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Association of Severe Intrahepatic Cholestasis of Pregnancy With Adverse Pregnancy Outcomes: A Prospective Population-Based Case-Control Study

Victoria Geenes,[1] Lucy C. Chappell,[2] Paul T. Seed,[2] Philip J. Steer,[3] Marian Knight,[4] and Catherine Williamson[1,2]

Intrahepatic cholestasis of pregnancy (ICP) is a pregnancy-specific liver disease, characterized by maternal pruritus and raised serum bile acids. Our objectives were to describe the epidemiology and pregnancy complications associated with severe ICP and to test the hypothesis that adverse perinatal outcomes are increased in these women. A prospective population-based case-control study with national coverage was undertaken using the UK Obstetric Surveillance System (UKOSS). Control data for comparison were obtained from women with healthy pregnancy outcome through UKOSS (n = 2,232), St Mary's Maternity Information System (n = 554,319), and Office for National Statistics (n = 668,195). The main outcome measures investigated were preterm delivery, stillbirth, and neonatal unit admission. In all, 713 confirmed cases of severe ICP were identified, giving an estimated incidence of 9.2 per 10,000 maternities. Women with severe ICP and a singleton pregnancy (n = 669) had increased risks of preterm delivery (164/664; 25% versus 144/2200; 6.5%; adjusted odds ratio [OR] 5.39, 95% confidence interval [CI] 4.17 to 6.98), neonatal unit admission (80/654; 12% versus 123/2192; 5.6%; adjusted OR 2.68, 95% CI 1.97 to 3.65), and stillbirth (10/664; 1.5% versus 11/2205; 0.5%; adjusted OR 2.58, 95% CI 1.03 to 6.49) compared to controls. Seven of 10 stillbirths in ICP cases were associated with coexisting pregnancy complications. These differences remained significant against national data. Risks of preterm delivery, meconium-stained amniotic fluid, and stillbirth rose with increasing maternal serum bile acid concentrations. *Conclusion*: In the largest prospective cohort study in severe ICP to date, we demonstrate significant increased risks of adverse perinatal outcomes, including stillbirth. Our findings support the case for close antenatal monitoring of pregnancies affected by severe ICP. (HEPATOLOGY 2014;59:1482-1491)

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Intrahepatic cholestasis of pregnancy (ICP) is a pregnancy-specific liver disease, characterized by maternal pruritus and raised serum bile acids. It typically presents in the third trimester with rapid resolution of symptoms and biochemical abnormalities postpartum. Previous retrospective case series have suggested that ICP is associated with an increased risk of adverse fetal outcomes, including spontaneous preterm labor, meconium staining of the amniotic fluid, low Apgar scores, and sudden intrauterine death, but there have been concerns over publication bias and case ascertainment when considering the risk against that of the general pregnant population.[1,2]

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma glutamyl transferase; ICP, intrahepatic cholestasis of pregnancy; SMMIS, St Mary's Maternity Information System; UKOSS, UK Obstetric Surveillance System

From the [1]Institute of Reproductive and Developmental Biology, Imperial College London, London, UK; [2]Women's Health Academic Centre, King's College London, London, UK; [3]Academic Department of Obstetrics and Gynaecology, Division of Cancer, Imperial College London, Chelsea and Westminster Hospital, London, UK; [4]National Perinatal Epidemiology Unit, University of Oxford, Oxford, UK.

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There are accumulating data indicating that the risk of fetal complications relates to high levels of circulating bile acids.[3-10] One prospective cohort study from Sweden examined the relationship between adverse fetal outcomes and maternal serum bile acid levels; there was a 1%-2% increased risk of spontaneous preterm delivery, asphyxial events, and meconium-stained amniotic fluid with every 1 μmol/L increase in maternal serum bile acids, but this was not statistically significant until the fasting maternal serum bile acid level exceeded 40 μmol/L. However, only 96 women (19%) had bile acids exceeding this level.[3] Although these findings have been partially replicated in other populations,[6-8,10] no studies have been large enough to evaluate the relationship between maternal serum bile acids and stillbirth. Furthermore, there have been no prospective studies examining the relationship between maternal nonfasting serum bile acid levels and perinatal outcomes.

We hypothesized that the risks of adverse perinatal outcomes, including stillbirth, are increased in women with nonfasting serum bile acid levels $\geq$40 μmol/L, and that the extent of the rise in bile acid level can be used to predict the likelihood of specific adverse outcomes.

