CLINICO-EPIDEMIOLOGICAL STUDY OF PHENYLKETONURIA IN INFANTS AND CHILDREN: A RETROSPECTIVE STUDY

By

Ghada Abd El-Kawi Mahmoud*, Mostafa Abd El-Azeem Hassan* and Mohammed A. Al-Adawy*

*Pediatrics Department, Faculty of Medicine, Al-Azhar University (Assiut)

***Corresponding Author: Ghada Abd El-Kawi Mahmoud**

E-mail:

ABSTRACT

Background: *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, developmental delay, hyperactivity, autistic symptoms, blue eyes, rough and dry skin, albinism or blond hair and skin and a ''musty odor '' to the baby's urine and sweat.*

Aim of the Work: *To evaluate management of phenylketonuria in infants and children.*

Program and methods: *All patients diagnosed as phenylketonuria based on screening program, clinical and laboratory findings by measuring phenyl alanine level in the blood were retrospectively assessed through checking the files of patients at Assiut Genetic Counseling Centre and Al-Azhar Assiut University Hospital from 1st January 2016 to 28th February 2021, All files of patients were evaluated through full history taking, general and complete neurological examination and developmental assessment, serum phenyl alanine and tetrahydrobiopterin loading test was done in some cases. Intelligent Question (IQ), Electroencephalogram (EEG), Childhood Autistic Rating Scale (CARS), Attention Deficit Hyperactivity Disorder (ADHD) test and Brain Magnetic Resonance Imaging (MRI) were done in some cases.*

Results: *In our study we estimated 400 cases retrospectively with phenylketonuria, 240 of them were found to be less than 6 months old (60%) diagnosed by screening while 160 of them ages more than 6 months (40%) diagnosed with clinical suspicion plus elevated serum phenyl alanine with mean age ± SD (1.99± 1.32) years. 204 cases were males (51%) and 196 cases were females (49%). 276 cases (69%) lived in rural areas and 124 cases (31%) lived in urban areas. 230 cases (57.5%) were offsprings of consanguineous parents. 250 cases (62.5%) had similar condition in their families. 160 cases (40%) were diagnosed by clinical presentation which included blond hair and other findings as follows: blond hair and autism in 24 cases (15%), blond hair and mental retardation in 60 cases (37.5%), blond hair and hyperactivity in 34 cases (21.25%), blond hair and seizures 29 in cases (18.125%), blond hair and bad odor*

urine 13 cases (8.125%). 132 cases (33%) had Phenyl alanine (Phe) level more than 1200µmol/L (classical PKU), 107 cases (26.8%) had Phe level between 900&1200µmol/L (moderate PKU) and most of them 161 cases (40.3%) had Phe level between 600&900µmol/L (mild PKU). 308 cases (77%) were compliant to dietary supplement and healthcare recommendations and 92 cases (23%) were non-compliant.

Conclusion: *Mild PKU was the most common form (40%) followed by the classic form (33%). Most of diagnosed cases of PKU asked for dietary supplementation and health care recommendations (77%). The development of Egyptian neonatal screening programs demonstrated how effective treatment can lead to a near normal outcome for affected individuals.*

Key words: *phenylketonuria, Egyptian screening programs, inborn error of metabolism.*

INTRODUCTION

Phenylketonuria is an autosomal recessive metabolic genetic disorder characterized by mutation in the gene for the hepatic enzyme phenyl alanine hydroxylase (PAH) **(Williams, et al., 2008)**. This enzyme is necessary to metabolize the amino acid tyrosine. When PAH activity is reduced, phenylalanine accumulates and is converted into phenyl pyruvate (also known as phenyl ketone), which is detected in the urine **(Gonzalez. Willis and Ivarasbjornfolling., 2010)**.

Newborn screening for PKU began with the use of a bacterial bioassay. Subsequently greater sensitivity has been achieved by the implementation of fluorometric methods and tandem mass spectrometry. This methodology makes PKU screening effective, reliable, and efficient **(Abdel –Salam, et al., 2005)**.

PKU was first described by Asbjørn Følling, one of the first Norwegian physicians to apply chemical methods to the study of medicine **(Centerwall and Centerwall., 2000)**.

