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Original article

Vitamin/mineral and micronutrient status in patients with classical phenylketonuria

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SUMMARY

Background & aims: Strict low-phenylalanine diet is associated with an increased risk of developing micronutrient deficiencies in patients with phenylketonuria (PKU). The primary objective of this single-center, case–control study was to assess the nutritional parameters of patients with PKU on strict low-phenylalanine diet without vitamin and mineral supplementation compared to a healthy control group. Secondary objective was to identify the adequacy of vitamin/mineral supplementation in phenylalanine-free (Phe-free) amino acid formulas.

Methods: A total of 112 age- and sex-matched patients with PKU and 36 controls who did not take vitamin or mineral supplementation at least for the last 6 months were enrolled in the study. Biochemical and hematological markers including hemoglobin, serum vitamin B12, folic acid, iron, ferritin, transferrin saturation, copper, prealbumin, albumin, total protein, phosphorus, calcium, 25-hydroxy vitamin D, zinc, vitamin A and vitamin E levels were screened from fasting morning blood samples.

Results: One hundred and twelve patients with classical PKU (53 females, 47.3%) and 36 healthy controls (18 females, 50.0%) were enrolled in the study. The mean age of patients with PKU was 136.8 ± 82.1 months (18–377). Median serum vitamin B12 level of patients with PKU was found to be higher than the control group ($p = 0.002$). Vitamin B12 deficiency was 15.2% and 30.6% in patients with PKU and healthy controls, respectively ($p = 0.040$). Mean serum folic acid level was higher in patients with PKU than the control group ($p < 0.0001$). In 55.4% of patients with PKU, and 2.8% of the control group, serum folic acid level was above the reference range ($p < 0.0001$). The frequency of ferritin and prealbumin values above the reference range was found to be higher in patients with PKU compared to the control group (44.4% vs 16.9%, $p = 0.001$; 38.8% vs 22.1%, $p = 0.020$, respectively). 25-Hydroxy vitamin D deficiency was detected in 53.6% and 47.2% of patients with PKU and the control group, respectively. Mean serum copper level was higher in the well-controlled (114.3 ± 26.7 μg/dL) group than the poorly controlled group (101.0 ± 29.1 μg/dL) ($p = 0.022$).

Conclusions: Phe-free amino acid formulas provide adequate vitamin A and zinc levels in patients with PKU, and result in excess folic acid, vitamin B12, copper and vitamin E values that are higher than required levels. Our results demonstrate a high percentage of vitamin D deficiency in patients with classical PKU and also in healthy controls in Turkey.

1. Introduction

Phenylketonuria (PKU) is a congenital disorder of the phenylalanine (Phe) metabolism caused by mutations in the liver enzyme, phenylalanine hydroxylase, encoded by the PAH gene (OMIM 261600). Individuals with PKU must maintain a life-long Phe-restricted diet [1]. High-biological-protein food are replaced by phenylalanine-free (Phe-free) amino acid formulas in these patients. Unless the affected child is maintained on a strict low-phenylalanine diet, PKU leads to mental retardation, seizures, behavioral difficulties, and other neurological symptoms [2].

In PKU, a strict low-phenylalanine diet is associated with the risk of developing vitamin and mineral deficiencies [3]. Due to the

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vitamin/mineral deficiency associated with restriction of natural proteins in the early days of diet treatments, vitamin/mineral supplementation has become a part of the treatment. Unfortunately, vitamin/mineral supplementation does not have higher bioavailability than natural sources. For example, heme iron and milk calcium have relatively higher bioavailability due to specific absorption with specific transport system and formations [4,5]. On the other hand, with the increasing trend of adding vitamins, minerals and trace mineral supplements to Phe-free amino acid formulas over the last 20 years, over supplementation of vitamin/mineral has become a matter of debate as well as vitamin/mineral deficiency in patients with PKU [6]. There is no consensus on the amount of vitamin/mineral supplementation for Phe-free amino acid formulas.

