# Non-visualisation of fetal gallbladder in a Chinese cohort

YH Ting, PL So, KW Cheung, TK Lo, Teresa WL Ma, TY Leung *


## ABSTRACT

**Introduction:** Non-visualisation of fetal gallbladder (NVFGB) is associated with chromosomal abnormalities, biliary atresia, cystic fibrosis, and gallbladder agenesis in Caucasian fetuses. We investigated the outcomes of fetuses with NVFGB in a Chinese cohort.

**Methods:** This retrospective analysis included cases of NVFGB among Chinese pregnant women at five public fetal medicine clinics in Hong Kong from 2012 to 2019. We compared the incidences of subsequent gallbladder visualisation, chromosomal abnormalities, biliary atresia, cystic fibrosis, and gallbladder agenesis between cases of isolated NVFGB and cases of non-isolated NVFGB.

**Results:** Among 19 cases of NVFGB detected at a median gestational age of 21.3 weeks (interquartile range, 20.0-22.3 weeks), 10 (52.6%) were isolated and nine (47.4%) were non-isolated. Eleven (58.0%) cases had transient non-visualisation, four (21.0%) had gallbladder agenesis, three (15.8%) had chromosomal abnormalities (trisomy 18, trisomy 21, and 22q11.2 microduplication), one (5.2%) had biliary atresia, and none had cystic fibrosis. The incidence of serious conditions was significantly higher in the non-isolated group than in the isolated group (44.4% vs 0%; P=0.029); all three cases with chromosomal abnormalities and the only case of biliary atresia were in the non-isolated group, while all four cases with gallbladder agenesis were in the isolated group. The incidences of transient non-visualisation were similar (55.6% vs 60.0%; P=1.000).

**Conclusion:** Isolated NVFGB is often transient or related to gallbladder agenesis. While investigations for chromosomal abnormalities and biliary atresia are reasonable in cases of NVFGB, testing for cystic fibrosis may be unnecessary in Chinese fetuses unless the NVFGB is associated with consistent ultrasound features, significant family history, or consanguinity.


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**New knowledge added by this study**

- Non-visualisation of fetal gallbladder (NVFGB) is associated with chromosomal abnormalities, biliary atresia, cystic fibrosis, and gallbladder agenesis in Caucasian fetuses. A similar pattern of associated conditions was observed in Chinese fetuses with NVFGB, but none had cystic fibrosis.
- While the incidences of chromosomal abnormalities and biliary atresia were significantly higher in cases of non-isolated NVFGB, isolated NVFGB was generally transient or related to gallbladder agenesis; the risks of chromosomal abnormalities and biliary atresia are presumably low in cases of isolated NVFGB.
- The chromosomal abnormalities associated with NVFGB include common aneuploidies, microdeletions, and microduplications.

**Implications for clinical practice or policy**

- Considering the association with chromosomal abnormalities, amniocentesis is recommended in cases of NVFGB. Chromosomal microarray analysis is more appropriate than karyotyping for the detection of associated microdeletions and microduplications.
- Amniotic fluid gamma-glutamyl transpeptidase (AFGGT) assay may be useful because low AFGGT level is reportedly a marker for biliary atresia; it is sensitive but not specific, particularly after 22 weeks of gestation.
- Further testing for cystic fibrosis may be unnecessary in Chinese fetuses unless the NVFGB is associated with consistent ultrasound features, significant family history, or consanguinity.

 

## 華人隊列中的胎兒膽囊不顯示

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**引言：**胎兒膽囊不顯示（NVFGB）與白人胎兒的染色體異常、膽道閉鎖、囊性纖維化和膽囊發育不全相關。我們檢視中國隊列中NVFGB胎兒的結局。

