Clinical Neurology and Neurosurgery 113 (2011) 727–730

Contents lists available at ScienceDirect

Clinical Neurology and Neurosurgery

journal homepage: www.elsevier.com/locate/clineuro

Phenylketonuria is still a major cause of mental retardation in Tunisia despite the possibility of treatment

Sameh Khemir[a], Monia El Asmi[a], Haifa Sanhaji[a], Moncef Feki[a], Riadh Jemaa[a], Neji Tebib[b], Jean Louis Dhondt[c], Marie Françoise Ben Dridi[b], Abderraouf Mebazaa[a], Naziha Kaabachi[a,*]

[a] Biochemistry Laboratory, La Rabta Hospital, 1007 Jebbari, Tunis, Tunisia
[b] Department of Paediatrics, La Rabta Hospital, Tunis, Tunisia
[c] Clinical Biology Laboratory, Lomme, France

ARTICLE INFO

Article history:
Received 14 July 2010
Received in revised form 13 July 2011
Accepted 20 July 2011
Available online 8 September 2011

Keywords:
Phenylketonuria
Mental retardation
Motor difficulty
Behaviour disturbance
Frequency
Treatment

ABSTRACT

Background and objective: Accumulation of phenylalanine following a deficiency of phenylalanine hydroxylase activity generates a brain damage with mental retardation: phenylketonuria (PKU).

In the developing countries, where PKU systematic neonatal screening program is not established yet, the management of PKU handicap is not properly carried out.

The aim of this study was to estimate the frequency of the PKU diagnosed following clinical features anomalies, to provide information about the untreated PKU patients profile in Tunisia not covered by neonatal screening. Also it is stressed that treated patients have a normal development.

Patients and methods: This is a retrospective study of 156 cases of PKU detected in Tunisia over 20 years following symptoms suggestive of inherited metabolic disease. Phenylalaninemia level was performed by fluorometric method. Among them 9 patients were treated.

Results: The PKU estimated frequency was 1/7631. The diagnosis mean age was 4 years. The phenylalaninemia mean was 1680 μmol/L; the classical PKU form accounted for 85.3% of cases and the dominant clinical symptoms were: mental retardation (88.2%), motor delays (87.7%), speech difficulties (83.2%) and pigmentation anomalies (61.7%). The treated patients responded to treatment and showed a normal development.

Conclusion: The establishment of neonatal screening should be a priority to avoid cases of mentally retardation.

© 2011 Elsevier B.V. All rights reserved.

1. Introduction

Phenylketonuria (OMIM 261600) (PKU) is an autosomal recessive disease caused by phenylalanine hydroxylase (PAH) (EC1.14.16.1) deficiency. PAH is a liver-specific enzyme that catalyses the hydroxylation of L-phenylalanine (Phe) to L-tyrosine in the presence of the cofactor, tetrahydrobiopterin. Accumulation of phenylalanine generates a brain damage and consequently irreversible mental retardation. If PKU were diagnosed early at birth and the patients maintained on a strict and controlled low-phenylalanine diet, the symptoms of this disease could be prevented [1,2].

PAH deficiency is diagnosed by detecting an elevation of serum phenylalanine concentration which is persistently above 240 μmol/L in the untreated state [3].

The incidence of PKU is variable, being high in Turkey (around 1 in 2600 births) and low in Japan (1 in 120,000 births). The incidence in Caucasians, where the disease has a neonatal screening, is approximately 1:10,000[4].

In the developing countries and in Tunisia in particular, the rate of consanguinity is rather high (32%) and PKU systematic neonatal screening program is lacking similarly to other North African countries [5], therefore PKU diagnosis is established late, following detection of clinical features and typical anomalies. For patients having a PKU family history, new cases are diagnosed at birth. In fact the PKU handicap management is emphasized.

The aim of this study was to estimate the frequency of the PKU diagnosed following detection of clinical features anomalies observed in Tunisia, over 20 years, in order to provide information about the untreated PKU patients profile in a country not covered

* Corresponding author. Tel.: +216 71 561 912; fax: +216 71 570 506.
E-mail addresses: naziha.kaabachi@rns.tn, naziha.kaabachi@gmail.com (N. Kaabachi).

0303-8467/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.clineuro.2011.07.016

Table 1
Different classes of PKU in the Tunisian patients according to the French consensus [7].

