Clinical evaluation of isolated nonvisualized fetal gallbladder

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Objective Isolated nonvisualized fetal gallbladder (INVFG) is relatively rare. In most cases, the gallbladder will eventually be detected. In some cases however, INVFG may be associated with serious abnormalities, cystic fibrosis (CF), aneuploidy, and agenesis of the gallbladder. We describe a clinical evaluation of prenatally diagnosed INVFG.

Methods Cases of nonvisualized gallbladder were first evaluated by serial scans. Cases with no additional malformations were designated as INVFG, and were further evaluated by mutation analysis for CF, and amniocentesis for karyotype and microvillar membrane enzymes (MME).

Results A total of 22 cases of nonvisualized gallbladder were detected. Of these, 2 had additional malformations, and 3 were excluded because of incomplete evaluation. Of the remaining 17 cases, 3 (17.6%) had adverse outcomes: 1 case of CF, 1 case of 47,XXX, and 1 case of multiple congenital anomalies detected only postnatally. Abnormal levels of MMEs were detected in 3 cases, 1 of which was diagnosed with CF. In 2 cases, the gallbladder was not detected even after birth, but development is normal.

Conclusion Evaluation of INVFG should include genetic counselling, amniocentesis for karyotype and MME analysis, CFTR mutation analysis and repeated ultrasound scans. Copyright © 2007 John Wiley & Sons, Ltd.

KEY WORDS: isolated nonvisualized gallbladder (INVFG); microvillar membrane enzymes (MME); cystic fibrosis (CF); aneuploidy; ultrasound scan

INTRODUCTION

Visualization of the fetal gallbladder on ultrasound scan is considered to be part of the routine gastrointestinal examination. The gallbladder can usually be visualized on early second-trimester fetal ultrasound scan, in most cases (Hata et al., 1987; Hertzberg et al., 1996; Blazer et al., 2002). If the fetal gallbladder is not visualized on the early scan, it will eventually be detected, in the majority of the cases, later in pregnancy or even after delivery (Hertzberg et al., 1996; Blazer et al., 2002). In some cases, associated abnormalities can be detected, and the prognosis usually depends on their severity. Isolated nonvisualized fetal gallbladder (INVFG) is a relatively rare finding, with an incidence of 1:330–1:875 in the second-trimester. The etiology of INVFG is varied and includes agenesis of the gallbladder, chromosomal anomalies, cystic fibrosis (CF), intestinal obstruction, extrahepatic biliary duct atresia (EHBA) and other severe abnormalities (Fryns et al., 1982; Hatamaka et al., 1984; Abramson et al., 1987; Bronshtein et al., 1993; Duchatelet et al., 1993; Nothen et al., 1993; Singh et al., 1999; Ben-Ami et al., 2002; Gangbo et al., 2004). Therefore, when INVFG is encountered, further evaluation is warranted to distinguish between the different possible etiologies, as the prognosis for each of these conditions is quite different.

In this study, we suggest a clinical evaluation of INVFG, based on our experience. The evaluation includes repeated fetal ultrasound scans throughout gestation, molecular analysis for CF and amniocentesis for chromosomal analysis. In addition, we propose the analysis of amniotic fluid microvillar membrane enzymes (MME), as low levels thereof were reported in fetuses with intestinal obstruction, CF, extrahepatic biliary atresia, and other gastrointestinal tract abnormalities (Carbars et al., 1983; Brock et al., 1984, 1988; Muller et al., 1988; Dechecchi et al., 1989; Muller et al., 1991, 1994; Ben-Ami et al., 2002).

METHODS

During a 5-year-period (2000–2005), 22 cases of nonvisualized fetal gallbladder were referred to the Prenatal Diagnosis Unit at the Genetic Institute, Tel Aviv Sourasky Medical Center. A nonvisualized fetal gallbladder (NVFG) was defined as the inability to depict it on two targeted ultrasound examinations within a 1-week period. An isolated nonvisualized fetal gallbladder (INVFG) was defined as NVFG in the absence of additional abnormal sonographic findings.
All cases underwent genetic counselling and evaluation, which consisted of the following:

