Cardiology in the Young (2016), **26**, 901–908
doi:10.1017/S104795111500150X

Original Article

Comparison of atherogenic risk factors among poorly controlled and well-controlled adolescent phenylketonuria patients

Mehmet Gündüz,[1] Sevim Çakar,[2] Pınar Kuyum,[2] Balahan Makay,[2] Nur Arslan[2,3]

[1]*Division of Metabolism, Ankara Children Education and Research Hospital, Ankara;* [2]*Department of Pediatrics, Faculty of Medicine, Dokuz Eylul University;* [3]*Department of Molecular Medicine, Izmir, Turkey*

Abstract *Background:* Previous studies investigating the known risk factors of atherosclerosis in phenylketonuria patients have shown conflicting results. The primary aim of our study was to investigate the serum atherogenic markers in adolescent classical phenylketonuria patients and compare these parameters with healthy peers. The secondary aim was to compare these atherogenic markers in well-controlled and poorly controlled patients. *Methods:* A total of 59 patients (median age: 12.6 years, range: 11–17 years) and 44 healthy controls (median age: 12.0 years, range: 11–15 years) were enrolled in our study. Phenylketonuria patients were divided into two groups: well-controlled (serum phenylalanine levels below 360 μmol/L; 24 patients) and poorly controlled patients (serum phenylalanine levels higher than 360 μmol/L). *Results:* The mean high-density lipoprotein cholesterol levels of well-controlled patients (1.0 ± 0.2 mmol/L) were significantly lower compared with poorly controlled patients and controls (1.1 ± 0.2 mmol/L, $p = 0.011$ and 1.4 ± 0.2 mmol/L, $p < 0.001$, respectively). Poorly controlled patients had lower high-density lipoprotein cholesterol levels than healthy controls ($p = 0.003$). Homocysteine levels of both well-controlled (9.8 ± 6.4 μmol/L) and poorly controlled (9.2 ± 5.6 μmol/L) patients were higher compared with controls (5.8 ± 1.8 μmol/L, $p < 0.01$). The mean platelet volume of well-controlled patients (9.5 ± 1.1 fL) was higher than that of poorly controlled patients and controls (8.9 ± 0.8 fL, $p = 0.024$ and 7.7 ± 0.6 fL, $p < 0.001$, respectively). *Conclusion:* Lower high-density lipoprotein cholesterol and higher homocysteine and mean platelet volume levels were detected in phenylketonuria patients. In particular, these changes were more prominent in well-controlled patients. We conclude that phenylketonuria patients might be at risk for atherosclerosis, and therefore screening for atherosclerotic risk factors should be included in the phenylketonuria therapy and follow-up in addition to other parameters.

Keywords: Phenylketonuria; low-density lipoprotein cholesterol; high-density lipoprotein cholesterol; mean platelet volume; homocysteine

Received: 20 March 2015; Accepted: 13 July 2015; First published online: 17 August 2015

Phenylketonuria (OMIM: 261600) is an autosomal recessive disorder caused by hepatic phenylalanine hydroxylase (EC 1.14.16.1) deficiency. Phenylketonuria is the most prevalent inborn error of amino acid metabolism. Increased phenylalanine levels cause irreversible neurological damage in almost all children if they are not treated with a phenylalanine-restricted diet, commenced soon after birth. Adequate and continuous dietary treatment results in good neurological outcome in early diagnosed patients.[1]

Atherosclerotic risk factor assessment relies mainly on the evaluation of dietary habits, anthropometric measurements and blood pressure, blood lipid and homocysteine levels, and other inflammatory biomarkers, as well as genetic factors. Natural protein-restricted and phenylalanine-restricted diets, even if they are rich in fruits and vegetables (antioxidants), represent a serious risk for nutritional

Correspondence to: Dr N. Arslan, MD, PhD, Department of Pediatrics, Division of Pediatric Gastroenterology, Nutrition and Metabolism, Faculty of Medicine, Department of Molecular Medicine, Dokuz Eylul University, Izmir, Turkey. Tel: +0090 2324126107; Fax: +0090 2324126005; E- mail: nur.arslan@deu.edu.tr

deficiencies – for example, selenium, zinc, ubiquinone-10, and L-carnitine;[2] however, despite this vegan diet, the risk for inflammation and oxidative stress increases in phenylketonuria patients.[3–5] Moreover, despite decreased saturated lipids in this non-atherogenic green diet, atherogenic blood changes such as altered lipid profile[6–8] or elevated homocysteine levels[3,9] have been observed in some studies. Inflammation, dyslipidaemia, and hyperhomocysteinaemia were shown to be important risk factors for atherosclerosis.[10,11] Atherosclerosis, beginning in childhood, may be predictive of coronary artery disease in adulthood.[12,13] Therefore, early diagnosis of atherosclerosis is essential to prevent its future complications.

There are few studies that have investigated the known risk factors of atherosclerosis, such as blood lipids, homocysteine, and mean platelet volume in phenylketonuria patients, in the literature.[3,6–9,14–22] In most of these previous studies, these factors were evaluated independently, and only one of them included mean platelet volume levels.[22] The primary aim of the present study was to investigate the serum atherogenic markers in adolescent phenylketonuria patients and compare these parameters with healthy peers. The secondary aim of this study was to compare the well-controlled and poorly controlled patients regarding these atherogenic markers.