The objective of this single-center, case–control study was to assess the nutritional parameters (growth parameters, vitamin, mineral and hematological status) of patients with PKU on strict low-phenylalanine diet without vitamin and mineral supplementations compared to a healthy control group. The second objective was to compare the differences of nutritional status between poorly controlled and well-controlled patients with PKU to identify the adequacy of vitamin mineral supplementation in phenylalanine free amino acid formulas.

2. Methods

A single-center, retrospective case–control study was conducted between January and May 2016. Patients with acute infection and those with any chronic disease other than PKU (two patients with hypothyroidism, one patient with thalassemia major, one patient with growth hormone deficiency), acute infection (while sample received) and receive any medication were excluded from the study. Patients treated with sapropterin dihydrochloride and large neutral amino acids were not included in the study. The Phe-free amino acid formulas of patients with PKU were PKU Anamix Infant, PKU 2-prima, 2-secunda, 3, PKU Lophlex LQ 20 (Nutricia Metabolics), PKU Cooler 10, 15 (Nestlé Health Science), and Comida PKU A, B, C (Comidamed). The control group consisted of healthy subjects. Children and adults who referred to hospital for routine control were evaluated to be included in the study. A total of 112 age- and sex-matched patients with PKU and 36 controls who did not receive vitamin or mineral supplementation for at least 6 months were enrolled in the study.

Patients with classical PKU were divided into two subgroups as low and high adherence to diet. High adherence to diet was defined as annual mean plasma Phe level <360 μmol/L for patients under 6 years of age; <480 μmol/L for children between 6 and 10 years; ≤600 μmol/L for older patients [7]. Demographics (age, gender) and anthropometric measurements (weight SDS, height SDS and BMI [body mass index] SDS, W/H [weight for height]) were recorded.

Biochemical and hematological parameters including serum vitamin B12, folic acid, serum iron, ferritin, transferrin saturation, copper, prealbumin, albumin, total protein, phosphorus, calcium, 25-hydroxy vitamin D, zinc; and plasma vitamin A, vitamin E, and hemoglobin levels were measured from fasting morning blood samples.

Reference ranges for laboratory parameters were determined as follows: vitamin B12: 200–1900 pg/mL; folic acid: 3–17.5 ng/mL; serum iron: 50–150 μg/dL; ferritin: 14–325 ng/mL; transferrin saturation: 15–50%; copper: 64–140 μg/dL; prealbumin: 21–41 mg/dL; 25-hydroxy vitamin D: 20–42 ng/mL; zinc: 65–140 μg/dL. Vitamin A and vitamin E: reference range was determined according to the age [1,8–11].

High-performance liquid chromatography was used to measure plasma Phe, and vitamin A and vitamin E levels with Prominence SPD-20A and Prominence SIL-20AHT (Shimadzu, Kyoto, Japan), respectively. Serum vitamin B12, folic acid and ferritin levels were determined by the immunoassay method with Unicel DxI800 (Beckman Coulter, CA. USA). Serum iron, copper, zinc, prealbumin, albumin, total protein, calcium and phosphorus levels were analyzed by the spectrophotometric method with AU5800 analyzer (Beckman Coulter, CA. USA). Immunoassay method was utilized to analyze 25-hydroxy vitamin D with Advia Centaur XP Analyzer (Siemens Healthcare Diagnostics, Erlangen, Germany).

3. Statistical analysis

Data were analyzed with the Statistical Package for the Social Sciences (SPSS) computer software (version 21.0; SPSS, Chicago, IL). Categorical variables were assessed by means of the Chi-square test and expressed as number and percentage (%). Parametric [mean ± standard deviation (minimum–maximum)] and non-parametric [median (minimum–maximum)] continuous variables were assessed using Mann–Whitney U-test and t-test, respectively. Kolmogorov–Smirnov and Shapiro–Wilk tests were performed to examine the normality of parameters. Pearson's correlation coefficient was used to analyze the associations between continuous variables. A two-tailed p-value <0.05 was considered significant.