In the untreated classic PKU cases, mental retardation is severe, precluding speech and toilet training. seizures, common in the more severely retarded cases, usually start before 18 months of age, during infancy they often take the form of infantile spasm later changing into tonic- clonic attacks. the untreated phenyketoneuric 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, also hyperactivity and autistic features are not unusual. microcephaly

may be present as well as mild increase in muscle tone, particularly in the lower extremities. Coma and irregular tremors of the outstretched hand are seen in approximately 30 % of the patients. Parkinsonian – like extrapyramidal symptoms also have been encountered. the planter response is often extensor **(Deroche and Welsh., 2008)**.

PKU Patients need to maintain normal, physiological levels of phenylalanine and tyrosine for life. Studies have shown that of elevated phenylalanine affect brain development. Newborns diagnosed as PKU should begin dietary treatment as soon as possible. Commercial PKU formulas and various phenylalanine –restricted foods are available. phenylalanine (and tyrosine) should be measured on a regular basis to follow dietary control, because the diagnosis and therapy of PKU are complex, the pediatrician is advised to manage the patient in close collaboration with a consulting pediatric metabolic disease specialist and dietician. It is recommended that parent travel with a letter of treatment guidelines from the patient's physician **(Karimzadeh and Tabarestani., 2010)**.

Magnetic resonance imaging (MRI) enables observing abnormalities in patients with PKU. The typical brain MRI findings of PKU are T2-weighted hyperintense lesions, located in parieto-occipital regions **(Scarabino, et al., 2009)**. In previous studies, it was stated that with early diagnosis and good dietary compliance, the development of white matter lesions could be prevented and even may resolve **(Manara, et al., 2009)**. But recent studies based on diffusion tensor imaging (DTI) stated that even in early-diagnosed PKU patients with dietary compliance, the macroscopic and microscopic white matter damage process may persist **(González, et al., 2018)**.

STUDY METHODS

Ethical consideration:

1. A written informed consent was obtained from patients or their legal guardians.
2. An approval by the local ethical committee was obtained before the study.
3. The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.
4. All the data of the patients and results of the study are confidential, and the patients have the right to keep it.

5. The authors received no financial support for the research, authorship, and/or publication of this article.

Inclusion criteria: All Phenylketonuria Patients (infants and children) below 16 years old, in the period between 1st of January 2016 to 28th of February 2021 (400 PKU cases).

Exclusion criteria:

- Phenylketonuria patients older than 16 years old.
- Patients with other metabolic or genetic diseases associated with hyperphenylalaninemia.

Diagnosis of phenylketonuria was established based mainly on screening program and to less extent to clinical and laboratory finding by measuring phenyl alanine level in the blood.

All studied patients were subjected to the following:

Full history including:

- Name, birth date, type of delivery, weight at birth, father name, age and work, mother name, age and work, residence, phone number, antenatal care, delivery place, birth complication, birth order, consanguinity and presenting symptoms like seizures and developmental history, autistic symptoms, hyperactivity symptoms, family history of similar condition, presence of epilepsy, mental retardation or global developmental delay are all things to consider.

Clinical examination: which include:

- General, systematic, detailed neurological examination, and developmental assessment.

Lab. Evaluation including:

- Measuring serum phenylalanine and tyrosine levels routinely done in the first week of life in Egypt since 2015, follow up of serum phenylalanine –tyrosine levels at regular times to evaluate the response to special formula, it is type and quantity. Tetrahydrobiopterin loading test was done in some cases with hyperphenylalaninemia that not clinically responsive to a phenylalanine-restricted diet Lastly. IQ, EEG, neuroimaging, ADHD test, Brain Magnetic Resonance Imaging (MRI) may be done if needed in some cases.

Statistical analysis:

(SPSS) version 24.was used to analyze the data (Quantitative data were expressed as mean ±SD). Qualitative data were expressed as frequency and percentage. Mean (average): the central value of a

discrete set of numbers, specifically the sum of values divided by the number of values. Standard deviation (SD): is the measure of dispersion of a set of values. A low SD indicates that the values tend to be close to the mean of the set, while a high SD indicate that the values are spread out over a wider range furthermore, numerical data was equated using the Independent - samples t-test of significance and P-value < 0.05 was considered statistically significant, while P-value < 0.001 was considered as highly significant.