Patients and methods

Study population

This study was conducted in Dokuz Eylül University Faculty of Medicine, Department of Paediatrics, Division of Paediatric Metabolism, Izmir, Turkey, between June 2013 and December 2013. Participants included adolescent phenylketonuria patients and healthy controls between 11 and 18 years of age. In total, 89 adolescent phenylketonuria patients were screened. Patients with a disease other than phenylketonuria, such as diabetes mellitus (one patient) and hypothyroidism (one patient), who used other medications such as anti-inflammatory, antipyretic, or anticonvulsive drugs or herbal medicines (three patients), and smokers (two patients) were excluded. Patients with acute infections (two patients) were also excluded from the study. Patients with obesity (15 patients) or protein–energy malnutrition (five patients) were not included in this study. In total, 59 patients were included in this study. None of the adolescent girls in the study group or the control group were using oral contraceptive drugs. All the patients were diagnosed as having classical phenylketonuria by mass screening in the neonatal period. Moreover, all the patients had pre-treatment phenylalanine levels greater than 1200 μmol/L between 3 and 7 days of life, and classical phenylketonuria was diagnosed in these patients by the analysis of dihydropteridine reductase activity in erythrocytes and pteridine analysis in urine.[23] All the patients were on phenylalanine-restricted diet since the diagnosis. According to our standard treatment protocol, adolescent patients had monthly visits to our outpatient clinic. All the patients were given amino acid mixtures. The phenylalanine tolerance and diet regulations of all patients were adjusted according to serum phenylalanine levels during every visit. Patients' total protein intakes were 0.8–1.2 g/kg/day – 0.20–0.40 g/kg/day from natural sources and 0.6–0.8 g/kg/day from amino acid mixture. Daily phenylalanine intakes ranged between 300 and 900 mg/day according to phenylalanine tolerance and the patient's age and weight. Patients who consumed vitamins, minerals, or fish oils in the last three months were not included in the study.

Physical examinations and anthropometric measurements of all patients and controls were performed. The body mass index was calculated as weight divided by height squared (kg/m^2). Obesity was defined as a body mass index exceeding the 95th centile based on the patient's age and sex.[24] Relative weight was defined as the ratio of the actual weight of the patient to the weight of a child in the 50th centile of the same height and gender × 100. All patients and control children had normal anthropometric values.

Systolic blood pressure and diastolic blood pressure were measured by a mercurial sphygmomanometer using the right arm in sitting position in all the adolescents. Blood pressure higher than the 95th percentile of blood pressure according to age-, sex-, and height-specific norms was accepted as hypertension.[25] Patients – one patient was obese – or controls with hypertension were not included.

Patients were divided into two subgroups according to their mean serum phenylalanine levels of the previous year, which were calculated from the data from the monthly visits of the last year: well-controlled patients (group 1) and poorly controlled patients (group 2). The upper limit for the recommended range of blood phenylalanine levels in phenylketonuria patients in this age group who received the phenylalanine-restricted diet varies between countries.[26] We used 360 μmol/L (6 mg/dl) as the upper limit of cut-off for both children and adolescents in our institution. On the other hand, some of the patients, especially adolescents, did not follow this diet strictly, as in other countries.[26,27] Mean serum phenylalanine levels between 120 and 360 μmol/L were accepted as well-controlled for classical phenylketonuria patients. Mean serum phenylalanine levels greater than 360 μmol/L were defined as poorly controlled in this study.

The control group (group 3) consisted of asymptomatic healthy adolescents who were admitted to the

https://doi.org/10.1017/S104795111500150X Published online by Cambridge University Press

well-child outpatient clinic of our hospital for medical screening, which included screening for hepatitis, thyroid function, lipid profile, coeliac disease, anaemia, etc. All the screening test results were normal. All the adolescents in this group were not taking any medications, vitamin or mineral supplementation, and did not use tobacco. All of them were on an omnivorous diet. None of them had an acute or chronic infectious disease. None of them had a history of familial hypercholesterolaemia, hypertension, or chronic disease. Anthropometric values of the controls were in the normal range. Complete blood count, phenylalanine and glucose level tests, lipid profiles, and liver function tests of the control group were performed and the results were found to be in normal ranges.

Laboratory analyses of atherosclerotic biomarkers

All the measurements were performed using blood samples collected on the same morning in each child. Blood was obtained by venipuncture in the forearm in the morning after an overnight fast. Standard tubes without an anti-coagulant (BD Vacutainer, Becton Dickinson Company, New Jersey, United States of America) were used for biochemical analysis. Samples were centrifuged at 3000 *g* at 4°C for 15 minutes. We measured the levels of fasting serum glucose, total cholesterol, high-density lipoprotein cholesterol, triglycerides, vitamin B12, and folic acid. Low-density lipoprotein cholesterol level was calculated using the Friedewald formula (LDL-C = total cholesterol – HDL-C – triglycerides/5).[28] Serum vitamin B12 and folic acid concentrations were measured by chemiluminescence immunoassay method (Abbott Architect i2000). Serum was used for measuring blood phenylalanine levels, which were measured by flourometric analysis (RF-5301PC, Shimadzu).

