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Extremely low level of serum pigment epithelium-derived factor is a special biomarker of Chinese osteogenesis imperfecta patients with *SERPINF1* mutations

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ABSTRACT

Backgrounds: *SERPINF1* mutations caused deficiency of pigment epithelium-derived factor (PEDF) and would lead to osteogenesis imperfecta (OI) type VI. However, serum PEDF levels were unclear in Chinese OI patients who had clear molecular diagnosis.
Objective: To assess PEDF levels in different genotypes of OI, to evaluate the influencing factors of PEDF in Chinese OI patients with clear molecular diagnosis.
Methods: Known candidate genes of OI were examined by a targeted next generation sequence. Serum PEDF levels were measured by ELISA in 6 OI patients with *SERPINF1* mutations, 6 carriers of one copy of the *SERPINF1* mutation, 88 OI patients with *COL1A1*, *CLO1A2*, *IFITM5* and other pathogenic mutations of OI and 24 healthy controls. We compared the differences in serum PEDF levels among different OI patients and normal controls.
Results: Serum PEDF levels were extremely low in OI patients with *SERPINF1* mutations (0.66 ± 1.60 μg/ml) than in OI patients with other pathogenic mutations (4.88 ± 1.43–7.07 ± 2.43 μg/ml), carriers of one copy of *SERPINF1* mutation (4.94 ± 2.35 μg/ml), and normal controls (7.29 ± 2.31 μg/m) ($P < 0.001$). No significant differences in serum PEDF concentrations were found among patients with OI type I, III or IV, and between patients with or without bisphosphonate treatment. Serum PEDF level was positively correlated with Z-score of weight (r = 0.310, $P = 0.004$), BMI (r = 0.253, $P = 0.020$) and alanine aminotransferase (r = 0.291, $P = 0.007$).
Conclusions: Extremely low level of PEDF was demonstrated as a specific, convenient, and inexpensive diagnostic biomarker for OI patients with *SERPINF1* mutations, but it could not provide information regarding the clinical severity of OI and the efficacy of bisphosphonates treatment.

1. Introduction

Osteogenesis imperfecta (OI) was a clinically and genetically heterogeneous disorder characterized by decreased bone mineral density (BMD), propensity to fracture, progressive bone deformities, and growth deficiency [1]. Extra-skeletal manifestations of OI included blue sclera, hearing loss, dentinogenesis imperfecta, and joint hypermobility [1]. According to Sillence classification, OI was divided into four types based on clinical phenotype, of which type I was mild, type II was perinatal lethality, type III was the most severe type among the surviving patients, and type IV was of moderate severity [2]. In addition to phenotypic differences, recent research continued to identify various genetic causes of OI.

OI type VI (MIM #610968) was a rare autosomal recessive form of

Abbreviations: OI, osteogenesis imperfecta; BPs, bisphosphonates; PEDF, pigment epithelium-derived factor; PUMCH, Peking Union Medical College Hospital; ELISA, enzyme-linked immunosorbent assay; Ca, calcium; P, phosphate; ALP, alkaline phosphatase; ALT, alanine aminotransferase; Cr, creatinine; β-CTX, beta cross-linked carboxy-terminal telopeptide of type I collagen; 25OHD, 25-hydroxyvitamin D; PTH, parathyroid hormone; NGS, next-generation sequencing; PCR, polymerase chain reaction; BMD, bone mineral density; LS, lumbar spine; FN, femoral neck; DXA, dual-energy X-ray absorptiometry; MSC, mesenchymal stem cells

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OI caused by inactivating mutations in *SERPINF1* [3–6]. OI type VI was characterized by defects in bone mineralization and early onset multiple fractures [7]. Bone histology revealed a large amount of unmineralized osteoid and fish scale lamellation, indicating a severe mineralization defect in the setting of normal collagen in patients with OI type VI [8,9]. Bisphosphonates (BPs) appeared to have poor effects on bone of OI type VI patients in comparison with other types of OI [10].

The pathogenic gene implicated in OI type VI had been identified as *SERPINF1*, which was located on chromosome 17p13.3 and encoded pigment epithelium-derived factor (PEDF) [3]. PEDF was a 50 kDa secreted glycoprotein that was firstly identified from human fetal retinal pigment epithelium cells [11]. PEDF belonged to the serpin superfamily, and had numerous functions, including antiangiogenic, antitumor, anti-inflammatory, neurotrophic properties and fat metabolism [12–15]. Moreover, PEDF gene expression was active in adult bone, particularly at sites of active bone growth [16]. PEDF contained binding sites for type I collagen, which regulated osteoblastogenesis and osteoclast function through osteoprotegerin and sclerostin [17–19].

The relationship between serum PEDF level and different genotypes of OI was not evaluated because of the limitation of accurate molecular typing of OI. On the basis of next-generation sequencing, we evaluated serum PEDF levels in Chinese OI patients with *SERPINF1* mutations, as well as in a large sample of Chinese OI patients with other pathogenic mutations and healthy controls.