**方法：**這項回顧性分析包括2012年至2019年在香港5間公立醫院胎兒醫學診所的中國孕婦NVFGB病例。我們比較孤立性NVFGB和非孤立性NVFGB病例引致膽囊不顯示、染色體異常、膽道閉鎖、囊性纖維化和膽囊發育不全的發生率。

**結果：**檢測到的19例NVFGB中，胎齡中位數為21.3週（四分位距，20.0-22.3週）。10例（52.6%）為孤立性NVFGB，9例（47.4%）為非孤立性NVFGB。11例（58.0%）為短暫性膽囊不顯示，4例（21.0%）為膽囊發育不全，3例（15.8%）有染色體異常（18-三體症、21-三體症和22q11.2區微重複），1例（5.2%）有膽道閉鎖，沒有病例發現囊性纖維化。非孤立性NVFGB的重症發生率明顯高於孤立性NVFGB（44.4% vs 0%；P=0.029）；3例染色體異常和1例膽道閉鎖均在非孤立性NVFGB組，4例膽囊發育不全均在孤立性NVFGB組。兩組的短暫性膽囊不顯示發生率相若（55.6% vs 60.0%；P=1.000）。

**結論：**孤立性NVFGB通常是短暫或與膽囊發育不全相關。雖然在NVFGB病例中檢查染色體異常和膽道閉鎖是合理的，但在中國胎兒中可能不需要檢測囊性纖維化，除非NVFGB與超聲特徵一致、有明顯家族史或血緣關係相關。

## Introduction

The fetal gallbladder can be observed by antenatal ultrasound scan at 14 weeks of gestation.[1] During the morphology scan at approximately 20 weeks of gestation, >99% of fetal gallbladders can be observed; in 75% of cases of non-visualisation of fetal gallbladder (NVFGB), a gallbladder is clearly present during subsequent scans.[2] However, the visualisation rate drops to 75% to 85% after 32 weeks of gestation when the gallbladder becomes contractile.[3,4]

Although sonographic examination of the fetal gallbladder is not technically difficult, the International Society of Ultrasound in Obstetrics and Gynecology and other professional bodies have not yet included the gallbladder as a routine component of the mid-trimester anatomical survey.[5-8] The problem with routine examination of the fetal gallbladder is that non-visualisation of the gallbladder can lead to challenging counselling and antenatal diagnosis because NVFGB is related to a wide spectrum of fetal conditions. While NVFGB may be a transient phenomenon in a normal fetus or the result of gallbladder agenesis (a benign congenital anomaly), it can also be associated with more serious underlying conditions such as biliary atresia, cystic fibrosis, or chromosomal abnormalities. While it is generally simple to identify chromosomal abnormalities and cystic fibrosis by amniocentesis, the antenatal diagnosis of biliary atresia is challenging because no diagnostic antenatal test is currently available. Because biliary atresia can be fatal without early postnatal intervention and may eventually require liver transplantation, uncertainty regarding the antenatal diagnosis of such a condition may cause significant parental anxiety; some parents may even consider termination of pregnancy to avoid the risk of a severe abnormality in their child.[9,10]

A systematic review of isolated NVFGB in Western populations revealed that the incidences of transient non-visualisation, gallbladder agenesis, biliary atresia, cystic fibrosis, and chromosomal abnormalities were 69.4%, 24.7%, 3.5%, 2.4%, and 1.4%, respectively. The incidences of biliary atresia, cystic fibrosis, and chromosomal abnormalities were higher in cases of non-isolated NVFGB with additional sonographic abnormalities: 18.2%, 23.1%, and 20.4%, respectively.[11] However, the incidences may differ considerably among Chinese women with NVFGB, as cystic fibrosis is uncommon in Asian populations, while biliary atresia is more prevalent in Chinese individuals.[12-16] The aim of this study was to investigate the outcomes of fetuses with NVFGB in a cohort of Chinese women; the findings may provide guidance for the management of NVFGB.

## Methods

This retrospective review included cases of NVFGB among Chinese pregnant women at five public fetal medicine clinics in Hong Kong from 2012 to 2019. In these clinics, fetal morphology scans were limited to high-risk cases and fetal gallbladder assessment was not routinely performed, in accordance with guidelines from the International Society of Ultrasound in Obstetrics and Gynecology.[5,17] When cases of NVFGB were detected incidentally or referred from private clinics, the pregnant women were provided counselling regarding possible differential diagnoses and offered amniocentesis for chromosomal analysis. In cases of serious fetal abnormalities where parents decided for legal termination of pregnancy before 24 weeks of gestation, post-mortem examinations were arranged with parental consent. After birth, babies with NVFGB were referred to paediatricians for further evaluation.

