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● *Original Contribution*

PHALANGEAL QUANTITATIVE ULTRASOUND IN CHILDREN WITH PHENYLKETONURIA: A PILOT STUDY

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Abstract—Bone alterations in phenylketonuria (PKU) have been detected, especially with increasing age, in several studies by using different radiologic techniques. Quantitative ultrasound (QUS) assesses skeletal status by measuring the amplitude-dependent speed of sound (AD-SoS) and the bone transmission time (BTT), mainly dependent on mineral density and cortical thickness. Bone condition in 30 children and adolescents (mean age 15.1 ± 6.4 y) affected by PKU was evaluated by phalangeal QUS, considering its relationship with their clinical, biochemical and therapeutic features. Measured AD-SoS Z-Score and BTT Z-Score were 0.27 ± 1.42 and −0.26 ± 1.21, respectively. In patients with previous fractures, the two QUS parameters were lower than in patients without history of fracture ($p < 0.001$ and $p = 0.006$, respectively). AD-SoS Z-Score and BTT Z-Score were negatively correlated with plasma phenylalanine (Phe) concentration in the year before QUS ($p = 0.005$ and $p < 0.001$, respectively) and with age ($p < 0.001$ for both parameters). These results parallel the previous findings obtained by different radiologic tools and suggest phalangeal QUS as an attractive option for the regular evaluation and longitudinal monitoring of bone condition in children and adolescents affected by PKU. (E-mail: porta.franc@gmail.com) **© 2008 World Federation for Ultrasound in Medicine & Biology.**

Key Words: **Phenylketonuria, Bone, Quantitative ultrasound.**

INTRODUCTION

Phenylketonuria (PKU; OMIM 261600) is an autosomal recessive inborn error of phenylalanine (Phe) metabolism caused by phenylalanine hydroxylase deficiency (PAH, EC 1.14.16.1) (Scriver et al. 2001). Early diagnosis and treatment with a protein-restricted diet, by avoiding high plasma Phe-related neurotoxicity during the early developmental period, allows for the prevention of irreversible mental retardation and neurologic disorders. The integration with Phe-free medical mixtures supplies the majority of protein equivalents, minerals and vitamins in the diet of treated PKU patients. Poor compliance to Phe-restricted diet with consequent raising of plasma Phe level is a well known problem in adolescents affected by PKU (MacDonald et al. 2006), and long-term consequences of chronically elevated blood Phe on different tissues, including bone, are still a debated issue. Reduced bone mineralization in PKU have been described in previous reports by means of different radiologic techniques (Allen 1994; Al-Qadreh 1998; Hillman 1996; McMurry 1992; Schwahn 1998).

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Recently, quantitative ultrasound (QUS) devices have been applied more often for assessment of bone condition, because they explore not only bone mineral density, but also structural and architectural skeletal properties (Barkmann et al. 2000; De Terlizzi et al. 2000; Sakata et al. 2004). These aspects could be considered of paramount importance in metabolic diseases (Dalle Carbonare et al. 2004), such as PKU, in which bone damage has been related not only to the restricted diet, but also to a possible direct toxic effect of metabolic derangement, as Phe overload (Yannicelli et al. 2002). Moreover, QUS devices are portable, cheaper than other instruments and nonionizing, making this method an attractive option for the regular evaluation and longitudinal long-term monitoring of skeletal impairment, especially in pediatrics.

We used phalangeal QUS to assess the bone condition in a group of patients with PKU, taking into account their clinical, biochemical and therapeutic features.

MATERIALS AND METHODS

Patients

Thirty patients affected by PKU (13 males and 17 females, mean age 15.1 ± 6.4 y) were enrolled in this study to undergo bone evaluation by phalangeal QUS.

All patients were diagnosed at newborn screening and treated early with a normocaloric hypoproteic diet able to reduce the Phe intake, supplemented the with the same Phe-free amino acid mixture at a mean dose of 1.20 ± 0.57*g*/kg, according to the age-related recommendations (Scriver et al. 2001).