RESULTS

Our result will be demonstrated in the following Tables and figures.

Table (1): Demographic data of studied patients with phenylketonuria

		No of patients	Percent (%)
Age at time of diagnosis	Less than 6 months	240	60 %
	6 months - 2 years	20	5%
	2 years - 6 years	60	15%
	6 years - 12 years	65	16.25%
	12 years - 16 years	15	3.75%
Gender	Male	204	51%
	Female	196	49%
Residence	Urban	124	31%
	Rural	276	69%
Consanguinity	+ve	230	57.5%
	-ve	170	42.5%
Similar conditions	+ve	250	56.5%
	-ve	150	37.5%

Table (1) Showed that PKU diagnosis is most common in age less than 6 months. Both male and female are nearly affected equally. Also, PKU is more common in rural than urban population. Positive consanguinity and similar familial conditions were founded in 57.5%, 56.5 % respectively.

Table (2): Clinical presentation of non-screened phenylketonuria at time of diagnosis no= 160

Clinical presentation of non-screened phenylketonuria at time of diagnosis		
Blond hair association	No of patients	Percent (%)
Autism	24	15%
Mental retardation	60	37.5%
Hyperactivity	34	21.25%
Seizure	29	18.125%
Bad urine odor	13	8.125%
Total	160	100%

In this study (160) cases (40%) diagnosed as phenyl ketonuria by clinical presentation and confirmed by measuring of serum phenyl alanine level, they presented with blond hair and either autism, mental retardation, hyperactivity, seizures and bad urine odor in 24 cases (15%), 60 cases (37.5%), 34 cases (21.25%), 29 cases (18.125%) and 13 cases (8.125%) respectively.

Table (3): Serum phenylalanine level at time of diagnosis and severity of PKU of all studied patients

Serum phenylalanine level	No of patients	Percent (%)
>1200 µmol/L(classical PKU)	132	33
900-1200 µmol/L(moderate PKU)	107	26.8
600-900 µmol/L(mild PKU)	161	40.2
Total		100

Phenylketonuria (PKU)

As shown from table (3) the 400 Phenylketonuria cases in this study could be classified as mild, moderate and severe according to serum phenylalanine levels at time of diagnosis which were 600-900µmol/L, 900-1200µmol/L and >1200 µmol/L in 40.2%, 26.8% and 33% respectively.

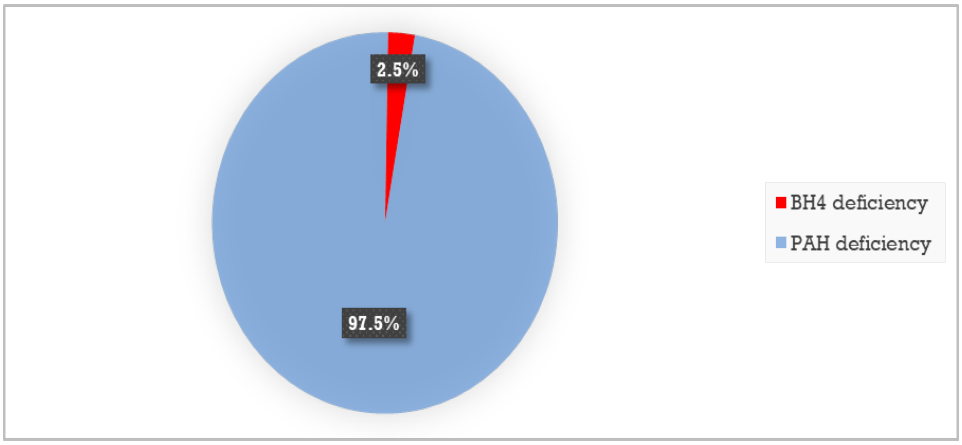

Figure (1): Percentage of phenylketonuria according to enzymatic deficiency

Table (4): Dietary compliance in PKU cases

	Number of patients	Percent (%)
Compliant	308	77%
not compliant	92	23%
Total	400	100%

Table (4) Showed that 308 out of 400 cases of PKU (77%) followed the dietary restriction were founded to be compliant while the remaining 92 cases (23%) were founded to be non-compliant.

