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Behavioral and Emotional Problems in Early-Treated Brazilian Children and Adolescents with Phenylketonuria

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Background: Phenylketonuria (PKU) is an inborn error of metabolism caused by mutations in the phenylalanine hydroxylase (PAH) gene. When untreated, PKU leads to a significant intellectual deficiency. Although early initiation of dietary therapy allows normal cognitive development, low adherence to treatment may result in neuropsychological deficits, including attention problems. This study was performed to evaluate emotional and behavioral problems in early-treated children and adolescents with PKU using the Child Behavior Checklist – CBCL/6–18 answered by parents.

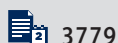
Material/Methods: The study included 36 PKU patients. The mean scores of internalizing, externalizing, and total problems, syndrome scales, and DSM-IV-oriented scales of patients were compared with those of controls. An analysis to evaluate the importance of adherence to treatment and presence of intellectual disability was also performed.

Results: There were no significant differences between patients and controls for almost all CBCL/6–18 scales, with the exception of the Attention Problem Scale – CBCL-APS. The mean (\pm SD) of the CBCL-APS scores of patients (7.86 ± 5.33) was considerably higher than the mean of the controls (6.07 ± 4.37 ; $p=0.016$), but not different from the mean of a matched control subsample (6.69 ± 4.46 ; $p=0.316$). The difference between the mean of the scores of DSM-IV/ADHD scale of patients (6.72 ± 4.07) and controls (5.73 ± 3.56 ; $p=0.102$) was not significant. Non-adherence to treatment and intellectual disability had a negative impact on both CBCL-APS and DSM-IV/ADHD scale scores.

Conclusions: Our findings indicate a significant prevalence of parents' complaints of attention problems and hyperactivity in non-adherent to treatment and intellectually low performing patients with PKU.

MeSH Keywords: **Attention • Child Behavior • Cognition • Patient Compliance • Phenylketonurias**

Full-text PDF: <https://www.medscimonit.com/abstract/index/idArt/909146>



Background

Phenylketonuria (PKU) is an inborn error of metabolism caused by mutations in the gene that encodes the enzyme phenylalanine hydroxylase (PAH). These mutations affect the enzyme's activity, blocking the conversion of phenylalanine (Phe) to tyrosine. When left untreated, the majority of patients develop profound intellectual disability and other psychiatric and neurological disorders (e.g., epilepsy) as a consequence of the protracted neurotoxic high Phe blood and brain levels that interfere with myelin formation and maintenance, inducing extensive structural damage to the central nervous system [1]. Agoraphobia, autistic symptoms, aggression towards others and self-aggression, and extreme irritability are among the symptoms described in these non-treated patients [2].

The introduction of newborn screening allowing the early institution of a diet restricted in natural proteins and phenylalanine, supplemented with an amino acid mixture formula, profoundly modified the natural course of the disease. Newborn screening for PKU was introduced in Brazil in 1976, but became widely available as a public government sponsored program since 2001 [3]. The incidence of PKU in this Latin American country is reported as 1 in 25 326 live newborns in 2005 [3].

Although the early institution of dietary treatment prevents the most severe psychiatric and neurological symptoms, especially intellectual disability, behavioral and emotional problems are still described in many continuously treated children and adolescents. It is postulated that these problems are caused by a decrease of the transport of tyrosine and tryptophan across the blood-brain barrier, which brings about a reduction in the concentration of the neurotransmitters dopamine and serotonin in the brain [4]. Dopamine is an important neurotransmitter for the dorsolateral prefrontal cortex neurons responsible for executive functions, attention, and abstract reasoning. The impairment of these mental processes leads to inattention, hyperactivity, and impulsiveness in early-treated PKU pediatric patients [5]. Moreover, internalizing behavioral problems, such as anxiety, phobias, depression, social isolation, and psychosomatic complaints, have also been described in these patients [6,7].

Low adherence to treatment with consequent fluctuating or higher than the target levels of Phe may be partially responsible for these neuropsychological symptoms. In a previous article, we described inadequate adherence to dietary treatment, determined by blood Phe levels, that worsens with age in early-treated children and adolescents with PKU in Brazil [8]. Moreover, mild intellectual disability, which is not as evident as the severe disease found in untreated patients, can also be found in early-treated PKU children in Brazil [9].

The aim of this article was to evaluate behavioral and emotional problems, including signs and symptoms of inattention and Attention Deficit Hyperactivity Disorder (ADHD), in early-treated children and adolescents with PKU from Brazil using the Child Behavior Checklist questionnaire CBCL/6–18, using as controls a Brazilian normative population of non-referred children. The roles of adherence to treatment, evaluated as the attainment of target levels of Phe, and of intellectual capacity on these outcomes were also investigated.