Exclusion criteria were: age <4 y, short stature (defined as height below –2 standard deviation for their age), history of immobility or high physical activity; treatment with drugs influencing bone metabolism, deformities or previous fractures of the phalanges, and concomitant diseases impairing bone status. Informed consent was obtained from patients or from their parents if they were too young to give consent. Height and pubertal development were assessed according to Tanner's standards and criteria. Body mass index (BMI) was calculated using the weight/height^2 (kg/m^2) formula, corrected for sex and age, and expressed as BMI Z-Score, calculated according to Italian reference charts (Cacciari et al. 2006). Daily dietary regimen and dosage of substitutive Phe-free mixture supplementation were recorded. Moreover, for each patient, we collected plasma Phe concentrations recorded monthly in the last year before QUS evaluation.

Quantitative ultrasound

Bone status was assessed by the same operator with phalangeal QUS, by using the DBM Sonic Bone Profiler (Igea, Carpi, Modena, Italy). The device uses two flat transducers with a 16-mm diameter and a frequency of 1.25 MHz, assembled on a high precision electronic caliper measuring the distance between emitting and receiving probes (Njeh et al. 1999). This device was positioned on the lateral and medial surfaces at the distal diaphysis of the first phalanges, in the proximity of the condyles of II-V digits of the dominant hand. To ensure the accuracy and the reproducibility of QUS readings, the DBM Sonic Bone Profiler was provided by the manufacturer with a Plexiglas phantom for daily calibration (performed by the operator) and periodic quality assurance (performed every 6 mo by the manufacturer). Furthermore, the manufacturer controlled the device every two years for periodic maintenance, as recommended (Njeh et al. 1999). Parameters measured by the device were the following: amplitude-dependent speed of sound (AD-SoS) in m/s, measured considering the first signal with an amplitude above 2 mV at the receiving probe, and the bone transmission time (BTT) in microns, calculated by subtracting the instant corresponding to the arrival time of the fastest ultrasound-received signal from the time of transmission of an ultrasound pulse at 1700 m/s velocity were determined. Because the calculation relies only on the bone tissue between the two probes, BTT is completely independent of soft tissue thickness (Barkmann et al. 2000). Intraoperator coefficients of variation for AD-SoS and BTT were 0.69% and 0.89%, respectively.

Statistical analysis

Statistical analyses were performed with SPSS ver. 12.0 (Chicago, IL, USA). AD-SoS and BTT were compared with reference values assessed in a large control group of young Italian subjects (Baroncelli et al. 2006), female (Wuster et al. 2000) and male (Montagnani et al. 2000) adults, as provided by the manufacturer, and expressed as Z-Score calculated considering age- and sex-matched healthy controls. We defined reduced values as those below –2 SD, whereas values between –1 and –2 SD were considered to be in the lower normal range. The Shapiro-Wilk test was used to check normal distribution of data. To evaluate whether a parameter was significantly different from the control group, the difference of the mean Z-Score to zero was assessed. Differences between groups were established with Student's *t*-test. Pearson's correlation coefficients were used to check univariate associations of Ad-SoS and BTT Z-Scores with the patient's age, BMI Z-Score, mean Phe in the previous year and b.w. dosage of Phe-free amino acid supplementation. The statistical significance for all calculations was considered achieved when 2-tailed *p*-value was less than 0.05.

RESULTS

The mean values of AD-SoS Z-Score and BTT Z-Score were 0.27 ± 1.42 and −0.26 ± 1.21, respectively. AD-SoS and BTT Z-Scores were highly correlated ($r = 0.887$, $p < 0.001$). Five patients (16.7%) had a previous bone fracture. They showed lower AD-SoS Z-Score ($p < 0.001$) and BTT Z-Score ($p = 0.006$) and higher mean plasma Phe concentration ($p < 0.001$), with respect to subjects without history of fractures, whereas no significant differences between age and Phe-free protein integrative supplementation were found. All fractures were the result of high-impact trauma, according to the Landin classification (Landin et al. 1983). Z-Scores were lower in males than in females, but the comparison did not reach statistical significance. Patients aged >8 y showed higher plasma Phe concentration ($p = 0.035$) and lower AD-SoS Z-Score ($p = 0.015$) and BTT Z-Score ($p = 0.043$) than younger patients. Characteristics of different subgroups are summarized in Table 1. Mean BMI Z-score was normal (−0.26 ± 1.26, $p = 0.275$).

In the whole study group, QUS parameters were correlated negatively with age (AD-SoS Z-Score: $r = -0.618$; $p < 0.001$; BTT Z-Score: $r = -0.635$; $p < 0.001$) (Fig. 1) and with mean plasma Phe concentration in the previous year (AD-SoS Z-Score: $r = -0.501$; $p = 0.005$ and BTT