and 111 individuals with a biochemical phenotype of PKU, defined as serum phenylalanine (Phe) ≥10mg/dL, or non-PKU HPA, defined as Phe levels of 3.5 to 9.9mg/dL (Table 1)[11]. The diagnosis of atypical PKU was made by measurement of biopterin levels in blood and urine and BH4 loading tests[12]. We studied patients diagnosed through neonatal screening and those with a late diagnosis suggested by clinical symptoms.

Demographic and clinical data, collected from medical charts, were sex, place of birth, parental consanguinity, other individuals affected in the family, age at beginning of treatment, ancestry according to phenotype classification made by trained professionals using the criteria defined by Krieger[13], form of diagnosis (neonatal screening or clinical symptoms), biochemical phenotype and signs and symptoms at diagnosis (irritability, skin and hair depigmentation, delayed neurological, psychological and motor development [NPMD], behavioral disorders and musty urine odor). This descriptive cross-sectional study used the SPSS® 11.0 to prepare and analyze its database. Frequency distribution, measures of central tendency (means and medians) and dispersion (standard deviation and interquartile range) were used for statistical analysis.

The number of live births in the state of Bahia from 2007 (year when the state completed municipal inclusion in the program) to 2009 was obtained from the database of the Brazilian Unified Health System (DATASUS) and the Health Department of the State of Bahia (SESAB). The number of screening tests was obtained from the SRTN database.

This study was approved by the Ethics in Research Committee of the Gonçalo Moniz Research Center of Fundação Oswaldo Cruz (Fiocruz, Bahia) and the legal guardians of all patients signed an informed consent term.

Results

From 2007 to 2009, there were 653,375 live births in Bahia (DATASUS), and the neonatal screening program reached 90.8% of all LB. During the study, SRTN diagnosed 40 cases of hyperphenylalaninemia, at a cumulative incidence of 1 case for each 16,333 LB.

This study evaluated individuals in 99 families, at a total of 111 patients with classical PKU, mild PKU, atypical PKU and non-PKU HPA; 58 (52.3%) of the patients were girls. The diagnosis was made through neonatal screening in 91 (82%) of the cases and by clinical symptoms in 20 (18%). The major symptom was mental retardation, which ranged from mild to severe. In 11 (11.1%) families, there was more than one affected individual: in one family, both cases were diagnosed due to clinical symptoms; in two other families, cases were diagnosed after population screening in a region of high prevalence. In three families, the second individual affected was born after the first had already been diagnosed through neonatal screening; in another family, the sibling was a twin who also received an early diagnosis and treatment. In the other cases, late diagnoses were made after neonatal screening of the younger sibling.

Table 1 - Biochemical classification of hyperphenylalaninemia

Biochemical phenotype	Serum Phe (mg/dL)	Estimated enzyme activity	Treatment
Classical PKU	>20	<1	Yes
Mild PKU	10-20	1-3	Yes
Non-PKU HPA	3,5-10	>3	No

Source: Adapted from Koch & Wenz. Phenylketonuria[11] Phe: phenylalanine; HPA: hyperphenylalaninemia; PKU: phenylketonuria.

Table 2 - Age at beginning of treatment in days of life of 85 patients with a diagnosis of HPA made through neonatal screening in SRTN - Bahia

Age at 1st consultation	n	Minimum	Maximum	Mean±sd	Median (25p-75p)
Overall	85*	22	202	60±36	49 (35–76)
1998 – 2001	07	32	150	71±40	73 (40–84)
2002 – 2004	23	22	202	70±48	53 (39–84)
2005 – 2007	28	23	129	57±31	48 (34–74)
2008 – 2009	27	25	109	51±23	42 (31–69)
Classical PKU	10	25	74	40±15	35 (29–46)
Mild PKU	05	26	92	51±26	52 (29–72)
Non-PKU HPA	11	38	109	64±22	65 (39–81)

*Includes patient with diagnosis of atypical PKU; PKU: phenylketonuria; HPA: hyperphenylalaninemia.