Material and Methods

Participants

All parents of 6–18-year-old patients with early-treated PKU, at a reference center for the disease in Rio de Janeiro, Brazil, were invited to participate in the study. The recruiting period was from March 2012 to July 2014. About 150 PKU patients of all ages, early and late-treated, regularly attend the reference center's outpatient clinics. Patients younger than 6 years of age, older than 18 years of age, late-treated, and with another severe or progressive underlying diagnosis other than PKU were excluded. As a result, 63 children and adolescents met the inclusion criteria. Parents of 36 of the 63 (57%) potentially eligible patients for this study enrollment actually accepted to participate and to complete the questionnaire. The principal reason for declining to participate was an allegation by parents of lack of time to fill out the questionnaire. An early-treated PKU patient was defined as an affected child or adolescent with a diagnosis of PKU of any severity phenotype established prior to 183 days of age, and since then submitted to a dietary restriction of Phe supplemented with a Phe-free formula containing essential amino acids, vitamins, minerals, and other micronutrients. Twenty-seven classical PKU and 9 mild or moderate PKU patients were enrolled: 14 females (38.9%) and 22 males (61.1%) aged 7 to 17 years (mean 11.3 years; SD 2.7 years). The age at diagnosis ranged from 14 to 182 days (mean 54, SD 39), and the mean age for beginning a dietary restriction of Phe was 60 (SD 46) days. Demographic and clinical characteristics of the PKU sample of this study are summarized in Table 1.

Ethics approval

Ethics approval was obtained from the Research Ethics Review Board of the State Institute of Diabetes and Endocrinology "Luiz Capriglione" (IEDE). The study was performed in accordance with the Guidelines and Standards for Research in Human Beings, established by the Brazilian National Health Council [10]. Written informed consent was obtained from the parents or guardians of all children and adolescents enrolled in this study.

Table 1. Demographic and clinical characteristics of the PKU sample.

| Patient ID | Gender | Age (years) | Age at diagnosis (days) | Age at diet onset (days) | Biochemical phenotype | Genotype | |
|------------|--------|-------------|-------------------------|--------------------------|-----------------------|-----------------------------|-----------------------------|
| | | | | | | Allele 1 | Allele 2 |
| 1 | Female | 8 | 55 | 60 | Classical PKU | c.1042C>G (p.L348V) | c.1045T>C (p.S349P) |
| 2 | Female | 7 | 18 | 21 | Moderate PKU | IVS10-11G>A (c.1066-11G>A) | c.1241A>G (p.Y414C) |
| 3 | Female | 10 | 37 | 38 | Classical PKU | c.1055delG (p.G352Vfs*48) | IVS10-11G>A (c.1066-11G>A) |
| 4 | Male | 9 | 57 | 57 | Classical PKU | c.194T>C (p.I65T) | IVS7+1G>A (c.842+1G>A) |
| 5 | Female | 11 | 37 | 37 | Classical PKU | IVS2+5G>C (c.168+5G>C) | IVS2+5G>C (c.168+5G>C) |
| 6 | Female | 11 | 50 | 50 | Classical PKU | c.194T>C (p.I65T) | c.204A>T (p.R68S) |
| 7 | Female | 11 | 62 | 62 | Classical PKU | – | – |
| 8 | Female | 9 | 114 | 68 | Moderate PKU | c.1045T>C (p.S349P) | c.1162G>A (p.V388M) |
| 9 | Male | 9 | 35 | 35 | Mild PKU | c.1222C>T (p.R408W) | c.1223G>A (p.R408Q) |
| 10 | Male | 11 | 19 | 67 | Classical PKU | c.967_969delACA (p.T323del) | c.1045T>C (p.S349P) |
| 11 | Female | 10 | 21 | 21 | Classical PKU | c.782G>A (p.R261Q) | IVS10-11G>A (c.1066-11G>A) |
| 12 | Male | 8 | 37 | 38 | Classical PKU | c.526C>T (p.R176*) | c.1162G>A (p.V388M) |
| 13 | Male | 7 | 140 | 140 | Moderate PKU | c.754C>T (p.R252W) | c.842C>T (p.P281L) |
| 14 | Male | 8 | 32 | 33 | Classical PKU | c.842C>T (p.P281L) | c.1162G>A (p.V388M) |
| 15 | Male | 10 | 40 | – | Classical PKU | c.994G>A (p.G332R) | c.1162G>A (p.V388M) |
| 16 | Female | 10 | 23 | 27 | Classical PKU | c.116_118delTCT (p.F39del) | c.1162G>A (p.V388M) |
| 17 | Male | 11 | 26 | 40 | Classical PKU | c.116_118delTCT (p.F39del) | c.1162G>A (p.V388M) |
| 18 | Male | 11 | 30 | 32 | Classical PKU | IVS2+5G>C (c.168+5G>C) | IVS10-11G>A (c.1066-11G>A) |
| 19 | Male | 9 | 42 | 42 | Classical PKU | c.782G>A (p.R261Q) | IVS10-11G>A (c.1066-11G>A) |
| 20 | Male | 8 | 107 | 198 | Classical PKU | c.194T>C (p.I65T) | IVS10-11G>A (c.1066-11G>A) |
| 21 | Male | 11 | 14 | 14 | Classical PKU | c.1162G>A (p.V388M) | c.1162G>A (p.V388M) |
| 22 | Male | 12 | 49 | 82 | Moderate PKU | c.782G>A (p.R261Q) | c.1045T>C (p.S349P) |
| 23 | Male | 13 | 56 | 56 | Classical PKU | c.842C>T (p.P281L) | c.967_969delACA (p.T323del) |
| 24 | Male | 13 | 70 | 70 | Classical PKU | c.842C>T (p.P281L) | IVS10-11G>A (c.1066-11G>A) |
| 25 | Female | 15 | 28 | 39 | Classical PKU | c.194T>C (p.I65T) | IVS12+1G>A (c.1315+1G>A) |
| 26 | Male | 16 | 35 | 35 | Moderate PKU | c.782G>A (p.R261Q) | c.1045T>C (p.S349P) |
| 27 | Female | 17 | 40 | 40 | Classical PKU | c.754C>T (p.R252W) | c.1162G>A (p.V388M) |