PKU classes	Number of patients (%)	Phe[b] concentration (μmol/L)	Means of Phe concentration ± SD[c] (μmol/L)	Means age at diagnosis
Classic PKU[a]	110 (85.3%)	>1200	1806 ± 660	4 years and 1month
Moderate PKU	17 (13.2%)	600 < Phe < 1200	996 ± 144	4 years
Mild PKU	2 (1.5%)	<600	282 ± 78	1 years and 6 months

[a] Phenylketonuria.
[b] Phenylalanine.
[c] Standard deviation.

by neonatal screening. Also stressed the possibility to treat late diagnosed patients that subsequently show good developments.

2. Materials and methods

This is a retrospective study of 156 cases of PKU from 106 families detected in Tunisia between 1988 and 2008 and originating from different departments: pediatrics (64%), neurology (25%) and neonatology (2%).

A total of 142 patients were referred to our laboratory for diagnosis of inherited metabolic disease and 14 with PKU family history for PKU diagnosis. The treatment of newborns diagnosed early in the families at risk was initiated and 9 patients are followed at pediatric department of La Rabta since the acceptance of the management of PKU cost refunding by social security a decade ago. The oldest is 10 years.The treatment consisted of a controlled diet in phenylalanine with the objective to maintain the rate of phenylalanine between 120 and 300 μmol/L by reducing dietary intake of phenylalanine and adding mixtures of amino acids adapted to local diets.

Each sample was associated with a demand of analysis comprising the anamnesis characteristics and the clinical information of the patient. The samples included random urines and plasma or dried blood spots taken in blood cards (Schleicher & Schuell 903).

In order to exclude other aminoacidopathies: plasma and urine amino acids quantification were performed on amino acids analyzer (Beckman 6300) and also to eliminate organic acidurias: urinary organic acids analysis were performed by liquid gas chromatography–mass spectrometry (GC–MS) (Hewlett Packard 5890/HP 5972).

Since 2000, phenylalanine level was determined by fluorimetric method on dried blood spots. For high values (>240 μmol/L), tetrahydrobiopterin disorders were excluded by measurement of pterins in urine and analysis of dihydropterin reductase in blood [6,7].

Collected data has been analyzed using Epi Info software and for the estimate of the frequency of PKU, the Hardy–Weinberg formula was adapted in view of the birth rate and the consanguinity factor [8].

3. Results

The totals of 156 PKU diagnoses were made and the estimated frequency was 1/7631.

The age range of diagnosis was from 1 day to 31 years with an average of 4 years. Only 9.4% of patients were diagnosed before 3 months; 8.7% between 3 months and one year but 32.9% were diagnosed between 2 and 3 years and nearly half the cases (49%) after 3 years.

The sex ratio (M/F) was 0.79.

The rate of consanguinity among PKU cases and those having previous similar familial disorder were respectively, 94.3% and 80.4%. Nine families were consanguineous and 37 of the families had two or three affected child.

According to the origin of PKU patients, we noted an equivalent distribution of patients from North and Centre of Tunisia, respectively 50% and 46% but only 4.5% originated from the South. The mean phenylalanine blood level was 1680 ±690 μmol/L.

Table 1 represents the different classes of PKU according to the French consensus on the care of children diagnosed with hyperphenylalaninemia. These guidelines specify the minimal diagnosis procedures leading to an optimal treatment of all patients. The classical and moderate forms require a low-phenylalanine diet while the third class does not require special arrangements [9].

In this study, the classic PKU form, accounted for more than two-thirds of the total cases (85.3%). The majority of patients (98.5%) that required treatment were diagnosed late (age > 4 years). For 27 patients, plasma Phe levels were not available because their diagnosis were performed by the detection of accumulation of urinary phenolic acids (pyruvic phenyl acids, 2-hydroxy phenylacetic acids, phenylacetic and phenylactic) by GC/MS. In addition they had an accumulation of plasma Phe on thin layer chromatography.

Mental retardation represented a constant clinical symptom and was pronounced in most cases (88%). Motor delay and speech difficulties were present respectively in 88 and 83%, hypotonia in 51% and convulsions in 43% of patients. Abnormal attitude, behavior and reactions and rage crises were found in 68% of patients. Pigmentation anomalies were observed in 62% and the particular odor in 44% of PKU cases.

