

# Phenylketonuria is not a risk factor for gut mucosa inflammation: a preliminary observation

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**Abstract. – BACKGROUND:** Phenylketonuria (PKU) is an inborn error of amino acid metabolism in which high phenylalanine (Phe) concentrations in the central nervous system adversely affect its development and functioning. In PKU high oxidative stress and inefficiency of free radical scavenging may lead to systemic chronic inflammation. We hypothesised that in PKU gut mucosa is chronically inflamed and that this leads to release of calprotectin from neutrophils and monocytes.

**AIM:** The aim of this study was to compare intestinal mucosa inflammation status, as measured using fecal calprotectin, in patients with PKU irrespective of compliance, and healthy controls.

**PATIENTS AND METHODS:** Forty-four patients with classical PKU were included in the study (21 male, 23 female; aged 0-41 years; mean  $\pm$  SEM: 16.5  $\pm$  1.7 years). Forty-eight healthy subjects (HS) aged 9-68 years (29.4  $\pm$  2.6 years) comprised the control group, of whom 21 were male and 27 female. Among PKU patients 25 had normal Phe blood concentrations and in 19 they were elevated. In all subjects calprotectin stool concentrations were assessed (PhiCal ELISA, Calpro, Lysaker, Norway).

**RESULTS:** Normal FC (fecal calprotectin) concentrations were found in 43 (97.7%) PKU patients and 46 (95.8%) HS. No correlation between dietary control of Phe blood concentrations and FC levels in PKU patients was found.

**CONCLUSIONS:** No detectable intestinal inflammation occurs in phenylketonuria. Lack of dietary control and elevated Phe levels do not seem to be risk factors for inflammation of the mucosa of the gut.

*Key Words:*

Phenylketonuria, Intestinal inflammation, Phenylalanine.

## Introduction

Phenylketonuria (PKU) is an inborn error of amino acid metabolism, in which elevated levels of phenylalanine (Phe) lead to toxic injury of the central nervous system<sup>1</sup>. As the treatment of PKU is based on restriction of natural protein intake, supplementation of amino acids must be provided. The diet and supplementation which enable normal brain development and functioning in PKU may lead to weakening of anti-radical mechanisms and alterations in gut microflora<sup>2-6</sup>. There is also evidence that points to a direct link between increased Phe concentrations and oxidative stress in patients with PKU<sup>7</sup>. This is why Rocha and Martins<sup>8</sup> recently suggested that more research on consequences of oxidative stress in PKU patients with insufficient dietary control is needed.

Calprotectin is a S100 family calcium-binding protein that is present in neutrophils, monocytes and other cells of the immune system. Fecal calprotectin (FC) is a biomarker of intestinal inflammation of established value<sup>9</sup>.

We hypothesized that PKU itself as well as its treatment promote chronic mucosal inflammation in the gut, leading to increased fecal calprotectin concentrations.

The aim of this work was to compare intestinal mucosa inflammation status using FC in patients with classical PKU and healthy controls. The study was designed to assess if there is a correlation between metabolic control in PKU patients and FC concentrations.

## Patients and Methods

The study group comprised of 44 patients with classical PKU, most of whom were diagnosed within the framework of the Polish national neonatal screening program. The group included 21 male (48%) and 23 female (52%) patients aged 0-41 years (mean  $\pm$  SEM: 16.5  $\pm$  1.7 years; median, 1<sup>st</sup>-3<sup>rd</sup> quartile: 17.4, 6.5-22.6). The patients underwent physical examination and daily dietary register control. Actual Phe level was determined in dry blood spot. The study group was divided into two subgroups, depending on Phe control status. Group A consisted of 25 (57%) compliant patients with Phe blood concentrations between 1 and 6 mg/dL in children younger than 12 years and 1-12 mg/dL in patients aged 12 years and older. Nineteen patients (43%) with poor dietary control of Phe levels were assigned to group B. The control group comprised of 48 healthy siblings and parents of PKU patients (HS; 21 male, 44%; 27 female, 56%), aged 9-68 years (mean  $\pm$  SEM: 29.4  $\pm$  2.6 years; median, 1<sup>st</sup>-3<sup>rd</sup> quartile: 31.5, 12-38.7).

All eligible subjects were asked to provide a single stool sample. The samples were frozen without delay and stored for subsequent investigation. Analyses were carried out using PhiCal ELISA Test (Calpro, Lysaker, Norway). Calprotectin concentrations up to 10 mg/L were considered normal, and larger than 15 mg/L were referred to as elevated.