Table continued 1. Demographic and clinical characteristics of the PKU sample.

| Patient ID | Gender | Age (years) | Age at diagnosis (days) | Age at diet onset (days) | Biochemical phenotype | Genotype | |
|------------|--------|-------------|-------------------------|--------------------------|-----------------------|----------------------------|----------------------------|
| | | | | | | Allele 1 | Allele 2 |
| 28 | Male | 14 | 37 | 47 | Mild PKU | c.250G>T (p.D84Y) | IVS11+17G>A (c.1199+17G>A) |
| 29 | Female | 14 | 182 | 209 | Classical PKU | c.842C>T (p.P281L) | c.1162G>A (p.V388M) |
| 30 | Male | 14 | 36 | 54 | Classical PKU | c.782G>A (p.R261Q) | c.782G>A (p.R261Q) |
| 31 | Female | 16 | 43 | 43 | Mild PKU | IVS10-11G>A (c.1066-11G>A) | c.1162G>A (p.V388M) |
| 32 | Male | 14 | 150 | 150 | Classical PKU | IVS2+5G>C (c.168+5G>C) | c.745C>T (p.L249F) |
| 33 | Male | 13 | 73 | 73 | Mild PKU | IVS2+5G>C (c.168+5G>C) | c.1243G>A (p.D415N) |
| 34 | Female | 12 | 67 | 67 | Classical PKU | – | – |
| 35 | Male | 12 | 23 | 23 | Classical PKU | c.136G>A (p.G46S) | c.1162G>A (p.V388M) |
| 36 | Male | 14 | 43 | 43 | Classical PKU | c.194T>C (p.I65T) | c.1045T>C (p.S349P) |

Behavioral and emotional evaluation

The most recent Brazilian Portuguese version of the Child Behavior Checklist – CBCL/6–18 [11] was used for evaluation of the behavioral and emotional status of children and adolescents with PKU based on information provided by parents or caregivers. This version of the original American English questionnaire [12] consists of 120 items: the first 7 relating to the social competence of the child or adolescent and the other 113 relating to behavior problems. Social competence was not evaluated in this study due to the lack of a reliable normative Brazilian sample. The 113 items with statements about behaviors that may be present or absent in the life of the child or adolescent are grouped into 8 syndromes that constitute the behavioral profile: anxious/depressed, withdrawn/depressed, somatic complaints, social problems, thought problems, attention problems, rule-breaking behavior, and aggressive behavior [12]. Likewise, the responses to the items are converted into 6 scales based on the diagnostic criteria of the fourth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV): affective problems, anxiety problems, somatic problems, attention deficit/hyperactivity problems, oppositional defiant problems, and conduct problems [12]. CBCL/6–18 was applied by trained interviewers to low-educated (educational attainment less than high school) parents and was self-administered by parents that were at least high school graduates. The Assessment Data Manager (ADM) software (Burlington, VT, USA) was employed to enter the responses from the CBCL/6–18 forms for scoring.

Normative data from 1228 school children recruited in 6 Brazilian cities, with 475 from Rio de Janeiro, by different researchers of child behavior were assembled by the authors of the Brazilian Portuguese version of CBCL/6–18. From the initial pool of 2369 children, the authors excluded those identified with any genetic disease, neurological damage, or diagnosed psychiatric disorder, subsamples with <90 children, children >11 years, and children referred for psychological treatment. The normative sample comprised 608 females (49.5%) and 620 males (50.5%), with a mean age of 8.1 (SD 1.3) years [13]. Raw mean scores and SD from this normative sample were used in the statistical analyses.

To avoid distortions in the results of the statistical tests due to the large difference in size between the general population sample and the PKU sample, 36 individual cases matching the PKU cases for age and gender were drawn from the general population sample. This sample is henceforth called the matched control subsample.

Intellectual capacity assessment

To evaluate intellectual capacity, the Brazilian versions of the non-verbal (fluid) intelligence tests developed by John C. Raven [14] were employed. Raven's Colored Progressive Matrices – CPM [15] was used for assessing 6- to 11-year-old children, and Raven's Standard Progressive Matrices - SPM for 12- to 18-year-old adolescents [16]. These tests were designed to minimize influences of culture, nationality, and educational level on measuring non-verbal intelligence. The Brazilian standardization of CPM is based on the results of

1547 students, aged 5 to 11.5 years, from kindergartens and elementary schools in the city of São Paulo [17], and of SPM is based on 366 individuals from the city of Rio de Janeiro, participating in employee selection processes or vocational guidance sessions, with backgrounds ranging from primary to higher education [16].

