Adult Phenylketonuria: Evaluation of the neurocognitive and hormonal system

PhD thesis

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1. Introduction

Phenylketonuria (PKU) is one of the most common inborn errors of metabolism caused by the defective functioning of the phenylalanine hydroxylase enzyme (PAH). PAH is mainly found in the liver and catalyzes the hydroxylation of the essential amino acid phenylalanine (Phe) to tyrosine (Tyr). The toxic accumulation of Phe in the blood and central nervous system causes severe mental retardation (IQ $\leq$50), behavioural disorders, and epileptic seizures. The implementation of neonatal screening and the early initiation of lifelong therapy can prevent these serious complications. The lifelong therapy consists of a natural protein-restricted diet combined with Phe-free amino acid mixtures (AAM). Supplementation of vitamins and micronutrients using AAM is vital to compensate for the low protein diet.

Neonatal screening of PKU in Hungary is compulsory since the beginning of the 70s. Due to this fact, PKU care is no longer limited to a pediatric setting. The goal of the transition of patients with PKU from pediatric to adult care is to provide quality care and treatment in an adult care setting.

Neuropsychiatric symptoms caused by elevated levels of Phe, significantly impact the life of PKU patients. The relationship between blood Phe levels and the severity of the symptoms was the basis of the establishment of international guidelines regarding safe ranges of blood Phe levels. Nevertheless mild neurocognitive symptoms, mainly impacting executive function, and working

memory are seen in patients with Phe levels within the recommended ranges. Adherence to therapy can decrease during adolescence and especially in adulthood. Recent studies have shown that in childhood shortcomings in diet, can lead to an IQ deficit and executive function disorders in adulthood.

The early diagnosis of mild neurocognitive impairment is essential to monitor the effectiveness of the diet of PKU patients. Unfortunately, specific tests are currently not available.

CANTAB (Cambridge Neuropsychological Test Automated Battery, Cambridge Cognition, Cambridge, UK) tests are computer-based tests used in a variety of neurocognitive disorders. CANTAB test used in adolescent and adult PKU patients has highlighted the relationship between adherence to low protein diet and specific neurocognitive deficits.

Iodine is essential in the synthesis of thyroid hormones thyroxine (T4) and triiodothyronine (T3). Recent studies have shown improvement in the iodine status of the general population in Hungary. In the western diet, the main source of iodine is processed foods, dairy products and seafood. However, due to the high protein content of these foods, they can only be consumed in minimal amounts in the case of PKU patients. To compensate for this AAM contain the daily recommended amount of 150 μg of iodine recommended by the WHO. Studies based on dietary diaries suggest that patient keeping a loose diet, with AAM consumption below the recommended amount have significantly lower iodine

intake compare to patients with adequate diet and the general population.

Catecholamines, adrenaline, noradrenaline and dopamine, are synthesized from tyrosine, and function as monoamine neurotransmitters and play an essential role in stress response. Lower levels of monoamine neurotransmitters and catecholamines have been previously observed in PKU patients. One of the potential explanation for this finding is the competitive inhibition of tyrosine hydroxylase enzyme (TYH) by Phe. Sympathetic stimulation increases the activity of TYH and DOPA decarboxylase, which results in a surge of catecholamines from the adrenal glands and paravertebral ganglia. Catecholamines in the bloodstream bind to the cells adrenergic receptors and increase blood pressure, cardiac output, induce mydriasis, lower the tone of bronchial and intestinal non-striated muscles, increase blood sugar and free fatty acid levels and induce protein catabolism. Very few studies are available that measured plasma catecholamine levels in PKU patients.

2. Aims

The aims of the studies of my thesis were the following:

a) To evaluate the cognitive function of adult PKU patients and compare them to healthy controls using the computer-based CANTAB neuropsychological tests. Furthermore, we aimed to evaluate the effect of actual and lifelong Phe values on the performance during the neuropsychological tests.

b) To determine the iodine status and thyroid function of PKU patients. To investigate the possible connection of iodine status and thyroid function to the diet and blood amino acid levels.

c) To determine the catecholamine, metabolic and physiologic response of PKU patients compared to healthy controls during mild-medium and intense sympathetic stimulation tests.