Standard tubes with constant amount of K3-ethylenediaminetetraacetic acid (BD Vacutainer, Becton Dickinson Company) were used for complete blood count analyses and for testing plasma homocysteine levels. Plasma homocysteine was measured using a kit (IC2801, ImmunoChrom) by high-performance liquid chromatography with fluorescence detection (Shimadzu). Complete blood count analyses were performed using a Coulter analyzer (LH-780, Beckman Coulter, Brea, California, United States of America) by the impedance method (intra-assay variation coefficient 1.6%, inter-assay variation coefficient 1.6%), which was routinely checked every month in the central laboratory of our institution at regular intervals of 1 hour.

Ethical approval

The study protocol was designed in compliance with the Declaration of Helsinki. Informed consent was obtained from both the adolescents and their parents on enrolment to the study. The study was started after the approval of the Ethics Committee of the Dokuz Eylul University Faculty of Medicine was obtained (Ethics approval number: 2013/29-11)

Statistical analysis

Statistical analysis was performed using the Statistical Package for Social Sciences version 15.0. Data are presented as mean ± standard deviation and median [range, 25P–75P]. Student's t-test was used for comparing the phenylketonuria group with the control group regarding age, relative weight, phenylalanine level, and concentrations of lipids, vitamin B12, homocysteine, and folic acid. The Kruskal–Wallis test was used for analysing group averages among the three groups (well-controlled, poorly controlled patients, and healthy controls). The Mann–Whitney U-test was used for comparing two group averages as a post-hoc test (well-controlled patients versus poorly controlled patients; well-controlled patients versus controls; and poorly controlled patients versus controls). The χ^2 test was used for comparing group ratios. All the p values are two-tailed, and group differences or correlations with $p < 0.05$ were considered to be statistically significant.

Results

A total of 59 phenylketonuria patients (32 male, mean age 13.7 ± 2.4 years, median: 12.6 [11–17] years) and 44 healthy controls (19 male, mean age 13.0 ± 2.0 years, median: 12.0 [11–15] years) were enrolled in this study. There were no significant differences between phenylketonuria patients and controls regarding age, gender, and relative weight ($p = 0.125$, 0.321, and 0.594, respectively).

Haemoglobin levels, leucocyte and platelet counts, triglyceride, total cholesterol, low-density lipoprotein cholesterol, and vitamin B12 levels did not differ significantly between patients and controls. On the other hand, mean high-density lipoprotein cholesterol levels were significantly lower in patients than in controls (1.1 ± 0.2 versus 1.4 ± 0.2 mmol/L, $p < 0.001$). Homocysteine (9.5 ± 5.9 versus 5.8 ± 1.8 μmol/L, $p < 0.001$), folic acid (32.7 ± 9.2 versus 22.5 ± 7.2 nmol/L, $p < 0.001$), and mean platelet volume (9.1 ± 1.0 versus 7.7 ± 0.6 fL, $p < 0.001$) were higher in patients than in healthy peers. Homocysteine levels were >15.0 μmol/L in five children (8.5%, mean homocysteine level in this group was 23.7 ± 9.6 μmol/L, median 19.0 [15.6–34.1] μmol/L) in the patient group. There was no case of hyperhomocysteinaemia in the control group. High-density lipoprotein cholesterol levels of the

https://doi.org/10.1017/S104795111500150X Published online by Cambridge University Press

hyperhomocysteinaemic group were significantly lower (0.9 ± 0.07 mmol/L, median 0.9 [0.8–1.0] mmol/L) than in patients with homocysteine levels <15.0 μmol/L (1.1 ± 0.2 mmol/L, median 1.0 [0.9–1.3] mmol/L) and in controls ($p = 0.044$ and $p < 0.001$, respectively). No significant difference was observed between triglyceride and low-density lipoprotein cholesterol levels in all the three groups.

Among all, 24 phenylketonuria patients (41%, 8 girls) were well-controlled and 35 patients (59%, 19 girls) were poorly controlled. Clinical characteristics, serum phenylalanine concentrations, and haematological and biochemical parameters of patients and controls are presented in Table 1. Mean serum phenylalanine levels were 306.1 ± 78.0 μmol/L in well-controlled and 720.8 ± 196.7 μmol/L in poorly controlled patients ($p < 0.001$). In control adolescents, mean serum phenylalanine levels were 48.8 ± 12.2 μmol/L ($p < 0.001$ for both phenylketonuria groups). None of the adolescents in the control group had serum phenylalanine levels >120 μmol/L.

The mean high-density lipoprotein cholesterol levels of well-controlled patients (1.0 ± 0.2 mmol/L) were significantly lower compared with poorly controlled patients and controls (1.1 ± 0.2 mmol/L, $p = 0.011$ and 1.4 ± 0.2 mmol/L, $p < 0.001$, respectively). Moreover, poorly controlled patients had lower high-density lipoprotein cholesterol levels than healthy controls ($p = 0.003$). Homocysteine levels of both well-controlled and poorly controlled patients were significantly higher compared with control adolescents (Table 1). On the other hand, there was no statistically significant difference regarding homocysteine levels between the two patient groups (Table 1). Mean platelet volume levels showed no difference between girls and boys in the patient (9.1 ± 0.9 versus 9.1 ± 1.1 fL, $p = 0.970$) and control groups (7.7 ± 0.7 versus 7.8 ± 0.4 fL, $p = 0.704$). The mean platelet volume levels of well-controlled patients (9.5 ± 1.1 fL) were higher compared with the poorly controlled patients and controls (8.9 ± 0.8 fL, $p = 0.024$ and 7.7 ± 0.6 fL, $p < 0.001$, respectively). Furthermore, poorly controlled patients had higher mean platelet volume levels than healthy controls ($p < 0.001$) (Table 1).