**Serial fetal ultrasound scans:** Initial scans were performed at 14–17 weeks of gestation. A repeat detailed scan was performed at 20–24 weeks of gestation. Thereafter, patients were scheduled for additional targeted scans at 3–6 week intervals, until the gallbladder was visualized or until birth. The detailed scan included specific focus on the gastrointestinal tract to detect echogenic bowel and/or dilated loops of bowel. In addition, fetal echocardiography was performed in all cases. **Mutation analysis for cystic fibrosis:** The mutation panel included cystic fibrosis transmembrane conductance regulator (CFTR) mutations that are common in the Israeli population, including F508del, W1282X, N1303K, G542X, and D1152H. It was recommended that both parents undergo CF mutation screening. Complete CFTR gene sequencing was suggested in cases highly suspicious of CF, when one parent was found to carry a common pathological mutation, while the other did not.

**Chromosome analysis:** Amniocentesis was performed at 17 to 20 weeks of gestation for chromosome analysis by standard cytogenetic techniques.

**Amniotic fluid MME levels:** Some of the amniotic fluid was sent for biochemical analysis of MMEs at Biochimie Hormonale, Hopital Robert Debre, Paris, France. Assays were performed for gamma glutamyl transpeptidase (GGTP), aminopeptidase M, total and intestinal alkaline phosphatase activities (Muller et al., 1988).

Patients were followed-up until delivery and additional data were obtained after the delivery or birth of the child.

The study was approved by the local Institutional Review Board IRB.

**RESULTS**

Of the 22 cases with nonvisualized fetal gallbladder, 2 were excluded because of subsequent major malformations detected on scans (one case with clubfoot and the other with micropenis, facial dysmorphism, and cardiac anomalies). Three other cases were also excluded because of incomplete evaluation. Complete evaluation was performed in 17 cases of INVFGB, as presented in Table 1. Of these, 3 (17.6%) had adverse outcomes, including 1 case of thyroid aplasia, 1 case of aneuploidy (47,XXX), and 1 case of cystic fibrosis:

Case 1: Thyroid aplasia: In this case, MME levels were within normal range. Chromosome analysis was normal, 46,XX. Cystic fibrosis mutation analysis was negative. After delivery, hypotonia was noted. Complete agenesis of the thyroid gland was diagnosed, and thyroid replacement therapy was initiated. Brain ultrasound detected mild intraventricular hemorrhage with periventricular leukomalacia. Patent ductus arteriosus was diagnosed and treated with cardiac catheterization. Development is currently normal at 14 months.

Case 2: Aneuploidy, 47,XXX. In this case, MME levels were within normal range. The father was found to be a carrier of a cystic fibrosis mutation (W1282X), but the mother was mutation-negative. Chromosome analysis revealed a 47,XXX karyotype. The pregnancy was terminated. Ultrasound performed before the termination revealed a gallbladder.

Case 6: Cystic fibrosis: In this case, amniotic fluid MME levels were abnormally low (GGTP = 1st percentile; intestinal alkaline phosphatase <1st percentile; aminopeptidase <1st percentile). These results suggested intestinal atresia or obstruction. Mutation analysis for cystic fibrosis demonstrated the ΔF508 mutation in the father, but no mutation was detected in the mother on initial screening. Following extensive genetic counselling, the pregnancy was terminated. Autopsy demonstrated a small atrophic gallbladder (Figure 1A) and meconium ileus of the distal gastrointestinal tract, suggestive of CF (Figure 1B). Subsequently, complete CFTR gene sequencing demonstrated a novel pathogenic mutation in the mother (3121 − 1G > A), confirming the diagnosis of CF.

In 2 other cases, the MME levels were abnormal but the pregnancies were continued, and resulted in normal delivery of healthy newborns:

Case 12, amniotic fluid levels of intestinal alkaline phosphatase were abnormally low (0.2 MoM), but GGTP and aminopeptidase levels were within the normal range. Chromosome analysis was normal 46,XY. Pregnancy resulted in a normal delivery of a healthy newborn. Abdominal scan was performed shortly after delivery. The gallbladder was not visualized.

Case 13, abnormally high GGTP and aminopeptidase levels (3.2 MoM and 2.8 MoM, respectively. Both levels are >99st percentile), were detected in the amniotic fluid. These findings were suggestive of fetal 'vomiting', perhaps due to high intestinal obstruction. Chromosome analysis was normal 46,XY. Pregnancy resulted in a normal delivery of a healthy newborn. The gallbladder was detected on a postnatal scan.