3. Methods

3.1. The evaluation of the neurocognitive function of PKU patients

3.1.1. Participants

In a monocentric, cross-sectional study, 46 adult patients with PKU and 31 healthy controls were consecutively enrolled between September 2013 and April 2015 at Semmelweis University, Budapest, Hungary. All patients were diagnosed during neonatal screening, and their treatment was initiated from birth without discontinuation. In the case of 25 patients, lifelong Phe values were available. PKU patients were divided into two groups according to the recommended upper target Phe-level 600 μmol/l. The "on diet" group included patients whose blood Phe-level was under 600 μmol/l (n = 20). The "loose diet" group included patients whose blood Phe-level was above 600 μmol/l (n = 26).

3.1.2. Motor Screening Test (MOT)

MOT is a test that measures two different brain functions, speed of response and the accuracy of pointing. A series of white crosses show up in different locations on the screen. The participants have to touch it in the middle as quickly and accurately as possible. This test is used to determine the ability of the participant to use a touchscreen.

3.1.3. Spatial Working Memory (SWM)

SWM is a test that assesses the patient's ability to retain spatial information and to manipulate remembered items in working memory. This test measures the frontal lobe and "executive" dysfunction. By touching the coloured boxes on the screen to open them, participants must find the small blue squares that are hidden inside and use them to fill up an empty column on the right side of the screen. The most difficult aspect of the task is that the patient must not open a box where a blue square has been found before. The test becomes more complicated as the number of boxes increases. Outcome measures include errors and strategy.

3.1.4. Stockings of Cambridge (SOC)

SOC is a test that shows the speed of the participant's response. The patient sees the screen split into two. The two displays include three coloured balls (a red, a blue, and a green one) held in stockings floating from a bin. In the lower display, the participant must copy the pattern which is shown in the upper display. The number of moves and the time taken to complete the task measures the patient's planning ability. Stockings of Cambridge has three outcome measures, containing the number and percentage of correct trials, and latency (speed of participant's response).

3.2. The evaluation of thyroid function and iodine status in adult PKU patients.

3.2.1. Participants

In this monocentric, prospective case-control study, 77 adult ETPKU patients, and 50 healthy controls were included. Exclusion criteria were 1. previously known thyroid dysfunction 2. no data regarding Phe levels. Daily recommended AAM intake (g/day) was calculated using the formula recommended by the European Guidelines.

3.2.2. Patient classification

The patients were classified into subgroups based on their adherence to a low protein diet and AAM consumption. According to their adherence to a low protein diet for the 12 months before the study, patients were classified as having either good adherence or low adherence. Based on their consumption of AAM, PKU patients were classified as having either “adequate AAM consumption” or “reduced AAM consumption”.

3.2.3. Blood, Urine Samples and Thyroid Ultrasound

From every participant serum concentrations of free T3 (fT3), free T4 (fT4), thyroid-stimulating hormone

(TSH), and thyroid peroxidase antibodies (TPOAb) were determined using chemi-immunometric assay. Serum thyroglobulin (Tg) and Tg antibody (TgAb) were measured with electrochemiluminescence immunoassay method. Urinary iodine concentration was determined using Sandel Kolthoff reaction adapted to microplate.
Thyroid ultrasound was also performed by an expert in thyroid ultrasonography

3.3. Stress-induced catecholaminergic and metabolic response in adult PKU

3.3.1. Participants and baseline measurements

In this monocentric study, 12, early-treated adult PKU (ETPKU) male patients and 10 healthy controls. Subjects were then asked to rest in a supine position for 30 min, in a quiet dimly lit room. After this resting period, basal heart rate and blood pressure were measured and resting blood samples were collected. The first day included two mild sympathetic stimuli: the cold pressor test (CPT) and the isometric handgrip test (HGT). On a separate day, an intense sympathetic test, namely, the peak treadmill test to exhaustion, was performed.

3.3.2. Cold pressor test

The subjects were asked to immerse their non-dominant hand into a bucket containing ice water (2 °C)

for as long as they could tolerate the cold (maximum of 2 min). Heart rate was monitored continuously and blood pressure before the completion of the test. When the subject pulled his hand from the ice water, a blood sample for catecholamines and amino acids measurement was collected.

3.3.3. Isometric handgrip test

The subjects were then asked to perform the isometric handgrip exercise at 30% of Tmax for 3 min. Heart rate was monitored continuously and blood pressure was measured before the completion of the exercise. After the termination of the test, blood samples for catecholamines and amino acid measurement were collected.