Discussion

The results of this study showed that adolescent phenylketonuria patients had significantly higher homocysteine and mean platelet volume levels and lower high-density lipoprotein cholesterol levels compared with healthy controls. Furthermore, lower high-density lipoprotein cholesterol levels and higher mean platelet volume levels were detected in well-controlled patients compared with the poorly controlled ones. Furthermore, mean homocysteine levels were higher in both phenylketonuria groups compared with healthy peers; however, they were similar in both well-controlled and poorly controlled patient groups.

Previous studies investigating high-density lipoprotein cholesterol status in phenylketonuria patients have shown conflicting results. Some studies in the literature did not find any difference between phenylketonuria patients and controls[14,15] and also between well-controlled and poorly controled phenylketonuria patients and healthy controls regarding high-density lipoprotein cholesterol levels.[3,9,16,17] On the contrary, few other studies have found lower high-density lipoprotein cholesterol levels in phenylketonuria patients than in controls, similar to our findings.[6–8] Different mechanisms might be involved for the lower high-density lipoprotein cholesterol levels in phenylketonuria patients. First, depressed apolipoprotein E (Apo E) synthesis in PAH-deficient patients might cause high-density lipoprotein cholesterol deficiency.[6] Nagasaka et al[6] showed that phenylketonuria patients who were treated with phenylalanine-restricted diet had diminished Apo E and high-density lipoprotein cholesterol levels compared with hyperphenylalaninaemia patients who did not receive restricted diet and healthy controls. They concluded that the diminished Apo E levels were due to supressed high-density lipoprotein cholesterol synthesis;[6] however, the exact mechanism is not known. We did not measure Apo E levels or perform Apo E genotyping in our patients. Second, phenylketonuria patients generally consume a vegetarian diet, especially well-controlled patients who strictly comply with this low-cholesterol diet.[8,22] Our results support this hypothesis. Numerous studies have shown that people consuming vegetarian and also vegan diet had lower high-density lipoprotein cholesterol levels compared with omnivorous healthy people.[29–31] Although the exact mechanism is not clear, a recent public-based study showed that Apo A-1 levels were significantly lower in vegan women than in their carnivorous peers when adjusted for body mass index.[32] As Apo A-1 is an important structural apolipoprotein of the high-density lipoprotein cholesterol molecule, diminished Apo A-1 levels might be a causative factor for lower high-density lipoprotein cholesterol levels in vegetarians. Another possible mechanism is the effect of elevated homocysteine levels on high-density lipoprotein cholesterol trafficking in phenylketonuria patients. Holven et al[33] demonstrated that hyperhomocysteinaemic patients had impaired ability to induce cholesterol efflux from lipid-loaded macrophages and had dysfunctional high-density lipoprotein particles

https://doi.org/10.1017/S104795111500150X Published online by Cambridge University Press

Table 1. Comparison of clinical and laboratory parameters between well-controlled (WC) and poorly controlled (PC) PKU patients and healthy controls (data are presented as mean ± standard deviation and median [range]).

