Environmental Toxicology and Pharmacology 45 (2016) 302–308

Contents lists available at ScienceDirect

Environmental Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/etap

Evaluation of trace element and mineral status and related to levels of amino acid in children with phenylketonuria

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ARTICLE INFO

Article history:
Received 20 March 2016
Received in revised form 9 June 2016
Accepted 11 June 2016
Available online 14 June 2016

Keywords:
Phenylketonuria
Trace element
Minerals
Amino acids

ABSTRACT

The aim of the present study was to examine trace elements (Zn, Cu, Mn, Se, Fe, Co, Cr, Ni, Cd, Pb), minerals (Ca, Mg, K), amino acids status in children with phenylketonuria and also whether they were correlated with each other in phenylketonuric patients.

It has been found out that the HPA group was significantly lower than the control group with regards to Zn, Se, K, Ca, Mg and Zn/Cr levels ($p < 0.001$, $p < 0.01$, $p < 0.001$, $p < 0.01$, $p < 0.01$ and $p < 0.001$ respectively). In the patients with HPA, significantly strong positive correlations were observed between magnesium and calcium ($r = 0.791$; $p = 0.001$), also, indicates negative significant correlation between the concentrations of magnesium and phenylalanine ($r = -0.591$; $p = 0.026$).

The results of this study showed that, in the HPA group, phenylalanine-Mg relationship found, the presence of disease will in the evaluation of phenylalanine and other amino acids, together with the value of magnesium is required to consider.

1. Introduction

Phenylketonuria (PKU) is a genetic metabolic disorder resulted from a deficiency in activity of hepatic phenylalanine hydroxylase enzyme (PAH; EC 1. 14.16.1) (Okano and Nagasaka, 2013; Weigel et al., 2007; Knerr et al., 2013) which is responsible for the conversion of phenylalanine to another amino acid, tyrosine in the presence of molecular oxygen, iron and the essential cofactor tetrahydrobiopterin (BH_4) (Yew et al., 2013). Untreated PKU is typically characterized by elevated blood phe concentrations (Sawin et al., 2014). The high blood levels of phenylalanine disrupts the development of brain in childhood and brain functions at any age, making the central nervous system the most sensitive for hyperphenylalaninemia (HPA) (Okano and Nagasaka, 2013).

Treatment of PKU includes restricted phenylalanine intake through a natural protein restricted diet which is supplemented with a phenylalanine-free amino acid mixture (AAM) fortified with some essential micronutrients, such as trace elements, vitamins and minerals (Artuch et al., 2004).

Phenylalanine which is a biogenic small molecule and essential amino acid in humans, has been used as a diagnostic tool to identify phenylketonuria, a metabolic inherited autosomal recessive disorder (Schulpis et al., 2005). Phenylalanine is a precursor of numerous important biomolecules including thyroid hormone, melanin, and catecholeamines (dopamine, norepinephrine, and epinephrine) (Karam et al., 2013).

Trace elements can be classified in two separate categories as essential trace elements that play a crucial role in metabolic activities such as Cu, Zn, Mo and Se, and non-essential elements which may lead to serious intoxications and diseases such as Cd, As, Hg and Pb. Determination of trace elements in blood is crucial in forensic toxicology, clinical toxicology, environmental exposure and workplace analyses (Vacchina et al., 2014).

Trace elements are involved in tissue, cellular and subcellular functions and have an important role in health and disease. These functions include nerve conduction, muscle contraction, mitochondrial activity, membrane potential regulation and immunoregulation by both cellular and humoral mechanisms (Rania et al., 2013). Minerals are necessary for human nutrition, and their content in the body varies depending on their presence in drinking water, food and soil. Deficiency and excess of any chemical element may trigger adverse effects in the human body, particularly in children (Molska et al., 2014). Trace element content of body depends on the daily consumption and intestinal absorption which is affected by the interaction of different trace elements with each other as well as other nutrients (Dobbelaere et al., 2003).

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http://dx.doi.org/10.1016/j.etap.2016.06.014
1382-6689/

Selenium is a trace element with important antioxidant properties. Children in all age groups are susceptible to both deficiency and excess of this mineral (Athanasopoulou and Doulgeraki, 2013). In addition, selenium has additional crucial effects on health especially associated with immune response (Gać et al., 2015).

The biological roles of metallic trace elements especially iron, copper and zinc has been extensively studied in various pathologic conditions in many diseases (Rania et al., 2013). Copper is an essential trace element that is vital to health and play a role in the metabolisms of several key enzymes such as cytosolic superoxide dismutase and cytochrome oxidase of the mitochondrial electron transport (Rania et al., 2013). Zinc is an essential mineral which has a critical role in the stabilization of many biomembranes and in many physiologic processes, including protein synthesis and DNA synthesis, lymphocyte proliferation and cell division, and the production of several vital biologic elements including interleukin-2 helper T leukocytes. Deficiency of zinc element has been implicated in delayed or disrupted wound healing, loss of taste acuity, skin changes, poor appetite, cell-mediated immune and neurosensory disorders (Bogden, 2000).

Iron is implied in the constitution of enzymatic systems such as peroxidase, catalase and cytochromes that have an essential role in several cellular respiratory mechanisms, particularly in the mitochondrial respiratory channel (Rania et al., 2013). Manganese is an essential co-factor of metalloenzymes, especially mitochondrial superoxide dismutase. (Knerr et al., 2013).

Magnesium deficiency which clearly develops in people of all ages is most frequently caused by intestinal magnesium leaks, as from ileostomy losses or sprue. Ca bioavailability is influenced by endogenous factors such as age, gender, pregnancy and lactation and exogenous factors including amount of dietary levels of Ca, vitamin D, fat, lactose, phytate, fiber and oxalate. Numerous dietary elements may also affect urinary excretion of Ca, including alcohol, caffeine, proteins, sodium and phosphorus (Bogden, 2000).

