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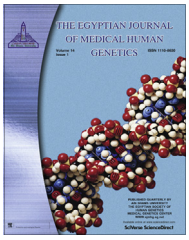

ORIGINAL ARTICLE

Clinicolaboratory profile of phenylketonuria (PKU) in Sohag University Hospital-Upper Egypt

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KEYWORDS

Phenylketonuria (PKU);
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Abstract Phenylketonuria (PKU) is an autosomal recessive metabolic genetic disorder characterized by a mutation in the gene for the hepatic enzyme phenylalanine hydroxylase (PAH). The disease may present clinically with seizures, albinism (excessively fair hair and skin), and a "musty odor" to the baby's sweat and urine. In the untreated classic case, mental retardation is severe, precluding speech and toilet training. Seizures are common in the more severely retarded, usually start before 18 months of age. This study aimed to identify clinical profile and impacts of newly diagnosed (untreated) PKU on children. Children presented to the Pediatric Department, or Pediatric Neurology Clinic, Sohag University Hospital in whom the diagnosis of Pheylketonuria was established based on measuring phenylalanine level in blood samples were eligible for this study. All studied patients were subjected to thorough history, full examination, and developmental assessment. Electroencephalography (EEG), computed tomography of the brain (CT), phoniatric and audiologic evaluations were also done. During the period of the study we diagnosed 24 cases with phenylketonuria, the main clinical presentations were global developmental delay, hyperactive symptoms, seizures, and autistic features. CT of the brain showed that 58.3% of cases had atrophic changes. EEG showed that 58.3% of cases had abnormal findings as generalized epileptic discharges, focal epileptic discharges, and hypsarrhythmia. We concluded that untreated phenylketonuria still represents a significant burden on children development and mental function in Upper Egypt. So we recommend establishment of national screening programs and pushing it forward as well as immediate development of specific metabolic centers in various universities and research institutes.

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1. Introduction

Phenylketonuria (PKU) is an autosomal recessive metabolic genetic disorder characterized by a mutation in the gene for the hepatic enzyme phenylalanine hydroxylase (PAH), rendering it nonfunctional [1]. This enzyme is necessary to metabolize the amino acid phenylalanine (Phe) to the amino acid tyrosine.

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When PAH activity is reduced, phenylalanine accumulates and is converted into phenylpyruvate (also known as phenylketone), which is detected in the urine [2]. The mean incidence of PKU varies widely in different human populations. In Turkey, 1 in 2600 births (the highest rate in the world); in Ireland, 1 in 4500 [3], in Norway 1 in 13,000, and fewer than one in 100,000 in Finland. In the United States, about 1 in 15,000 births show classical PKU. The incidence is relatively high in Italy, China, and Yemen [4–6]. PKU is commonly included in the newborn screening panel of most countries, with varied detection techniques. Most babies in developed countries are screened for PKU soon after birth [7]. However in Egypt screening practice for PKU does not include all neonates but sporadic studies were done. Screening for phenylketonuria among Egyptian newborns in Menoufiya governorate was conducted and revealed a prevalence of 1/3000 [8].

If a child is not screened during the routine newborn screening test, the disease may present clinically with seizures, albinism (excessively fair hair and skin), and a "musty odor" to the baby's sweat and urine (due to phenylacetate, one of the ketones produced). Untreated children are normal at birth. In untreated infants, vomiting which at times projectile and irritability are frequent during the first 2 months of life. By 4–9 months, delayed intellectual development becomes apparent [9]. In the untreated classic case, mental retardation is severe, precluding speech and toilet training. Seizures, common in the more severely retarded, usually start before 18 months of age. During infancy, they often take the form of infantile spasms, later changing into tonic–clonic attacks. The untreated phenylketonuric child is blond and blue-eyed, with normal and often pleasant features. The skin is rough and dry, sometimes with eczema. Significant neurologic abnormalities are rare, although hyperactivity and autistic features are not unusual. Microcephaly may be present as well as a mild increase in muscle tone, particularly in the lower extremities. A fine, irregular tremor of the outstretched hands is seen in approximately 30% of the patients. Parkinsonian-like extrapyramidal symptoms also have been encountered. The plantar response is often extensor [10].