The following data were reviewed: demographic information, gestational age at detection of NVFGB, findings during the morphology scan and subsequent scans, results of all amniotic fluid investigations if amniocentesis had been performed, pregnancy outcome, all neonatal imaging reports, operations performed on the baby and intra-operative findings, and autopsy findings in case of termination of pregnancy. The cases were segregated into isolated and non-isolated groups according to the absence

or presence of additional sonographic findings. The incidences of subsequent visualisation of gallbladder, gallbladder agenesis, biliary atresia, cystic fibrosis, and chromosomal abnormalities were compared between the two groups.

The study protocol was approved by the Joint Chinese University of Hong Kong–New Territories East Cluster Clinical Research Ethics Committee on 3 March 2020 (CREC Ref. No.: 2020.060). This manuscript was written in accordance with STROBE reporting guidelines.

### Statistical analysis

Fisher's exact test was used for comparisons between the isolated and non-isolated groups. All statistical analyses were performed using SPSS software (Windows version 22.0; IBM Corp., Armonk [NY], United States). P values <0.05 were considered statistically significant.

## Results

Among 19 cases of NVFGB detected at a median gestational age of 21.3 weeks (interquartile range, 20.0-22.3 weeks), 10 (52.6%) were isolated and nine (47.4%) were non-isolated. Eleven (58.0%) cases had transient non-visualisation, four (21.0%) had gallbladder agenesis, three (15.8%) had chromosomal abnormalities (trisomy 18, trisomy 21, and 22q11.2 de novo microduplication), and one (5.2%) had biliary atresia. There were no cases with features suggestive of cystic fibrosis (Tables 1 and 2).

### Non-isolated non-visualisation of fetal gallbladder

Amniocentesis was performed in eight of the nine non-isolated cases; three chromosomal abnormalities (33.3%) were found, including trisomy 18 (case 1), trisomy 21 (case 2), and 22q11.2 de novo microduplication (case 3). Case 1 ended in neonatal death and the parents declined post-mortem investigation. Case 2 was a dichorionic twin pregnancy; selective feticide was performed on the fetus with trisomy 21, but post-mortem investigation could not be performed because of the long interval between selective feticide and delivery of the normal co-twin. Termination of pregnancy was performed in case 3 and gallbladder agenesis was confirmed at autopsy. Termination of pregnancy was also performed in case 4 because of multiple structural abnormalities; chromosomal microarray (CMA) findings were normal and post-mortem investigation revealed normal gallbladder. Live birth occurred in the remaining five cases; in one case, the gallbladder was observed during a subsequent antenatal scan and the postnatal outcome was normal, while NVFGB persisted in the other four cases. Among the four cases with persistent NVFGB, one (11.1%) had biliary atresia that required liver transplantation (case 6); antenatal scans had shown a hepatic hilar cyst (Fig 1), which was highly suggestive of cystic biliary atresia.[18] Postnatal examination showed a normal gallbladder in the other three cases. None of the five live births had features suggestive of cystic fibrosis (Table 1 and Fig 2).

### Isolated non-visualisation of fetal gallbladder

Among the 10 cases of isolated NVFGB, amniocentesis was performed in one, while chorionic villi sampling was performed in the first trimester because of positive Down syndrome screening result in another; CMA findings were normal in both cases. In four cases, gallbladders were observed during subsequent antenatal scans at a mean follow-up interval of 1.0 week (range, 0.3-2.0); NVFGB persisted in the other six cases. Among the six cases with persistent NVFGB, gallbladder agenesis was confirmed in four (66.7%) and gallbladders were observed after birth in two (33.3%). None of the 10 cases had features suggestive of cystic fibrosis after birth (Table 1 and Fig 2).

