JBACHS
Journal of Basic and Clinical Health Sciences

J Basic Clin Health Sci 2017; 3:79-81
DOI: 10.5152/jbachs.2017.240

Original Article

The Effect of Large Neutral Amino Acids on Blood Phenylalanine Levels in Patients with Classical Phenylketonuria

Pelin Teke Kısa[1], Engin Köse[1], Nusret Ören[2], Nur Arslan[1]

[1]Department of Pediatric Metabolism and Nutrition, Dokuz Eylül University, İzmir, Turkey
[2]Department of Pediatrics, Dokuz Eylül University, İzmir, Turkey

Address for Correspondence: Nur Arslan **E-mail:** nurarslan@deu.edu.tr
Received: 21.07.2017; **Accepted:** 02.08.2017

Cite this article as: Kısa PT, Kose E, Oren N, Arslan N. The Effect of Large Neutral Amino Acids on Blood Phenylalanine Levels in Patients with Classical Phenylketonuria. J Basic Clin Health Sci 2017; 3: 79-81.

Abstract

Purpose: Phenylketonuria (PKU) is an inherited metabolic disease caused by low levels of the enzyme phenylalanine hydroxylase. Treatment includes dietary restriction of phenylalanine (Phe) and supplementation with tetrahydrobiopterin and large neutral amino acids (LNAAs). The purpose of this study is to evaluate the effect of LNAA therapy on blood Phe levels in patients undergoing treatment for at least 6 months.

Methods: Blood Phe levels in 34 patients with classical PKU receiving LNAA supplementation for longer than 6 months were compared before the treatment and during the first 3 years of treatment.

Results: The mean age of patients was 20.7±6.6 years, and the mean age at the beginning of LNAA therapy was 16.0±6.1 years. The median duration of LNAA use was 32 months (minimum-maximum: 8-171 months). The mean blood Phe level before the use of LNAA supplementation was 23.1±5.9 mg/dL, whereas the first blood Phe level 1 month after the start of LNAA therapy was 18.9±5.5 mg/dL (p=0.000). The mean blood Phe levels in the first (n=34), second (n=33), and third year (n=28) after the beginning of LNAA supplementation were 21.1±5.0 mg/dL, 21.2±6.4 mg/dL, and 21.3±5.8 mg/dL, respectively. There was no significant variation between these and pre-treatment values (p>0.05).

Conclusion: There were no significant decreases in the blood Phe levels of patients receiving LNAA supplementation observed in this study. This may be due to poor dietary compliance. Nevertheless, since LNAA supplementation reduces the passage of Phe through the blood-brain barrier, it is still recommended in all adolescent and adult patients with PKU not complying with diet therapy, even if blood values do not change.

Keywords: Phenylalanine, classical phenylketonuria, LNAA supplementation

INTRODUCTION

Phenylketonuria (PKU) is an autosomal recessive metabolic disorder caused by a deficiency in the enzyme phenylalanine hydroxylase that converts the essential amino acid phenylalanine (Phe) into tyrosine. Phenylalanine hydroxylase deficiency leads to varying degrees of hyperphenylalaninemia (HFA). The prevalence of PKU is approximately 1/4500 in Turkey, and it is included in the neonatal screening program (2). If untreated, high blood Phe concentrations may lead to severe cognitive function disorder, seizures, and psychiatric problems (3). The aim of PKU treatment is to maintain blood Phe levels below 6 mg/dL. A Phe-restricted diet and tetrahydrobiopterin, a cofactor of phenylalanine hydroxylase in responsive patients, are the principal therapeutic options.

Large neutral amino acids (LNAA) are made up of the amino acids valine, leucine, isoleucine, histidine, lysine, methionine, threonine, tryptophan, and tyrosine. The use of LNAAs in mice has been shown to reduce Phe levels in the central nervous system (CNS) (4, 5 ,6). LAT-1 is a transport protein found in the intestine and blood-brain barrier, and it is a competitive transporter for LNAA. If non-Phe LNAA concentrations in the environment are higher, these LNAAs cross the blood-brain barrier, whereas if Phe concentrations are higher, Phe crosses the barrier. LNAA supplementation without Phe reduces Phe and increases non-Phe LNAA levels in the CNS (6, 7). It also increases neurotransmitter levels in the CNS (8). LNAA supplementation is particularly recommended in non-diet compliant, late diagnosed or previously undiagnosed adolescents and adults (9). In literature, there are a limited number of studies showing the effect of long-term LNAA supplementation on blood Phe levels in patients. The aim of this study was to evaluate clinical findings and blood Phe levels in patients with PKU receiving LNAA supplementation for at least 6 months.