A variety of electroencephalographic (EEG) abnormalities have been found, but hypsarrhythmic patterns, recorded even in the absence of seizures, and single and multiple foci of spike and polyspike discharges are the most common [11]. MRI of the brain is abnormal in almost every patient, regardless of when treatment was initiated [12]. As PKU is the leading cause for severe morbidity and mental retardation although a preventable disease and to the best of our knowledge, there are few studies done on PKU in Upper Egypt, this research aimed to identify clinical profile and impacts of newly diagnosed (untreated) PKU on children in Sohag University Hospital.

2. Patients and methods

2.1. Study design

This is a single center observational Cohort Study, done in Pediatric Department, and Pediatric Neurology Clinic at Sohag University Hospital, Upper Egypt, during the period from January 2009 through June 2012. All infants and children clinically suspicious of having PKU during the study period were included. The diagnosis of PKU was established based on clinical manifestations and laboratory confirmation by measuring phenylalanine level in the blood sample. Consent was taken from the family to conduct this research and approved by the Faculty of Medicine, Sohag University Ethics Committee.

2.2. Methods

Phenylalanine assay in blood was done by using the Bio-Rad Laboratories products, Microplate Neonatal PHE/GAL(galactose)–Assay-(Test Kit 532-6053); for quantitative microplate assay of phenylalanine and galactose in the newborn blood to screen for phenylketonuria, galactosemia on Whatman 903 paper and level $\geqslant 3$ mg/dl was considered elevated. All studied patients were subjected to thorough clinical history including detailed history of the presenting symptoms like seizures and developmental history. Autistic symptoms, hyperactivity symptoms, and family history of similar condition, presence of epilepsy, mental retardation or global developmental delay were also clarified.

Full clinical examination (general, systematic, and detailed neurological examinations), and developmental assessment were done. Computed tomography of the brain (CT) and electroencephalography (EEG) were done for all patients. All patients were referred to the Phoniatric Unit and were subjected to Ear, Nose, and Throat (ENT) examination, language evaluation as well as assessment of passive and active vocabulary were done. Also evaluation of the autistic features using the American Psychiatric Association diagnostic criteria for autism [13] and Childhood Autism Rating Scale (CARS) was done; the degree of autistic disorders was set 30 serving as a cut off for the diagnosis of autism, mild-moderate autism (30–37) and severe autism (> 37) [14]. Psychometric evaluation was done using Vineland adaptive Behavior Scales [15] and Stanford–Binet Intelligence Scales [16]. Complete audiological evaluation was done in the Audiologic Unit.

3. Results

3.1. Descriptive data of the studied patients

This study was done on newly diagnosed cases of PKU and they did not receive any treatment or dietary management before. The cornerstone for diagnosis of our cases was measuring the blood phenylalanine level. The mean phenylalanine level in the studied cases was 15.80 ± 8.84 mg/dl, range 3.8–28.1. The total number of patients confirmed to have pheylketonuria (PKU) was 24 cases, fifteen of them (62.5%) were boys and nine (37.5%) were girls. Twelve cases (50%) had classic PKU (Phe level > 20 mg/dl), seven cases (29.2%) had mild PKU (Phe level 10–20 mg/dl), and five cases (20.8%) had mild hyperphenylalaninemia (MHP) (Phe level < 10 mg/dl) The age of presentation ranged from 0.08 to 11 years with mean age of presentation of 3.37 ± 3 years while the median age was 2.8 years.

3.2. Clinical features

Clinical features of the studied cases showed that the majority 16 of 24 cases (66.7%) had global developmental delay (affection of more than two developmental domains; motor, language, and social development), while 11 cases had

Figure 1 A 5.5 month old girl presented by motor delay, blond features, and microcephaly.

Figure 2 A 5.5 year old girl with global developmental delay and blond features.

Figure 3 An 11 year old boy presented by delayed language development in addition to other autistic and blond features.

