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Is deoxypyridinoline a good resorption marker to detect osteopenia in phenylketonuria?

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Abstract

Objectives: To evaluate deoxypyridinoline as a resorption marker in phenylketonuria (PKU) and to search for a relationship between deoxypyridinoline, calcium/creatinine index (Ca/Cr I), osteocalcin and bone alkaline phosphatase (BAP).

Methods: This was a transversal analytical study of 46 PKU patients [17.5 (4–38) years]. Deoxypyridinoline and osteocalcin were measured with a chemiluminescent assay and BAP was measured with an immunoradiometric assay.

Results: Deoxypyridinoline was significantly increased in patients aged 7–14 and >18 years old, being associated with age ($r = -0.724$, $P < 0.001$). Adult patients showed significantly higher Ca/Cr I, which correlates with Phe values for the year prior to the study ($P = 0.014$). Serum BAP was significantly increased in pediatric patients (9–13 years), while it was decreased in adult patients ($P = 0.003$). Decreased osteocalcin levels were found in patients >15 years ($P = 0.028$). Altered deoxypyridinoline and BAP values were related ($P = 0.042$).

Conclusion: PKU patients excreted increased D-Pyr, suggesting high bone resorption. Bone formation seems active in childhood but deteriorates in adult PKU patients. Periodic measurement of D-Pyr and BAP may be useful in the prevention of osteopenia in PKU patients.

Keywords: Osteopenia; Phenylketonuria; Deoxypyridinoline; Bone alkaline phosphatase; Osteocalcin; Calcium/creatinine index; Bone remodeling markers

Introduction

Phenylketonuria (PKU; OMIM 261600) is an inborn error of phenylalanine (Phe) metabolism caused by a deficiency of phenylalanine 4-monooxygenase (phenylalanine hydroxylase, PAH; EC 1.14.16.1) which causes an accumulation of Phe in biological fluids and tissues. Treatment is based on a dietary Phe restriction, which means a low natural protein intake offset by a special formula including a Phe-free amino acid mixture, supplemented with mineral salts, trace elements and vitamins. Early treatment prevents neurological impairment and mental retardation [1]. Despite recent improvements in the composition of this diet, poor mineralization in the bone mass of several PKU patients has been described, resulting in growth failure, fractures and a high incidence of osteopenia in adulthood [2,3]. Whether these observations are caused by malnutrition owing to the artificial diet of treated patients, or rather reflect a toxic effect of the disease itself, has not been clarified [4].

Osteopenia can be measured by different osteodensitometric methods [5,6]. However, some biochemical parameters can also be analyzed, since they are useful to identify patients at risk and to monitor therapy [7–9]. Among these, some biochemical markers of bone formation, such as osteocalcin (OSTEO) and bone alkaline phosphatase (BAP), and markers of bone resorption, including calcium/creatinine index (Ca/Cr I), hydroxyproline and pyridinium collagen cross-links, have been introduced in clinical practice [10,11].

Deoxypyridinoline (D-Pyr) is a small cross-linking peptide of type I collagen molecules that is released into the circulatory system and excreted when bone is resorbed [12–15]. D-Pyr is thought to be more specific than other classical resorption markers [16]. Unlike hydroxyproline, D-Pyr is not influenced

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by the diet [17], and it is stable in stored urine [18]. The measurement of total urinary pyridinium cross-links in adults has shown good correlation with bone turnover as it is assessed by radioisotopic and histomorphometric techniques [19,20]. However, D-Pyr shows variable levels in childhood [21] and there are few data on urinary pyridinium cross-link excretion in healthy children [22]. In contrast to adults, the clinical potential of D-Pyr as a marker of bone resorption in pediatric patients is still controversial and there are no data in PKU patients [23], although D-Pyr has been analyzed in animal models of the disease [24]. We previously reported a study of some osteopenia markers in a reduced group of PKU patients [3] and we therefore saw fit to try to evaluate D-Pyr as a biochemical marker of bone resorption in a larger group of PKU patients. As a first approach, we established the reference values for D-Pyr in a healthy pediatric and adolescent population, so as to be able to compare D-Pyr values obtained in our PKU patients with these reference values. Moreover, we assessed the value of D-Pyr as a resorption marker in PKU patients and tried to search for a possible relationship with the calcium/creatinine index as well as with serum bone formation markers (osteocalcin and bone alkaline phosphatase) and also with the results of the densitometric study.

