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Neurodevelopmental Outcome of Children with Phenylketonuria after Early Neonatal Screening

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ABSTRACT

Background: Phenylketonuria (PKU) is an autosomal recessive (AR) disorder of phenylalanine (Phe) metabolism. Increased blood Phe levels could be associated with harmful actions on the cerebral function. Newborn screening (NBS) programs allow proper recognition and management of PKU with low-Phe diet.

Objective: This study aimed to assess neurological development, behavioral and dietary regimen of patients with phenylketonuria with positive neonatal screening compared to patients with phenylketonuria without neonatal screening.

Patients and methods: This observational study was conducted on a total of 40 children with PKU and were divided into two groups: Screening group (n=27) and non-screening group (n=13). All patients were subjected to complete history taking, special dietary regimen, developmental milestone examination, neurological examination, IQ test assessment, behavior change evaluation and laboratory investigations.

Results: There were significant increases in motor affection hyperreflexia and developmental millstones in non-screening group. ADHD demonstrated insignificant difference between both groups. There was significant increase in phenylalanine level in non-screening group compared to screening one before treatment and 6 months after treatment, while no significant difference was recorded at 12 months after treatment. There was a statistically significant correlation between Phe level and IQ before and 12 months after treatment. IQ was higher among non-ADHD cases compared to attention deficit hyperactivity disorder (ADHD) ones.

Conclusion: The current study concluded that PKU still has adverse effects on children in the context of motor function, developmental milestone, ADHD development and IQ affection. Early screening seemed to be associated with promising outcomes.

Keywords: Phenylketonuria, Early neonatal screening, Phenylketonuria, Stanford–Binet fifth edition,

INTRODUCTION

Phenylketonuria (PKU) has been considered as common disease caused by an inborn error in amino acid metabolism (AAM). It happens owing to mutation in the phenylalanine hydroxylase (PAH) gene. It has many forms, which differ from a mild increase in blood Phe values to a severe phenotype with extensive hyperphenylalaninaemia, which if left without proper treatment results in extensive and permanent mental disability [1].

It is the commonest autosomal recessive (AR) disorder of AAM and is caused by hepatic PAH deficiency [2]. In PKU cases, Phe accumulates in their blood and brain, and, if left without treatment, they may complain of extensive mental retardation [3]. PKU is a heterogeneous diseases ranging from mild hyperphenylalaninemia to severe PKU [1].

Various mutations in the PAH gens are responsible for different phenotypes in PAH deficiency. More than 830 variants in the therapeutic range of the PAH gene, primarily attained by a diet low in natural protein and synthetic amino acid supplementation without Phe [4]. PKU cases, if left without treatment, could acquire several forms of impairment, such as behavioral, mental, neurologic, and physical manifestations [5].

Newborn screening (NBS) is a public health program of screening for situations, which are treatable, but not clinically obvious in the newborn period. Robert introduced the 1st NBS strategy for PKU [6]. Congenital hypothyroidism was the 2nd disorder widely added in the1970 [7].

In Egypt, the implementation of the screening program was expanded to involve all 27 Governorates by the end of 2003 [8]. If diagnosis and management of PKU initiated directly following labor, dietary treatment can prevent complication. On the other hand, if treatment has been improper for long periods, cases with PKU could acquire several consequences such as limb spasticity and ataxia, tremors, encephalopathy and blurred vision [9]. This work aimed to assess neurodevelopmental, behavioral outcome of patients with phenylketonuria with positive neonatal screening compared with patients with phenylketonuria without neonatal screening.

PATIENTS AND METHODS

This was a cross sectional observational study that was conducted on 40 cases aged less than 18 years old with PKU who were examined at Mansoura University Children's Hospital by clinical and Phe level through the period from Jan 2022 to Jan 2023. They were divided into two groups: screening group (n=27) and non-screening group (n=13).

Exclusion criteria: Patients with other metabolic disorders, with chromosomal disorders, with mental retardation of unknown origin and patients whose parents refused to participate.

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METHODS

All patients were subjected to complete history taking, which included age, sex, consanguinity, positive family history and dietary regimen (PKU 1, PKU 2, Mixed and normal). Developmental milestone was also examined (normal or delayed). Neurological examination included motor and sensory systems and reflexes. Behavior changes included depression, anxiety, phobic tendencies, and attention deficit hyperactivity disorder. Laboratory investigations included Neonatal screening and follow up of Phe. IQ test score that failed below seventy was considered low IQ, whereas a score more than 140 was considered high IQ.