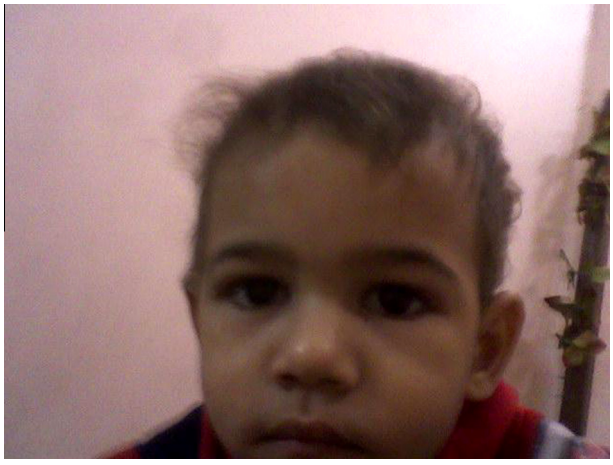

Figure 4 An 18 month old boy with global developmental delay, hyperactivity and blond features.

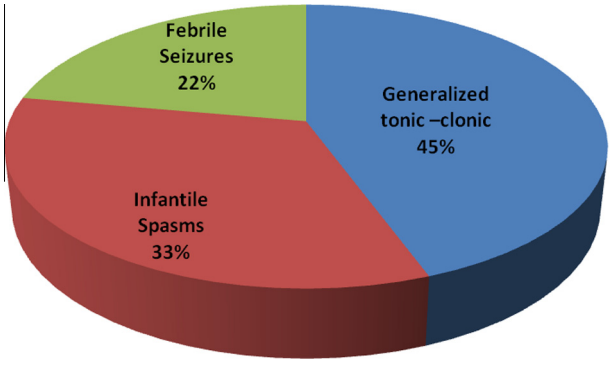

Figure 5 Chart showing types of seizures reported in the cases.

hyperactive symptoms. Seizures were reported in nine cases and included four cases with generalized tonic–clonic seizures, three cases with infantile spasms, and two cases with febrile seizures, and eight cases had autistic features. Isolated language delay without autistic features was found in two cases, moderate hearing loss was reported in two cases, microcephaly in three cases, while one case had delayed motor milestones, and one case had dysmorphic features (Figs. 1–5), (Table 1).

3.3. CT of the brain

CT study of the brain showed that 14 cases (58.3%) had brain atrophic changes, seven cases (29.2%) had normal imaging, two cases (8.3%) had white matter disease with brain atrophy while one case (4.2%) had brain atrophy with lissencephaly (Fig. 6), (Table 2).

3.4. EEG findings

EEG was done in all cases and showed that 10 cases (42.7%) had normal EEG finding, six cases had generalized epileptic discharges, five cases had focal epileptic discharges, and three cases had hypsarrhythmia pattern (Table 3).

Table 1 Clinical features of the included cases.

Clinical features	Number of cases	Percentages from total number (24) (%)
Global developmental delay	16	66.7
Hyperactivity symptoms	11	45.8
Seizures	9	37.5
Autistic features	8	33.3
Microcephaly	3	12.5
Isolated delayed language development	2	8.3
Hearing impairment	2	8.3
Dealyed motor development	1	4.2
Dysmorphic facial features	1	4.2

(It is to be noted that one case may have more than one presentation).

4. Discussion

In our study we prospectively diagnosed 24 cases with phenylketonuria. The disease was more common in boys than girls (62.5% versus 37.5%). The mean age of the studied patients was 3.37 years and this correlates with the study done in Tunisia by Khemir et al. [17], to evaluate the role of phenylketonuria in mental retardation. They found that, the PKU estimated frequency was 1/7631 in mentally retarded children with a mean age of 4 years. Our results showed that the mean phenylalanine level was 15.8 mg/dl while in Tunisia, the phenylalanine mean level was 28 mg/dl (1680 μmol/L), and in the Egyptian study done in the Menoufiya Governorate by El Araby et al. [8], the mean phenylalanine level was 3.19 mg/dl. Our results regarding mean age were lower than those of Karimzadeh et al. [18], as they evaluated 105 patients with the diagnosis of PKU and the mean age of the patients was 8.5 ± 6.2 years.