The classification of the biochemical phenotype, based on the levels of phenylalanine at diagnosis, was classical PKU in 63 (56.8%) patients, mild PKU in 25 (22.5%), non-PKU HPA in 22 (19.8%) and atypical PKU due to cofactor deficiency in one case. Age at the beginning of treatment was known for 85 patients diagnosed through neonatal screening, and is summarized in Table 2.

All patients with a late diagnosis had symptoms of the disease at diagnosis. Information about symptoms was available for 68 of the patients with mild or classical PKU diagnosed early through neonatal screening. Of these patients, 22 (32.4%) already had signs and symptoms of the disease, and the most frequent were irritability, described primarily as difficulty to fall asleep and feed and frequent crying. Delayed NPMD was not detected in the first consultation of patients diagnosed through neonatal screening. The 22 patients with a biochemical phenotype of non-PKU HPA had no symptoms, as expected; they have been regularly followed up and eat a non-restrictive diet, as recommended in the literature[2].

The only patient with a diagnosis of atypical PKU had an unfavorable clinical progression in the first months of life, evident NPMD delay after the third month, clinical symptoms and laboratory confirmation of the disease.

Patients came form 61 different municipalities; 9 (8.1%) were form Monte Santo, in the northeastern region of the state, and 16 (14.4%) from Salvador. Monte Santo was the municipality with the highest incidence of the disease because its population was 52,360 inhabitants in 2010[14]. Data about consanguinity were available for 90 families and reported as positive in 29 (32.2%) families. Ancestry by phenotype criteria was defined for 100 patients: 63 were classified as white, 32 as mulattos, two as native brazilians and two as black.

Data about socioeconomic status at the time when the child was registered in the service were available for 94 families: 10.6% had no fixed income, 40.4% had an income of less than one minimum wage (MW), 25.5% between one and two MW, and only 9.6% of the families had an income of more than 2 MW; 13.8% of the families had no income and survived on social benefits.

Table 3 - Schooling of parents with a diagnosis of PKU in SRTN - Bahia

Schooling	Mother		Father	
	n	%	n	%
No schooling	6	6.1	8	8.1
Incomplete elementary school	44	44.4	44	44.4
Complete elementary school	8	8.1	5	5.1
Secondary school	16	16.2	10	10.1
College	4	4.0	2	2.0
No data	21	21.2	30	30.3
Total	99	100.0	99	100.0

Data about parents' schooling were available for 78.8% of the mothers and 69.7% of the fathers (Table 3). In general, the level of schooling was low, and most parents had not finished elementary school.

Discussion

The Neonatal Screening Reference Service (SRTN) in the state of Bahia performs diagnoses, active search, treatment and follow-up of patients with PKU. Of the 61 different municipalities in Bahia in which cases have been diagnosed, Monte Santo and Uauá, two neighboring towns located in the northern region of the state and where neonatal screening has been effectively performed for a few years, concentrated patients being followed up. Monte Santo is currently the site of a study about population genetics because of its high number of individuals with autosomal recessive disorders, probably associated with a high rate of consanguinity in the population[15].

SRTN has screened about 90% of all infants born in Bahia. According to data of the Health Ministry in 2007, Bahia has the highest coverage among the states in the Northeastern region of Brazil, and its figures are above the national average[16]. The increase in coverage has become evident in the last few years, as only 71.5% of the children born in Bahia had the screening test in 2003[8]. A scarcity of studies, divergent findings, and lack of standardized calculations of PNTN coverage make it difficult to compare data between states in Brazil. However, the improvement in coverage in the state of Bahia has been well documented[8,9,16,17].

The incidence found in this study revealed a higher than expected frequency among ethnically mixed populations[18], also higher than the national mean value, previously described, which is one in each 25,294 LB[19]. The greater coverage may explain the higher number of diagnosed patients because the number of births has been stable in the last five years.

