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ORIGINAL ARTICLE

Factors Determining Bone Mineral Density in Patients with Biliary Atresia after a Successful Kasai Operation

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Key Words
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Background: Hepatic osteodystrophy is a common complication in patients with chronic liver disease, however, bone mineral status in patients with biliary atresia has rarely been investigated.
Methods: Twenty-nine children with biliary atresia were enrolled in our study and their demographic data, bone mineral density (BMD) of lumbar spine and bilateral femoral neck, and biochemical parameters were measured and analyzed.
Results: The majority of our patients had osteopenia or osteoporosis over at least one part of the skeleton although none had jaundice. Instead of T helper 1 cell cytokine, interleukin (IL)-4 had a significant negative correlation with BMD of the right femoral neck ($\beta = -0.251$, $p = 0.027$) and left femoral neck ($\beta = -0.299$, $p = 0.012$) independently by multiple linear regression analysis.
Conclusion: We conclude that chronic inflammation with increased expression of IL-4 may be an important factor for compromised bone health in patients with biliary atresia.

1. Introduction

Biliary atresia is an infantile disorder of progressive inflammation and obliteration of both extrahepatic and intrahepatic bile ducts that leads to cirrhosis.[1] The pathogenesis of biliary atresia is thought to be an autoimmune response against the biliary epithelial cells after an unknown viral infection. The life expectancy of patients with biliary atresia has improved greatly due to early surgical intervention by Kasai portoenterostomy and subsequent liver transplantation.[2,3] However, several complications developed in some of these patients, including osteopenia and osteoporosis, or so-called "hepatic osteodystrophy."[4]

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The mechanisms of bone disease in biliary atresia are not entirely understood. Chronic cholestasis, malabsorption of vitamin D and calcium, malnutrition, and decreased physical activity or sunlight exposure are possible factors contributing to bone loss in these patients.[5–7] However, compromised bone health was still identified in patients with biliary atresia even without these risk factors.[8] Previous studies have shown that CD4+ activated T helper (Th) cells, with different Th-cell cytokines playing an important role in the pathogenesis of biliary atresia. These include Th1 [interleukin (IL)-2, interferon gamma (IFN-γ)], Th2 (IL-4, IL-10), Th3 [transforming growth factor (TGF)-β1], and Th17 (IL-17) cytokines.[9–12] These cytokines could also influence bone health in patients with chronic inflammatory disease according to previous reports.[13–16]

Optimizing peak bone mass is of undoubted importance during childhood.[6] Nevertheless, data exploring the relationship between cytokines and bone health in children with biliary atresia are rare. In this article, we evaluated the demographic data, biochemical parameters, the concentrations of circulating cytokines, and bone mineral density (BMD) to analyze the determinants of bone health in children with biliary atresia.

2. Material and methods

2.1. Study population

Between August 2010 and August 2011, 29 biliary atresia patients (10 boys and 19 girls, age range 5–18 years) were recruited during routine follow up at our hospital. All patients had undergone a Kasai operation with short-duration (<6 months) steroid treatment following the surgery, and had no other chronic diseases. None of the patients received liver transplantation during enrollment. No fever or other acute illness was noted in any of our participants during blood sampling.

The study was approved by the institutional ethics committee of our hospital. All parents of children with biliary atresia and of the healthy controls were informed of the study's objectives. Informed consent from participating patients and/or parents was obtained before enrollment in the study, in accordance with the Helsinki Declaration.

2.2. Laboratory methods

Plasma was separated by centrifuging blood samples anticoagulated with EDTA at 3000*g* for 10 minutes. The plasma was stored at −70°C after an additional centrifugation at 13,000*g* for 10 minutes to ensure no contamination. We measured plasma IL-2, IL-4, IL-10, IL-17, IFN-γ, and TGF-β1 concentrations using a commercially available enzyme-linked immunosorbent assay kit (R&D Systems, Minneapolis, MN, USA). The concentrations were calculated from a standard curve according to the manufacturer's protocol. Plasma total bilirubin, direct bilirubin, aspartate aminotransferase (AST), alanine aminotransferase, gamma glutamyl transpeptidase, albumin, and calcium levels of biliary atresia patients were measured by the central laboratory using the Hitachi 7180 automated test system.

2.3. BMD measurements

BMD of biliary atresia patients was analyzed using dual energy X-ray absorptiometry (GE Lunar Prodigy Advance) over posterior–anterior lumbar spine (L1–L4) and bilateral femur neck areas. Control data were provided in the software of GE Lunar Prodigy Advance. BMD *Z*-scores in each part were obtained from comparisons of BMD with age- and ethnically-matched norms. Children were classified into normal, osteopenic, and osteoporotic by World Health Organization guidelines. Osteoporosis was defined as BMD $\geq$ 2.5 standard deviation (SD) below the average values (*Z* score ≤ -2.5). Osteopenia was defined as BMD $<$ 2.5 SD but $>$ 1 SD under the average values ($-2.5 <$ *Z* score < -1.0). Normal BMD was defined as BMD $\leq$ 1 SD under the average values (*Z* score ≥ -1.0). BMD was expressed as g/cm^2.

2.4. Statistical analyses

Results are expressed as mean $\pm$ SD. Analyses were performed using the statistical package for the social science (SPSS version 15.1; SPSS Inc., Chicago, IL, USA). The independent sample *t* test was employed for comparison of demographic and clinical data between groups. We applied the Pearson correlation test for correlation analysis and multiple linear regression analyses to investigate any relationships between BMD and other various independent parameters. A p value < 0.05 was considered statistically significant.

3. Results

BMD of the patients measured using dual energy X-ray absorptiometry is summarized in Table 1. The majority of children with biliary atresia (20/29, 69%) were identified to have osteopenia over at least one part of the skeleton in this study. BMD *Z*-scores were within the osteopenic/osteoporotic range in 6/1 (21/3%), 16/1 (55/3%), and 12/2 (41/7%) patients, respectively, over anterior–posterior lumbar spine (L1–L4), left femoral neck, and right femoral neck. Significant difference between genders was only found over BMD of the lumbar spine ($p = 0.010$) but there was no difference between BMD *Z*-scores. The mean age- and ethnically-matched BMD *Z*-scores over three areas were all below average value, approaching the osteopenic range over the bilateral femoral neck (−0.53, −0.97, and −0.90).

Comparison of demographic data, biochemical parameters, and plasma cytokine levels between osteopenic and nonosteopenic biliary atresia patients at the weight-bearing skeleton is shown in Table 2. All patients enrolled in the study were nonjaundiced. No significant difference was seen regarding age distribution, sex ratio, body height, body weight, biochemical parameters, or six plasma cytokine levels measured between the two groups.

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