In our series, 50% of cases had classic PKU and the dominant clinical manifestations were global developmental delay, hyperactivity symptoms, autistic features, and seizures while in the Tunisian study [17], 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%).

37.5% of our cases had seizures; the commonest seizure type was generalized tonic–clonic. In the study done by Karimzadeh et al. [18], 52.3% had seizure and 47.7% were clinically seizure-free. Also we found that abnormal EEG was more common as detected in 52.4% of patients. Of these, generalized epileptic discharges were the commonest. These findings agree with the study done by Karimzadeh et al. [18], as they found

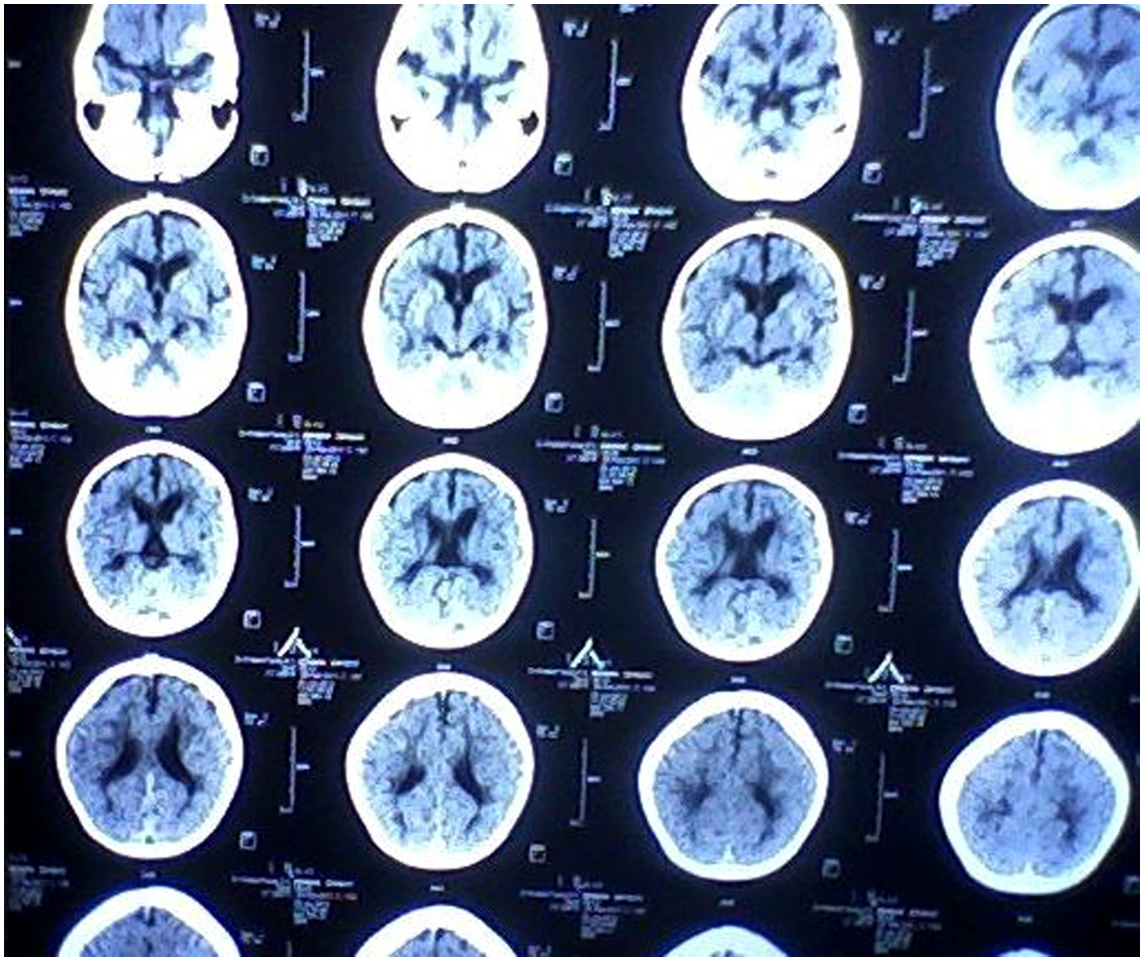

Figure 6 CT brain findings of one case showing diffuse brain atrophy.