4. Results

One hundred and twelve patients with classical PKU (53 females, 47.3%) and 36 healthy controls (18 females, 50.0%) were enrolled in the study. There were no differences between PKU and control groups in terms of age and gender distribution. The analysis of anthropometric measurements, weight SDS, height SDS, BMI SDS, and W/H did not reveal any difference between the two groups (Table 1).

In the analysis of laboratory findings of patients with PKU and the healthy control group, median serum vitamin B12 level of patients with PKU (396 pg/mL) was found to be higher than the control group (260 pg/mL) ($p = 0.002$). Vitamin B12 deficiency was 15.2% and 30.6% in patients with PKU and healthy controls, respectively ($p = 0.040$). Mean serum folic acid level was higher in patients with PKU than the control group ($p < 0.0001$). Folic acid deficiency was not detected in either group (data not shown in table). Median serum ferritin and mean serum prealbumin concentrations were found to be increased in patients with PKU compared to the control group (25.1 vs 13.4 ng/mL, $p = 0.007$; 24.1 ± 4.6 vs 21.9 ± 3.9 mg/dL, $p = 0.013$, respectively). The frequency of ferritin and prealbumin levels above the reference range was higher in patients with PKU than the control group. Mean serum phosphorus levels were lower in patients with PKU compared to the control group. On the contrary, median plasma vitamin A level was found to be higher in patients with PKU than the control group (55.0 vs 45.7 μg/dL, respectively, $p = 0.014$). There was no vitamin A or vitamin E deficiency in either group (data not shown in table). 25-Hydroxy vitamin D deficiency was detected in 53.6% and 47.2% of patients with PKU and the control group, respectively (Table 2).

When we categorized the patients with PKU into two groups as low adherence to diet (LAD) ($n = 71$) and high adherence to diet (HAD) ($n = 41$), no difference was seen with respect to age between the two groups. Females accounted for 57.7% and 29.3% of the patients in low adherence to diet and high adherence to diet groups, respectively ($p = 0.004$). In the analysis of anthropometric measurements, mean weight SDS did not differ between two groups. Mean height SDS was statistically higher in the HAD group than LAD ($p = 0.008$) and consistently, mean BMI SDS and median W/H

Table 1
Analysis of demographic and anthropometric findings of patients with phenylketonuria and control groups.

Parameters	Phenylketonuria group (n = 112)	Control group (n = 36)	p
Gender F/M, n (%)	53 (47.3)/59 (52.7)	18 (50.0)/18 (50.0)	0.780
Age, (month) Mean ± SD (min–max)	136.8 ± 82.1 (18–377)	119.7 ± 37.3 (73–206)	0.087
Weight SDS Median (min–max)	0.35 (−3.6 to 2.7)	0.34 (−1.65 to 1.7)	0.783[a]
Height SDS Mean ± SD (min–max)	−0.1 ± 1.2 (−3.2 to 2.5)	0.1 ± 0.9 (−1.7 to 1.6)	0.374
BMI SDS Mean ± SD (min–max)	0.2 ± 1.2 (−2.5 to 2.3)	0.2 ± 1.2 (−1.3 to 1.5)	0.988
W/H, (%) Median (min–max)	101 (82–201)	101 (89–123)	0.193[a]

F: female, M: male, SD: standard deviation, min: minimum, max: maximum, SDS: standard deviation score, BMI: body mass index, W/H: weight-for-height.
[a] Mann Whitney U test was performed.

Table 2
Analysis of laboratory findings of patients with phenylketonuria and control groups.