				p value		
	WC patients (n = 24)	**PC patients (n = 35)**	**Controls (n = 44)**	**WC versus PC**	**WC versus C**	**PC versus C**
Age (years)	13.1 ± 2.4 12.2 [11.4–14.0]	14.1 ± 2.9 13.1 [11.5–18.0]	13.0 ± 2.0 12.0 [11.0–15.0]		0.115*	
Gender (M/F)	16/8	16/19	19/25		0.743*	
RW (%)	102.5 ± 9.3 102.0 [91.0–109.0]	101.2 ± 11.4 101.9 [92.0–110.0]	102.8 ± 10.2 102.0 [98.0–111.0]		0.777*	
BMI (kg/m^2)	19.1 ± 2.1 19.0 [18.2–20.9]	18.9 ± 1.9 19.0 [18.9–21.0]	19.7 ± 2.0 19.2 [18.0–21.0]		0.757*	
SBP (mmHg)	107.9 ± 10.2 108.0 [104.0–117.0]	109.7 ± 10.6 109.3 [105.0–120.0]	108.8 ± 11.1 108.2 [103.9–118.2]		0.658*	
DBP (mmHg)	68.9 ± 8.6 69.1 [65.9–73.2]	67.8 ± 8.4 68.0 [64.0–74.3]	69.1 ± 8.0 69.0 [66.1–74.0]		0.549*	
Triglyceride (mmol/L)	1.0 ± 0.6 0.8 [0.6–1.3]	0.8 ± 0.3 0.8 [0.5–1.0]	0.8 ± 0.2 0.8 [0.7–1.0]		0.949*	
LDL-C (mmol/L)	1.9 ± 0.5 1.8 [1.4–2.2]	2.0 ± 0.5 1.9 [1.7–2.4]	2.0 ± 0.5 1.8 [1.5–2.7]		0.700*	
HDL-C (mmol/L)	1.0 ± 0.2 1.0 [0.8–1.1]	1.1 ± 0.2 1.0 [1.0–1.3]	1.4 ± 0.2 1.3 [1.2–1.6]	**0.011**	**0.001**	**0.003**
Glucose (mmol/L)	4.7 ± 0.3 4.6 [4.4–5.0]	4.6 ± 0.3 4.6 [4.4–4.8]	4.9 ± 0.3 4.7 [4.7–5.3]	0.665	0.057	**0.010**
Homocysteine (μmol/L)	9.8 ± 6.4 7.9 [5.4–13.4]	9.2 ± 5.6 8.3 [5.1–10.9]	5.8 ± 1.8 5.3 [4.4–6.6]	0.722	**0.006**	**0.007**
Folic acid (nmol/L)	32.4 ± 9.7 34.7 [26.1–40.6]	32.8 ± 9.0 36.3 [25.1–40.4]	22.5 ± 7.2 22.2 [17.0–26.7]	0.830	**0.001**	**0.001**
Vitamin B12 (pmol/L)	256.5 ± 139.3 209.5 [146.1–350.5]	308.8 ± 119.1 284.1 [230.9–344.6]	303.0 ± 85.8 298.8 [241.3–353.5]		0.141*	
Leucocyte (×10^3/μl)	6.9 ± 1.7 7.5 [5.2–8.0]	6.7 ± 1.4 6.5 [5.8–7.3]	6.8 ± 1.4 7.1 [5.6–7.8]		0.839*	
Thrombocyte (x10^3/μl)	245.2 ± 53.0 237.5 [206.2–300.2]	245.2 ± 50.5 241.0 [204.0–280.0]	266.7 ± 55.6 263.0 [223.0–297.0]		0.137*	
MPV (fL)	9.5 ± 1.1 9.2 [8.9–9.7]	8.9 ± 0.8 8.7 [8.2–9.6]	7.7 ± 0.6 7.8 [7.3–8.1]	**0.024**	**0.001**	**0.001**
Phe level (μmol/L)	306.1 ± 78.0 6.0 [270.4–360.0]	720.8 ± 196.7 702.2 [582.4–860.8]	48.8 ± 12.2 48.1 [24.0–96.0]	**0.001**	**0.001**	**0.001**

BMI = body mass index; DBP = diastolic blood pressure; HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol; MPV = mean platelet volume; Phe = phenylalanine; PKU = phenylketonuria; RW = relative weight; SBP = systolic blood pressure

*The Kruskal–Wallis test and the Mann–Whitney U-test were used

Statistically significant results were represented in bold characters

with attenuated anti-atherogenic activity. Hyperhomocysteinaemia was detected in five patients in our patient group. High-density lipoprotein cholesterol levels were significantly lower in this hyperhomocysteinaemic group than in patients with normal homocysteine levels and healthy controls. Our results support this hypothesis. Finally, the results of an experimental study by Castillo et al.[34] showed that hyperphenylalaninaemia might inhibit 3-hydroxy-3-methylglutaryl-CoA reductase and mevalonate-5-pyrophosphate decarboxylase, which are two of the main regulatory enzymes of cholesterogenesis. Although lower high-density lipoprotein cholesterol levels were seen in our study, total cholesterol and low-density lipoprotein cholesterol levels were similar between groups. Thus, whether the results of this animal study can be adapted to humans is unclear. Decreased high-density lipoprotein cholesterol levels were found to be associated with atherosclerosis in numerous adult studies.[35,36] On the other hand, new basic and clinical studies are needed to evaluate the atherogenic effect of diminished high-density lipoprotein cholesterol levels in phenylketonuria patients.

In this study, mean platelet volume in well-controlled patients were higher compared with poorly controlled patients and controls. Platelets play an important role in the pathogenesis of disorders associated with local or systemic inflammation.[37,38] During atherogenesis, platelet activation and aggregation along with migration of smooth muscle cells from the media to the endothelium and consequent proliferation are the early events. The status of mean platelet volume has been proposed as a marker for platelet activation, as larger-sized platelets have been associated with higher pro-thrombotic risk, which may also reflect atherosclerosis.[39–42] The role of mean platelet volume has been previously demonstrated in various systemic disorders related to inflammation and atherosclerosis, such as familial Mediterranean fever,[43] obesity,[44,45] diabetes,[46] and coronary artery disease.[47,48] To date, there are no data about the presence of atherosclerosis in phenylketonuria patients. On the other hand, several studies demonstrated that phenylketonuria patients had increased sub-clinical inflammation and oxidative stress.[4,5] In the previously mentioned study of Mutze et al,[22] which investigated the long-term effects of phenylketonuria diet on fatty acid metabolism, 12 phenylketonuria patients with good metabolic control and eight healthy controls were included. This study also investigated the status of mean platelet volume in phenylketonuria patients. The mean platelet volume levels of the patients were not significantly different compared with the controls in this study. In our study, we had a larger number of patients and we showed a significant difference between groups in terms of mean platelet volume. Furthermore, we compared the patients with good metabolic control and poor metabolic control.