Table (5) Relations between parent education level and diet compliance in PKU

Dietary compliance versus Parents education level			Parents education level		Total No 400	P. value
			Educated parents	Not educated		
Dietary compliance	Compliant	Number	260	48	308	P<0.05
		% within Dietary compliance	84.4%	15.6%	77 %	
	Not compliant	Number	0	92	92	
		% within Dietary compliance	0.0%	100.0%	23 %	
Total		Total Number	260	140	400	
		% within Dietary compliance	65.0%	35.0%	100.0%	

Table (5) Showed that the majority of studied cases 308 out of 400 (77 %) of cases of PKU found to be dietary compliant, of them 84.4% and 15.6% were belonged to educated and non-educated parents respectively, while 92 cases (23%) were belonged to non-educated parents were founded to be exclusively non dietary compliant.

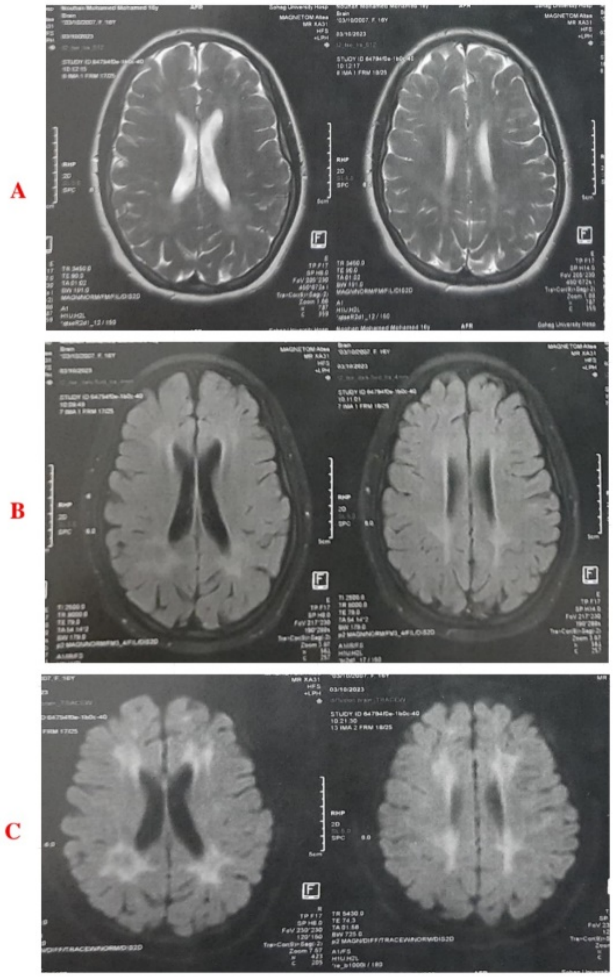

Figure (2): Brain Magnetic Resonance Imaging (MRI) findings of patient number (19): 16 years old female diagnosed as classic PKU at age of 4 years old with moderate mental delay, not compliant to low phenyl alanine diet. Axial brain T2 (A), FLAIR (B) and diffusion (C) at the ventricular level: The images show bilateral periventricular white matter increased T2/FLAIR signal, with corresponding diffusion restriction. Normal brain cortex and no other abnormalities.

DISCUSSION

In this study we had 400 cases diagnosed as phenylketonuria classified to five age groups, first group less than six months which diagnosed by screening included 240 cases (60%), second group 20 cases (5%) from six months to two years, third group from 2 to 6 years 60 cases (15%), fourth group from 6 to 8 years 65 cases (16.25%) and the last group more than 8 years 15 cases (3.75%), as shown at table (1) this agreed with the study done by **Yong-An Zhou, et al., 2012** who found that 38 patients out of 59 with phenylketonuria their ages less than 2 years old , 18 patients 2-6 years old and 3 patients their ages 6 -10 years old.

In our study we evaluated 400 cases retrospectively with phenylketonuria 204 cases were found to be males (51%) and 196 cases were found to be females (49%) as shown in **table (1)**, this agreed with the study done by **Yong-An Zhou, et al., 2012** who found that 30 out of 59 patients with phenylketonuria were males and 29 were females identified during treatment at the Neonatal Screening Center of the Shanxi Province Women and Children's Hospital in Taiyuan and came from various regions of Shanxi province.