3.3.4. Peak treadmill test to exhaustion

Each subject participated in a peak treadmill test using a treadmill ergometer coupled with O2 and CO2 gas analyser. The classic “vita maxima type” criteria was used to evaluate the plateau in oxygen uptake (VO2max). Tests were terminated when the subjects reached the maximum oxygen uptake criteria or had subjective complaints (fatigue, pain, or dizziness). Tests were terminated when the subjects reached the maximum oxygen uptake criteria or had subjective complaints (fatigue, pain, or dizziness). At the moment of the termination of the test, blood

samples for catecholamines and amino acid measurement were collected.

3.3.5. Catecholamine and amino acid level measurement

E and NE were assayed in plasma using liquid chromatography coupled with amperometric detection. Phe and Tyr levels were determined from dried blood spots using Tandem Mass Spectrometry (MS/MS).

3.5. Statistical analysis

Statistical analyses were performed using statistical package SPSS version 23 (IBM Corp. Armonk, NY, USA). The Shapiro Wilk test was used to check for normally distributed variables. Normally distributed results were reported as mean ± SD, and nonnormally distributed data were reported as median and interquartile range (IQR). Independent Student t-test and one-way analysis of variance (ANOVA) were used to test for differences between subgroups for normally distributed continuous data, whereas Mann-Whitney U test and Kruskal-Wallis H test were used for nonnormally distributed continuous data. Tukey's and Dunn's Multiple Comparison tests were used for post hoc comparisons. Due to the small sample size, Fisher's Exact test was used to test for associations between categorical data. Correlation analyses were performed using Pearson's correlation test for normally distributed variables and

Spearman's rank correlation test for non-normally distributed variables. p values <0.05 were considered significant.

4. Results

4.1 The evaluation of the neurocognitive function of PKU patients

CANTAB computer-based neuropsychological tests were used to evaluate the cognitive function of PKU patients. The advantage of the CANTAB tests is their ability to effectively measure executive function, which is frequently affected in patients with PKU. Our results have shown that excepting response speed and initial thinking time the performance of PKU patients was significantly worse compared to the healthy controls. The European guidelines recommend an upper limit for Phe of 600 μmol/L in adult PKU. Interestingly by grouping our patients based on the recommended cutoff value we did not observe a significant difference in the performance of the patients with adequate metabolic control compared to those on a loose diet. However, patients who had Phe values within the recommended ranges during 0-12 years (Phe values between 120-360 μmol/L), had fewer errors and a better strategic performance during the tests compared to the patients with a loose metabolic control.

4.2 The evaluation of thyroid function and iodine status in adult PKU patients.

In our next study, we report the Tyr derived thyroid hormone levels, thyroid function and iodine status of PKU patients. In line with previous studies, we found that thyroid hormone metabolism is not affected in PKU patients. However, the iodine status of PKU patients is influenced by the diet, specifically AAM consumption. Based on the WHO/UNICEF/ICCIDD criteria iodine status was optimal in both the PKU and control groups. (median UIC >100 μg/L, and UIC/Cr >100 μg/g). When divided according to their diet (low protein diet and AAM consumption), we found that PKU patients on a loose diet had significantly lower UIC and UIC/Cr levels compared to patients with adequate diet and controls. UIC and UIC/Cr levels were highest in patients with good adherence to the diet, nevertheless, the median UIC did not exceed the WHO criteria for excess iodine intake (median UIC ≥300 μg/L). This suggests that a low protein diet completed with regular AAM intake in adult PKU provides enough iodine to meet the recommended UIC ranges of iodine by WHO. Thyroid ultrasound of PKU patients showed normal thyroid structure (echogenic and homogenous), without signs of inflammation. Thyroid size and nodularity incidence were similar in the PKU group compared to healthy controls.

4.3 Stress-induced catecholaminergic and metabolic response in adult PKU

To evaluate the catecholamine metabolism and amino acid changes during exercise in PKU patients we conducted a series of dynamic tests with increasing intensity. During the CPT and HGT PKU patients had similar catecholaminergic, metabolic and physiologic response compared with the healthy controls. PKU patients performed significantly worse on the threadmill test, with lower VO2max and cumulative compered with the controls. However, the PKU groups performance expressed in MET, where body weight was accounted for, was not significantly different when compared to the control group. Baseline and stress-induced catecholamine levels were similar in both groups. Similarly, no significant difference in blood pressure and pulse rate was found between the PKU and control group. The measured catecholamine response to sympathetic stimuli supports an adequate catecholamine metabolism in PKU patients. During the intense exercise blood, Phe level increased significantly, in the PKU group compared to the healthy controls. This change compared to baseline levels was only 4.9%, which is similar to postprandial Phe values. Tyr levels were similar at baseline level and remained stable during and after the treadmill exercise in both the PKU and the control group.

