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Increased serum sclerostin in postoperative biliary atresia ☆

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ABSTRACT

Background: We determined the relationship between serum sclerostin and disease severity of biliary atresia (BA).
Methods: Seventy postoperative BA patients and 35 controls were recruited. Serum sclerostin, C-terminal telopeptide of type I collagen (CTX), and osteocalcin were analyzed using ELISA. Bone mineral density (BMD) of the lumbar spine was measured by dual energy X-ray absorptiometry.
Results: BA patients had significantly higher serum sclerostin than controls. Serum sclerostin was markedly elevated in jaundice patients compared with jaundice-free patients. Serum osteocalcin was not different, whereas serum CTX was greater in BA patients than controls. BMD of jaundice patients was significantly lower than jaundice-free patients. Additionally, serum sclerostin was correlated with biochemical parameters and BMD in BA.
Conclusion: Increased sclerostin levels were associated with liver dysfunction and the severity of BA, suggesting that sclerostin may reflect the deterioration of hepatic function and the outcome in postoperative BA.

1. Introduction

Biliary atresia (BA) is a serious neonatal liver disorder characterized by progressive, inflammatory, fibrosclerotic cholangiopathy resulting in the obliteration of bile ducts. BA patients are generally presented with a triad of obstructive jaundice, acholic stools, and hepatosplenomegaly. Currently, the Kasai procedure or hepatoportoenterostomy is the first line of surgical treatment to re-establish ductal continuity for adequate bile drainage [1]. Despite early diagnosis and a successful Kasai operation, a great majority of the BA patients inevitably develop recurrent cholangitis, persistent jaundice, progressive ascites, bleeding esophageal varices, and end-stage liver disease [2,3]. In addition, osteopenia and osteoporosis are common among patients with chronic liver diseases including BA [4–6]. The precise mechanisms leading to osteopenia and osteoporosis in BA patients remain unclear.

The canonical Wnt/β-catenin signaling pathway influences bone formation through direct effects on osteoblast proliferation, maturation, and progenitor differentiation; these actions are opposed by various intracellular and secreted factors [7]. Wnt signaling is modulated by soluble antagonists including dickkopf-1 (Dkk-1), secreted frizzled-related proteins (sFRPs), and sclerostin [8]. Sclerostin, encoded by the *SOST* gene, is an endogenous, osteocyte-derived, soluble inhibitor of Wnt signaling and a potent inhibitor of osteoblastic bone formation. The negative regulatory actions of sclerostin occur through the canonical Wnt/β-catenin signaling pathway by binding specifically to a seven-transmembrane domain-spanning frizzled receptor and to either of two co-receptors, low density lipoprotein-related proteins 5 and 6 (LRP5/6) and inhibiting their association with frizzled receptors [9,10]. Genetic defects that cause a lack of sclerostin expression and therefore result in significantly increased bone mass occur in sclerosteosis and van Buchem disease; whereas high sclerostin expression leads to low bone mass in mice [11–13]. Hence, sclerostin inhibition may provide a therapeutic target for conditions associated with low bone mass. In recent years, the role of circulating sclerostin has been documented in children with obesity [14], X-linked hypophosphatemic rickets, osteogenesis imperfecta [15], cow's milk allergy [16], and chronic liver diseases [17]. The relationship of serum sclerostin with cholestasis and bone remodeling has been previously demonstrated in primary biliary cirrhosis patients [18]. However, until recently, the study of correlations between serum sclerostin, biochemical parameters, and bone mineral density (BMD) in BA children has received little attention.

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Although the emerging knowledge of the biological effects of the sclerostin signaling pathway derived from *in vitro* and animal studies has generated a new comprehension of bone biology and has provided novel insights into the molecular and cellular basis of various metabolic bone diseases, the contribution of sclerostin to the pathogenesis of bone disease in BA is not entirely known as yet.

2. Methods

2.1. Study population

Seventy BA patients (40 girls and 30 boys with a mean age of 7.6 ± 0.5 y) and 35 healthy children (20 girls and 15 boys with a mean age of 7.8 ± 0.9 y) were recruited in this study. None of them had undergone liver transplantation or exhibited signs and symptoms of fever or ascending cholangitis at the time of blood sampling. None of the study patients had previously received exogenous steroid treatment. Healthy controls attending the Well Baby Clinic at King Chulalongkorn Memorial Hospital for vaccination had normal physical findings and no underlying disease. The serum specimens were collected and stored at −80 °C until analysis. BA patients were classified into two groups according to their serum total bilirubin (TB). Based on their jaundice status, BA children were divided into a jaundice-free group (TB < 2 mg/dl, n = 42) and a persistent jaundice group (TB ≥ 2 mg/dl, n = 28). Portal hypertension (PH) was validated by the presence of ascites and/or esophageal varices as diagnosed by endoscopy. Thirty patients had no PH, but the remaining 40 suffered with PH.

The present study was approved by the Institutional Review Board on Human Research of the Faculty of Medicine, Chulalongkorn University, and was conducted in agreement with the ethical guidelines of the Declaration of Helsinki. All parents of children were informed of the study's purpose and of any interventions involved in this study. Written informed consent was obtained from the parents prior to the children entering the study.