### Comparison between the isolated and non-isolated groups

The characteristics of isolated and non-isolated groups are compared in Table 2. The incidence of serious abnormalities (chromosomal abnormalities, biliary atresia) was significantly higher in the non-isolated group than in the isolated group (44.4% vs 0%; P=0.029). Notably, all serious conditions in the cohort (all three cases of chromosomal abnormalities and the only case of biliary atresia) were observed in the non-isolated group, while all benign conditions (all four cases of isolated gallbladder agenesis) were observed in the isolated group. The incidences of transient non-visualisation did not significantly differ between the isolated and non-isolated groups (60.0% vs 55.6%; P=1.000).

## Discussion

### Isolated non-visualisation of fetal gallbladder

In our cohort, cases of isolated NVFGB had a good overall prognosis, with a 60% probability that the gallbladder would be observed in a subsequent scan and a 40% probability of gallbladder agenesis. This incidence of transient non-visualisation (60%) is consistent with findings by Yayla and Bayik[2] (75%) and Di Pasquo et al[11] (69.4%). In our cases of transient NVFGB, the gallbladder was observed during a subsequent antenatal scan in 40%, and during the postnatal period in the remaining 20%. The mean interval between NVFGB and subsequent antenatal detection of the gallbladder was 1 week. Therefore, a second sonographic examination within 1 week after NVFGB would help to alleviate

TABLE 1. Details of the 19 cases of non-visualisation of fetal gallbladder

| Case | GA at DX, wk | Additional findings | GA at amnio or CVS, wk | Amnio result | GB at FU scan | GA at GB noted again, wk | AN remarks | Fetal outcome | Final DX on GB | PN remarks |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 29.4 | Polyhydramnios, VSD, absent DV, absent stomach, flexed wrist | 29.4 | T18 | - | - | - | NND | N/A | - |
| 2 | 18.7 | Absent DV, echogenic bowel | 18.6 | T21 | - | - | DCDA twin | Selective feticide | N/A | - |
| 3 | 22.1 | Hypospadias, underlapping toes | 22.1 | 22q11.21 duplication | Persistent NVFGB | - | - | TOP | PM GBA | - |
| 4 | 21.7 | TOF, small kidneys, oligohydramnios | 22.6 | CMA normal | Small GB | 23.6 | - | TOP | PM GB present | - |
| 5 | 21.3 | SUA, preaxial polydactyly | 22.6 | CMA normal | Normal GB | 22.3 | - | LB | No US | Normal at 6 years |
| 6 | 27.0 | LCDH, hepatic hilar cyst | 20.4 | CMA normal | Persistent NVFGB | - | - | LB | Biliary atresia | CDH repair, Kasai, liver transplant |
| 7 | 19.1 | Hydrops, echogenic bowel, anaemia | 19.0 | CMA normal B19 PCR +ve | Persistent NVFGB | - | Hydrops and anaemia resolved | LB | US small GB | EHIDA scan normal |
| 8 | 20.9 | Echogenic bowel | 20.9 | CMA normal | Persistent NVFGB | - | Echogenic bowel resolved | LB | US GB present | - |
| 9 | 22.3 | Ascites, peritoneal calcification | - | - | Persistent NVFGB | - | Ascites and calcification resolved | LB | US GB present | - |
| 10 | 21.5 | - | - | - | Persistent NVFGB | - | - | LB | US GBA | EHIDA scan normal |
| 11 | 20.7 | - | - | - | Persistent NVFGB | - | - | LB | US GBA | EHIDA scan normal |
| 12 | 22.7 | - | - | - | Persistent NVFGB | - | - | LB | US GBA | EHIDA scan normal |
| 13 | 20.0 | - | | - | Persistent NVFGB | - | - | LB | US GBA | Normal at 4 years |
| 14 | 19.3 | - | - | - | Persistent NVFGB | - | - | LB | US GB present | - |
| 15 | 22.0 | - | - | - | Persistent NVFGB | - | - | LB | US GB present | - |
| 16 | 21.0 | - | 21.1 | CMA normal | Normal GB | 23.0 | - | LB | US GB present | - |
| 17 | 20.0 | - | 13 | CMA normal | Normal GB | - | CVS for positive DSS | LB | US GB present | - |
| 18 | 19.7 | - | - | - | Normal GB | 20.6 | - | LB | No US | Normal at 2 months |
| 19 | 23.1 | - | - | - | Normal GB | 23.4 | - | LB | No US | Normal at 1 months |