Homocysteine levels were higher in both phenylketonuria groups compared with healthy peers in our study. To date, one adult and five paediatric studies have been reported investigating the serum/plasma homocysteine levels in phenylketonuria patients.[3,9,18–21] Lucock et al and Huemer et al did not find any differences between phenylketonuria patients and controls regarding homocysteine levels.[18,19] On the other hand, Schulpis et al[3,9] found higher homocysteine levels in well-controlled phenylketonuria patients compared with poorly controlled patients and healthy peers in their two different studies. Recently, two studies reported reduced homocysteine levels in phenylketonuria patients.[20,21] Elevation of homocysteine concentration has been shown to be related to atherosclerosis in previous case–control and prospective studies.[49] On the other hand, no data were found indicating hyperhomocysteinaemia and presence of atherosclerosis in phenylketonuria patients in the literature. Hyperhomocysteinaemia enhances vascular disease via endothelial dysfunction, smooth muscle cell proliferation, and vascular re-modelling.[50] It was demonstrated that homocysteine elevation enhanced platelet activation in both in vitro[51] and in vivo[52] studies. Moreover, it was demonstrated in animal studies that homocysteine elevation caused oxidative stress-diminished cardiac oxygen consumption, which might lead to cardiac metabolic disease.[53] All these mechanisms may contribute to the pathogenesis of atherosclerosis. Elevation of homocysteine levels in phenylketonuria patients could be a result of functional vitamin B12 deficiency due to a relatively low intake of vitamins B6 and B12 as well as folate.[3,54] On the other hand, few other studies have shown that phenylketonuria patients following a strict diet supplemented with phenylalanine-free amino acid formulae were not at risk of developing deficiencies in these micronutients.[55,56] More studies are required to clarify these conflicting results.

A major limitation of our study is its small sample size. The second limitation is the lack of the hyperphenylalaninaemia patients whose blood phenylalanine levels range between 120 and 600 μmol/L without any dietary treatment to compare the dietary effect on lipids, homocysteine, and mean platelet volume levels as well as other nutritional factors. Another limitation of this study is the absence of information regarding other known inflammation parameters such as C-reactive protein and tumour necrosis factor alpha as well as oxidative stress markers. Finally, we did not measure intima media

https://doi.org/10.1017/S104795111500150X Published online by Cambridge University Press

thickness of the patients to directly identify the presence or absence of atherosclerosis.

In conclusion, lower high-density lipoprotein cholesterol levels and higher homocysteine and higher mean platelet volume levels were detected in phenylketonuria patients in our study. These changes were more prominent in well-controlled phenylketonuria patients. We conclude that phenylketonuria patients might be at risk for atherosclerosis, and therefore screening for atherosclerosis and atherosclerotic risk factors should be included in the phenylketonuria therapy and follow-up in addition to other parameters, such as patients' history, examination, anthropometrical data, laboratory analyses, and further clinical diagnostics such as intima media thickness. The exact mechanism causing higher mean platelet volume and lower high-density lipoprotein cholesterol in well-controlled phenylketonuria patients needs to be investigated.

Acknowledgements

The authors thank all the patients and parents who contributed to this study.

Financial Support

This research received no specific grant from any funding agency, commercial, or not-for-profit sectors.

Conflicts of Interest

The authors declare no conflicts of interest.

Ethical Standards

The authors assert that all the procedures contributing to this work comply with the ethical standards of the relevant national guidelines on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008, and has been approved by the institutional committees of Dokuz Eylul University Faculty of Medicine.