Regarding residence, 276 cases (69%) lived in rural areas and 124 cases (31%) lived in urban areas as shown in **table (1)** and this agreed with the study done by **Fathollahpour, et al., 2019**, who showed that higher incidence of PKU in rural area than urban area and this may attributed to more consanguineous marriage in rural area with low socioeconomic status and low education level of parent and this may be disagreement with study in Kurdistan Epidemiology of phenylketonuria and its related factors in Kurdistan province during 2012-2014 The prevalence of PKU was higher in urban than in rural areas, which was not significant (P = 0.976) **(Fathollahpour, et al., 2019)**.

In the present study 230 cases (57.5%) and 250 cases (62%) of PKU had offspring of consanguineous parents and similar familial condition respectively, these results supported the finding of other works as **Mokhtari & Bagga., 2003** who stated that consanguinity, genetic disorders and malformations are common in the Iranian population, also **Shokri, et al., 2020** and **Treacy, et al., 1996** found that phenylketonuria were encountered exclusively in children of consanguineous couples.

In this study PKU cases due to PAH deficiency were the most frequent cases (97.5%) and (2.5%) of cases due to BH4 deficiency as shown in **Figure (1)** also we noticed a little improvement with therapeutic formula and this agreed with the study done by **Moradi, et al., 2013**. The proportion of tetrahydrobiopterin deficiency and PAH gene deficiency variants among cases with hyperphenylalaninemia in Western Iran as total of 30 patients showed PAH deficiency and two patients were diagnosed with BH4 deficiency (BH4/HPA ratio = 6.25%). Both of these two BH4-deficient patients were assigned to severe variant of dihydropteridine reductase (DHPR) deficiency. More than 75% of patients with PAH deficiency classified as classic phenylketonuria (PKU) according to their levels of pre-treatment phenylalanine concentrations **(Moradi, et al., 2013)**.

In the current study, 160 out of 400 cases (40%) presented by blond hair at time of diagnoses of PKU in association with either autism, mental retardation, hyperactivity, seizures and bad urine odor in 24 cases (15%), 60 cases (37.5%), 34 cases (21.25%), 29 cases (18.125%) 13 cases (8.125%) respectively as shown **table (2)** and this agreed with the study done by **(Deborah, et al., 2016)**.

In the present study, regarding phenylalanine level at time of diagnosis it showed that 132 cases (33%) had Phe level (>1200 μmol/L) (classical PKU), 107 cases (26.8%) had Phe level (900-1200μmol/L) (moderate PKU) and the majority of them 161 cases (40.2%) had Phe level 600-900μmol/L (mild PKU) as shown table (3) and this agreed with the study done by **(Foreman, et al., 2021)**.

Also, an obvious relationship were found between parent's education level and diet recommendation compliance for their offspring's as 260 cases (84.4%) had well educated parents were compliant for dietary recommendation otherwise 92 cases (23%) who were non-compliant had non educated parents with significant (p value (<0.05) as shown in table (5) and this agreed with the study done by **Alaei et al., et al., 2011**, which showed that there is positive correlation with parent education & dietetic recommendation compliance **(Alaei, M et al., 2011)**.

CONCLUSION

Mild PKU was the most common form (40%) followed by

the classic form (33%) Most of diagnosed cases of PKU ask for dietary supplementation and health care recommendations (77%). The development of Egyptian neonatal screening programs demonstrated how effective treatment can lead to a near normal outcome for affected individuals.

RECOMMENDATION

- Ealy and restrict screening of PKU in all neonates.
- Education of parents of PKU patients about dietetic treatment of their infants.
- Continuous follow up of PKU patients.

LIMITATIONS

- Difficulties in obtaining approval of MOH for this study.
- Difficulties in doing some investigation e.g, MRI., genetic studies.
- Expenses of some investigations e.g. genetic (whole exome sequencing).