Materials and Methods

A prospective population-based cohort study was carried out over 12 months (June 2010 to May 2011). Cases of severe ICP were identified through the UK Obstetric Surveillance System (UKOSS). UKOSS is a national system that allows collection of information about specific uncommon disorders of pregnancy (i.e., conditions that affect no more than 1 in 2,000 births) from all hospitals with consultant-led maternity units in the UK; 209 of 213 eligible units reported cases for this study. Severe ICP was defined as serum bile acid levels $\geq$40 μmol/L at any time during pregnancy. Exclusion criteria included women with pruritus but no elevation in serum bile acids, and pregnancies ending before 24 weeks' gestation. Biochemical, management and outcome data were collected (www.npeu.ox.ac.uk/ukoss/dcf). Data were anonymized and double-entered into a customized database. The overall incidence with 95% confidence intervals (CIs) of severe ICP was calculated using the most recently available national birth data[11-13] as a denominator for the number of maternities during the study period (n = 798,634), less the number of maternities at the four nonparticipating units (n = 21,922). Control data were obtained from three sources. A cohort of women with uncomplicated singleton (n = 2,205) or twin (n = 27) pregnancies identified from the UKOSS database of control women from other studies was used for the majority of the comparisons (https://www.npeu.ox.ac.uk/ukoss/completed-surveillance). All cases meeting the definition are reported to the central data collection unit by a clinician without requiring determination of past exposure, while controls were taken from the UKOSS database, in order to minimize information bias. Outcomes were restricted to those that could be reported without subjective interpretation to minimize interviewer bias. Response bias should also be minimized by the use of a national survey tool (UKOSS), with 98% of maternity units contributing data. As the UKOSS control database does not have information about meconium-stained amniotic fluid, the St Mary's Maternity Information System (SMMIS) was used; routine maternity data were collected prospectively from all pregnancies (585,291) booked at 15 maternity units in North West London, from 1988 to 2000.[14,15] National data were obtained from the Office of National Statistics, England, for 2010-2011 (n = 668,195).[16] Ethnicity was categorized into groups as defined by the Office for National Statistics (UK).

The perinatal outcomes studied were gestational age at delivery, iatrogenic and spontaneous preterm delivery, stillbirth, mode of delivery, birthweight and birthweight centile, 5-minute Apgar score $\leq$7, neonatal unit admission, and meconium-stained amniotic fluid. Data on maternal demographics, obstetric, and medical history were collected for all women; data on serum biochemistry, management, and monitoring were collected for the ICP cases. Data missing due to incomplete reporting are indicated in the footnotes of the relevant tables.

The study number of 700 cases was prespecified in the protocol as being of sufficient size to enable estimation of uncommon adverse perinatal outcomes

Address reprint requests to: Professor Catherine Williamson, Women's Health Academic Centre, 10th floor North Wing, St Thomas' Hospital, London SE1 7EH, UK. E-mail: catherine.williamson@kcl.ac.uk

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(with intrauterine death estimated at 0.5% to 3%). Statistical analysis was performed using Stata v. 11.2 (StataCorp, College Station, TX). Unadjusted and adjusted odds ratios or mean differences and 95% CIs were calculated. Perinatal outcome data were adjusted for maternal age, body mass index (BMI), occupation, ethnicity, and parity. Data were available on >99% of cases for all demographic variables except BMI (91.7%) and occupational group (83%). As BMI was missing in 247 (24 cases and 223 controls) of 2,963 women, multiple imputation (50 repeats) and proxy indicator analysis were used to estimate the association between ICP and perinatal outcomes, based on birthweight, delivery mode, ethnic group, and parity. Only data from the multiple imputation model are presented here, as the results from both methods were very similar. No imputation was used for occupational group. The numbers of values for biochemical tests are provided. Logistic regression (LR) was used to establish a relationship between adverse perinatal outcomes and maternal biochemical parameters (peak level of serum bile acids, alanine transaminase [ALT], aspartate transaminase [AST], bilirubin, and gamma glutamyl transferase [GGT]). Correlations between different maternal biochemical parameters were examined using Spearman's correlation.

Results

Incidence and Maternal Characteristics. During the study period there were 713 confirmed cases of severe ICP (Fig. 1) in an estimated 776,712 deliveries, giving an estimated incidence of 9.2 per 10,000 maternities (95% CI 8.5-9.9 per 10,000).

For singleton pregnancies, the mean gestation was 32 ± 4 weeks (SD 5.3 days) for onset of symptoms and 33 ± 4 weeks (SD 5.2 days) for diagnosis. Three percent (23 cases) were diagnosed before 20 weeks' gestation. The mean gestation for both onset of symptoms and diagnosis were earlier in twin pregnancies (31 ± 0 and 31 ± 6, weeks respectively; SD 4.7 and 6.1 days).