References

1. Jahja R, Huijbregts SC, de Sonneville LM, van der Meere JJ, van Spronsen FJ. Neurocognitive evidence for revision of treatment targets and guidelines for phenylketonuria. J Pediatr 2014; 164: 895–899.
2. Rocha JC, Martins MJ. Oxidative stress in phenylketonuria: future directions. J Inherit Metab Dis 2012; 35: 381–398.
3. Schulpis KH, Papassotiriou I, Tsakiris S, Vounatsou M, Chrousos GP. Increased plasma adiponectin concentrations in poorly controlled patients with phenylketonuria normalize with a strict diet: evidence for catecholamine-mediated adiponectin regulation and a complex effect of phenylketonuria diet on atherogenesis risk factors. Metabolism 2005; 54: 1350–1355.
4. Okano Y, Nagasaka H. Optimal serum phenylalanine for adult patients with phenylketonuria. Mol Genet Metab 2013; 110: 424–430.
5. Sanayama Y, Nagasaka H, Takayanagi M, et al. Experimental evidence that phenylalanine is strongly associated to oxidative stress in adolescents and adults with phenylketonuria. Mol Genet Metab 2011; 103: 220–225.
6. Nagasaka H, Tsukahara H, Okano Y, et al. Changes of lipoproteins in phenylalanine hydroxylase-deficient children during the first year of life. Clin Chim Acta 2014; 433C: 1–4.
7. Rocha JC, van Spronsen FJ, Almeida MF, et al. Dietary treatment in phenylketonuria does not lead to increased risk of obesity or metabolic syndrome. Mol Genet Metab 2012; 107: 659–663.
8. Colomé C, Artuch R, Lambruschini N, Cambra FJ, Campistol J, Vilaseca M. Is there a relationship between plasma phenylalanine and cholesterol in phenylketonuric patients under dietary treatment? Clin Biochem 2001; 34: 373–376.
9. Schulpis KH, Karikas GA, Papakonstantinou E. Homocysteine and other vascular risk factors in patients with phenylketonuria on a diet. Acta Paediatr 2002; 91: 905–909.
10. Ait-Oufella H, Sage AP, Mallat Z, Tedgui A. Adaptive (T and B cells) immunity and control by dendritic cells in atherosclerosis. Circ Res 2014; 114: 1640–1660.
11. Perk J, De Backer G, Gohlke H, et al. European guidelines on cardiovascular disease prevention in clinical practice (version 2012): the fifth joint task force of the European society of cardiology and other societies on cardiovascular disease prevention in clinical practice (constituted by representatives of nine societies and by invited experts). Int J Behav Med 2012; 19: 403–488.
12. Li S, Chen W, Srinivasan SR, et al. Childhood cardiovascular risk factors and carotid vascular changes in adulthood: the Bogalusa Heart Study. JAMA 2003; 290: 2271–2276.
13. Raitakari OT, Juonala M, Kähönen M, et al. Cardiovascular risk factors in childhood and carotid artery intima-media thickness in adulthood: the cardiovascular risk in young finns study. JAMA 2003; 290: 2277–2283.
14. Agostoni C, Scaglioni S, Bonvissuto M, Bruzzese MG, Giovannini M, Riva E. Biochemical effects of supplemented long-chain polyunsaturated fatty acids in hyperphenylalaninemia. Prostaglandins Leukot Essent Fatty Acids 2001; 64: 111–115.
15. Nagasaka H, Okano Y, Kimura A, et al. Oxysterol changes along with cholesterol and vitamin D changes in adult phenylketonuric patients diagnosed by newborn mass-screening. Clin Chim Acta 2013; 416: 54–59.
16. Schulpis KH, Karakonstantakis T, Bartzeliotou A, Karikas GA, Papassotiriou I. The association of serum lipids, lipoproteins and apolipoproteins with selected trace elements and minerals in phenylketonuric patients on diet. Clin Nutr 2004; 23: 401–407.
17. Schulpis KH, Bartzeliotou A, Tsakiris S, Gounaris A, Papassotiriou I. Serum paraoxonase/arylesterase activities in phenylketonuric patients on diet. Eur J Clin Nutr 2007; 61: 803–808.
18. Lucock M, Yates Z, Hall K, et al. The impact of phenylketonuria on folate metabolism. Mol Genet Metab 2002; 76: 305–312.
19. Huemer M, Födinger M, Bodamer OA, et al. Total homocysteine, B-vitamins and genetic polymorphisms in patients with classical phenylketonuria. Mol Genet Metab 2008; 94: 46–51.
20. Huemer M, Simma B, Mayr D, et al. Free asymmetric dimethylarginine (ADMA) is low in children and adolescents with classical phenylketonuria (PKU). J Inherit Metab Dis 2012; 35: 817–821.
21. Colomé C, Artuch R, Sierra C, et al. Plasma thiols and their determinants in phenylketonuria. Eur J Clin Nutr 2003; 57: 964–968.
22. Mütze U, Beblo S, Kortz L, et al. Metabolomics of dietary fatty acid restriction in patients with phenylketonuria. PLoS One 2012; 7: e43021.
23. Walter JH, Lachmann RH, Burgard P. Hyperphenylalaninemia. In: Saudubray JM, van den Berge G, Walter JH (eds). Inborn Metabolic Diseases: Diagnosis and Treatment, 5th edn. Springer-Verlag, Berlin Heidelberg, 2012: 253–264.