The cases identified due to clinical symptoms account for 17% of all diagnoses so far, and this number has decreased in the last few years (21.6% in 2003), which suggests a greater coverage of the screening test. Brandalize and Czeresnia[20] reported that 30% of the patients with PKU followed up in the state of Paraná from 1996 to 2001 had a late diagnosis. In Bahia, the number of known cases reveals an estimated prevalence of one case in each 131,869 inhabitants. As SRTN is the only service authorized to provide treatment and, therefore, follow up all cases diagnosed in Bahia, even those tested in private laboratories, this frequency might not reflect the reality. Phenylketonuria is a genetic disease whose frequency is expected to remain stable along the years; considering the population of Bahia in 2010 (14,021,432 inhabitants)[14], the real number of cases must be around 858, and this disease seems to be underdiagnosed. Explanations for this fact may include the low coverage of the screening test before 2001 associated with the absence of clinical suspicion[21]. Although few studies discuss it, there seems to be a consensus about the existence of a large number of undiagnosed cases in Brazil[22].

Recurrence among siblings is in agreement with the autosomal recessive pattern of inheritance, as well as with the high frequency of consanguinity between parents. The examination of siblings with suggestive signs and symptoms may clarify the diagnosis of children with mental retardation of unknown etiology and lead to the beginning of their treatment, with good results in promoting growth, reducing neurological symptoms and improving social behaviors, although it is not possible to reverse cognitive impairments[23,24].

Mean age at first consultation was 39.5 days (median = 34.5 days) for cases of classical PKU in the last few years, which is still high in comparison with the recommendation to start treatment within the first month of life[7]. The standard deviation of 15 days indicates that there are still patients that have a significant delay in starting treatment within the recommended limits of time. However, results already suggest an improvement: up to 2004, mean patient age at first consultation was 57±38 days, and median age was 45 days (range: 17 days to 6 months)[9]. Compared with other states, means were lower than in Mato Grosso[25] and Sergipe[26], but higher than those found for the city of Ribeirão Preto[27].

In the fist consultation, no NPMD delay was observed, which was expected because this is a symptom usually detected only later on in children with untreated PKU. However, other signs and symptoms attributable to hyperphenylalaninemia were present. Although these data may be overestimated by the fact that the examiner was previously aware of the diagnosis, it suggests that there might be clinically evident impairment, although usually reversible, before six months of age, as described in classical studies in the literature[28,29]. There was a reduction in the percentage of symptomatic patients in comparison with previous findings[9], which might be explained by the reduction of age at first consultation along the years. This information is relevant because an association between higher age at treatment initiation and neurological deficits has been described among Brazilian patients[20].

The greater prevalence of patients classified as white according to phenotype may suggest European or Caucasian ancestors, which would be in agreement with current knowledge about the genetic basis of phenylketonuria[5].

Socioeconomic data revealed a poor population for which PNTN, a program that is entirely sponsored by the Brazilian Unified Health System, gains special importance. Among parents, 64.1% of the mothers and 75.4% of the fathers for whom the level of education was known had not finished elementary school. These data are close to those described by the Brazilian Institute of Geography and Statistics (IBGE), which found that 60.8% of the population in Bahia has seven or fewer years of schooling[30]. These rates are higher in the state of Paraná, where 37.5% of the fathers and 21.8% of the mothers finished high school or had a college education[20].

Data about income of the families included in the study revealed that 69.5% earned up to two minimum wages per month. Most families had at least four members, which results in a per capita income of half a minimum wage. These data

are compatible with the reality of the state of Bahia, where, according to IBGE, 66.8% of the families with children up to six years of age have a per capita income of up to half a minimum wage[30]. These findings draw attention to the need to provide regular and multidisciplinary care, which should include social assistance and health education, as a way to reduce potential damage to health due to low education levels and family income. Parental schooling and income are important factors; several studies showed that there may be an association between low income, low parental schooling and lower motor scores among affected children[20].

This study described the clinical and demographic characteristics of a group of patients that included all the known diagnoses of phenylketonuria and hyperphenylalaninemia in the state of Bahia, Brazil, up to 2009. Results confirmed consanguinity and familial recurrence and drew attention to the importance of a thorough family investigation to detect cases not diagnosed through neonatal screening that may benefit, though partially, from treatment. Higher age at the beginning of the treatment is also fundamental to establish strategies to adapt the program and to provide multidisciplinary support to the families whose income and schooling are usually low, two known risk factors for health problems.

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