In all remaining cases, MME levels were within normal range, no other abnormalities were detected on subsequent scans and pregnancy outcomes were normal.

In 2 cases (cases 8 and 12) the fetal gallbladder was not visualized even on postnatal scans. Both of these children were healthy and developing normally at 8 months and 14 months, respectively.

**DISCUSSION**

The gallbladder can be demonstrated on ultrasound scans as early as 14–15 weeks (Hata et al., 1987). In some cases, the gallbladder may only be visualized later in pregnancy and in ~95% of the cases it will eventually be detected by 24–32 weeks of gestation (Hertzberg et al., 1996). Inability to demonstrate the gallbladder on ultrasound scans may also be the result of 'technical difficulties' such as, aberrant location of the...
### Table 1—Clinical characteristics of 17 cases with isolated nonvisualized fetal gall bladder (INVFGB)

<table>
<thead>
<tr>
<th>Case</th>
<th>Initial U/S scan (weeks)</th>
<th>Subsequent scans (weeks)</th>
<th>Subsequent findings</th>
<th>Amniotic fluid MME*</th>
<th>Karyotype</th>
<th>CF mutation analysis</th>
<th>Pregnancy outcome</th>
<th>Gallbladder detected</th>
<th>Diagnosis</th>
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<tr>
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<td>17,19</td>
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<td>Normal</td>
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<td>17</td>
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<td>47,XXX</td>
<td>Paternal carrier (W1282X)</td>
<td>TOP</td>
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<td>Normal X</td>
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<tr>
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<td>Delivery</td>
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<td>Normal</td>
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<tr>
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<td>Delivery</td>
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<td>6</td>
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<td>17,21</td>
<td>FEB</td>
<td>All low</td>
<td>46,XX</td>
<td>Fetus affected</td>
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<td>17,19,24</td>
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<td>Low iALKP</td>
<td>46,XY</td>
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<td>Delivery</td>
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<td>Normal</td>
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<tr>
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<td>18</td>
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<tr>
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<td>24</td>
<td>None</td>
<td>Normal</td>
<td>46,XX</td>
<td>Normal</td>
<td>Delivery</td>
<td>Yes</td>
<td>Normal</td>
</tr>
</tbody>
</table>

*low levels: <1st percentile, high levels >99th percentile. U/S, ultrasound; MME, microvillar membrane enzymes; TOP, termination of pregnancy; VSD, ventricular septal defect. FEB, fetal echogenic bowel; iALKP, intestinal alkaline phosphatase.
gallbladder (which can be intra- or retrohepatic), a left-sided gall bladder, or rarely within the lesser omentum or falciform ligament (Singh et al., 1999). In addition, transvaginal sonography demonstrated the gallbladder in 99.9%, whereas transabdominal scans demonstrated the gallbladder in only 82.5% (Blazer et al., 2002).

True INVFGB is usually associated with a favorable outcome, particularly if the gallbladder is subsequently detected on repeated scans or even postnatally. However, INVFGB may be associated with some poor pregnancy outcomes. There are sporadic case reports of associated chromosome aberrations in cases of absent gallbladder, particularly trisomies (Fryns et al., 1982; Hatanaka et al., 1984; Matheson et al., 2003; Gangbo et al., 2004). Hertzberg et al. reported one case of trisomy 21 among 80 cases of INVGB (Hertzberg et al., 1996). In 34 cases of nonvisualized fetal gallbladder, Blazer et al. detected 5 (17%) with abnormal karyotypes (Blazer et al., 2002). Likewise, in our series, there was 1 case of trisomy X. These data suggest that nonvisualized fetal gallbladder is an indication for fetal karyotyping.