Parameters	Phenylketonuria group (n = 112)	Control group (n = 36)	p
Serum vitamin B12 level, (pg/mL) Median (min–max)	396 (74–986)	260 (110–601)	0.002[a]
Vitamin B12 (<200 pg/mL), n (%)	17 (15.2)	11 (30.6)	0.040
Serum folic acid level, (ng/mL) Mean ± SD (min–max)	19.7 ± 9.3 (2.5–49.0)	9.7 ± 3.6 (6.2–16.5)	<0.0001
Folic acid (>17.5 ng/mL), n (%)	62 (55.4)	1 (2.8)	<0.0001
Serum iron level, (μg/dL) Mean ± SD (min–max)	79.2 ± 35.6 (12–175)	76.9 ± 33.6 (10–142)	0.732
Iron (<50 μg/dL), n (%)	22 (19.6)	6 (16.7)	0.594
Serum ferritin level, (ng/mL) Median (min–max)	25.1 (1.7–82.4)	13.4 (3.2–32.9)	0.007[a]
Ferritin (<14 ng/mL), n (%)	19 (16.9)	16 (44.4)	0.001
Transferrin saturation level, (%) Mean ± SD (min–max)	23.3 ± 10.4 (3–57)	21.6 ± 10.1 (2–43)	0.404
Transferrin saturation (<15%), n (%)	24 (21.4)	11 (30.6)	0.341
Serum copper level, (μg/dL) Mean ± SD (min–max)	105.9 ± 28.9 (31.3–195.6)	115.5 ± 21.6 (52.4–156.3)	0.074
Copper (<64 μg/dL), n (%)	6 (5.4)	1 (2.8)	N/A
Serum prealbumin level, (mg/dL) Mean ± SD (min–max)	24.1 ± 4.6 (10.5–35.5)	21.9 ± 3.9 (15.9–29.8)	0.013
Prealbumin (<21 mg/dL), n (%)	21 (21.1)	14 (38.8)	0.020
Serum albumin level, (g/dL) Mean ± SD (min–max)	4.5 ± 0.3 (3.8–5.4)	4.5 ± 0.3 (3.7–5.1)	0.745
Serum total protein level, (g/dL) Mean ± SD (min–max)	7.1 ± 0.5 (5.7–8.1)	7.2 ± 0.5 (5.9–7.9)	0.732
Serum phosphorus level, (mg/dL) Mean ± SD (min–max)	4.4 ± 0.8 (2.4–6.6)	4.8 ± 0.6 (3.8–5.8)	0.004
Serum calcium level, (mg/dL) Mean ± SD (min–max)	9.8 ± 0.4 (8.7–10.9)	9.7 ± 0.5 (8.6–10.6)	0.514
Serum 25-hydroxy vitamin D level, (ng/mL) Mean ± SD (min–max)	19.2 ± 7.8 (2.6–39.3)	19.9 ± 6.3 (4.9–32.2)	0.600
25-Hydroxy vitamin D (<20 ng/mL), n (%)	60 (53.6)	17 (47.2)	0.445
Serum zinc level, (μg/dL) Median (min–max)	90.5 (67–165)	92 (64–121)	0.128[a]
Zinc (<65 μg/dL), n (%)	1 (0.9)	2 (5.6)	N/A
Plasma vitamin A level, (μg/dL) Median (min–max)	55.0 (28.9–132.8)	45.7 (28.6–103.2)	0.014[a]
Elevated plasma vitamin A level n (%)	30 (26.8)	5 (13.9)	0.107
Plasma vitamin E level, (mg/dL) Median (min–max)	1.2 (0.46–3.3)	1.1 (0.7–2.9)	0.284[a]
Elevated plasma vitamin E level n (%)	15 (13.4)	4 (11.1)	0.705
Hemoglobin level, (g/dL) Mean ± SD (min–max)	12.8 ± 1.2 (9.0–11.9)	12.6 ± 1.2 (10.3–14.5)	0.485

SD: standard deviation, min: minimum, max: maximum, SDS: standard deviation score, N/A: statistical analysis not performed.
[a] Mann Whitney U test was performed.

were significantly higher in the LAD group than HAD ($p = 0.049$; $p < 0.0001$) (Table 3).