According to the PKU classes, the clinical characteristics of phenylketonuria are reported in Table 2.The clinical signs for classical and moderate forms were similar; the mild PKU presented only motor delay.Nine patients were diagnosed at birth because of a history of PKU; the clinical examination was normal in all cases. The mean age at diagnosis was 19 days (min: 2 days; max: 5 months)The dietary treatment consisted in a phenylalanine-controlled diet that allows the reduction of systemic phenylalanine concentration, satisfactory tyrosine provision, and optimal growth and development.

Table 2
Clinical symptoms according to PKU classes.

PKU classes	Number of patients	Clinical symptoms							
		Mental retardation	Motor delay	Speech difficulties	Anomalous attitude	Pigmentation anomalies	Hypotonia	Particular odor	Convulsions
Classic PKU	110	87%	89%	84%	67%	63%	52%	45%	40%
Moderate PKU	17	85%	75%	67%	70%	50%	50%	28%	40%
Mild PKU	2	0	50%	0	0	0	0	0	0

PKU, phenylketonuria.

This was done under the supervision of metabolic pediatrician and nutritionist. Serial monitoring of blood phenylalanine levels is an essential element of treatment; the control was carried out periodically by the dosage of phenylalanine in blood cards (Schleicher & Schuell 903). For the treated patients, the mean age of treatment start was 40 days (min: 9 days; max: 5 months). After 10 years, these patients showed normal psychomotor development and had normal good schooling.

4. Discussion

The incidence of PKU in Tunisia is estimated at 1/7631; this frequency is rather underestimated since many cases remain unfortunately undiagnosed. PKU disease was the most frequent aminoacidopathies disorder in Tunisia: Maple syrup urine disease (1/17825), Tyrosinemia (1/22831), Nonketotic hyperglycinemia (1/26/109), Homocystinuria (1/32679) [10]. According to Ben Dridi et al., the frequency among maghrebian PKU children living in France was 1/3432 in North Pas de Calais and 1/3291 in Rhone Alpes; it was 1/3571 in Belgium too [11].

The incidence of PKU in Caucasian population is reported between 1/10,000 and 1/15,000. It seems to be lower in Finland (1/200,000) and in Japan (1/125,000) and higher in Turkey (1/6,200) [12].

The frequency of PKU in Tunisia seems to be higher probably due to the elevated rate of consanguinity in the population (32%). In fact, the real incidence will be known only after 12 years when neonatal screening program will be implanted.

The majority of cases in this study were addressed by pediatric departments (64%); this percentage is related to the late diagnosis of this pathology (>4 years), Fan et al. reported a higher result in Mainland China (91.7%), where the neonatal screening was started late and was incomplete [13].

A difference in the number of PKU diagnosed from the North and the Central Tunisia was observed; the Centre region is closer to Tunis where the Biochemistry Laboratory of La Rabta hospital is located and serves as a referral centre for diagnosis of inherited metabolic diseases, and also accepts patients from all the country geographical areas. In the Centre many clinicians specialized in the study of inherited metabolic disorders are found and the routing of samples is not a problem. However, the low frequency of PKU from the South can also be explained by the lower population density in this area.

Occurrence of consanguineous marriages in Tunisian population might partially be responsible for the high incidence of PKU (94.3% of cases). This situation is similar to the one of Turkey and Kuwait where the consanguinity is also high [14,15].

The majority of PKU patients are detected late when severe and irreversible damage has already occurred. Marholin et al. reported that if the treatment was initiated with children less than 6 months of age, damage to the central nervous system may be totally prevented; even providing a low Phe diet as late as 2 years of age may be beneficial; however, after 2 years, the lower diet cannot prevent severe retardation. Although diet treatment started after 2 years of age is ineffective in preventing or correcting structural changes in the central nervous system; when administered to older PKU children and adults, desirable changes in behavior have been reported [16].

In an epidemiological report of Turkey, Coskun et al. reported 471 Turkish hyperphenylalninemic cases of which 408 (89.3%) had classical form and 49 (10.7%) moderate form; this profile is similar to the results of this study (85.3% and 13.2%, respectively) [17].

Mental retardation, the dominant disease phenotype, was present in most cases and in different classes of PKU. Güttler et al. reported untreated PKU patients with moderate to profoundly mental retardation. The degree of mental retardation was not related to the serum Phe level [18].

In China, where studies were carried out between 1984 and 2002, the cases treated late had some signs and symptoms similar to those of untreated PKU. Mental retardation was found in the totality of patients and various patterns of seizures in 25.48% of cases [19]. Next to intellectual deficit, behavioural disturbance has been described as the most characteristic symptom of PKU. Hyperactivity, irritability, destructiveness, rage and fear reactions, and autistic behaviour have been described repeatedly [2,4,20].

