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Nonvisualization of the fetal gallbladder by second-trimester ultrasound scan: strategy of clinical management based on four examples

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Objective When the fetal gallbladder is not seen at ultrasound (US) scan, to propose a diagnostic method of differentiating fetuses who are healthy or have minor anomalies from fetuses with severe anomalies requiring intensive management.

Method We present four clinical cases illustrating this variability, together with additional examinations: karyotyping, screening for cystic fibrosis mutations, amniotic fluid digestive enzyme activities.

Results The four examples we present-biliary duct atresia, biliary agenesis, gallbladder reveal at birth, and cystic fibrosis-illustrate the difficulties of making both diagnosis and prognosis prenatally when the gallbladder is not visualized. Laboratory assays allowed prenatal management.

Conclusion Failure to visualize the gallbladder prenatally may indicate fetal diseases of highly variable prognosis, but may also sometimes be followed by postnatal visualization in a child free of any disease. Prenatal management could help in defining diagnosis and prognosis.

KEY WORDS: prenatal diagnosis; ultrasound examination; gallbladder; biliary duct atresia; cystic fibrosis

INTRODUCTION

Nonvisualization of the fetal gallbladder may indicate abnormalities ranging from gallbladder agenesis without serious clinical sequelae to biliary atresia, which carries a poor prognosis (Bronshtein *et al*., 1993; Chardot, 2006; Ochshorn *et al*., 2007). In such cases, a gallbladder undetected prenatally is sometimes visualized postnatally in a disease-free child (Hertzberg *et al*., 1996; Blazer *et al*., 2002).

The gallbladder starts to develop around the third week of embryonic life as a cystic diverticulum of endodermal origin, located under the hepatic diverticulum, at the duodenal level. The diverticulum develops into the gallbladder and cystic duct, which joins the hepatic duct. Growth of the bile duct progressively distances the gallbladder from the duodenum.

At prenatal ultrasound (US), the gallbladder is visualized below the liver in the right anterosuperior quadrant of the abdomen. It is round or oblong, roughly pear-shaped, with echogenic walls and anechogenic contents. Smaller than the stomach, its shape and volume vary from one fetus to another, depending on its fullness. It is visible by US from the 14th week of gestation, and is normally examined at 16 weeks, as part of a standard morphological examination.

The aim of this study was to propose a diagnostic method of differentiating between fetuses that are healthy or have minor anomalies and fetuses with severe anomalies requiring intensive management. We illustrate our approach through four clinical cases of nonvisualization of the gallbladder by second-trimester US.

Clinical case no. 1

A 20-year-old primipara was referred to our department at 26 weeks of gestation because of nonvisualization of the gallbladder. Pregnancy monitoring started at 16 weeks, and the gallbladder was not mentioned in the US findings. At 22 weeks, the US scan was normal, but the gallbladder could not be visualized. At 25 weeks, US again failed to visualize the gallbladder, but the small intestine was too clearly delineated, with no real hyperechogenicity. Cystic fibrosis screening of the parents found none of the 36 mutations. Amniocentesis at 27 weeks indicated a normal fetal karyotype (46,XY). Amniotic fluid gamma-glutamyltranspeptidase (GGTP) and leucine-aminopeptidase (LAP) activities were very low. At 33 weeks, US still failed to visualize the gallbladder. At 39 weeks, the patient gave birth to a 4460-g boy. Clinical examination was normal, as was routine cystic fibrosis neonatal screening by the immunoreactive trypsinogen test (IRT). Mother and child were discharged on day 5. On day 16, the child developed an occlusive syndrome associated with jaundice. Abdominal US showed hepatomegaly, a small gallbladder, ascites, gastrointestinal stasis, and intrahepatic

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bile ducts were not visible. On day 30, peritonitis and necrosis of the ileum were diagnosed, and the ileum was resected. The gallbladder was small and very fibrous. Liver biopsy indicated secondary biliary cirrhosis compatible with biliary duct atresia. The Kasai procedure (hepatoportoenterostomy) was performed (Kasai *et al*., 1968), surgical follow-up was simple, but jaundice persisted.