24. Cole TJ, Lobstein T. Extended international (IOTF) body mass index cut-offs for thinness, overweight and obesity. Pediatr Obes 2012; 7: 284–294.
25. National High Blood Pressure Education Program Working Group on Hypertension Control in Children and Adolescents. Update on the 1987 Task Force Report on High Blood Pressure in Children and Adolescents: a working group report from the National High Blood Pressure Education Program. Pediatrics 1996; 98: 649–658.
26. Blau N, van Spronsen FJ, Levy HL. Phenylketonuria. Lancet 2010; 376: 1417–1427.
27. Das AM, Goedecke K, Meyer U, et al. Dietary habits and metabolic control in adolescents and young adults with phenylketonuria: self-imposed protein restriction may be harmful. JIMD Rep 2014; 13: 149–158.
28. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 1972; 18: 499–502.
29. Jian ZH, Chiang YC, Lung CC, et al. Vegetarian diet and cholesterol and TAG levels by gender. Public Health Nutr 2015; 18: 721–726.
30. Huang YW, Jian ZH, Chang HC, et al. Vegan diet and blood lipid profiles: a cross-sectional study of pre and postmenopausal women. BMC Womens Health 2014; 14: 55.
31. Chiang JK, Lin YL, Chen CL, et al. Reduced risk for metabolic syndrome and insulin resistance associated with ovo-lacto-vegetarian behavior in female Buddhists: a case-control study. PLoS One 2013; 8: e71799.
32. Bradbury KE, Crowe FL, Appleby PN, Schmidt JA, Travis RC, Key TJ. Serum concentrations of cholesterol, apolipoprotein A-I and apolipoprotein B in a total of 1694 meat-eaters, fish-eaters, vegetarians and vegans. Eur J Clin Nutr 2014; 68: 178–183.
33. Holven KB, Aukrust P, Retterstøl K, et al. The antiatherogenic function of HDL is impaired in hyperhomocysteinemic subjects. J Nutr 2008; 138: 2070–2075.
34. Castillo M, Zafra MF, Garcı´a-Peregrin E. Inhibition of brain and liver 3-hydroxy-3-methylglutaryl-CoA reductase and mevalonate-5-pyrophosphate decarboxylase in experimental hyperphenylalaninemia. Neurochem Res 1988; 13: 551–555.
35. Yu S, Yarnell JW, Sweetnam P, Bolton CH. High density lipoprotein subfractions and the risk of coronary heart disease: 9-years follow-up in the Caerphilly Study. Atherosclerosis 2003; 166: 331–338.
36. Jacobs DR, Mebane IL, Bangdiwala SI, Criqui MH, Tyroler HA. High density cholesterol as a predictor of cardiovascular disease mortality in men and women: the follow-up study of the lipid research clinics prevalence. Am J Epidemiol 1990; 131: 32–47.
37. Ware J, Corken A, Khetpal R. Platelet function beyond hemostasis and thrombosis. Curr Opin Hematol 2013; 20: 451–456.
38. Rondina MT, Weyrich AS, Zimmerman GA. Platelets as cellular effectors of inflammation in vascular diseases. Circ Res 2013; 112: 1506–1519.
39. Chesnutt JK, Han HC. Platelet size and density affect shear-induced thrombus formation in tortuous arterioles. Phys Biol 2013; 10: 056003.
40. Martin JF, Trowbridge EA, Salmon G, Plumb J. The biological significance of platelet volume: Its relationship to bleeding time, thromboxane B2 production and megakaryocyte nuclear DNA concentration. Thromb Res 1983; 32: 443–460.
41. Berger JS, Eraso LH, Xie D, Sha D, Mohler ER. Mean platelet volume and prevalence of peripheral artery disease, the National Health and Nutrition Examination Survey, 1999-2004. Atherosclerosis 2010; 213: 586–591.
42. Chu SG, Becker RC, Berger PB, et al. Mean platelet volume as a predictor of cardiovascular risk: a systematic review and meta-analysis. J Thromb Haemost 2009; 8: 148–155.
43. Makay B, Turkyilmaz Z, Unsal E. Mean platelet volume in children with familial Mediterranean fever. Clin Rheumatol 2009; 28: 975–978.
44. Arslan N, Makay B. Mean platelet volume in obese adolescents with nonalcoholic fatty liver disease. J Pediatr Endocrinol Metab 2010; 23: 807–813.
45. Arslan N, Makay B, Hızlı S, et al. Assesment of atherosclerosis in obese adolescents: positive corelation of mean platelet volume and carotid intima media thickness. J Paediatr Child Health 2013; 49: 963–968.
46. Dolasık I, Sener SY, Celebi K, Aydın ZM, Korkmaz U, Canturk Z. The effect of metformin on mean platelet volume in diabetic patients. Platelets 2013; 24: 118–121.
47. Khode V, Sindhur J, Kanbur D, Ruikar K, Nallulwar S. Mean platelet volume and other platelet volume indices in patients with stable coronary artery disease and acute myocardial infarction: a case control study. J Cardiovasc Dis Res 2012; 3: 272–275.
48. Verdoia M, Camaro C, Barbieri L, et al. Mean platelet volume and the risk of periprocedural myocardial infarction in patients undergoing coronary angioplasty. Atherosclerosis 2013; 228: 136–141.
49. Ahluwalia N, Blacher J, Szabo de Edelenyi F, et al. Prognostic value of multiple emerging biomarkers in cardiovascular risk prediction in patients with stable cardiovascular disease. Atherosclerosis 2013; 228: 478–484.
50. Steed MM, Tyagi SC. Mechanisms of cardiovascular remodeling in hyperhomocysteinemia. Antioxid Redox Signal 2011; 15: 1927–1943.
51. Signorello MG, Pascale R, Leoncini G. Reactive oxygen species accumulation induced by homocysteine in human platelets. Ann N Y Acad Sci 2002; 973: 546–549.
52. Holven KB, Aukrust P, Pedersen TM, Ose L, Nenseter MS. Enhanced platelet activation in hyperhomocysteinemic individuals. J Thromb Haemost 2007; 5: 193–195.
53. Becker JS, Adler A, Schneeberger A, et al. Hyperhomocysteinemia, a cardiac metabolic disease: role of nitric oxide and the p22phox subunit of NADPH oxidase. Circulation 2005; 111: 2112–2118.
54. Vugteveen I, Hoeksma M, Monsen AL, et al. Serum vitamin B12 concentrations within reference values do not exclude functional vitamin B12 deficiency in PKU patients of various ages. Mol Genet Metab 2011; 102: 13–17.
55. Thiele AG, Weigel JF, Ziesch B, et al. Nutritional changes and micronutrient supply in patients with phenylketonuria under therapy with tetrahydrobiopterin (BH(4)). JIMD Rep 2013; 9: 31–40.
56. Rohde C, von Teeffelen-Heithoff A, Thiele AG, et al. PKU patients on a relaxed diet may be at risk for micronutrient deficiencies. Eur J Clin Nutr 2014; 68: 119–124.

https://doi.org/10.1017/S104795111500150X Published online by Cambridge University Press