Adherence to treatment

Median blood Phe measurements in the years immediately before this study (2010–2012) were used as a proxy of adherence to treatment. The adequacy of median blood Phe was assessed according to the target range of the Brazilian Ministry of Health's Clinical Protocol and Therapeutic Guidelines for PKU [18]. The target ranges for blood Phe in these Guidelines are ≤ 6 mg/dL for children and adolescents < 13 years of age, and ≤ 10 mg/dL for adolescents ≥ 13 years of age. The percentage of blood Phe results in the target range was also evaluated as an adherence to treatment parameter. Blood Phe long-term fluctuation was assessed by the standard deviation of measurements in the years immediately before this study.

Statistics

The statistical software package Stata/SE 12.1 for Mac (StataCorp, USA) was used to analyze the data. The independent samples *t* test was used for comparing the Brazilian general population sample mean CBCL/6–18 scores and the mean scores of the behavior profile (syndrome scales) and DSM-IV oriented scales of children and adolescents with PKU in this study. This test was also used to evaluate the statistical significance of differences in behavioral and emotional status between adherent, non-adherent, intellectually satisfactory, and intellectually low performing PKU patients and the Brazilian general population sample. The correlation between medians of blood Phe measurements and CBCL/6–18 scores was investigated by linear regression. Multivariate analysis of covariance (MANCOVA) was used with age and gender as covariates for each set of CBCL/6–18 scales (dependent variables): the sets of 8 Syndrome scales, 6 DSM-oriented scales, and Internalizing and Externalizing problems. A test of equality of group (adherent and non-adherent; intellectually satisfactory and intellectually low performing) effects adjusted for the covariates was performed. An analysis of covariance (ANCOVA) analysis with the same covariates was used for the Total Problems scale. The level of significance for the independent-samples *t* test and correlation test by linear regression was ≤ 0.05 , and for the MANCOVA and ANCOVA analyses ≤ 0.10 .

Results

Behavioral and emotional status of the total PKU sample

The mean (\pm SD) scores for CBCL/6–18 scales of children and adolescents with PKU and the Brazilian general population sample can be found in Table 2. There were no significant differences between patients and Brazilian children from the general population sample for almost all CBCL/6–18 scales, with the exception of the Attention Problem Scale – CBCL-APS. The mean (\pm SD) of the CBCL-APS scores was $7.86 (\pm 5.33)$, higher than the mean of the controls (6.07 ± 4.37 ; $p=0.016$). This difference faded away when the comparison was done with the matched control subsample (6.69 ± 4.46 ; $p=0.316$).

A total of 9 patients (25.0%) were in the clinical range, and 6 (16.7%) in the borderline range for CBCL-APS, according to the output of the ADM software, which uses a US normative population for comparison.

The differences between the mean of the scores of DSM-IV/ADHD scale of patients (6.72 ± 4.07) and controls (5.73 ± 3.56 ; $p=0.102$) or the matched control subsample (5.28 ± 3.49 ; $p=0.112$) were not statistically significant.

Intellectual capacity

The intellectual capacity of 5 of the 36 (13.9%) children and adolescents with PKU was classified by CPM and SPM as Grade IV negative (IV–, definitely below average in intellectual capacity – score $> 5^{\text{th}}$ percentile and $\leq 10^{\text{th}}$ percentile) and 9 (25.0%) as Grade V (intellectually defective – score $\leq 5^{\text{th}}$ percentile). These 14 (38.9%) patients were considered as intellectually low performing. Patients whose intellectual capacity was classified from Grade IV+ to I were considered as intellectually satisfactory.

The mean (\pm SD) scores for CBCL/6–18 scales of intellectually satisfactory and intellectually low performing children and adolescents with PKU and the statistical significance of the difference between the mean scores of each group and those of the Brazilian children from the general population sample can be found in Table 3. Intellectually low performing children and adolescents with PKU presented significantly higher mean CBCL-APS scores (mean 10.43, SD 4.78; $p<0.001$) and DSM-IV/ADHD scale scores (mean 8.86, SD 3.70; $p=0.001$) than controls. These differences persisted when the assessments were done against the matched control subsample – $p=0.012$ for CBCL-APS scale and $p=0.002$ for DSM-IV/ADHD scale. There were no significant differences between this group of patients and controls for all the other CBCL/6–18 scales. Intellectually satisfactory children and adolescents with PKU behavioral and emotional status did not differ significantly from that of controls (Table 3).