Stanford–Binet Fifth Edition (SB5) is utilized for diagnosis of developmental or intellectual deficiencies in young children, contrasting to the Wechsler Adult Intelligence Scale (WAIS). The test assesses 4 weighted factors and is composed of verbal and non-verbal subtests [10].

Attention-deficit/hyperactivity disorder (ADHD):

ADHD is classified into three forms with various rates of prevalence. PKU and ADHD are highly heritable situations, though those with PKU experience ADHD at a rate that is about twofold higher than that of the general population [11].

Diagnostic criteria of ADHD:

1. Cannot stay seated when needed, 2. Feels restless, 3. Has no ability to play in a quiet manner, 4. Often "on the go", 5. Talks excessively, 6. Impatiently blurts out answers without finishing question, 7. Cannot await turn, 8. Interrupts the others, 9. Has no ability to pay attention to details, 10. Has minimal attention when working or playing, 11. Doesn't seem to listen when spoken to, 12. Cannot follow instructions and has no ability to finish work, 13. Cannot organize tasks and activities, 14. Avoid tasks, which need concentration, 15. Misses things required for tasks and 16. External stimuli such as unrelated beliefs may distract the patient [12].

Assessment of Phe level: We classified PAH deficiency into mild hyperphenylalaninemia (Phe level 120–360 μmol/l; not need treatment) and PKU (> 360 μmol/l), that can be further classified as BH4-responsive PKU or BH4-non-responsive PKU [13]. In NBS, on Whatman 903 filter paper, blood from a heel stick was detected and dried in preparation for screening. The sample period fell between the third and seventh day of life. Following the instructions provided by the NeoBaseTM none-derivatized MS/MS kit, dried blood spots were preprocessed before being examined using the TQD MS/MS system and the NeoBaseTM non-derivatized system. The analyses involve Phe, tyrosine, and acylcarnitines [14]. The positive cases were undergone plasma amino acid analysis by utilizing chromatography to detect Phe values. Detection of urinary pterin values could be utilized to assess BH4 deficiencies [15].

Ethical approval: All patients' data were confidential. Appropriate de-identification of the records were implemented. Data were only used for the purpose of this research. Informed verbal consents were taken from all patients' caregivers and the patients themselves when appropriate before any history taking or investigations. The Helsinki Declaration was observed throughout the study's operations.

Statistical analysis

Data analysis was conducted by SPSS software version 25.0. Qualitative data were described using number and percent. Quantitative data were defined by utilizing median for non-normally distributed data and mean ± SD for normally distributed data. Normality was measured by utilizing Kolmogorov-Smirnov test. Chi-Square and Fisher exact tests were utilized to compare qualitative data between groups. Mann Whitney U and Kruskall Wallis tests were utilized to compare between 2 studied groups and more than 2 studied groups, respectively. Wilcoxon signed Rank test and Friedman tests were utilized to compare between more than two studied periods. The Spearman's correlation was utilized to determine the strength and direction of a linear relationship. In terms of all the previously utilized tests. P was considered significant when its value ≤ 0.05.

RESULTS

Table **(1)** demonstrated demographic characteristics of the studied cases of phenylketonuria. The mean age was 5.03 ± 2.37 years. Male to female (M/F) ratio was 1.2/1. Positive consanguinity and positive family history were recorded in 60% and 37.5% of cases respectively. Regarding motor function development, most of the studied cases were normal (82.5%), while convulsion and hypotonia were recorded in 5% and 12.5% respectively. All cases had normal sensory function development. Regarding reflexes, Hyperreflexia was recorded in 15% of cases. Delayed developmental milestones were recorded in 32.5% of cases. The mean IQ of the studied cases was 79.8 ± 14.44. The distribution of the studied cases according to behavior, ADHD was recorded in 52.5% of cases, while 47.5 of which were normal.

Table (1): Demographic characteristics and neurological criteria of the studied cases of phenylketonuria

	n=40	%
Age / years	5.03±2.37 (2-9)	
Sex		
male	22	55.0
female	18	45.0
Consanguinity		
-ve	16	40.0
+ve	24	60.0
Family History		
-ve	25	62.5
+ve	15	37.5
Motor		
Normal	33	82.5
Convulsion	2	5.0
Hypotonia	5	12.5
Sensory (normal)	40	100
Reflex		
Normal	34	85.0
Hyperreflexia	6	15.0
Developmental Milestones		
Normal	27	67.5
Delayed	13	32.5
IQ	79.8±14.44 (55-109)	
Behaviour		
Normal	19	47.5
ADHD	21	52.5

Table **(2)** revealed demographic characteristics between screened cases and non-screened cases. There was a significant decrease in age among screening group compared to non-screening one (P<0.05), while no significant differences were recoded as regards the remaining parameters (sex, consanguinity and family history) (P > 0.05).