METHODS

Study Design

In this study, there were 34 patients with classical phenylketonuria recruited from the Division of Pediatric Metabolism and Nutrition at Dokuz Eylül University. All patients had diet programs appropriate to LNAA supplementation. The mean daily protein consumption was 1 gr/kg/day. All patients received LNAA therapy at a dose of 685 mg/kg/day. LNAA represented 40% of total daily protein intake and protein from natural food sources 60%.

Statistical Analysis

The data derived from a normally distributed population (Kolmogorov-Smirnov test, $p>0.05$) were reported as mean±standard deviation. Variables that were not normally distributed (Kolmogorov-Smirnov test, $p<0.05$) were expressed as median values (minimum-maximum). Categorical variables were expressed as numbers and percentages (%).

Univariate repeated measure analysis of variance (ANOVA) with Greenhouse-Geisser correction was performed to analyze changes in plasma Phe levels over time (prior to and at 1, 2, and 3 years after LNAA therapy). Post-hoc analysis was performed using the Bonferroni test to identify the source of significant differences among mean values. A significance level of 0.008 was set for post-hoc multiple comparisons. Statistical Package for Social Sciences version 22.0 (IBM Corp.; Armonk, NY, USA) for Windows was used for all statistical analyses.

Ethical Approval

All procedures performed were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The study received ethical approval from the Dokuz Eylül University School of Medicine Ethical Committee.

RESULTS

The mean age of the patients was 20.7±6.6 years (minimum-maximum: 11-37). The mean age at the start of LNAA therapy was 16.0±6.1 years. The median duration of LNAA use was 32 months (minimum-maximum: 8-171). Eleven (33%) patients were female and 23 (67%) were male (Table 1). Twelve (36%) received LNAA therapy in powder form and 22 (64%) in tablet form.

The mean final blood Phe level before LNAA therapy was 23.1±5.9 mg/dL, whereas the first blood Phe level after the commencement of LNAA supplementation was 18.9±5.5 mg/dL ($p<0.001$) within the first 3 months. The mean plasma Phe levels in the first year (34 patients), second year (33 patients), and third year (28 patients) after the commencement of LNAA therapy were 21.1±5.0 mg/dL, 21.2±6.4 mg/dL, and 21.3±5.8 mg/dL, respectively (Figure 1). No statistically significant difference was determined for any of the three values compared to pre-LNAA values ($p=0.077$, $p= 0.978$, and $p= 0.152$, respectively). In addition, no significant difference was determined between plasma Phe levels at the first, second, and third year after treatment ($p>0.05$).

DISCUSSION

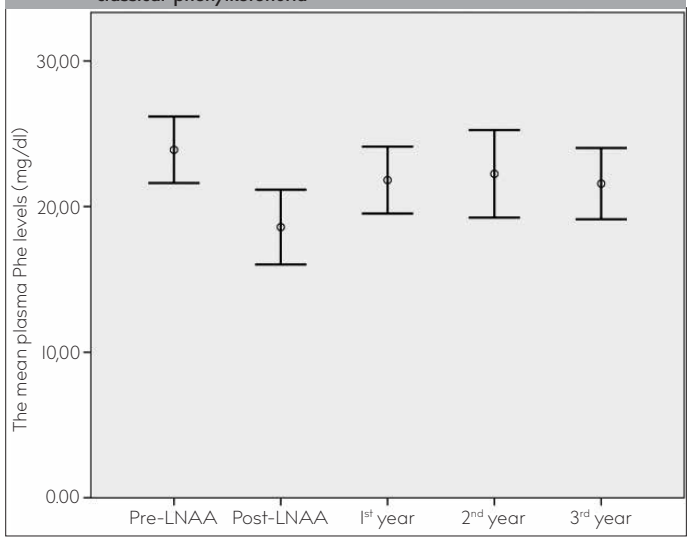
This study investigated the effect of LNAA supplementation on blood Phe levels in patients with classical PKU, exhibiting poor compliance with a Phe-restricted diet and receiving long-term LNAA supplementation. Although a significant decrease in plasma Phe values was observed 1 month after LNAA use, no significant variation compared to baseline was determined at long-term follow-up. Studies of adult patients with PKU have determined decreases between 24% and 52% in blood Phe values 4 weeks after LNAA (10-13). A decrease of only 5% in plasma Phe levels, albeit a statistically significant one, was determined on the 4th week of treatment in our patients. This may be attributed to our patients' inadequate diet compliance.