In the assessment of laboratory findings, mean plasma phenylalanine level was 943.2 ± 314.0 and 369.7 ± 102.8 μmol/L in LAD and HAD groups, respectively ($p < 0.0001$). Mean serum vitamin B12 level was statistically higher in the HAD than the LAD group. Mean serum copper level was also higher in the HAD group compared to LAD. Although frequency of copper deficiency was

Table 3
Analysis of demographic and anthropometric findings of PKU patients with low and high adherence to diet.

Parameters	Phenylketonuria N = 112		p
	Low adherence to diet n = 71	High adherence to diet n = 41	
Gender F/M, n (%)	41 (57.7)/30 (42.3)	12 (29.3)/29 (70.7)	0.004
Age, (month) Mean ± SD (min–max)	138.9 ± 80.1 (18–377)	133.1 ± 84.4 (18–207)	0.790
Weight SDS Median (min–max)	0.2 ± 1.1 (−2.2 to 2.7)	0.0 ± 1.2 (−3.6 to 2.4)	0.319
Height SDS Mean ± SD (min–max)	−0.3 ± 1.3 (−3.2 to 2.5)	0.3 ± 1.0 (−1.8 to 2.5)	0.008
BMI SDS Mean ± SD (min–max)	0.3 ± 1.2 (−2.2 to 2.3)	−0.1 ± 1.1 (−2.5 to 2.3)	0.049
W/H, (%) Median (min–max)	108 (83–201)	98 (82–146)	0.001[a]

F: female, M: male, SD: standard deviation, min: minimum, max: maximum, SDS: standard deviation score, BMI: body mass index, W/H: weight-for-height.
[a] Mann Whitney U test was performed.

higher in LAD (7.0%) than HAD (2.4%), this difference was not statistically significant. Serum prealbumin level was lower in the HAD group (22.5 ± 4.4 mg/dL) than the LAD group (24.9 ± 4.6 mg/dL) ($p = 0.011$). Compared to the LAD group, higher serum phosphorus levels were seen in HAD. However, median plasma vitamin E levels did not differ between the LAD and HAD groups, and frequency of plasma vitamin E levels above the reference range was higher in HAD than the LAD group (21.9% vs 8.5%, $p = 0.030$). While mean serum folic acid and 25-hydroxy vitamin D levels were higher in the HAD group compared to LAD, these differences were not statistically significant (Table 4).

Correlation analysis of laboratory parameters revealed negative correlations between plasma phenylalanine level and serum phosphorus ($r = -0.477$, $p < 0.0001$), 25-hydroxy vitamin D ($r = -0.309$, $p = 0.001$), copper ($r = -0.274$, $p = 0.005$) and vitamin B12 levels ($r = -0.265$, $p = 0.005$). Also, there was a positive correlation between plasma phenylalanine level and serum prealbumin level ($r = 0.256$, $p = 0.003$) as well as between age and prealbumin level in PKU ($r = 0.556$, $p < 0.0001$) and control groups ($r = 0.682$, $p < 0.0001$).

5. Discussion

In this study, we evaluated patients with PKU and a healthy control group who had not received vitamin and/or mineral supplementation at least during the last 6 months. Exclusion of PKU patients treated with sapropterin dihydrochloride and large neutral amino acids and evaluating a larger study population than previous studies [12–15] allowed us to eliminate confounding factors and provided insight about the effect of vitamin/mineral supplemented Phe-free amino acid formulas on nutritional status of patients with PKU in routine clinical practice. We found higher serum vitamin B12, folic acid, ferritin and plasma vitamin A levels in patients with PKU compared to the control group. Furthermore, high adherence to diet was associated with higher serum vitamin B12, copper, phosphorus levels, and lower serum prealbumin levels than low adherence to diet.