Table 2 CT Brain findings of the included cases.

CT findings	Number of cases	Percentages
Normal findings	**7**	29.17
Abnormal Findings	**17**	70.83
Brain atrophic changes	14	82.35
White matter disease with brain atrophy	2	11.76
Brain atrophy with lissencephaly	1	5.88

Table 3 EEG findings of the included cases.

EEG findings	Number of cases	Percentages (%)
Normal	10	41.7
Abnormal	14	58.3
Generalized epileptic discharge	6	42.86
Focal epileptic discharge	5	35.71
Hypsarrhythmia	3	21.43

that 66.6% of their patients had abnormal EEG. About 45% of the patients had an abnormal EEG and nearly 30% had a normal EEG in the beginning which became abnormal later as stated by Gross et al. [11]. In an Egyptian study done by Abdel-Salam et al. [19], 25% of the patients had seizure, but more than 50% had an abnormal electroencephalogram, which means some PKU patients had an abnormal EEG without any clinical seizure. There is evidence that subclinical discharges can cause psychocognitive impairment and behavioral disturbance [18]. Karimzadeh and Tabarestani [20] reported negative effects of this epileptic discharge on the choice reaction time, verbal and nonverbal communication and behavioral disorder.

Behavioral abnormalities were also studied in our series as they represented significant clinical presentations of PKU. Hyperactivity manifestations were the commonest (45.8%) while autistic features were defined in 33.3%. In the study of Karimzadeh et al. [18], the behavioral–emotional scale evaluation showed that the frequency of behavioral disorder was 85.7% of cases and 42.8% in control patients. Some neuropsychological damage occurs even in treated PKU as reported by DeRoche and Welsh [21]; reaction times are delayed in PKU and this relates to concurrent elevated phe levels. They also said that there are other behavioral and psychiatric symptoms attributed to PKU. Poor dietary control early in life results in anxiety, hyperactivity and social withdrawal, and those with satisfactory early treatment still appear to have a higher risk of low self-esteem and possibly depression.

In our study CT of the brain showed that the majority (70.8%) of cases had abnormal findings, mainly brain atrophic changes and white matter disorders. These radiological findings obtained by CT were in agreement with radiological findings obtained by MRI of the brain in other studies [12,22–25].

5. Conclusion

We concluded that PKU still has adverse effects on children in Upper Egypt leading to developmental problems, mental retardation, and behavioral abnormalities. We recommend establishment of national screening programs and pushing them forward, immediate development of specific metabolic centers in various universities and research institutes especially in Upper Egypt.

References

[1] Waisbren S, Noel K, Fahrbach K, Cella C, Frame D, Dorenbaum A, et al. Phenylalanine blood levels and clinical outcomes in phenylketonuria: a systematic literature review and meta-analysis. Mol Genet Metab 2007;92:63–70.

[2] Gonzalez J, Willis MS. Ivar Asbjorn Folling discovered phenylketonuria (PKU). Lab Med 2010;41(2):118–9.

[3] Guldberg P, Henriksen KF, Sipila I, Guttler F, de la Chapelle A. Phenylketonuria in a low incidence population: molecular characterization of mutations in Finland. J Med Genet 1995;32(12):976–8.

[4] DiLella AG, Kwok SCM, Ledley FD, Marvit J, Woo SLC. Molecular structure and polymorphic map of the human phenylalanine hydroxylase gene. Biochemistry 1986;25(4):743–9.