There were significant changes in motor affection (Either convulsion or hypotonia), hyperreflexia and developmental milestones in non-screening group compared to screening one. However, ADHD demonstrated insignificant difference between both groups. There was a significant increase in IQ in screening group compared to non-screening one.

There were significant increases in Phe level in non-screening group compared to screening one at basal and 6 months, while no significant difference was recorded at 12 months.

Table (2): Demographic characteristics, Neurological criteria and Phenylalanine level between screened cases and non-screened cases

	Screening (n=27)	Non screening (n=13)	Test of significance
Age / years	3(2-6) (2.4-4.5)	8(7-9) (7-8.5)	z=5.09 p=0.001*
Sex Male Female	14(51.9) 13(48.1)	8(61.5) 5(38.5)	x^2=0.333 P=0.564
Consanguinity -ve +ve	13(48.1) 14(51.9)	3(23.1) 10(76.9)	x^2=2.29 P=0.130
Family history -ve +ve	17(63) 10(37)	8(61.5) 5(38.5)	x^2=0.008 P=0.931
Motor Normal Convulsion Hypotonia	27(100) 0 0	6(46.2) 2(15.4) 5(38.5)	MC=17.62 P<0.001*
Reflex Normal Hyperreflexia	27(100) 0	7(53.8) 6(46.2)	x^2=14.66 P<0.001*
Developmental milestones Normal Delayed	27(100) 0	0 13(100)	
Age at diagnosis	birth	4 years (3-6) (3.75-5.5)	
Behaviour Normal ADHD	15(55.6) 12(44.4)	4(30.8) 9(69.2)	x^2=2.16 p=0.141
IQ	88 (66-109)	65 (55-75)	z=4.78 P<0.001
Phenylalanine level (mmol/L) Basal	416 (118-1448)	700 (300-1050)	z=2.56, p=0.01*
6 months	360 (300-1440)	712 (300-900)	z=2.79, p=0.005*
12 months	330 (200-1440)	600 (212-1150)	z=1.64, p=0.102

*Statistically significant.

Table (3) displayed Phe level (mmol) change from basal, after 6 & 12 months follow up. There were no statistically significant differences either between basal and after 6 months or between basal and after 12 months, while significant decrease in Phe level was recorded between 6 & 12 months follow up.

Table (3): Phenylalanine level change from basal, after 6 & 12 months follow up

	Basal	**after 6 months**	**after 12 months**	**Test of significance**
Phenylalanine level (mmol/L)	492.5 (118-1448)	450 (300-1440)	348.5 (200-1440)	p1=0.811 p2=0.102 p3=0.005*

Z: Wilcoxon signed rank test, *statistically significant, p1: difference between basal and after 6 months, p2: difference between basal and after 12 months, p3: difference between 6 & 12 months follow up, Phe: phenylalanine.

Table (4) demonstrated demographic characteristics between non-ADHD and ADHD cases. There were no statistically significant differences between both groups regarding all demographic characteristics (age, sex, consanguinity and family history) (P>0.05). There were no statistically significant differences between both groups regarding motor functions, reflexes, developmental milestones. However, IQ was significantly increased among non-ADHD cases compared to ADHD ones (P<0.001).

Table (4): Demographic characteristics and Neurological criteria between Non-ADHD and ADHD cases

	Non-ADHD (n=19)	**ADHD (n=21)**	**Test of significance**
Age / years	4.1(2-9) (3-6)	6(2-9) (2.5-8)	z=0.803 p=0.422
Sex Male Female	 12(63.2) 7(36.8)	 10(47.6) 11(52.4)	 x^2=0.973 P=0.324
Consanguinity -ve +ve	 9(47.4) 10(52.6)	 7(33.3) 14(66.7)	 x^2=0.819 P=0.366
Family history -ve +ve	 13(68.4) 6(31.6)	 12(57.1) 9(42.9)	
Motor Normal Convulsion Hypotonia	 16(84.2) 1(5.3) 2(10.5)	 17(81) 1(4.8) 3(14.3)	 x^2=0.131 P=0.937
Reflexes Normal Hyperreflexia	 17(89.5) 2(10.5)	 17(81) 4(19)	 FET=0.568 p=0.451
Developmental milestones Normal Delayed	 15(78.9) 4(21.1)	 12(57.1) 9(42.9)	 x^2=2.16 P=0.141
IQ	90(65-109) (77-95)	75(55-87) (60-81)	z=3.89 p<0.001*

Z: Mann Whitney U test, *statistically significant, x2=Chi-Square test.