The aim of a PKU treatment is to maintain blood Phe levels at below 6 mg/dL. A Phe-restricted diet and tetrahydrobiopterin, a cofactor of phenylalanine hydroxylase, are the principal therapeutic options used. A Phe-restricted diet is a highly limited (meat, milk, cheese, yogurt, eggs, nuts, beans, seafood, etc. must be entirely eliminated from the diet) form of treatment, and compliance is very difficult. Although, dietary compliance is good in the early periods of life, compliance rates decrease in adolescence and adulthood worldwide, and also in Turkey (14, 15). In addition to compliance problems, this diet is low in natural foodstuffs and can lead to deficiencies in various micronutrients (15, 16). It also impairs families' quality of life and exacerbates anxiety levels (17, 18). Moreover, even if very good adherence to the diet is achieved, conditions such as impairment of cognitive functions, anxiety, and depression may be observed (19, 20). For these reasons, there is an ongoing search for alternative treatment options to reduce Phe levels in the blood or brain of patients with PKU. One

Table 1. Characteristics and plasma phenylalanine levels in patients with classical phenylketonuria (mean±SD)

Characteristics	
Gender (n,%)	
Male/Female	23 (67.0)/11 (33.0)
Age (years)	20.7±6.6
Age at LNAA therapy (years)	16.0±6.1 years
Initial phenylalanine level	23.1±5.9 mg/dL
First phenylalanine levels after LNAA treatment	18.9±5.5 mg/dL
Phenylalanine levels after LNAA treatment	
1st year	21.1±5.0 mg/dL
2nd year	21.2±6.4 mg/dL
3rd year	21.3±5.8 mg/dL

Figure 1. Blood phenylalanine levels after LNAA treatment in patients with classical phenylketonuria



such is LNAA use, which has been shown to reduce the absorption of intestinal Phe and to prevent to some degree the passage of Phe into the brain. LNAA preparations contain non-Phe amino acids acting as neurotransmitters in the brain that have been shown to decrease in patients with PKU (21). LNAA supplementation reduces Phe levels in the brain (22, 23) and increases non-Phe LNAA levels and neurotransmitter levels (19, 22, 24). An improvement in concentration, response to external stimuli, socialization, emotional tolerance and psychological state, and a decrease in behavior harmful to self and others have been found in late diagnosed mentally retarded PKU patients (25). LNAA therapy is therefore recommended even if dietary adherence is poor, in order to reduce the Phe entering the brain and to increase neurotransmitter synthesis.

There are a number of limitations to this study. The first is that different age groups could not be compared due to the low patient numbers. The second limitation is the absence of a patient group with good dietary compliance. The effect of LNAA use on blood Phe levels might have been assessed more accurately in the presence of such a group. The third limitation is that no neurocognitive assessment was performed. Showing the type of changes in cognitive functions before and after treatment may be important in establishing the long-term effects of treatment. The final limitation is that the levels of neurotransmitter metabolites were not measured.

In conclusion, LNAA supplementation does not affect blood Phe levels in patients with classical PKU and poor dietary compliance. Nevertheless, since LNAA supplementation reduces the passage of Phe through the blood-brain barrier, its use is recommended in all patients with PKU not complying with diet therapy, even if blood values do not change.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Dokuz Eylül University School of Medicine (Decision No: 2017/19-19, 3484-GOA).

Peer-review: Externally peer-reviewed.

Author contributions: Concept - N.A.; Design - P.T.K., N.A., L.G.P.; Supervision - N.A.; Resource - P.T.K., N.Ö.; Materials - E.K., P.T.K.; Data Collection and/or Processing - E.K., N.Ö.; Analysis and /or Interpretation - P.T.K.; Literature Search - P.T.K.; Writing - P.T.K.; Critical Reviews - N.A.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study has received no financial support.

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