Abbreviations: Amnio = amniocentesis; AN = antenatal; B19 = parvovirus B19; CDH = congenital diaphragmatic hernia; CMA = chromosomal microarray; CVS = chorionic villi sampling; DCDA = dichorionic diamniotic; DSS = Down syndrome screening; DV = ductus venosus; DX = diagnosis; EHIDA = hepatobiliary $^{99}Tc^{m}$-diethyl-iminodiacetic acid; FU = follow-up; GA = gestational age; GB = gallbladder; GBA = gallbladder agenesis; LB = live birth; LCDH = left congenital diaphragmatic hernia; N/A = not available; NND = neonatal death; NVFGB = non-visualisation of fetal gallbladder; PCR = polymerase chain reaction; PM = post-mortem; PN = postnatal; SUA = single umbilical artery; TOF = tetralogy of Fallot; TOP = termination of pregnancy; US = ultrasound; VSD = ventricular septal defect

parental anxiety and avoid the need for further investigations in nearly half of such cases. Even in cases with persistent isolated NVFGB, the prognosis remains good, because the gallbladder is likely to be observed after birth in one-third of cases; while gallbladder agenesis is likely in the remaining cases. Our findings are similar to the results of a recent systematic review of seven studies in Western populations, including 217 cases of isolated NVFGB; most cases had transient non-visualisation (69.4%)

TABLE 2. Comparison of characteristics between isolated and non-isolated cases of non-visualisation of fetal gallbladder*

| | Whole cohort | Isolated | Non-isolated | P value† |
|---|---|---|---|---|
| Total | 19 | 10 (52.6%) | 9 (47.4%) | |
| Transient non-visualisation | 11 (58.0%) | 6 (60.0%) | 5 (55.6%) | 1.000 |
| Gallbladder agenesis‡ | 4 (21.0%) | 4 (40.0%) | 0 | 0.087 |
| Serious abnormalities | 4 (21.0%) | 0 | 4 (44.4%) | 0.029 |
| Chromosomal abnormalities | 3 (15.8%) | 0 | 3 (33.3%) | 0.087 |
| Biliary atresia | 1 (5.2%) | 0 | 1 (11.1%) | 0.474 |
| Cystic fibrosis | 0 | 0 | 0 | - |

* Data are shown as No. (%), unless otherwise specified

† Fisher's exact test

‡ One case of gallbladder agenesis with 22q11.2 microduplication was classified as a chromosomal abnormality and was excluded from isolated gallbladder agenesis count

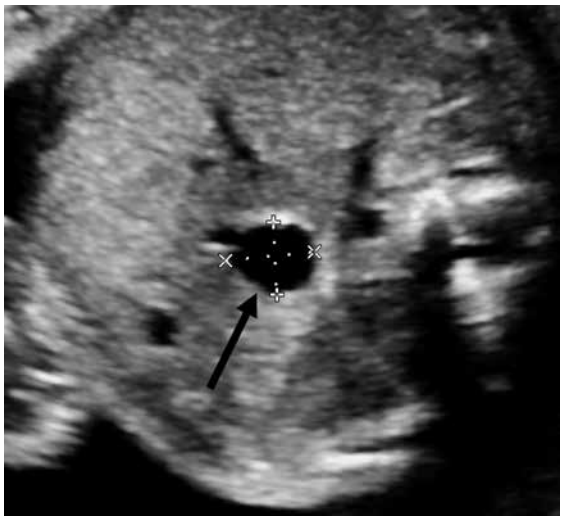

FIG 1. Antenatal ultrasonogram of Case 4 showing a hepatic hilar cyst (arrow), which was confirmed to be cystic biliary atresia after birth

and gallbladder agenesis (24.7%), but some cases had serious conditions (biliary atresia [3.5%], cystic fibrosis [2.4%], and chromosomal abnormalities [1.4%]).[11] Therefore, further investigations to rule out such serious abnormalities remain important in cases of isolated NVFGB.