During early years of PKU treatment, desire to achieve normal phenylalanine concentrations with very restrictive diet therapy had led to growth impairment. In our study, there were no differences between healthy controls and patients with PKU with respect to height, weight, BMI SDS and W/H. Studies in Dutch children emphasized that mean height z-scores deteriorated from birth to 3 years of age [16], and no relationship was found between growth and the severity of blood phenylalanine control [17]. Although no differences were observed between anthropometric measurements of patients with PKU and the healthy control group, our study revealed decreased height SDS and increased weight SDS, BMI SDS and W/H in the LAD group compared to the HAD group.

Vitamin B12 deficiency is mainly reported in adolescents or adult patients who have stopped or relaxed their diets and who are less adherent to Phe-free amino acid formulas [18,19]. In contrast, we determined high serum vitamin B12 levels and a lower frequency of vitamin B12 deficiency in patients with PKU than the healthy control group. Moreover, in the LAD group serum vitamin B12 level was significantly lower than HAD, and serum vitamin B12 level had a negative correlation with plasma Phe level. These findings may be explained with the high cobalamin content of Phe-free amino acid formulas, so the risk of vitamin B12 deficiency is minimal if adherence to Phe-free amino acid formula is adequate [20]. The high rate of vitamin B12 deficiency in our control group (30.6%) is consistent with previous studies performed in Turkey [21–23].

In our study, serum folic acid level was higher in patients with PKU than the healthy controls. The higher serum folate level in the HAD group compared to LAD supports the high folic acid content of Phe-free amino acid formulas. In previous studies, similar to our findings, high serum and/or red cell folate levels were documented in both adult and pediatric patients with PKU [14,20,24].

Prealbumin is a much more sensitive predictor of plasma protein status than albumin and total protein, and low prealbumin levels in children with PKU have been reported previously [1,25,26]. In our study, serum prealbumin levels were diminished in 21.1% of patients with PKU. Also, high adherence to treatment and lower plasma phenylalanine levels were associated with the decline in prealbumin levels. However, in the comparison of patients with PKU and the healthy group, prealbumin level was significantly higher in the PKU group. This finding is inconsistent with the previous studies [27,28] and may be related to the relatively younger (without a significant difference) mean age of the control group than the PKU group as prealbumin levels showed a positive correlation with the age in PKU and control groups in our study, as reported in a previous study [1]. Although the difference between prealbumin levels of the two groups do not indicate considerable clinical significance, further studies with the same age group are warranted to determine the effect of low-phenylalanine diet on prealbumin levels.

There have been conflicting reports about zinc status in patients with PKU [29–35]. Inflammatory stress, acute infections and fasting status may influence the results [36,37]. We assessed the zinc level of patients without any medication and in fasting morning blood samples to reduce false results. In our study, there was no

Table 4
Analysis of laboratory findings of PKU patients with low and high adherence to diet.