The aim of the present study was to investigate whether altered trace elements (Zn, Cu, Mn, Se, Fe, Co, Cr, Ni, Cd, Pb), minerals (Ca, Mg, K) and amino acids levels had interactive connection in children with PKU and also whether they were correlated with each other in Phenylketonuric patients.

2. Materials and methods

2.1. Study design

The study included 40 PKU patients (23 girls and 17 boys) and 20 control subjects (9 girls and 11 boys). PKU patients were divided into HPA (hyperphenylalaninemic) and AAM (amino acid mixture phenylalanine-free) groups based on the initial level of plasma phenylalanine before and after treatment: before treatment PKU patients (Phe concentrations >600 μmol/L) and after treatment PKU patients (Phe concentrations <600 μmol/L). The study protocol was approved by the Yuzuncu Yil University Faculty of Medicine ethics committee and conducted in accordance with the Helsinki Declaration. Parents were properly informed and signed an informed consent. Patients examined in the Department of Pediatrics, Division of Pediatric Endocrinology, Yuzuncu Yil University. Study was conducted from Jun 2014 to March 2015

Five ml of venous blood were drawn after an overnight fasting and blood was centrifuged for 10 min at 2500 rpm. The serum was separated and kept in covered polypropylene tubes and stored at −86 °C, until trace element and mineral were measured. Determinations of concentrations of trace elements (Zn, Cu, Mn, Se, Fe, Co, Cr, Ni, Cd, Pb), minerals (Ca, Mg, K) were performed by ICP-OES, which were made with an Thermo ICP-OES iCAP 6300 DUO (Thermo Fisher Scientific, Inc UK). Multi-Element reference materials (Inorganic ventures IV-Stock-8) were used. Plasma amino acid levels were determined by using ion exchange chromatographic method with Aracus amino acid analyzer (membraPure GmbH, Neuendorfstraße 20a; 16761 Hennigsdorf/Berlin, Germany). Measurements of biochemical parameters (ALT, AST, glucose, creatinine) were analyzed by standard procedures at the central laboratory of the Medical Faculty of YYU using the Architect CI-16200 (Abbott Diagnostics, Abbott Park, IL, USA). The dietary formula used by children with phenylketonuria was PKU2 (Milupa, Friedrichsdorf, Germany), without phenylalanine with vitamin, trace element and mineral supplementation.

Table 1
Characteristics of Phenylketonuric Patients and Control Subjects (Mean ± SEM).

	Control	HPA	AAM
Gender (M/F)	11/9	12/8	11/9
Age (years)	8.73 ± 0.77	7.50 ± 1.10	6.44 ± 1.69
BMI (kg/m^2)	16.60 ± 0.74	16.01 ± 0.27	15.93 ± 0.60

HPA: hyperphenylalaninemia, AAM: phenylalanine-free Amino Acid Mixture.

2.2. Statistical analysis

The results are expressed as the arithmetic mean ± SEM. The statistical analysis was performed using analysis of variance (ANOVA) followed by post-hoc comparison of means Tukey test. The Pearson test was used for correlation studies in the PKU and AAM groups. The statistical analysis was carried out utilizing the SPSS®, version 22.0 (SPSS Inc., Chicago, Illinois, USA) statistical software.

3. Results

The characteristics of HPA (hyperphenylalaninemic) and AAM (amino acid mixture without phenylalanine) and control subjects included into study are shown in Table 1.

As a result of the analysis, it was found that the value of BMI in HPA and AAM groups were decreased according to control group ($p > 0.05$). We measured serum phenylalanine concentrations (945,61 ± 80,99 μmol/l) and tyrosine concentrations (39,27 ± 4,54 μmol/l) in HPA group, level of phenylalanine and tyrosine (191.86 ± 32.96 μmol/l and 54.68 ± 8.40 μmol/l) in AAM group.

Table 2 show the concentration of serum, Cu, Zn, Fe, Co, Mn, Se, Ni, Pb, Cd, Cr, K, Ca, Mg, Cu/Zn, Cu/Fe, Zn/Cr, Zn/Se, Fe/Zn, ALT, AST, glucose and creatinine groups of HPA, AAM and control.

Trace elements and minerals were determined in serum samples. Statistical analysis showed that the HPA group was significantly lower than the control group with regards to Zn, Se, K, Ca, Mg and Zn/Cr levels ($p < 0.001$, $p < 0.01$, $p < 0.001$, $p < 0.01$, $p < 0.01$ and $p < 0.001$ respectively). On the other hand the AAM group was also significantly lower than the control group regarding Zn, Se, K, Ca, Mg and Zn/Cr levels ($p < 0.01$, $p < 0.001$, $p < 0.05$, $p < 0.001$, $p < 0.001$, $p < 0.001$ respectively), whereas the HPA group had increased levels of Cd, Cu/Zn, Fe/Zn and AST according to control group ($p < 0.05$, $p < 0.01$, $p < 0.05$ respectively). Similarly, AAM group had significantly higher levels of Cr, Fe/Zn and AST levels than control group ($p < 0.05$, $p < 0.05$ and $p < 0.001$ respectively). However, AAM group had significantly higher level of Co level than HPA group ($p < 0.05$) (Figs. 1–3). Moreover, no significant differences in Cu, Fe, Mn, Ni, Pb, Cu/Fe, Zn/Se, ALT, glucose, creatinine levels were observed in the HPA and AAM groups, as compared to the control group ($p > 0.05$) (Table 2).

The mean plasma amino acid levels of the HPA (hyperphenylalaninemic), AAM (amino acid mixture without Phenylalanine) and control groups are shown in Table 3.