5. Conclusions

The evaluation of the neurocognitive function of PKU patients

1.1 CANTAB neurocognitive tests can be a useful tool to evaluate the cognitive functions of PKU patients in an ambulatory setting.

1.2 Except for the response speed, the cognitive functions of PKU patients (fine motor coordination, working memory, planning and organizing skill, searching strategy, problem-solving and planning skills) are affected regardless of metabolic control.

1.3 Actual Phe levels do not influence the neurocognitive test results of PKU patients.

1.4 PKU patients with good metabolic control had better performance in certain neurocognitive tests (working memory, searching strategy, problem-solving and planning skills) compared to patients with worse metabolic control during this age period.

The evaluation of thyroid function and iodine status in adult PKU patients.

2.1 The incidence of clinically significant thyroid dysfunctions in PKU was similar to that found in the general population.

2.2 Low protein diet along with regular AAM consumption provides adequate iodine intake in an adult population.

2.3 In a region with sufficient iodine intake, such as Hungary, PKU patients with a low protein diet and inadequate AAM intake are at risk of iodine deficiency.

Stress-induced catecholaminergic and metabolic response in adult PKU

3.1 Stress-induced hormonal changes in PKU patients were similar to the control population, which suggest adequate catecholamine metabolism in PKU.

3.2 High Phe values do not seem to influence the Tyr dependent catecholamine levels in adult PKU.

3.3 Protein catabolism during intensive exercise did not lead to significant Phe level elevation, therefore this type of exercise can be considered safe in PKU patients.

6. List of publications related to the PhD thesis

1. **Sumánszki Cs**, Kiss E, Simon E, Galgóczi E, Soós A, Patócs A, Kovács B, Nagy EV, Reismann P, (2019). The Association of Therapy Adherence and Thyroid Function in Adult Patients with Phenylketonuria. Annals of Nutrition And Metabolism 75: 1 pp. 16-23. , 8 p.

   IF 2019: 2,848

2. **Sumánszki Cs**, Kovács K, Karvaly GB, Kiss E, Simon E, Patócs A, Tóth M, Komka Zs, Reismann P, (2020). Metabolic and catecholamine response to sympathetic stimulation in early-treated adult male patients with phenylketonuria. Hormones (Athens). 19: 3 pp. 395-402. , 8 p.

   IF 2019: 1,962

3. Bartus A, Palasti F, Juhasz E, Kiss E, Simon E, **Sumánszki Cs,** Reismann P, (2018). The influence of blood phenylalanine levels on neurocognitive function in adult PKU patients. Metabolic Brain Disease 33 : 5 pp. 1609-1615. , 7 p.

   IF 2018: 2,411

7. List of publications not directly linked to the PhD thesis

1. **Sumánszki Cs**, Barta AG, Reismann P, (2017). Phenylketonuria felnőttkorban [Adult phenylketonuria]. Orvosi Hetilap 158 : 47 pp. 1857-1863. , 7 p.

   IF 2017: 0,322

2. Barta AG, **Sumanszki Cs,** Reismann P, (2017). Csontanyagcsere felnőtt phenylketonuriás pácienseknél – hazai adatok [Bone metabolism in adults with phenylketonuria - Hungarian data]. Orvosi Hetilap 158 : 47 pp. 1868-1872. , 5 p.

   IF 2017: 0,322

3. Barta AG, **Sumanszki Cs,** Turgonyi Zs, Kiss E, Simon E, Serfozo Cs, Reismann P, (2020). Health Related Quality of Life assessment among early-treated Hungarian adult PKU patients using the PKU-QOL adult questionnaire. Molecular Genetics and Metabolism Reports 23: 100589, 7 p.
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4. Serfozo Cs, Barta AG, Horvath E, **Sumanszki Cs**, Csakany B, Resch M, Nagy ZZ, Reismann P (2020). Altered visual functions, macular ganglion cell and papillary retinal nerve fiber layer thickness in early-treated adult PKU patients. Molecular Genetics and Metabolism Reports. 25 p. 100649.

   IF 2019: 2,020

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