REFERENCES

1. **Abdel–Salam, Abdelkader and Inical (2005):** Electroencephalographic sneuroradiological and molecular (pku)patients Egypt neurol psychiatry neurosurg ,42(2) pp.390-406.
2. **Alaei, M., Asadzadeh-Totonchi, G., Gachkar, L., & Farivar, S. (2011):** Family social status and dietary adherence of patients with phenylketonuria. Iranian Journal of Pediatrics, 21(3), 379.
3. **Centerwall SA and Centerwall WR (2000);** The discovery of phenylketonuria: the story of a young couple, two retarded children, and a scientist. Pediatrics (105) pp 89-103.
4. **Deborah A. Bilder, J. Kay Noel, Erin R. Baker, William Irish, Yinpu Chen, Markus J. Merilainen, Suyash Prasad & Barbara J. Winslow, B. J. (2016):** Systematic review and meta-analysis of neuropsychiatric symptoms and executive functioning in adults with phenylketonuria. Developmental neuropsychology, 41(4), 245-260.
5. **Deroche and Welsh (2008):** twenty-five years of researching 504 neurocognitive outcomes in early treated phenylketonuria intelligenceppn. 747.
6. **Fathollahpour, A., Ataee, P., Touzandeh Jani, S., Akbari, I., Fathollahpour, A., & Khazaei, Z. (2019):** Epidemiology of phenylketonuria and its related factors in Kurdistan province during 2012-2014. International Journal of Biomedicine and Public Health, 2(2), 37-40.
7. **Foreman, P. K., Margulis, A. V., Alexander, K., Shediac, R., Calingaert, B., Harding, A., & Landis, S. (2021):** Birth prevalence of phenylalanine hydroxylase deficiency: a systematic literature

review and meta-analysis. Orphanet Journal of Rare Diseases, 16(1), 253.

8. **González MJ, Polo MR, Ripollés P, et al (2018):** White matter microstructural damage in early treated phenylketonuric patients. Orphanet J Rare Dis;13(1):1 12.
9. **Gonzalez. Willis and Ivarasbjornfolling (2010):** discovered phenylketonuria lab med ,41 (2) pp.118-119.
10. **Karimzadeh And Tabarestani (2010):** promising medical treatment for child hood psycho-cognitive problems neural regen res, 5(21) pp.1663-1667 hydroxylase gene in patients with phenylketonuria in Shanxi, China. Genetics and molecular biology, 35, 709-713.
11. **Manara R, Burlina AP, Citton V, et al. (2009):** Brain MRI diffusion-weighted imaging in patients with classical phenylketonuria. Neuroradiology;51(12):803 812. (10.1007/s00234-009-0574-z).
12. **Mokhtari, R., & Bagga, A. (2003):** Consanguinity, genetic disorders and malformations in the Iranian population. Acta Biologica Szegediensis, 47(1-4), 47-50.
13. **Moradi, K., Alibakhshi, R., & Khatami, S. (2013):** The proportion of tetrahydrobiopterin deficiency and PAH gene deficiency variants among cases with hyperphenyalaninemia in Western Iran. Indian journal of human genetics, 19(4), 454.
14. **Scarabino T, Popolizio T, Tosetti M, et al (2009):** Phenylketonuria: white-matter changes assessed by 3.0-T magnetic resonance (MR) imaging, MR spectroscopy and MR diffusion. Radiol Med;114(3):461 474. (10.1007/s11547-009-0365-y)
15. **Shokri, M., Karimi, P., Zamanifar, H., Kazemi, F., Badfar, G., & Azami, M. (2020):** Phenylketonuria screening in Iranian newborns: a systematic review and meta-analysis. BMC pediatrics, 20(1), 1-16.
16. **Treacy, E., Pitt, J. J., Seller, K., Thompson, G. N., Ramus, S., & Cotton, R. G. H. (1996):** In vivo disposal of phenylalanine in phenylketonuria: a study of two siblings. Journal of inherited metabolic disease, 19(5), 595-602.
17. **Williams, R. A., Mamotte, C. D., & Burnett, J. R. (2008):** Phenylketonuria: an inborn error of phenylalanine metabolism. The Clinical Biochemist Reviews, 29(1), 31.
18. **Yong-An Zhou, Yun-Xia Ma, Quan-Bin Zhang, Wei-Hua Gao, Jian-Ping Liu, Jian-Ping Yang, Gai-Xiu Zhang, Xiao-Gang Zhang, and Liang Yu (2012):** Mutations of the phenylalanine hydroxylase gene in patients with phenylketonuria in Shanxi, China, Genet Mol Biol v.35(4); Dec PMC3526075.