Study participants aged 16 years and older, and parents of all children provided informed consent for participation in the study. The study was approved by the Bioethics Committee at Poznan University of Medical Sciences (Decision no. 85/12).

### Statistical Analysis

Data analysis was performed using Statistica software suite v. 9 (StatSoft, Tulsa, OK, USA). The main methods employed were: U Mann-Whitney test, Fisher's test, logistic regression and calculation of Spearman's rank correlation coefficient. Values are expressed as median (interquartile range; mean  $\pm$  SEM). The level of significance was set at  $p < 0.05$ .

## Results

Mean FC concentration was 0.3 mg/L (1.4  $\pm$  0.4; 0.2-1.0 mg/L) in PKU patients and 0.5 mg/L

(0.2-1.0, 1.5  $\pm$  0.5 mg/L) in HS. Normal FC levels were found in 43 (97.7%) PKU patients and 46 (95.8%) HS. FC concentrations did not differ between patients with good and insufficient dietary Phe levels control. FC concentration was intermediate (between 10 and 15 mg/L) in 1 PKU patient and 1 healthy control and abnormal in 1 healthy subject.

Median Phe concentration in PKU patients was 6.9 mg/dL (4.4-14.4; 9.4  $\pm$  1.4 mg/dL). No correlation between Phe blood levels and FC concentrations was found in PKU patients. There was no correlation between age and FC concentrations in patients with PKU and HS. In logistic regression analyses in PKU patients no relations between FC concentrations and age, sex, dietary compliance and Phe level control were identified.

## Discussion

This is the first specific study to investigate the intestinal inflammation in patients with PKU. The question that this research addressed was whether PKU is related to gut inflammation that is detectable using fecal calprotectin.

The inflammation could result from PKU-related increase in oxidative stress and decrease in capabilities of protection against free radicals. Indeed, the evidence for increased oxidative stress and reduced cellular potential of free radical scavenging in PKU is convincing<sup>8,10</sup>. It has been shown that even in compliant patients with good Phe levels control alpha-tocopherol, beta-carotene, coenzyme Q10<sup>2,7</sup>, zinc, selenium and L-carnitine<sup>5,11</sup> deficits exist. Reduced glutathione deficiency was observed in PKU patients as well<sup>6</sup>. At the same time oxidative stress is systemically increased in PKU, producing strain on weakened protection mechanisms. As it was pointed out by Sitta et al<sup>12</sup>, the oxidative stress in PKU may result not only directly from Phe concentration increase, but also from other factors. Among noteworthy indicators of oxidative stress the increase in free radical activity in PKU patients is reflected by thiobarbituric acid-reactive species (TBARS) that indicate lipoperoxidation<sup>6,12</sup>. It is an open question whether PKU-related anti-oxidant deficiencies stem from dietary restrictions.

Calprotectin (CP) is a calcium and zinc binding protein that is mainly present in neutrophils and monocytes. It was implicated in immune re-

actions as well as cell growth and differentiation<sup>13</sup>. Its concentration can be measured in most organic fluids, including plasma, saliva and urine. Presence of calprotectin in feces results from its release from inflammatory cells within the gut mucosa. FC allows for identification of chronic low-grade inflammation<sup>14</sup> in patients with obesity<sup>15-17</sup> and cystic fibrosis<sup>18,19</sup>. In turn, chronic low-grade intestinal inflammation may increase the risk of colorectal cancer<sup>20</sup>.

FC concentrations did not differ between patients with PKU and healthy subjects. This may support the view that neutrophil and monocyte activity within the intestinal mucosa in patients with PKU is not greater than in healthy persons. It is, therefore, unlikely that chronic low-grade inflammation is a problem in PKU patients.

There are several criticisms to this study that could provide clues for further research. Firstly, although FC sensitivity in intestinal inflammation detection is high, tissue biopsy and histopathological assessment remain its golden standard. Secondly, the study was observational and only in part it will help us in answering the main question that remains: should antioxidant supplementation or other specific interventions be undertaken in patients with PKU exhibiting gastrointestinal symptoms?

## Conclusions

Phenylketonuria is not a risk factor for intestinal mucosa inflammation as can be detected by fecal calprotectin.

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## Conflict of Interest

The Authors declare that there are no conflicts of interest.

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