Neurological problems increase with age of diagnosis, profound mental retardation, epilepsy, spasticity, and severe behavioural disorders are typical across the disorder's life course [21–23].

Mental retardation is attributed to a toxic effect of excess phenylalanine on brain development and functions. Patients can have very different cognitive phenotypes, even when their metabolic phenotype (phenylalanine level) is similar. Such discordance between metabolic and cognitive phenotypes appears to be explained by differences in the function of the blood–brain barrier and the modulation of free phenylalanine content of the brain [12].

Corsello et al. reported a history of a late diagnosed PKU patient: a 17 years old boy with previously undiagnosed classical PKU: the psychomotor development has been poor since the first year of live, the QI was 55, the skin was dry with eczematous spots on the face and hands; thin and blond hair were noted and extra pyramidal movements and ataxic gait were noted. Speech was very poor with elementary vocabulary [20].

PKU is often associated with a mousy odor resulting from the excretion of phenylpyruvic acid. Reduced hair, skin and iris pigmentation is a consequence of reduced melanin synthesis [24]. Unfortunately, these signs continue to be the main elements of diagnosis of PKU in countries that do not have a systematic neonatal screening.For the 9 cases treated before the age of 5 months, they presented subsequently a normal psychomotor development and a satisfactory education. These results have been reported in several studies: Burgard reported that children with Phe levels below 400 μmol/l in early and middle childhood were near normal [25,26].These patients had been treated by a specific diet adapted to the Tunisian food traditions and using only mixtures of amino acids because low protein foods are quite expensive and not reimbursed by social security funds; the procedure in developed countries where a wide range products are used to diversify the supply of patients is not an economically available option.With only few resources, we could nevertheless have a satisfactory child development; this should encourage authorities to implement systematic neonatal screening.In addition we have set up an association: The ATEMMH (Tunisian association for the study of inherited metabolic diseases), founded in April 2004 by physicians, biologists and biochemists interested in metabolic disorders, which recognize that PKU is an important public health problem in Tunisia and militate for the establishment of a routine neonatal screening program.ATEMMH organizes every 2 years a meeting for PKU patients, their families and for clinicians. All participate in the debate in the screening organization of newborns in all maternity centers.

To sensitize the authorities, these meetings are co-supervised by the Ministry of Social Affairs and the Ministry of Public Health.

5. Conclusion

This disease should be suspected whenever a fair-skinned child is affected by severe or non severe psychomotor retardation, seizures together with language and behavioural disorders.

The high frequency of the PKU and the important rate of consanguinity in Tunisia stress the necessity of a neonatal screening

program for PKU. It should benefit patients and their families, as well as the general population in order to obtain the prevalence of mental retardation decrease.

The effective treatment of PKU to allow normal development is possible in Tunisia and all underdeveloped countries that do not enjoy for this screening.

Acknowledgments

The authors thank the physicians of Pediatric Departments in Tunis, Sousse, Monastir, Sfax, Gabes, Kairouan, Bizerte and biochemistry laboratory technicians: S. Ben Ayed, S. Skhiri, D. Bouhadra and M. Mzoughi for their technical assistance.