Clinical case no. 2

A 24-year-old primipara was referred because of nonvisualization of the gallbladder. US scan at 13 weeks of gestation was normal. At 22 and 25 weeks the gallbladder could not be visualized. Amniocentesis indicated a normal fetal karyotype (46,XY). None of the 31 most frequent mutations of cystic fibrosis was found. The gallbladder could still not be visualized by US at 31 weeks. Magnetic resonance imaging at 34 weeks failed to visualize the gallbladder, but no hepatosplenic or digestive anomaly was noted and the expected signal of the meconium was observed, indicating the presence of bile salts in the meconium. The patient gave birth to a 2950-g boy by cesarean section at 42 weeks. Clinical examination was normal and US on day 1 revealed a small gallbladder. Laboratory findings were normal. No biliary or digestive disease was observed at 6 months of age.

Clinical case no. 3

A 30-year-old Japanese primipara with Graves' disease (propylthiouracil treatment) was referred to our institution. The first US examination at 12 weeks of gestation was normal. US examinations at 22, 26, 29, and 37 weeks showed no fetal malformation, but the gallbladder was never visualized. Additional investigations (amniocentesis, cystic fibrosis screening of the parents) were refused by the patient. The patient gave birth at 38 weeks of gestation to a 2965-g girl. Clinical examination at birth was normal, but at day 3 the child presented with jaundice. Neonatal screening for cystic fibrosis (IRT test) was negative. At day 11, clinical examination was normal, but US failed to detect the gallbladder. There was no dilatation of the intra or extrahepatic bile ducts. Free bilirubin concentration was 128 μmol/L, and conjugated bilirubin 11 μmol/L, with normal transaminases, but elevated GGTP (308 IU/L). At 1 month, the clinical examination, gastrointestinal transit, stool coloration, and laboratory findings were all normal, and were confirmed at 4 months. At age 2 years, the child was healthy, clinical examinations and laboratory findings were normal but the gallbladder could still not be visualized, and so agenesis of the gallbladder was diagnosed.

Clinical case no. 4

A 33-year-old patient was referred at 25 weeks of gestation because of nonvisualization of the fetal gallbladder, associated with localized hyperechogenicity of the intestinal mass predominant in the right iliac fossa. Amniocentesis was performed for fetal karyotyping and viral screening. Screening for cystic fibrosis mutations revealed the presence of two mutations F508del indicating cystic fibrosis. Levels of amniotic fluid digestive enzymes (GGTP, LAP, and ALP) were below the first percentile, a profile compatible with cystic fibrosis. The pregnancy was terminated at the parents' request.

DISCUSSION

Nonvisualization of the fetal gallbladder is rare and occurs in 0.1% to 0.15% of pregnancies (Bronshtein *et al*., 1993; Blazer *et al*., 2002), Hertzberg *et al*. (1996) reported a frequency of 7.1%, which may be explained by the small number of patients included between 20 and 24 weeks of gestation (n = 84).

When nonvisualization of the gallbladder is confirmed by a second US examination performed 7 to 15 days later, the etiology should be investigated because of the association with various abnormalities, such as renal agenesis, hydronephrosis, cerebral ventricular dilatation, chromosomal anomalies (particularly trisomy 21), cystic fibrosis, biliary atresia, and multiple malformation (Bennion *et al*., 1988; Bronshtein *et al*., 1993; Duchatel *et al*., 1993; Albayram *et al*., 2002; Ben-Ami *et al*., 2002; Simon-Bouy *et al*., 2002; Blazer *et al*., 2002; Ochshorn *et al*., 2007). However, the absence of indications in the US does not exclude severe abnormalities, such as cystic fibrosis or biliary duct atresia. In cystic fibrosis, nonvisualization of the gallbladder may be isolated or associated with bowel dilatation, or hyperechogenic fetal bowel, ascites, or meconium peritonitis (Duchatel *et al*., 1993; Blazer *et al*., 2002). Biliary atresia is either syndromic (10% of cases), and if so associated with anomalies (intestinal malrotation, situs inversus, malposition of the spleen, cardiac defect), or nonsyndromic (Chardot, 2006). The etiology of biliary duct atresia is unknown, although various hypotheses have been formulated, such as persistence of fetal bile ducts, which release bile into the hepatic parenchyma, leading to an inflammatory reaction that causes fibrosis (Tan *et al*., 1994). A viral cause has been posited, but the data are contradictory (Steele *et al*., 1995), and familial cases suggest that a genetic component may be implicated (Lachaux *et al*., 1988), but no gene has yet been identified.