2.2. Laboratory methods

Serum sclerostin levels were quantitatively determined from venous fasting blood samples using a commercial sandwich enzyme-linked immunosorbent assay (ELISA) kit (R&D Systems) according to the manufacturer's instructions. The intra-assay coefficient of variation (CV) was 2.0% and inter-assay CV was 9.5%. The limit of detection for the sclerostin ELISA was 1.7 pg/ml. Serum osteocalcin, a marker of bone formation secreted by osteoblasts, was measured by an osteocalcin ELISA kit (Nordic Bioscience Diagnostics). Intra-assay and inter-assay CVs were 2.6% and 4.7%, respectively, with the detection limit at 0.5 ng/ml. Serum C-terminal telopeptide of type I collagen (CTX) as a marker of bone resorption reflecting osteoclast activity was detected by a CrossLaps ELISA kit (Nordic). Intra-assay and inter-assay CV values were 5.2% and 6.7%, respectively, and the detection limit was 0.01 ng/ml. Additionally, liver function tests including serum albumin, total bilirubin (TB), direct bilirubin (DB), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and γ-glutamyl transpeptidase (GGT) were measured by the central laboratory using the Hitachi 912 analyzer.

2.3. Bone density measurements

All children underwent BMD and bone mineral content (BMC) measurements of the lumbar spine (anteroposterior lumbar vertebrae L1–L4) with dual-energy X-ray absorptiometry (DEXA) using a Hologic QDR 2000. BMD was expressed in absolute values (g/cm^2) and Z-scores. Z-scores of BMD were defined as numbers of SDs from the mean BMD of age matched norms. Control data were from Caucasian children provided in the Hologic 2000 software. No significant differences in spine BMD between Asian and Caucasian children have been documented [19]. According to WHO guidelines, osteoporosis was defined as a spinal BMD ≥2.5 SD below the average values (Z score ≤ −2.5). Osteopenia was defined as a BMD below 2.5 SD but >1 SD under the average values (−2.5 < Z score < −1.0). Normal BMD was defined as a spinal BMD ≤1 SD under the average values (Z score ≥ −1.0).

2.4. Statistical analyses

All values are expressed as mean ± standard error of the mean (SEM). Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) software, ver 16.0. Tests of normality and test of homogeneity of variances were employed to analyze the subject's age, serum sclerostin, biochemical parameters, and BMD. When the populations from which the samples were normally or approximately normally distributed and the variances of the populations were equal, Student's unpaired *t*-test was performed to compare the means of 2 independent groups and 1-way analysis of variance (ANOVA) was employed to compare the means of >2 independent groups. Comparisons between groups were made using Mann–Whitney *U* test (for 2 groups) or Kruskal–Wallis test (>2 groups) when the variances were not equal among the groups. The correlation among numerical data was analyzed using the Spearman correlation test. A $P < 0.05$ was considered to indicate statistical significance for differences and correlations. Multiple regression models were performed with the BMD as the dependent parameter and serum sclerostin, osteocalcin, and CTX as the independent parameters.

3. Results

The demographic data, liver function tests, markers of bone metabolism, and serum sclerostin levels of BA patients without jaundice compared to BA patients with persistent jaundice are displayed in Table 1. Seventy BA patients and 35 healthy controls were recruited in the current study. There were no significant differences in age and gender between the BA patients and the controls. All BA patients had undergone Kasai portoenterostomy. There were 42 BA patients without jaundice and 28 BA patients with persistent jaundice. BA patients with persistent jaundice had significantly lower albumin levels than those without jaundice, whereas serum TB, AST, ALT, ALP, and GGT were remarkably elevated in the BA patients with jaundice compared to those without jaundice.

As demonstrated in Fig. 1, serum sclerostin levels of BA patients were significantly higher than those of the healthy controls ($P < 0.0005$). Among the BA patients, serum sclerostin levels were significantly elevated in patients with persistent jaundice compared with patients without jaundice ($P < 0.0005$) and healthy controls ($P < 0.0005$). Furthermore, jaundice-free BA patients had higher serum sclerostin levels compared with the controls ($P = 0.01$). Subsequent analyses showed that serum sclerostin levels were significantly increased in the BA patients with PH compared with those without PH (2476 ± 191 vs 1309 ± 198 pg/ml, $P < 0.0005$).

Additionally, we investigated the serum levels of osteocalcin and CTX, which are markers of bone turnover. Although the mean serum levels of osteocalcin in patients with BA were higher than those in healthy controls, the difference was not statistically significant (19 ± 3 vs 16 ± 2 ng/ml, $P = 0.2$) (Fig. 1). There was no significant difference in serum osteocalcin levels between the jaundice group, jaundice-free group, and the controls (21 ± 4, 18 ± 4, and 16 ± 2 ng/ml, respectively, $P = 0.3$).

The mean serum CTX levels in the healthy controls were 210 ± 40 pg/ml, whereas serum CTX levels in BA children were 377 ± 65 pg/ml. Thus, the serum CTX values were significantly increased in the BA patients in relation to those of the controls ($P = 0.02$). In the BA patients, serum CTX levels in the jaundice group were significantly higher than those in the jaundice-free group (524 ± 142 vs 280 ± 42 pg/ml, $P = 0.02$) and the healthy controls ($P = 0.01$) (Fig. 1).