Patients and methods

Patients

A group of 46 PKU patients [median age: 17.5 years old (range 4–38); 17 males and 29 females] monitored clinically and biochemically in our hospital (Reference Centre for PKU in Catalonia) were selected. Inclusion criteria were age higher than 6 years and adhesion to a Phe-restricted diet. We excluded patients with a history of immobility or one compatible with any other disease known to influence bone mineral status. We also excluded patients treated with calcitriol or any form of steroid hormones and those involved in any high-activity sports. All patients were on Phe-restricted diet at the time of the study. This diet consists of a Phe-free formula containing a balanced amino acid mixture, carbohydrates, minerals, trace elements and vitamins. Protein and mineral intake in the PKU patients met the RDAs, according to data calculated through a 3-day dietary questionnaire using the Sanutrin V2.0 program (Novartis) (data not shown) [3].

Patients' samples were obtained in accordance with the Helsinki Declaration of 1964, as revised in 2000, and the study was approved by the Ethics Committee of our hospital. Reference values for D-Pyr and Ca/Cr I were obtained from urine samples of a healthy population including 180 children, aged [mean (SD)] 8.99 (7.09) years (range: 4 days to 26 years) and sex: 89 boys and 91 girls. Children suffering from any illness or receiving long-term medication were excluded from the study. Blood samples for reference values of bone formation markers (BAP and OSTEO) were obtained from healthy children referred to our laboratory for minor pre-surgical analytical control (age range: 6 to 18 years). Moreover, a control group for the older patients (age range: 18 to 37 years) was obtained from among students, young clinicians and laboratory staff.

Methods

Specimens

Serum and urine samples were collected during the biochemical and nutritional monitoring periodically performed on our patients. Venous blood samples were collected after an overnight fast, centrifuged and analyzed or stored at −40°C until the moment of the analysis. Morning second void urine samples were collected for patients and controls and analyzed (calcium and creatinine) or frozen (−40°C) until D-Pyr analysis. Urine samples with leukocytes, blood, proteins or nitrites were rejected.

Biochemical analysis

Plasma phenylalanine was analyzed by ion exchange chromatography with a Biochrom 20 analyzer (Pharmacia Biotech, Cambridge, England). Index of Dietary Control (IDC) was calculated as the median of plasma Phe concentrations during the year prior to the study (minimum of 12 Phe values). We also considered the plasma Phe concentrations at the moment of the biochemical and densitometric study.

Calcium, creatinine and Ca/Cr I were determined by standard procedures with a Cobas Integra analyzer (Roche Diagnostic Systems, Switzerland). Bone remodeling markers, OSTEO and D-Pyr, were calculated using a chemiluminescent assay [25,26] with the Immulite One analyzer (Diagnostic Products Corporation, USA). BAP was determined by an Immunoradiometric assay (IRMA), Tandem®-R Ostease®-Beckman Coulter. The results of D-Pyr are expressed as D-Pyr/creatinine ratio following recommendations for the optimum specimen to avoid biological variation in the urinary excretion of pyridinium cross-links [27].

Densitometry study

Bone mineral density (BMD, g/cm^2) was measured in lumbar spine (L2, L3, L4) and calculated by dividing bone mineral content (g) by the cross-sectional area of bone measured (cm^2) by a dual energy X-ray bone densitometer (LUNAR DXP LPLUS, version 4.7A). Bone densitometric pediatric software with data for healthy control children living in Catalonia was used. BMD measurements were expressed as the BMD *Z* score, i.e., the difference between the BMD of the patient and the average BMD of sex- and age-matched controls, divided by the standard deviation of the control group. Osteopenia was defined as a *Z* score value below −1.

Statistical analysis

The SPSS program 12.0 version was used for statistical analysis. The distribution of D-Pyr according to age and sex was studied in a healthy population (Kruskal–Wallis test), and