Table 2. Mean (\pm SD) scores for CBCL/6–18 scales of children and adolescents with PKU and the Brazilian general population sample.

| Scale | Children and adolescents with PKU (n=36) | Brazilian general population sample (n=1228) [10] | p* |
|--|--|---|----------------|
| Total problems | 41.97 (\pm 26.54) | 43.34 (\pm 25.25) | 0.749 |
| Internalizing problems | 9.97 (\pm 6.06) | 11.43 (\pm 7.53) | 0.249 |
| Externalizing problems | 11.08 (\pm 8.95) | 12.35 (\pm 8.82) | 0.395 |
| Syndrome scales | | | |
| Anxious/depressed | 5.67 (\pm 3.46) | 6.21 (\pm 4.07) | 0.431 |
| Withdrawn/depressed | 2.50 (\pm 2.30) | 2.85 (\pm 2.61) | 0.427 |
| Somatic complaints | 1.81 (\pm 1.67) | 2.37 (\pm 2.69) | 0.215 |
| Social problems | 4.75 (\pm 3.29) | 4.87 (\pm 3.42) | 0.836 |
| Thought problems | 2.72 (\pm 3.84) | 2.87 (\pm 3.11) | 0.777 |
| Attention problems | 7.86 (\pm 5.33) | 6.07 (\pm 4.37) | 0.016** |
| Rule-breaking behavior | 2.61 (\pm 3.00) | 2.80 (\pm 2.74) | 0.683 |
| Aggressive behavior | 8.36 (\pm 6.49) | 9.55 (\pm 6.66) | 0.291 |
| DSM-oriented scales | | | |
| Affective problems | 2.78 (\pm 2.67) | 3.79 (\pm 3.38) | 0.076 |
| Anxiety problems | 3.33 (\pm 2.06) | 3.44 (\pm 2.29) | 0.776 |
| Attention deficit/hyperactivity problems | 6.72 (\pm 4.07) | 5.73 (\pm 3.56) | 0.102 |
| Conduct problems | 3.08 (\pm 4.45) | 3.12 (\pm 3.73) | 0.950 |
| Oppositional defiant problems | 3.42 (\pm 2.60) | 3.37 (\pm 2.47) | 0.905 |
| Somatic problems | 0.89 (\pm 0.95) | 1.15 (\pm 1.82) | 0.394 |

* p value of a t-test for two independent samples; ** patients with PKU mean significantly higher than controls.

MANCOVA analysis, adjusted for age and gender as covariates, for the set of 6 DSM-oriented scales demonstrated that intellectually satisfactory and low performing groups were not equal, with a Wilks' lambda value of 0.6505 and a p value of 0.0532. The same analysis, for the 8 Syndrome scales, and Internalizing and Externalizing problems, was not able to show that the 2 groups were not equal, with Wilks' lambda values of 0.6481 and 0.9489, and p values of 0.1486 and 0.4437, respectively. ANCOVA analysis with the same covariates for the Total Problems scale was not able to demonstrate that the 2 groups were not equal, with a Wilks' lambda value of 0.9547 and a p value of 0.2267.

Low intellectual capacity was significantly associated with median blood Phe levels in the first year of life ($\chi^2=10.24$, $p=0.001$) but not to median blood Phe levels in the years immediately before this study ($\chi^2=0.94$, $p=0.332$).

Adherence to treatment

Fourteen of the 34 children and adolescents with PKU whose laboratory data were available had adequate median blood Phe levels in the years immediately before this study. The mean of the median blood Phe levels of this group was 4.42 mg/dL (SD 1.61). They were considered as adherent to treatment. Twenty patients had inadequate median blood Phe levels (mean 11.24 mg/dL, SD 3.38) and were considered as non-adherents. The mean (\pm SD) scores for CBCL/6–18 scales of adherent and non-adherent to treatment children and adolescents with PKU can be found in Table 4. Non-adherent to treatment children and adolescents with PKU presented significantly higher mean CBCL-APS scores (mean 8.60, SD 4.87; $p=0.011$) and DSM-IV/ADHD scale scores (mean 8.00, SD 3.87; $p=0.005$) than controls. The mean for DSM-IV/ADHD scale scores of non-adherents was also significantly higher than the respective mean of the matched control subsample, with a $p=0.009$, but the mean CBCL-APS scale scores of this subgroup was not different from the mean

Table 3. Mean (\pm SD) scores for CBCL/6–18 scales for intellectually satisfactory (Grades IV+ to I of CPM or SPM) and intellectually low performing (Grades IV– or V) children and adolescents with PKU.