## Non-isolated non-visualisation of fetal gallbladder

In the aforementioned review, the incidences of biliary atresia, cystic fibrosis, and chromosomal abnormalities were much higher when NVFGB occurred in combination with other ultrasound abnormalities (18.2%, 23.1%, and 20.4%, respectively).[11] Copy number variants were observed in one of three cases with chromosomal abnormalities in our study and two of 11 such cases in the study by Di Pasquo et al[11]; these findings support the recommendation for the use of CMA, rather than karyotyping.[19-21] However, our results differ from the findings reported by Di Pasquo et al[11] in that none of our cases had cystic fibrosis, which is unsurprising because cystic fibrosis is rare in Chinese individuals; moreover, our incidence of biliary atresia (11.1%) was much lower than expected, considering that biliary atresia is reportedly threefold more common in Chinese individuals than in Caucasian individuals.[12-16]

## Non-visualisation of fetal gallbladder and biliary atresia

Based on the data described above, in cases of non-isolated NVFGB or persistent isolated NVFGB, amniocentesis may help to rule out chromosomal abnormalities; this approach is generally simple with current CMA technology.[19,20] However, the antenatal diagnosis of biliary atresia is challenging because fetal bile duct patency cannot be determined by sonographic examination; NVFGB may be the only suggestive sign of biliary atresia. When NVFGB is associated with a hepatic hilar cyst or heterotaxy, a diagnosis of biliary atresia is likely.[18] However, it is difficult to differentiate biliary atresia from gallbladder agenesis in cases of isolated NVFGB. Thus, a low amniotic fluid gamma-glutamyl transpeptidase (AFGGT) level has been proposed as an indicator of biliary atresia.[22-24] Gamma-glutamyl transpeptidase (GGT) is initially derived from the fetal biliary tract, passed into the gastrointestinal tract, and finally excreted into the amniotic fluid. The AFGGT level decreases with increasing gestational age because progressive maturation of the anal sphincter impairs the passage of GGT from the gastrointestinal tract into the amniotic fluid.[22,25,26] The anal sphincter muscles become fully mature by 20 weeks of gestation, and the AFGGT level becomes very low after 22 weeks of gestation. Therefore, it may be difficult to distinguish between a low level related to biliary atresia and a low level