Management depends on the presence or otherwise of associated malformations at US scan, parental screening for cystic fibrosis mutations, fetal karyotyping, and assay of amniotic fluid digestive enzymes. The difficulty will be to distinguish between agenesis of the gallbladder (without major clinical impact) and biliary duct atresia (a severe disease).

Ben-Ami *et al*. (2002) report a case of nonvisualization of the gallbladder associating low amniotic fluid digestive enzyme levels and the absence of *CFTR* gene mutations, suggested biliary duct atresia, a diagnosis confirmed at autopsy. In three prospective studies, isolated agenesis of the gallbladder explained 12

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to 28% of nonvisualization of the gallbladder (Bronshtein *et al*., 1993; Blazer *et al*., 2002; Ochshorn *et al*., 2007). In the present study, amniotic fluid digestive enzymes assays were performed in two of the four cases (amniocentesis not performed in one case and enzymes were not assayed in the other). The value of amniotic fluid digestive enzymes has been previously described (Carbarns *et al*., 1983; Muller *et al*., 1988). These digestive enzymes, which are synthesized by the biliary epithelium (GGTP) and by enterocytes [intestinal alkaline phosphatase (ALP)], are present in the amniotic fluid from 12 to 13 weeks of gestation, a period corresponding to the resorption of the anal membrane, and then rise to a plateau at 17–18 weeks, before gradually decreasing to 24 weeks, because the progressive maturation of the anal sphincter muscles makes the anus impermeable to digestive secretions around 24 weeks of gestation. An obstacle to intestinal transit (atresia of the small intestine, anorectal atresia, meconium ileus, biliary atresia), impairs flow of digestive secretions into the amniotic fluid. But after 24 weeks of gestation, it is no longer possible to differentiate between abnormally low and physiologically low levels of the enzymes, although high values exclude biliary atresia and cystic fibrosis. In biliary atresia, amniotic fluid GGTP levels alone drop dramatically from 16 weeks of gestation (Muller *et al*., 1991), whereas in cystic fibrosis, or intestinal atresia, levels of all the digestive enzymes fall sharply (Brock, 1983; Carbarns *et al*., 1983; Muller *et al*., 1988; Ochshorn *et al*., 2007). The latter profile, typical of cystic fibrosis, was seen in case no. 4 (homozygous F508del mutation). Given the great diversity of mutations implicated in cystic fibrosis, assay of digestive enzymes in the amniotic fluid is a precious aid. When enzyme levels are very low, mutation screening can be undertaken, while normal enzyme levels are reassuring.

It has been shown that early management of biliary duct atresia improves prognosis (Chardot, 2006). However, prenatal diagnosis is very difficult, as illustrated by our case no. 1, in which amniotic fluid was sampled too late to give information on digestive enzymes. Assay of digestive enzymes in the amniotic fluid is currently the only additional test that can point to a diagnosis of biliary duct atresia, but very few cases have been reported. In France, second-trimester US is usually performed between weeks 22 and 24, and amniocentesis for digestive enzyme assays should not be delayed. However, the incidence of biliary duct atresia is too low to justify changing the timing of the US examination.

In summary, when the gall bladder is observed at US scan, this should be specified in the medical records. Nonvisualization should be confirmed by an experienced sonographer as soon as possible in order to organize management before 24 weeks. Confirmation should be followed by amniocentesis for karyotyping, *CFTR* mutation screening, and digestive enzyme assays. If laboratory findings are normal, US scans can be performed monthly because the visualization of gallbladder would be reassuring.

In genetic counseling, parents should be told that nonvisualization of the gallbladder may indicate a simple anatomical variant, but could also indicate a severe disease, therefore justifying an invasive procedure such as amniocentesis.

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