| Scale | Intellectually satisfactory children and adolescents with PKU (n=22) | Intellectually low performing children and adolescents with PKU (n=14) | Brazilian general population sample (n=1228) [10] | p1* | p2** |
|--|--|--|---|-------|-----------|
| Total problems | 37.59 (\pm 30.33) | 48.86 (\pm 18.09) | 43.34 (\pm 25.25) | 0.292 | 0.415 |
| Internalizing problems | 10.32 (\pm 6.85) | 9.43 (\pm 4.77) | 11.43 (\pm 7.53) | 0.493 | 0.322 |
| Externalizing problems | 10.36 (\pm 10.29) | 12.21 (\pm 6.48) | 12.35 (\pm 8.82) | 0.296 | 0.953 |
| Syndrome scales | | | | | |
| Anxious/depressed | 5.82 (\pm 3.90) | 5.43 (\pm 2.77) | 6.21 (\pm 4.07) | 0.656 | 0.475 |
| Withdrawn/depressed | 2.86 (\pm 2.38) | 1.93 (\pm 2.13) | 2.85 (\pm 2.61) | 0.986 | 0.189 |
| Somatic complaints | 1.64 (\pm 1.71) | 2.07 (\pm 1.64) | 2.37 (\pm 2.69) | 0.205 | 0.677 |
| Social problems | 4.09 (\pm 3.50) | 5.79 (\pm 2.72) | 4.87 (\pm 3.42) | 0.289 | 0.316 |
| Thought problems | 2.59 (\pm 4.53) | 2.93 (\pm 2.56) | 2.87 (\pm 3.11) | 0.679 | 0.943 |
| Attention problems | 6.23 (\pm 5.10) | 10.43 (\pm 4.78) | 6.07 (\pm 4.37) | 0.865 | <0.001*** |
| Rule-breaking behavior | 2.36 (\pm 3.37) | 3.00 (\pm 2.35) | 2.80 (\pm 2.74) | 0.457 | 0.786 |
| Aggressive behavior | 7.73 (\pm 7.32) | 9.36 (\pm 5.02) | 9.55 (\pm 6.66) | 0.205 | 0.915 |
| DSM-oriented scales | | | | | |
| Affective problems | 3.00 (\pm 2.93) | 2.43 (\pm 2.28) | 3.79 (\pm 3.38) | 0.276 | 0.134 |
| Anxiety problems | 3.27 (\pm 2.27) | 3.43 (\pm 1.74) | 3.44 (\pm 2.29) | 0.730 | 0.987 |
| Attention deficit/hyperactivity problems | 5.36 (\pm 3.76) | 8.86 (\pm 3.70) | 5.73 (\pm 3.56) | 0.629 | 0.001*** |
| Conduct problems | 3.00 (\pm 5.35) | 3.21 (\pm 2.64) | 3.12 (\pm 3.73) | 0.882 | 0.928 |
| Oppositional defiant problems | 3.27 (\pm 2.71) | 3.64 (\pm 2.50) | 3.37 (\pm 2.47) | 0.851 | 0.684 |
| Somatic problems | 0.73 (\pm 0.88) | 1.14 (\pm 1.03) | 1.15 (\pm 1.82) | 0.280 | 0.984 |

* p1 – t-test intellectually satisfactory patients with PKU versus controls; ** p2 – t-test intellectually low performing patients with PKU versus controls; *** mean significantly higher than controls.

of the matched control subsample ($p=0.1431$). There were no significant differences between this group of patients and the Brazilian children from the general population sample for all the other CBCL/6–18 scales. Adherent to treatment children and adolescents with PKU behavioral and emotional status did not differ significantly from that of controls (Table 4).

MANCOVA analysis, adjusted for age and gender as covariates, for the set of 8 Syndrome scales and the 6 DSM-oriented scales, demonstrated that adherent and non-adherent groups were not equal, with Wilks' lambda values of 0.4593 and 0.6390, and p values of 0.0103 and 0.0612, respectively. The same analysis, for Internalizing and Externalizing problems, was not able to show that the 2 groups were not equal, with Wilks' lambda value of 0.9988 and a p value of 0.9827. ANCOVA analysis with

the same covariates for the Total Problems scale was not able to demonstrate that the 2 groups were not equal, with a Wilks' lambda value of 0.9964 and a p value of 0.7429.

The mean (\pm SD) of the percentage of blood Phe results in the treatment target range for the adherent group was 81.1% (\pm 24.8%), while that of the non-adherent group was significantly much smaller, at 7.3% (\pm 13.6%; $p<0.001$). On the other hand, fluctuations of Phe levels measured by SD were not different between adherent (2.06 ± 1.00) and non-adherent patients (2.39 ± 1.07 ; $p=0.375$). There were strong correlations between medians of blood Phe measurements and 2 DSM-IV oriented scales, ADHD ($t=2.39$; $p=0.023$; $R^2=0.152$) and Somatic Problems ($t=2.13$; $p=0.041$; $R^2=0.124$) (Table 5).

Table 4. Mean (\pm SD) scores for CBCL/6–18 scales for adherent (adequate median blood Phe levels) and non-adherent (inadequate median blood Phe levels) to treatment children and adolescents with PKU.