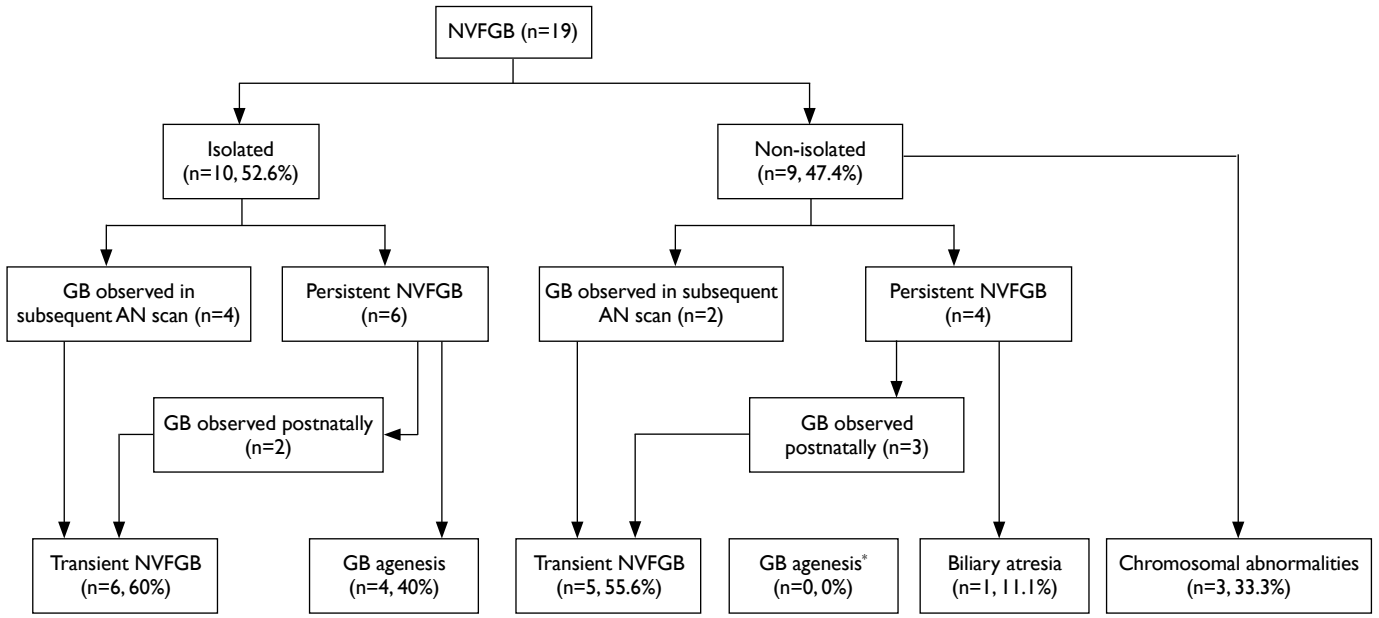


FIG 2. Diagram of the underlying diagnoses in 19 cases of non-visualisation of fetal gallbladder (NVFGB) in our Chinese cohort

Abbreviations: AN = antenatal; GB = gallbladder; NVFGB = non-visualisation of fetal gallbladder

* One case of gallbladder agenesis with 22q11.2 microduplication was classified as a chromosomal abnormality and was excluded from gallbladder agenesis count

TABLE 3. Reported efficacies of amniotic fluid enzymes for the prediction of biliary atresia in cases of non-visualisation of fetal gallbladder

| | Biliary atresia | Normal | Sensitivity* | False positive rate* | Positive predictive value* | Negative predictive value* |
|---|---|---|---|---|---|---|
| Bardin et al[23] 17-22 weeks of gestation† | | | | | | |
| Abnormal amniotic fluid enzyme§ | 3 | 1 | 100% | 4% | 75% | 100% |
| Normal amniotic fluid enzyme | 0 | 26 | | | | |
| Dreux et al[24] <22 weeks of gestation† | | | | | | |
| Abnormal amniotic fluid enzyme‡ | 3 | 4 | 100% | 20% | 43% | 100% |
| Normal amniotic fluid enzyme | 0 | 16 | | | | |
| Dreux et al[24] > 22 weeks of gestation† | | | | | | |
| Abnormal amniotic fluid enzyme‡ | 1 | 5 | 20% | 9% | 17% | 93% |
| Normal amniotic fluid enzyme | 4 | 52 | | | | |

* Calculation based on the number of cases reported in the corresponding paper

† Abnormalities other than biliary atresia were excluded

‡ Gamma-glutamyl transpeptidase and/or intestinal alkaline phosphatase <0.5 multiples of the median

§ Gamma-glutamyl transpeptidase levels <5th centile

related to normal development after 22 weeks of gestation.[25,26] Using an AFGGT level below the 5th centile, Bardin et al[23] reported 100% sensitivity in the detection of biliary atresia, with a false positive rate of 4%, between 17 and 22 weeks of gestation (Table 3). Using AFGGT level and/or intestinal alkaline phosphatase <0.5 multiples of the median, Dreux et al[24] also reported 100% sensitivity before 22 weeks of gestation; however, their false positive rate was 20%. Notably, when the test was performed after 22 weeks of gestation, the sensitivity decreased to 20%. Therefore, gestational age at amniocentesis is a critical consideration during the assessment of biliary atresia; if NVFGB is first detected near 22 weeks of gestation, amniocentesis should be performed immediately, rather than waiting for sonographic examination to be repeated. Another limitation of using the AFGGT level to identify

biliary atresia is that it has a moderately low positive predictive value: 43% to 75% before 22 weeks of gestation, and 17% thereafter.[23,24] Accordingly, a positive AFGGT test result is not diagnostic of biliary atresia, particularly in cases of isolated NVFGB where the incidence of biliary atresia is presumably low. Conversely, the negative predictive value of AFGGT is near 100%; a negative test result is very reassuring, which can help to alleviate parental anxiety and avoid unwarranted termination of pregnancy.[23,24] When NVFGB is detected after 22 weeks of gestation, the fetal blood GGT level may be useful for identification of biliary atresia.[27,28] However, cordocentesis may be unwarranted, as the procedure-related risk outweighs the possible diagnostic benefit, particularly in cases of isolated NVFGB where the risk of biliary atresia is presumably low.

