Histiocytic osteolysis secondary to hyperbilirubinemia: a case report

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ABSTRACT

A 6-year-old boy with Alagille syndrome, characterised by marked hyperbilirubinemia, presented with malunion of a pathological fracture of the femur with local bone atrophy and insufficient callus formation. During corrective osteotomy, it was noted that the femur was stained dark green, suggestive of bilirubin deposition. Histology of the resected bone revealed the presence of many histiocytes and osteoclast-like multinucleate giant cells containing bilirubin particles in the cytoplasm causing bone resorption. These findings suggest that bilirubin may activate macrophages to form osteoclast-like multinucleate giant cells, resulting in histiocytic osteolysis.

Key words: Alagille syndrome; cholestasis; histiocytosis; hyperbilirubinemia; jaundice; osteolysis

INTRODUCTION

Alagille syndrome is a multisystem disorder characterised by aplasia of the intrahepatic bile ducts and malformations of the cardiovascular system, the eyes, the vertebral column, and the facies. Major clinical features include jaundice, cholestasis, and congenital heart disease with peripheral pulmonary stenosis. It may result from heterogeneous gene mutations.

We report a 6-year-old boy with marked hyperbilirubinemia from birth, who presented with malunion of a pathological fracture of the femoral shaft. Hyperbilirubinemia inhibits osteoblast proliferation and induces osteoporosis. Our findings also suggest that hyperbilirubinemia activates histiocytes and induces formation of osteoclast-like multinucleate giant cells, resulting in histiocytic osteolysis.

CASE REPORT

In June 2006, a 6-year-old Indonesian boy with Alagille syndrome was referred to our hospital for orthotopic liver transplantation using a graft from his father. He had been diagnosed with the disease at age 9 months and had been treated with vitamins A, D, E, and K, ursodeoxycholic acid, and rifampin. Nonetheless, his liver dysfunction and hyperbilirubinemia worsened...
by the time he reached age 5 years. Two months prior to presentation, he sustained a pathological fracture of the left femoral shaft after a slip and was treated with a cast. Delayed bone union led to an anterolateral convex deformity.

On examination he was found to be malnourished with stunted growth (height, 80 cm; weight, 12 kg). He had all of the features of Alagille syndrome, including the characteristic face, mild peripheral pulmonary artery stenosis, butterfly vertebrae, posterior embryotoxon, and hyperbilirubinaemia. Blood tests revealed anaemia (haemoglobin, 92 g/l) and liver dysfunction with high serum aspartate transaminase (223 U) and alanine aminotransferase (151 U), hyperbilirubinaemia (218.9 µmol/l), high serum total cholesterol (23.5 mmol/l), and high serum alkaline phosphatase (2736 U).

Radiographs revealed an anterolateral convex deformity of the left femoral shaft with poor callus formation, cortical bone atrophy, osteoporosis, and osteolytic lesions (Fig. 1). Osteoporosis was also seen in the right femur and spine (Fig. 2). The patient was wheelchair-bound.

After successful liver transplantation, the patient underwent a corrective osteotomy and osteosynthesis using a plate and screws. Partial weight bearing was allowed after bone union at week 6 (Fig. 3), and the patient could walk without any support 2 months later.

During the osteotomy, coronal and cross-sections of the femoral cortex and callus around the fracture site showed dark green staining, suggestive of bilirubin deposition (Fig. 4). Histopathologically, numerous osteoclast-like multinucleate giant cells were observed at the surface of the cortical and callus lamellar bone, associated with marked histiocytic infiltration of the bone marrow. These cells contained brown pigment in the cytoplasm (Fig. 5). The pigment was identified as bilirubin based on negative Berlin-blue staining and positive staining using Hall’s method, which is specific for bilirubin (Fig. 6). Immunohistochemical staining confirmed the presence of phagocytic bone resorbing cells. Anti-CD68 (PG-M1), which labels macrophages immunologically, showed staining in these particles (Fig. 7).

**DISCUSSION**

Alagille syndrome is very rare, occurring in one in 100,000 births, affecting both genders equally. It is reported to be autosomally dominant with a low penetrance and highly variable expression. The Alagille gene has been identified in the 20p12 region.

![Figure 1](Image) Radiographs of the left femur, with an anterolateral convex deformity, poor callus formation, cortical bone atrophy, osteoporosis, and osteolytic lesions (arrows).

![Figure 2](Image) The (a) right femur and (b) lumbar spine are also osteoporotic.

![Figure 3](Image) Radiographs of the left femur after (a) corrective osteotomy and (b) bone union at 6 weeks.
The syndrome manifests as a multisystem disorder involving the liver, heart, eyes, face, and skeleton. One of the manifestations is hyperbilirubinaemia caused by cholestasis secondary to paucity of the interlobular bile ducts.\(^1\)\(^2\)

Patients with chronic liver disease have an increased prevalence of osteoporosis because of calcium malabsorption caused by low levels of 25-hydroxy vitamin D3 and hyperbilirubinaemia.\(^3\) Nonetheless, the underlying mechanism causing osteoporosis secondary to hyperbilirubinaemia remains unclear. Patients with primary biliary cirrhosis and osteoporosis have higher serum bilirubin levels than those without osteoporosis.\(^4\) A high serum bilirubin level is associated independently with increased bone loss at the femoral neck in patients with chronic liver disease.\(^5\) Exposure to excessive levels of bilirubin inhibits the proliferation of osteoblasts in cell cultures.\(^6\) Despite these findings, studies investigating the association between bilirubin levels and osteoclast function are lacking.

In our patient, long-term hyperbilirubinaemia was associated with systemic osteoporosis. The dark green staining around the fracture site looked like condensed bile. Histological examination revealed the
presence of numerous histiocytes in the bone marrow and osteoclast-like multinucleate giant cells lining the resorbed bone surface in the femoral cortex. Both cell types contained large amounts of brown pigment in the cytoplasm. A histochemical study revealed that these particles were bilirubin, not haemosiderin or other pigments derived from iron.

The multinucleate giant cells closely resembled osteoclasts, however, the number of nuclei and cell sizes were smaller than those seen in ordinary osteoclasts, and they were surrounded by numerous histiocytes. These giant cells also stained for CD68 (PG-M1), which binds immunologically to macrophages, so we considered them histiocytic in origin. It is speculated that the histiocytes first phagocytose serum bilirubin in the bone marrow and actively proliferate, with some of them fusing with each other to form multinucleate giant cells, causing bone resorption. This phenomenon suggests that bilirubin may induce osteoporosis by activating histiocytes, a mechanism similar to that seen in polyethylene-induced particle disease in which loosening of the stem occurs after artificial joint replacement.\(^7\)\(^8\) The major cause of bone resorption around a total joint prosthesis is the inflammatory response to the polyethylene wear debris. Particles derived from the wear debris cause macrophage activation and phagocytosis. Aseptic loosening and osteolysis after hip arthroplasty is caused predominantly by osteoclasts, mediated mainly by an osteoprotegerin ligand (RANKL) and TNF-\(\alpha\).\(^7\) It has been shown that RANKL is expressed by activated macrophages, osteoblasts, and lymphocytes.\(^7\) We speculate that bilirubin may behave in a similar manner to these particles.

REFERENCES