Table (5) demonstrated that, Phe level between non-ADHD and ADHD cases. There was no statistically significant difference between both groups at basal and 6 months, while after 12 months, non-ADHD group was associated with a significant decrease in Phe level compared to ADHD group.

Table (5): Phenylalanine (Phe) level between non-ADHD and ADHD cases of PKU

	Non-ADHD	**ADHD**	**Test of significance**
Phenylalanine (mmol/L)			
Basal	416(170-1448)	600(118-1200)	z=1.76, p=0.08
6 months	350(300-1210)	543(300-1440)	z=2.99, p=0.003
12 months	300(200-1010)	450(300-1440)	z=3.79, p<0.001*

Z: Mann Whitney U test, *statistically significant.

Table (6) demonstrated the correlation between Phe level (mmol) and IQ. There was a statistically significant negative correlation between Phe level and IQ at basal as well as at 12 months after treatment.

Table (6): Correlation between phenylalanine level (mmol/L) and IQ

	rs	p value
Phenylalanine		
Basal	-0.580	<0.001*
12 months	-0.672	<0.001*

Rs: Correlation coefficient, *statistically significant

DISCUSSION

Phenylketonuria (PKU), is an AR disorder of Phe metabolism, which is featured by impaired PAH activities. If left without proper treatment it results in extensive and permanent mental disability [13, 16]. Of note, low Phe diet could be considered as the primary treatment for PKU cases, which has to be started immediately without delay for life. Unluckily, it is very difficult to stick to this form of diet, particularly for adolescents [17].

Neonates are diagnosed with severe PKU, mild PKU, and hyperphenylalaninemia, if urine tyrosine and tetrahydrobiopterin readings are normal and Phe values are above 20 mg/dl, between 10 and 20 mg/dl, and between 2 and 10 mg/dl respectively [16]. In recent years, typical manifestations of PKU are infrequently recorded in developed nations, in which NBS is common. NBS has been considered as the main public screening strategy, which permits efficient recognition and management of PKU with low-Phe diet. Rapid treatment of PKU-affected cases has been demonstrated to be associated with normal results [18]. The first NBS program was emerged in in the US in the early 1960s [19] and after that spread to involve the majority of developed nations [20].

Of note, most of the previous research was mainly emphasized on the effect of phenylketonuria on neurological development. However, there was limited number of research that discussed the effect of phenylketonuria screening on neurological outcomes. The aim of the current study was to assess neurodevelopmental, cognitive and behavioral outcome in screened group compared to non-screened group in children with PKU.

Regarding demographic characteristics, our study revealed that the mean age was 5.03 ± 2.37 years. M/F ratio was 1.2/1. Positive consanguinity and positive family history were recorded in 60% and 37.5% of cases respectively. PKU is autosomal recessive inheritance disease, which support the increase in positive consanguinity. In addition, there was a significant decrease in age among screening group compared to non-screening one (P<0.05). The young age of screening group is due to early diagnosis at birth, while no significant differences were recoded as regards the remaining parameters (sex P=0.564, consanguinity P=0.130 and family history P=0.431) (P>0.05).

the current study demonstrated that regarding motor function development, in studied cases we found normal motor function (82.5%), while convulsion and hypotonia were recorded in 5% and 12.5% respectively. All cases had normal sensory function. Regarding reflexes, hyperreflexia was recorded in 15% of cases. Delayed developmental milestones were recorded in 32.5% of cases. In the same line, **Sadek *et al.*** [21] have demonstrated that 61 cases revealed a normal neurologic examination (54%), whereas hypotonia and exaggerated stretch reflexes were recorded in 50 cases (44.2%). Following two-year follow-up, the developmental assessment revealed that most of cases (79.6%) revealed improved motor functions, 15% had improved language skills. On the other hand, seventeen cases (15%) didn't fit the diagnosis of intellectual disability any longer. While, there has been no consensus agreement about the neuropsychological mechanisms of PKU. It has been demonstrated that the deficits are associated with individuals' Phe values at different stages throughout life [22].