### Non-visualisation of fetal gallbladder and cystic fibrosis

In Caucasian populations, the incidence of cystic fibrosis is 1:2500-3500 live births and the carrier rate is 1:50; in contrast, this hereditary disease is extremely rare among East Asian individuals (1:350000 people in Japan and 1:300000 live births in Hong Kong).[15,16,29] Unsurprisingly, we did not observe cystic fibrosis in either group of NVFGB cases. Therefore, in the absence of significant family history, consanguinity, or concurrent ultrasound features suggestive of cystic fibrosis (eg, echogenic or dilated bowel), amniocentesis for genetic testing for cystic fibrosis is not recommended in cases of NVFGB in Hong Kong. Assessment of the parental *CFTR* gene mutation status may be a useful alternative.

### Management protocol for non-visualisation of fetal gallbladder

Based on our findings and the results of previous studies, we propose the following approach for the management of NVFGB. When NVFGB is detected, a detailed morphology scan should be performed to identify associated abnormalities, such as hepatic hilar cyst and heterotaxy (indicative of biliary atresia) or echogenic and dilated bowel (suggestive of cystic fibrosis). A sonographic examination of the gallbladder should be repeated within 1 week. Considering the potential for chromosomal abnormalities (even in cases of isolated NVFGB), amniocentesis is recommended for CMA analysis in cases of persistent NVFGB. The AFGGT assay can also be performed before 22 weeks of gestation; counselling prior to the test should involve an explanation of the moderately low positive predictive value for identification of biliary atresia. Beyond 22 weeks of gestation, the AFGGT level is not useful for identifying biliary atresia, but cordocentesis for GGT level may be useful. However, cordocentesis is generally not recommended because the procedure-related risk outweighs the possible diagnostic benefit, particularly in cases of isolated NVFGB where the risk of biliary atresia is presumably low. Further testing for cystic fibrosis may be unnecessary in Chinese fetuses unless the NVFGB is associated with other ultrasound features suggestive of cystic fibrosis, significant family history, or consanguinity. Further research is needed concerning AFGGT reference values and the ability of the AFGGT level to identify biliary atresia in Chinese fetuses with NVFGB.

### Limitations and strength

Similar to other reports regarding NVFGB, our study was limited by its retrospective design and small cohort size. Because fetal gallbladder examination has not been a routine practice in Hong Kong, we cannot calculate the prevalence of NVFGB. To our knowledge, this is the first report of NVFGB in a Chinese cohort. Moreover, our results differed from findings in Caucasian populations in that we did not observe cystic fibrosis in our cohort; such information may be useful during antenatal counselling in cases of NVFGB.

## Conclusion

The prognosis of isolated NVFGB is generally good because the non-visualisation is either transient or related to gallbladder agenesis. While investigations of chromosomal abnormalities and biliary atresia are reasonable in cases of NVFGB, testing for cystic fibrosis may be unnecessary in Chinese fetuses unless the NVFGB is associated with consistent ultrasound features, significant family history, or consanguinity.

### Author contributions

Concept or design: YH Ting, TY Leung.
Acquisition of data: YH Ting, PL So, KW Cheung, TK Lo, TWL Ma.
Analysis or interpretation of data: YH Ting.
Drafting of the manuscript: YH Ting, TY Leung.
Critical revision of the manuscript for important intellectual content: All authors.

All authors had full access to the data, contributed to the study, approved the final version for publication, and take responsibility for its accuracy and integrity.

### Conflicts of interest

All authors have disclosed no conflicts of interest.

### Declaration

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## Ethics approval

The study protocol was approved by the Joint Chinese University of Hong Kong–New Territories East Cluster Clinical Research Ethics Committee on 3 March 2020 (CREC Ref. No.: 2020.060).

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