Congenital absence of the gallbladder is relatively rare, with an incidence of <1:6000 livebirths (Monroe, 1959). Nonvisualized fetal gallbladder has also been found in association with various congenital anomalies. The most frequently encountered associated malformations were cardiovascular (58.3%), gastrointestinal/genitourinary (25%), anterior abdominal wall (10.4%) and central nervous system (6.3%) (Bennion et al., 1988). In our series, 1 case of INVFGB (Case 1) was diagnosed after birth with complete agenesis of the thyroid gland. While the gallbladder was eventually detected, other abnormalities were found, including anisocoria, hypotonia, patent ductus arteriosus and periventricular leukomalacia, all of which can be attributed to congenital hypothyroidism (Siebner et al., 1992; Paul et al., 1998). In addition, absent fetal gallbladder has been associated with various syndromes, such as Steinfeld syndrome, Alagille syndrome, or cranio-microceral syndrome (Nothen et al., 1993; Barr et al., 1995; Krantz et al., 1999). In our series, only 1 case (Case 1) demonstrated an abnormality after birth (thyroid aplasia) following normal prenatal evaluation. We therefore believe that our series is too small to draw any conclusions regarding additional pediatric follow-up studies.

Intestinal obstruction or agenesis has been reported in cases of nonvisualized gallbladder. Intestinal obstruction usually develops at about 11 weeks of gestation but diagnosis by ultrasound may only be possible in the second half of pregnancy (Szabo et al., 1990). In contrast, decreased MME levels may suggest intestinal obstruction as early as 16–20 weeks of gestation (Morin et al., 1987). Another entity that has been associated with nonvisualized fetal gallbladder is EHBA, manifested by neonatal jaundice, hepatocellular damage, and cirrhosis. Without treatment, death usually occurs within 2 years. The etiology of this condition is unknown. Low levels of MME have been reported in association with EHBA (Mueller et al., 1988, 1991; Ben-Ami et al., 2002).

Nonvisualized gallbladder can also be the harbinger of cystic fibrosis. Duchatel et al. reported nonvisualized gallbladder in 9 out of 12 fetuses affected with CF (Duchatel et al., 1993). Additionally, abnormal amniotic fluid MME levels have been reported in CF including a significant decrease in GGTP and aminopeptidase (Brock et al., 1984, 1988; Muller et al., 1987).

In our series, 1 case of nonvisualized fetal gallbladder was diagnosed with CF. In this case, MME levels were very low (below 1st percentile). Initially, a CFTR mutation was found only in the father. Because the clinical manifestations were suggestive of CF, the pregnancy was terminated but the maternal CFTR mutation (3121-1G > A) was only detected several years later by complete CFTR sequencing. This mutation was subsequently detected in other Jews of Iraqi descent, and has since been incorporated into the mutation panel in Israel, for this ethnic group. Finally, more than 1000 different pathogenic CFTR mutations have been described. However, most CF mutation panels analyze less than 100 common mutations. Thus, in cases suggestive of CF, it may be prudent to perform complete sequencing of the CFTR gene. This service is now clinically available in several labs with a turnaround time of 1 month.

The MME include peptidases (GGTP), disaccharidases (trehease, lactase), and phosphatases (alkaline phosphatases).
phosphatase). These enzymes normally appear in the amniotic fluid at 12–13 weeks of gestation, the time when the fetal anal membrane disappears. Thereafter, the amniotic levels of these enzymes correlate with gestational age. However, their levels decline again from 18 to 24 weeks of gestation (Brocklehurst and Wilde 1980; Muller et al., 1988; Campbell et al., 1992). Various studies have shown that very low levels of GGTP and alkaline phosphatase may be associated with conditions characterized by slow intestinal transit, such as CF, intestinal tract obstruction, aneuaploidy, and EHBA (Brock et al., 1988; Jones and Evans 1988; Deecheci et al., 1989; Szabo et al., 1990; Muller et al., 1994; Ben-Ami et al., 2002). In contrast, increased levels of amniotic fluid MME can be observed in fetuses with upper gastrointestinal tract obstruction, below the ampulla of Vater, such as duodenal stenosis or atresia (Muller et al., 1988; Burc et al., 2001). This may be due to fetal ‘vomiting’. On the basis of these reports, testing of amniotic fluid MME has been suggested as a routine part of the clinical evaluation of pregnancies at risk for such conditions, including nonvisualized fetal gallbladder. Nevertheless, MME measurements may also be associated with false positive results. In our series, there were 2 cases of INVFGB with abnormal MME but with normal outcomes. It is therefore important to evaluate MME results in the light of the full clinical picture.

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REFERENCES