| Scale | Adherent children and adolescents with PKU (n=14) | Non-adherent children and adolescents with PKU (n=20) | Brazilian general population sample (n=1228) [10] | <i>p</i> 1* | <i>p</i> 2** |
|--|---|---|---|-------------|-----------------|
| Total problems | 40.57 (\pm 35.40) | 43.75 (\pm 19.06) | 43.34 (\pm 25.25) | 0.685 | 0.942 |
| Internalizing problems | 10.07 (\pm 5.41) | 10.05 (\pm 6.79) | 11.43 (\pm 7.53) | 0.501 | 0.416 |
| Externalizing problems | 10.43 (\pm 12.57) | 11.80 (\pm 5.48) | 12.35 (\pm 8.82) | 0.421 | 0.781 |
| Syndrome scales | | | | | |
| Anxious/depressed | 5.21 (\pm 2.94) | 6.10 (\pm 3.88) | 6.21 (\pm 4.07) | 0.360 | 0.905 |
| Withdrawn/depressed | 3.21 (\pm 2.36) | 2.15 (\pm 2.28) | 2.85 (\pm 2.61) | 0.608 | 0.234 |
| Somatic complaints | 1.64 (\pm 1.82) | 1.80 (\pm 1.61) | 2.37 (\pm 2.69) | 0.312 | 0.345 |
| Social problems | 5.79 (\pm 4.17) | 4.25 (\pm 2.49) | 4.87 (\pm 3.42) | 0.318 | 0.420 |
| Thought problems | 3.57 (\pm 5.52) | 2.20 (\pm 2.26) | 2.87 (\pm 3.11) | 0.408 | 0.338 |
| Attention problems | 6.86 (\pm 5.93) | 8.60 (\pm 4.87) | 6.07 (\pm 4.37) | 0.503 | 0.011*** |
| Rule-breaking behavior | 2.93 (\pm 4.39) | 2.50 (\pm 1.70) | 2.80 (\pm 2.74) | 0.861 | 0.626 |
| Aggressive behavior | 7.07 (\pm 8.45) | 9.40 (\pm 4.58) | 9.55 (\pm 6.66) | 0.168 | 0.920 |
| DSM-oriented scales | | | | | |
| Affective problems | 3.00 (\pm 3.14) | 2.70 (\pm 2.43) | 3.79 (\pm 3.38) | 0.384 | 0.151 |
| Anxiety problems | 3.29 (\pm 2.09) | 3.45 (\pm 2.11) | 3.44 (\pm 2.29) | 0.807 | 0.985 |
| Attention deficit/hyperactivity problems | 5.00 (\pm 3.62) | 8.00 (\pm 3.87) | 5.73 (\pm 3.56) | 0.446 | 0.005*** |
| Conduct problems | 3.86 (\pm 6.67) | 2.80 (\pm 2.19) | 3.12 (\pm 3.73) | 0.466 | 0.702 |
| Oppositional defiant problems | 2.50 (\pm 3.11) | 4.10 (\pm 1.94) | 3.37 (\pm 2.47) | 0.192 | 0.189 |
| Somatic problems | 0.71 (\pm 0.91) | 0.90 (\pm 0.91) | 1.15 (\pm 1.82) | 0.367 | 0.540 |

* *p*1 – t-test adherent patients with PKU versus controls; ** *p*2 – t-test non-adherent patients with PKU versus controls; *** mean significantly higher than controls.

Pearson's chi-squared test demonstrated that adherence to treatment was not significantly associated with intellectual capacity in our PKU sample ($\chi^2=0.941$, $p=0.332$).

Discussion

Using the CBCL/6–18, we found evidence for a higher prevalence of attention problems in early-treated children and adolescents with PKU when compared to a Brazilian general population sample. Attention problems have been previously reported as a common neuropsychological disorder in children and adolescents with PKU [19–23]. Weglage et al. [19] found that poor results in attention tests were significantly correlated with concurrent serum Phe levels but not with mean serum Phe

concentrations throughout life. The authors postulated that high concurrent serum Phe levels impaired frontal lobe functions due to dopamine deficiency. Our findings of significantly higher mean CBCL-APS scores in children and adolescents that had inadequate median blood Phe levels in the years immediately before this study may indirectly reflect high concurrent serum Phe levels and call attention to the importance of diet adherence in childhood and adolescence. High concurrent Phe levels have been associated with low frontal lobe dopamine concentrations, as well as with white matter abnormalities in early-treated PKU patients [23]. As patients were not submitted to brain magnetic resonance imaging (MRI), we cannot establish which neurophysiologic mechanism was underlying the linkage of recent metabolic control and attention in our study.

Table 5. Correlations between medians of blood Phe measurements in the years immediately before this study and scores for CBCL/6–18 scales by linear regression of 34 children and adolescents with PKU.

| Scale | t | p | R ² |
|--|-------|---------------|----------------|
| Total problems | 0.92 | 0.364 | 0.026 |
| Internalizing problems | 0.34 | 0.738 | 0.004 |
| Externalizing problems | 1.02 | 0.316 | 0.031 |
| Syndrome scales | | | |
| Anxious/depressed | 0.78 | 0.440 | 0.019 |
| Withdrawn/depressed | -0.99 | 0.328 | 0.030 |
| Somatic complaints | 1.00 | 0.324 | 0.030 |
| Social problems | -0.33 | 0.742 | 0.003 |
| Thought problems | -0.80 | 0.432 | 0.019 |
| Attention problems | 1.21 | 0.235 | 0.044 |
| Rule-breaking behavior | 0.31 | 0.761 | 0.003 |
| Aggressive behavior | 1.44 | 0.160 | 0.061 |
| DSM-oriented scales | | | |
| Affective problems | -0.40 | 0.689 | 0.005 |
| Anxiety problems | -0.22 | 0.829 | 0.002 |
| Attention deficit/hyperactivity problems | 2.39 | 0.023* | 0.152 |
| Conduct problems | -0.06 | 0.949 | <0.001 |
| Oppositional defiant problems | 1.67 | 0.105 | 0.080 |
| Somatic problems | 2.13 | 0.041* | 0.124 |

* Statistically significant correlation.