2. Materials and methods

2.1. Subjects

This cross-sectional study included a total of 6 patients with OI type VI, 88 patients with other types of OI (62 males, 32 females; aged 1.8–40.3 years old), 6 parents of patients with OI type VI carrying one copy of the *SERPINF1* mutation, and 24 healthy subjects who were age- and gender-matched to the patients with OI type VI. Of the patients with OI, 65 had previously received treatment (2 months–10 years) with intravenous zoledronic acid or oral alendronate.

Medical histories were collected and detailed physical examinations were performed. The clinical diagnosis of OI was made by the Endocrinology Department of Peking Union Medical College Hospital (PUMCH) from 2004 to 2015 year on the basis of the following criteria: early-onset and recurrent fractures under mild trauma; with or without extra-skeletal manifestations, such as blue sclera, hearing loss, dentinogenesis imperfecta, and hypermobility of joints; and BMD Z-score lower than − 2.0 [1]. Except OI type VI, all OI patients were clinically divided into three clinical types according to Sillence classification: OI type I (n = 24), OI type III (n = 35), and OI type IV (n = 29).

The study protocol was approved by the Scientific Ethics Committee of PUMCH. Written informed consents were obtained from all patients or their parents prior to participating in this study.

2.2. Biochemical measurements

Serum samples were obtained from all patients and normal controls between 8:00 and 10:00 AM after an overnight fast. Serum PEDF levels were measured using a DuoSet enzyme-linked immunosorbent assay (ELISA) kit (R & D Systems, Minneapolis, MN) in the laboratory of the Endocrinology Department of PUMCH. The test was performed according to a general double-antibody sandwich ELISA protocol. After the capture antibody bound to the surface of the solid support, the antigen bound to the capture antibody. Then the enzyme-labeled detection antibody bound to the antigen, and the antigen was quantitatively analyzed by color reaction through the enzyme-catalyzed substrate. The optical density was determined immediately using a microplate reader set to 450 nm and 540 nm. We averaged the duplicate readings for each standard, control, and sample and subtracted the average zero standard optical density. We calculated the concentrations of samples using a standard curve created by the concentrations and optical densities of standards. The intra-assay and inter-assay coefficients of variation were 7.7% and 8.1%, respectively. The detection range was 78.1–5000.0 pg/ml, the sensitivity and specificity were high, and no significant cross-reactivity was observed.

Serum calcium (Ca), phosphate (P), alkaline phosphatase (ALP, a bone formation marker), alanine aminotransferase (ALT), and creatinine (Cr) levels were measured using automated analyzers. Serum levels of beta cross-linked carboxy-terminal telopeptide of type I collagen (β-CTX, a bone resorption marker), 25-hydroxyvitamin D (25OHD), and intact parathyroid hormone (PTH) were quantified using an automated electrochemiluminescence system (E170; Roche Diagnostics, Switzerland). These serum biochemical parameters were measured in the central clinical laboratory of PUMCH.

2.3. DNA sequence analysis

Total genomic DNA was isolated from peripheral blood leukocytes using a QIAamp DNA Mini Kit (Qiagen, Germany). A targeted next-generation sequencing (NGS) capture panel was created to examine known OI-related genes. The methods of NGS and analysis of NGS results had been previously described [21]. To verify the NGS results, all fragments containing mutations were amplified by polymerase chain reaction (PCR). Sanger sequencing of PCR products was performed using an ABI 377 DNA automated sequencing kit with a dye terminator cycle (Applied Biosystems).

2.4. Anthropometric measurements

Height and weight were measured using a Harpenden stadiometer, which were converted to age- and gender-specific Z-scores according to the reference data published by the Chinese Journal of Pediatrics in 2009 [22].

2.5. Assessment of BMD

BMD at the lumbar spine 2–4 (LS) and femoral neck (FN) was determined in the anterior-posterior (AP) direction using a dual-energy X-ray absorptiometry (DXA, Lunar Prodigy, GE Healthcare, Madison WI, USA). The age- and sex-specific Z-scores for BMD were calculated according to the normal ranges for Chinese children [23–25].

2.6. Statistical analysis

ANOVA and chi-squared test were used to analyze continuous and categorical variables, respectively. General linear model analysis of covariance was applied to analyze the differences of serum PEDF levels among different groups with adjustment for gender, age and history of treatment with BPs. Spearman correlation analysis and partial correlation analysis were used to assess relationships between PEDF levels and anthropometric measures, biochemical markers, BMD, and OI severity with and without adjustment for gender, age and BP treatment history. Stepwise multiple regression analysis was used to assess independent predictors of serum PEDF levels. Nominal variables were coded as follows: gender: male = 1, female = 2; OI severity: OI type I = 1, OI type IV = 2, OI type III = 3. Two-tailed $P < 0.05$ indicated statistical significance. All statistical analyses were performed by SPSS Version 19.0 (SPSS, Inc., Chicago, IL, USA).

3. Results

3.1. Serum PEDF levels in OI patients with different gene mutations

The gene mutation spectrum of the 94 patients with OI included: *SERPINF1* (n = 6), *COL1A1* (n = 36), *COL1A2* (n = 28), *IFITM5* (n = 10), *FKBP10* (n = 4), *WNT1* (n = 4), *TMEM38B* (n = 3), *PLOD2*

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