Parameters	Phenylketonuria N = 112		P
	Low adherence to diet n = 71	High adherence to diet n = 41	
Plasma phenylalanine level, (μmol/L) Mean ± SD (min–max)	943.2 ± 314.0 (456–1762)	369.7 ± 102.8 (179–526)	<0.0001
Serum vitamin B12 level, (pg/mL) Mean ± SD (min–max)	372.4 ± 186.2 (88–821)	501.5 ± 224.8 (74–986)	0.001
Vitamin B12 (<200 pg/mL), n (%)	13 (18.3)	4 (9.8)	0.224
Serum folic acid level, (ng/mL) Mean ± SD (min–max)	18.4 ± 8.1 (2.5–49.0)	21.8 ± 10.9 (5.2–47.8)	0.082
Folic acid (>17.5 ng/mL), n (%)	37 (52.1)	25 (60.9)	0.363
Serum iron level, (μg/dL) Mean ± SD (min–max)	80.5 ± 36.4 (12–166)	77.0 ± 34.3 (27–175)	0.626
Iron (<50 μg/dL), n (%)	14 (19.7)	8 (19.5)	0.955
Serum ferritin level, (ng/mL) Median (min–max)	25.6 (1.7–78.0)	24.5 (6.3–82.4)	0.326[a]
Ferritin (<14 ng/mL), n (%)	11 (15.5)	8 (19.5)	0.657
Transferrin saturation level, (%) Mean ± SD (min–max)	23.8 ± 10.1 (3–44)	22.5 ± 11.1 (7–57)	0.552
Transferrin saturation (<15%), n (%)	13 (18.3)	11 (26.8)	0.246
Serum copper level, (μg/dL) Mean ± SD (min–max)	101.0 ± 29.1 (31.3–195.6)	114.3 ± 26.7 (38.1–174.3)	0.022
Copper (<64 μg/dL), n (%)	5 (7.0)	1 (2.4)	0.280
Serum prealbumin level, (mg/dL) Mean ± SD (min–max)	24.9 ± 4.6 (13.3–35.5)	22.5 ± 4.4 (10.5–30.1)	0.011
Prealbumin (<21 mg/dL), n (%)	12 (16.9)	9 (21.9)	0.374
Serum albumin level, (g/dL) Mean ± SD (min–max)	4.6 ± 0.3 (3.9–5.4)	4.5 ± 0.3 (3.8–5.0)	0.116
Serum total protein level, (g/dL) Mean ± SD (min–max)	7.2 ± 0.5 (6.0–8.1)	7.0 ± 0.5 (5.7–7.9)	0.210
Serum phosphorus level, (mg/dL) Mean ± SD (min–max)	4.2 ± 0.8 (2.4–6.3)	4.7 ± 0.6 (3.7–6.6)	<0.0001
Serum calcium level, (mg/dL) Mean ± SD (min–max)	9.8 ± 0.4 (8.7–10.9)	9.8 ± 0.3 (8.9–10.7)	0.695
Serum 25-hydroxy vitamin D level, (ng/mL) Mean ± SD (min–max)	18.2 ± 8.0 (2.6–38.1)	20.9 ± 7.1 (4.7–39.3)	0.075
25-Hydroxy vitamin D (<20 ng/mL), n (%)	40 (56.3)	20 (48.7)	0.469
Serum zinc level, (μg/dL) Median (min–max)	91 (61–146)	90 (72–165)	0.323[a]
Zinc (<65 μg/dL), n (%)	1 (1.4)	0 (0)	N/A
Plasma vitamin A level, (μg/dL) Median (min–max)	57.4 (33.1–111.9)	50.3 (28.9–132.8)	0.995[a]
Elevated plasma vitamin A level n (%)	16 (22.5)	14 (34.1)	0.121
Plasma vitamin E level, (mg/dL) Median (min–max)	1.2 (0.6–2.7)	1.3 (0.5–3.3)	0.166[a]
Elevated plasma vitamin E level n (%)	6 (8.5)	9 (21.9)	0.030
Hemoglobin level, (g/dL) Mean ± SD (min–max)	12.9 ± 1.3 (9.0–15.5)	12.7 ± 0.9 (10.8–14.8)	0.378

SD: standard deviation, min: minimum, max: maximum, SDS: standard deviation score, N/A: statistical analysis not performed.
[a] Mann Whitney U test was performed.

difference between the serum zinc level of patients with PKU and the control group. Dietary adherence did not have any effect on serum zinc levels. In only one patient in the PKU group, serum zinc level was below the reference range with no clinical findings.

There is limited evidence regarding blood copper level in patients with PKU. Gropper et al. [38] and Fisberg et al. [39] showed that there was no difference between the plasma copper concentration of patients with PKU and that of their control group. Our findings are consistent with the literature. While Schulpis et al. [30] revealed higher serum copper levels in PKU patients with poor adherence to diet compared to well-controlled patients, we determined higher copper levels in the HAD group than LAD. Trace element supplementation of Phe-free amino acid formulas in the last decades may explain this difference.