References

[1] Matalon R, Rouse B. Neonatal neurological assessment of offspring in maternal phenylketonuria. J Inherit Metab Dis 1998;21:39–48.
[2] Pietz J, Dunckelmann R, Rupp A, Rating D, Meinck HM, Schmidt H, et al. Neurological outcome in adult patients with early-treated phenylketonuria. Eur J Pediatr 1998;157:824–30.
[3] Walter JH, Lee JP, Burgard P. Hyperphenylalaninemia. In: Fernandes J, Saudubray JM, Van Den Berghe G, Walter JH, editors. Inborn metabolic diseases: diagnosis and treatment. 4th edition Springer; 2006. p. 221–43.
[4] Scriver CR. Hyperphenylalaninemia: phenylalanine hydroxylase deficiency. In: Scriver CR, Beaudet AL, Sly WS, Valle D, editors; Childs B, Kinzler KW, Vogelstein B, assoc. editors. The metabolic and molecular bases of inherited disease, 8th edition. New York: McGraw-Hill; 2001. p. 1667–1724.
[5] National Office for Family and Population. Survey of Demographic and Health in Tunisia 1988. Tunis; 1989.
[6] Mc Caman MW, Robins E. Fluorimetric method for the determination of phenylalanine in serum. J Lab Clin Med 1962;59:885–90.
[7] Dhondt JL. Strategy for the screening of teterahydrobiopterin deficiency among hyperphenylalaninemic patients: 15 years experience. J Inherit Metab Dis 1991;14:117–27.
[8] Monastiri K, Khairi H, Snoussi N. Biométrie du nouveau-né dans le sahel tunisien. Ann Pédiatr 1994;419:578–83.
[9] Abadie V, Berthelot J, Feillet F, Maurin N, Mercier A, Ogier De Baulny H, et al. Management of phenylketonuria and hyperphenylalaninemia: the French guidelines. Arch Pediatr 2005;12:594–601.
[10] Sanhaji H, Elasmi M, Souissi M. High frequency of aminoacidopathies and organic acidurias in Tunisia. In: European Congress of Clinical Chemistry and Laboratory Medicine. 2003. p. 1209–13. D601C1639/.
[11] Ben Dridi MF, Tebib N, Kaabachi N, Hessairi M, Ferchichi M, Mebazaa A. Frequency of phenylketonuria in the world and Maghreb. La Revue Maghrébine De Pédiatrie 1997;VII: 227–34.
[12] Williams RA, Mamotte C, Burnett DS, Phenylketonuria JR. An inborn error of phenylalanine metabolism. Clin Biochem Rev 2008;29(1):31–41.
[13] Fan GX, Qing LX, Jun Y, Mei Z. Molecular studies and prenatal diagnosis of phenylketonuria in Chinese patients. Southeast Asian J Trop Med Public Health 1999;30(Suppl. 2):63–5.
[14] Woolf LI. Phenylketonuria in Turkey, Ireland and West Scotland. J Inherit Metab Dis 1994;17(2):246–7.
[15] Teebi AS, Al-Awadi SA, Farag TI, Farag TI, Naguib KK, El-Khalifa MY. Phenylketonuria in Kuwait and Arab countries. Eur J Pediatr 1987;14(1):59–60.
[16] Marholin II D, Pohl III RE, Stewart RM, Touchette PE, Townsend NM, Kolodny EH. Effects of diet and behavior therapy on social and motor behavior of retarded phenylketonuric adults: an experimental analysis. Pediatr Res 1978;12(3):179–87.
[17] Coşkun T, Özgüç M, Tokatlı A, Yilmaz E, Erdem H, Dursun A, et al. Phenylketonuria in Turkey: epidemiological, clinical and genetic aspects. Dev Brain Dysfunct 1993;6:134–40. Special Issue.
[18] Güttler F, Guldberg P, Henreisken KF. Mutation genotype of mentally retarded patients with phenylketonuria. Dev Brain Dysfunct 1993;6:92–6. Special Issue.
[19] Yu WM, Xu L, Li XW, He C, Shen M, Zhang ZX, et al. An eighteen-year study on phenylketonuria. Zhongguo Yi Xue Ke Xue Yuan Xue Bao 2003;25(2):218–22 (in Japanese).
[20] Corsello G, Piccione M, Pirto E, Meli C, Romano V, Giuffrè B. Auditory and visual evoked potentials in a 17-year-old boy with undiagnosed classical phenylketonuria. Dev Brain Dysfunct (special issue) 1993;6:179–83.
[21] Blaskovics ME, Nelson TL. Phenylketonuria and its variations: a review of recent developments. Calif Med 1971;115(1):42–57.
[22] Yalaz K, Vanli L, Yilmaz E, Tokatli A, Anlar B. Phenylketonuria in pediatric neurology practice: a series of 146 cases. J Child Neurol 2006;21:987–90.
[23] Brenton DP, Pietz J. Adult care in phenylketonuria and hyperphenylalaninaemia: the relevance of neurological abnormalities. Eur J Pediatr 2000;159:114–20.
[24] Fölling A. Excretion of phenyl pyruvic acid in the urine as a metabolic anomaly in relation with imbecility. Hoppe Seylers Z Physiol Chem 1934;277: 169–76.
[25] Burgard P. Development of intelligence in early treated phenylketonuria. Eur J Pediatr 2000;159:S74–9.
[26] Burgard P, Rey F, Rupp A, Abadie V, Rey J. Neuropsychologic functions of early treated patients with phenylketonuria, on and off diet: results of a cross-national and cross-sectional study. Pediatr Res 1997;41:368–74.