[5] Ozalp I, Coşkun T, Tokatli A, Kalkanoğlu HS, Dursun A, Tokol S, et al. Newborn PKU screening in Turkey: at the present and organization for future. Turk J Pediatr 2001;43:97–101.

[6] Loeber JG. Neonatal screening in Europe; the situation in 2004. J Inherit Metab Dis 2007;30:430–8.

[7] Feillet F, Abadie V, Berthelot J, Maurin N, Ogier H, Vidailhet M, et al. Neonatal screening and long-term follow-up of phenylketonuria: the French database. Early Hum Dev 2001;65(2):149–58.

[8] El Araby H, Fateen E, Gouda A. Screening for phenylketonuria and galactosemia among Egyptian newborns in Menoufiya governorate, Egypt. Egypt J Med Hum Genet 2009;10(2):164.

[9] Partington MW. The early symptoms of phenylketonuria. Pediatrics 1961;27:465–73.

[10] MacLeod MD, Monroe JF, Ledingham JG, Farquhar JW. Management of the extrapyramidal manifestations of phenylketonuria with L-dopa. Arch Dis Child 1983;58:457–8.

[11] Gross PT, Berlow S, Schuett VE, Fariello RG. EEG in phenylketonuria. Attempt to establish clinical importance of EEG changes. Arch Neurol 1981;38(2):122–6.

[12] Cleary MA, Walter JH, Wraith JE, White F, Tyler K, Jenkins JP. Magnetic resonance imaging in phenylketonuria: reversal of cerebral white matter changes. J Pediatr 1995;127:251–5.

[13] American Psychiatric Association. Diagnostic and statistical manual of mental disorders. DSM-IV-TR. 4th ed., text revision. Washington, DC: American Psychiatric Association; 2000.

[14] Schopler E, Reichler R, Rochen B. The Childhood Autism Rating Scale (CARS). Los Angeles, CA: Western Psychological Service; 1988.

[15] Sparrow S, Balla D, Cicchetti D. Vineland adaptive behavior scales. Circle Pines, MN: American Guidance Service; 2004 [Statistics Norway].

[16] Terman LM, Merril MA. Stanford–Binet intelligence scale, manual for the third revision from L-M with revised IQ tables by Samuel R. Pinneau. Boston (MA): Houghton Mirfflin; 1960.

[17] Khemir S, El Asmi M, Sanhaji H, Feki M, Jemaa R, Tebib N, et al. Phenylketonuria is still a major cause of mental retardation in Tunisia despite the possibility of treatment. Clin Neurol Neurosurg 2011;113(9):727–30.

[18] Karimzadeh P, Alaee MR, Zarafshan H. The association between EEG abnormality and behavioral disorder: developmental delay in phenylketonuria. ISRN Pediatr 2012, 976206.

[19] Abdel-Salam GMH, Abdel-Kader AA, Inical LE. Electroencephalographic (EEG), neuroradiological and molecular (PKU) patients. Egypt J Neurol Psychiatry Neurosurg 2005;42(2):390–406.

[20] Karimzadeh P, Tabarestani S. Promising medical treatment for childhood psycho-cognitive problems. Neural Regen Res 2010;5(21):1663–7.

[21] DeRoche K, Welsh M. Twenty-five years of research on neurocognitive outcomes in early-treated phenylketonuria: intelligence and executive function. Dev Neuropsychol 2008;33:474–504.
[22] Thompson AJ, Smith I, Brenton D, Youl BD, Rylance G, Davidson DC, et al. Neurological deterioration in young adults with phenylketonuria. Lancet 1990;336:602–5.
[23] Cleary MA, Walter JH, Wraith JE, Jenkins JP, Alani SM, Tyler K, et al. Magnetic resonance imaging of the brain in phenylketonuria. Lancet 1994;344:87–90.
[24] Anderson PJ, Leuzzi V. White matter pathology in phenylketonuria. Mol Genet Metab 2010;99(1):S3–9.
[25] Pearsen KD, Gean-Marton AD, Levy HL, Davis KR. Phenylketonuria: MR imaging of the brain with clinical correlation. Radiology 1990;177:437–40.