Vitamin D and calcium levels of patients with PKU were assessed by D. Modan-Moses et al. [40] and were found to be better in PKU patients with high adherence to diet. This was associated with the vitamin D and calcium content of Phe-free amino acid formulas. Crujeiras et al. [1] reported that calcium and phosphorus levels were in normal range in patients with PKU, and 14% of their patients had vitamin D deficiency. In the same study, a negative correlation was seen between age and phosphorus, and also between plasma phenylalanine and phosphorus levels. In our study, almost all patients with PKU had serum calcium and phosphorus levels in normal range. Consistent with the literature, there was a negative correlation between plasma phenylalanine and phosphorus levels. Also, serum phosphorus level was significantly higher in patients with PKU than the control group. However, frequency of vitamin D deficiency was higher among patients with PKU (53.6%) compared to the control group (47.2%), and no relation was observed between adherence to diet and vitamin D status of patients with PKU, as reported in the literature. We believe that the

high rate of vitamin D deficiency is associated with the region where the study is performed. Recently, Gülez et al. [41] determined vitamin D deficiency in 31% of infants and 81.8% of mothers in the same region where we performed the present study. Our findings are consistent with the results of their study.

Colomé et al. [42] reported no significant differences in retinol and tocopherol concentrations between patients with PKU and the control group. Conversely, Schulpis et al. [43] showed higher plasma retinol and tocopherol concentrations in well-controlled patients with PKU compared to the control group. Additionally, positive correlations between adherence to diet and vitamin A and E levels were reported. In our study, while plasma vitamin A level was higher in patients with PKU than the control group, there was no difference in plasma vitamin E levels between the two groups. Although plasma vitamin A and vitamin E levels were similar between HAD and LAD groups, frequency of plasma vitamin E levels above the reference range was higher in HAD than the LAD group. We believe this is related to the vitamin E supplementation of Phe-free amino acid formulas. Neither vitamin A nor vitamin E deficiency was detected in any group.

Non-heme iron sources which have lower bioavailability than heme iron is the primary iron source of patients with PKU [15,44,45]. It is interesting to note that ferritin level of patients with PKU was higher than the control group in our study. Moreover, frequency of diminished ferritin level was observed to be higher in the control group than patients with PKU. Crujeiras et al. [1] and Schulpis et al. [44] also reported lower ferritin levels in poorly controlled patients compared to well-controlled patients with PKU, and these findings are related to the iron and ascorbic acid enriched formula supplementation, which improves non-heme iron absorption. On the contrary, there were no statistically significant differences between the HAD and LAD groups in terms of serum iron, ferritin, transferrin saturation and hemoglobin levels in our study.

6. Limitations

There are several limitations of the present study. Even if there is no statistical difference, the age range of the control group was lower than the PKU group, which may have affected the results. We did not assess dietary vitamin and micronutrient intake of patients with PKU, which may have resulted in overestimating the effect of Phe-free amino acid formulas on the nutritional status of patients with phenylketonuria. We included patients with PKU who did not receive vitamin or mineral supplementation for at least 6 months. This period may not be enough for an accurate evaluation of the effect of vitamin- and mineral-supplemented Phe-free amino acid formulas on nutritional status. Finally, serum vitamin B12 level is routinely used to determine the B12 status, but may be unreliable in some cases. Plasma homocysteine or serum methylmalonic acid analysis is necessary to better evaluate the vitamin B12 status. Unfortunately, we did not evaluate these parameters.

7. Conclusions

As a result, we concluded that Phe-free amino acid formulas provide more than necessary folic acid, vitamin B12, copper, vitamin E and adequate vitamin A and zinc for patients with PKU. Our results demonstrate that vitamin D deficiency is frequent in our patients with PKU compared to other studies, but mostly related to regional vitamin D insufficiency. These findings show that the nutritional status of patients with PKU varies widely between regions and countries, and biochemical monitoring of patients is important to identify vitamin/mineral deficiencies.

Conflicts of interest

The authors declared no conflicts of interest.

Acknowledgments

Ethical approval: The study protocol was designed in compliance with the Declaration of Helsinki, 1964. Ethics Committee of the University Hospital approved this study (Number of ethical approval: 2016/09-11).

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