Regarding neurological criteria between screening and non-screening, the current study explained that there were significant increases in motor affection (either convulsion or hypotonia) (p<0.001), hyperreflexia (p<0.001) and developmental millstones in non-screening group compared to screening one. However, ADHD demonstrated insignificant difference between both groups (p=0.141). There was a significant increase in IQ in screening group compared to non-screening one. In agreement, **Shokri *et al.*** [23] conducted a meta-analysis where dietary treatment was the most efficient treatment of PKU that could prevent its clinical manifestations if started early after detection by NBS.

In terms of Phe level, our study displayed that there were significant increases in phenylalanine level in non-screening group compared to screening one before treatment (P=0.01) and six months (P=0.005) after treatment, while no significant difference was recorded at 12 months (P=0.102) after treatment.

In the context of IQ, our study displayed that the mean IQ of the studied cases was 79.8 ± 14.44. Additionally, there was a statistically significant negative correlation between Phe level and IQ, which indicated that increase in Phe value was associated with decease in IQ. Thus it is very important to follow up Phe value to improve IQ outcome. Moreover, IQ was significantly increased among non-ADHD cases compared to ADHD ones (P<0.001). In the same line, **Sadek *et al.*** [21] revealed that the overall IQ and verbal IQ scores revealed that most of cases were moderately affected. Of note, most of their cases were diagnosed and treated late. Such outcomes were in accordance with a preceding study, which recorded a proportional association between blood Phe, during age's 0–12 and 0–18 years and IQ. For each 100 μm increase in blood Phe in PKU cases receiving early treatment, an average 2.6-point decline in IQ was predicted [24]. Much research conducted in adults established the prolonged effects of increased Phe value in children on cognitive function, even in cases had undergone early management and had a social environment conducive to good cognitive and psychiatric development [25]. Also, **Leuzzi *et al.*** [26] conducted their study on adolescents and demonstrated that two brain function measurements, the first for complicated cognitive skills and the second for certain network processing, were affected significantly with rapid elevation in Phe value. In addition, it was recorded that IQ is still stable over the 2nd decade of life. On the other hand, other cases demonstrated increases or decreased IQ scores, which wasn't clarified by the quality of metabolic control and could be linked to unknown subjective factors [27].

Related to the distribution according to behavior, the present study explained that ADHD was recorded in 52.5% of cases, while 47.5 were normal. There were no statistically significant difference between non-ADHD and ADHD cases regarding all demographic characteristics (age p=0.422, sex p=0.324, consanguinity p=0.366 and family history p=0.462) (P>0.05). Regarding neurological criteria between non-ADHD and ADHD cases, there were no statistically significant differences between non-ADHD and ADHD cases as regards Motor functions (p=0.937), reflex (p=0.451), developmental milestones (p=0.141), screening and age at diagnosis for non-screening cases. However, IQ was significantly increased among normal cases compared to ADHD ones (P<0.001). Regarding Phe level between non-ADHD and ADHD cases, there was no statistically significant difference between both groups at basal (p=0.08) and 6 months (p=0.003), while after 12 months non-ADHD group was associated with a significant decrease in Phe level in comparison with ADHD group (p<0.001).

In comparison with the prevalence confirmed in other research, this prevalence is significantly higher. In the overall population of children whose age ranges from six to twelve years old, 4 to 12% have ADHD [28, 29]. Lower prevalence was recorded by **Beckhauser *et al.*** [30] who have demonstrated the presence of manifestations of ADHD in thirteen cases (38.2%), with the majority of them having the hyperactive or impulsive form of ADHD. Such lower prevalence in that study could be clarified by the fact that all their studied cases were treated early. On the other hand; higher incidence was recorded by **Sadek *et al.*** [21] who have demonstrated that super average and average probability of ADHD was diagnosed in 88.5% of their studied phenylketonuria patients. Such higher prevalence in this study may be due to the fact that the majority of these cases were managed late.

The discrepancies between the current study and previous studies may be due the fact that they did not give a comment on screening, which reinforce our fact that screening was associated with a marked decrease in ADHD and the overall behavior.

CONCLUSION

The current study concluded that PKU still has adverse effects on children in the context of motor function, developmental milestone, ADHD development and IQ affection. Early screening seemed to be associated with promising outcomes with regard to neurological and developmental affection.

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