The intellectual capacity of children and adolescents with PKU has been reported as having no influence on attention [21, 20]. Moreover, there is a longstanding controversy about the attention performance of individuals with intellectual developmental disabilities (IDD) [24]. Nevertheless, we found that intellectually low performing children and adolescents with PKU, but not intellectually satisfactory patients, had higher mean CBCL-APS scores than the normative sample. This difference remained even when the comparison was performed against a matched control subsample. Intellectual capacity is an outcome related to lifetime Phe levels, especially Phe levels before age 12 [25]. Leuzzi et al. [26] found that early-treated PKU patients younger than age 13 were more impaired in some neurocognitive skills and more vulnerable than older adolescents to a Phe overload. These results are in agreement with the hypothesis of de Sonneville et al. [27,28] of the existence of windows of vulnerability to Phe exposure for different neurocognitive functions in early-treated PKU children. Consequently, attention problems can be the result of suboptimal dietary control in different age periods.

Due to the cross-sectional design of our study, we cannot firmly establish whether Phe levels in the most critical period of neurological development – the first year of life – affected intellectual capacity and adversely influenced the appearance of attention problems later in life, or vice versa.

While the mean of the scores of DSM-IV/ADHD scale of the entire assemblage of patients was not statistically different from controls, intellectually low performing patients and patients with inadequate median blood Phe levels in the last year of treatment had means that were higher than the medians of a Brazilian general population sample and of a matched control subsample. It has not been firmly established if ADHD symptoms affect verbal intelligence or performance/fluid intelligence, which is the intellectual capacity measured by CPM and SPM [29,30]. Again, the cross-sectional design of our study does not warrant assertions concerning the direction of effects between ADHD symptoms, especially its inattentive component, and performance/fluid intelligence.

Antshel and Waisbren [31,32] reported a 2.5 times greater prevalence of ADHD, using strict DSM-IV diagnostic criteria, in early-treated classical PKU children than that of the general population. All children with PKU in their study who met formal diagnostic criteria for ADHD were diagnosed with ADHD-inattentive type. Even though the relationship between attention problems, ADHD symptoms, and intellectual capacity, especially fluid intelligence measured by CPM and SPM, is full of controversy, the mean that was higher than controls' for CBCL-APS and DSM-IV/ADHD scale scores found in our study among non-adherent and intellectually low performing children and adolescents with PKU disclose the importance of inattention in this population.

We could not find in our sample the extensive internalizing behavioral problems, including depression, anxiety, social withdrawal, and somatic complaints, reported by previous studies that also used CBCL for assessing behavior and emotional status of early-treated children and adolescents with PKU [6,33,34]. Explanations for these differences may be methodological, as Weglage et al. [6] and Jusiene et al. [33] used an older version of CBCL – the CBCL/4–18 – and Cappelletti et al. [34] did not state clearly if they used a national (Italian) normative population as controls. If the American national non-referred normative sample, embedded in ADM software calculation of T-scores, had been used, extensive internalizing behavioral problems would also have been detected in our PKU population (data not shown).

Limitations of the present study recommend that our results should be considered as preliminary. The number of PKU patients followed at the reference center's outpatient clinics is approximately 150, but the age restrictions of the CBCL/6–18 and the exclusion of late-diagnosed patients reduced the eligible population to 63 children and adolescents, of which 36 parents filled out the questionnaire. As a consequence, our results may not be generalized to the whole Brazilian PKU population. CBCL/6–18 evaluation of children's behavior and emotional status is based on parents' information, and so is prone to be influenced by the parents' reaction – positive or

negative – to their children's illness [33]. Especially for the screening for ADHD, CBCL/6–18 does not allow us to conclude if the several inattentive or hyperactive-impulsive symptoms are present in 2 or more settings (e.g., at home, school, or work; with friends or relatives; and in other activities), and if the symptoms interfere with, or reduce the quality of, social, academic, or occupational functioning, which are criteria essential for the diagnosis of ADHD according to the DSM-5 [35].

Conclusions

We found evidence of a significantly higher prevalence of parents' complaints of attention problems in early-treated children and adolescents with PKU. Concurrent and early in life high blood Phe levels both appear to contribute to the appearance of attention problems. This evidence should be further corroborated by reliable, validated neuropsychological tests capable of assessing executive functions, such as working memory and inhibitory control. The inclusion of neuroimaging findings and academic outcomes data (e.g., School Achievement Test [SAT] scores and teacher's reports) should also be considered. A multicenter study is also urgently needed to overcome problems of sample size of a rare disease like PKU, which will enable evaluation of the generalizability of our findings. However, our findings warn PKU reference centers in Brazil of the necessity to incorporate behavior and emotional status screening tools into the routine interdisciplinary care for early-treated children and adolescents with PKU.

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