The demographic and obstetric characteristics of women with severe ICP are shown in Table 1. Women with ICP were more likely to be Asian (OR 1.93, 95% CI 1.50 to 2.49) and there were more twin pregnancies (6.2%, 44 women) than the control population (1.2%, 27 women). Given that the rates of the perinatal outcomes of interest were likely to be higher in twin pregnancies, we included only data from singleton pregnancies in all subsequent analyses (ICP n = 669, control n = 2,205). Data comparing

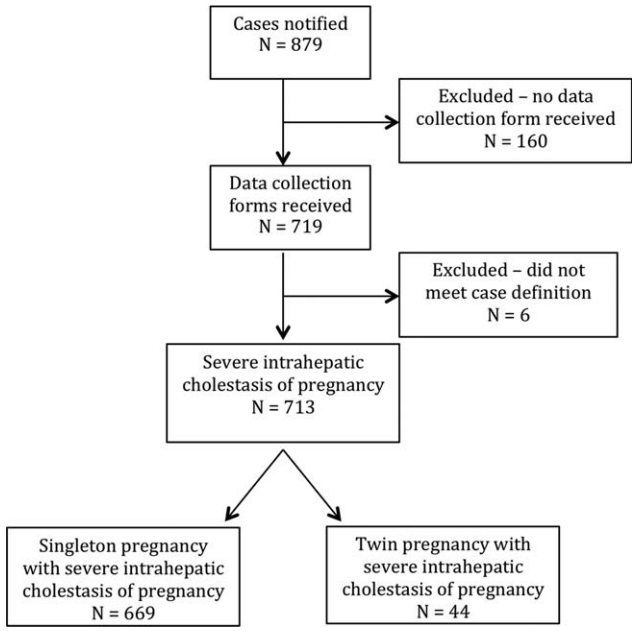

Fig. 1. Flow diagram showing the case ascertainment and completeness of reporting.

demographic and clinical characteristics of ICP twin, ICP singleton, and control twin pregnancies are given in Supporting Tables 1 and 2 and Supporting Fig. 1.

Of the remaining 669 singleton pregnancies, 4.9% (33 women) had a history of gallstones and 1.2% (8 women) had known coexistent hepatitis B infection. There was one woman with documented hepatitis C infection and one woman reported hepatic impairment following use of the oral contraceptive pill. Of the 349 multiparous women, 42% (145 women) had a history of ICP and 9.7% (34 women) had previous preeclampsia or HELLP (hemolysis, elevated liver enzymes, low platelets) syndrome.

The maternal serum biochemistry from ICP cases is shown in Table 2 and Supporting Table 3. There were no significant correlations between markers of liver function (ALT, AST, bilirubin, and GGT) and maternal serum bile acids (data not shown).

Management of Severe ICP. Ursodeoxycholic acid treatment was given to 72% (481 women) of the cases (Table 3); the starting doses used ranged from 150 mg to 2 g per day. The second most common drug used was vitamin K, given to 55% (368 women) of cases. Rifampicin was used in seven cases for symptoms or worsening biochemical markers despite treatment with UDCA. Further details of antenatal fetal monitoring, pharmacological and nonpharmacological management of ICP are shown in Table 3.

Pregnancy Outcomes in Severe ICP. Women with severe ICP delivered earlier than controls (mean difference in gestational age −2.12 weeks; 95% CI −1.98

Table 1. Sociodemographic and Obstetric Histories of Women With Severe ICP and Healthy Controls With Singleton Pregnancies

	ICP Population n (%)	UKOSS Controls n (%)	Comparison*
Total	669[†]	2,205[‡]	
Sociodemographic features			
Maternal age (mean + SD)	29.6 (6.3)	29.0 (6.1)	MD 0.55 (0.0 to 1.09)
Ethnicity			
White	524 (78%)	1771 (80%)	–
Black	14 (2.1%)	130 (5.9%)	OR 0.36 (0.21 to 0.64)
Asian	111 (17%)	194 (8.8%)	OR 1.93 (1.50 to 2.49)
Other	20 (3.0%)	110 (5.0%)	OR 0.61 (0.38 to 1.00)
Body mass index (mean + SD)	25.1 (4.9)	25.7 (5.5)	MD −0.54 (−0.09 to −0.99)
< 18.5	15 (2.2%)	56 (2.5%)	OR 0.80 (0.45 to 1.43)
18.5 - 25	353 (52.7%)	1050 (47.6%)	–
25 - 30	184 (27.5%)	537 (24.4%)	OR 1.02 (0.83 to 1.25)
30 - 35	63 (9.4%)	227 (10.3%)	OR 0.83 (0.61 to 1.12)
> 35	30 (4.5%)	139 (6.3%)	OR 0.64 (0.42 to 0.97)
Occupation			
Managerial	173 (25.9%)	556 (25.2%)	OR 1.26 (0.97 to 1.65)
Intermediate	140 (20.9%)	468 (21.2%)	OR 1.21 (0.92 to 1.60)
Manual and unskilled	202 (30.2%)	591 (26.8%)	OR 1.39 (1.07 to 1.79)
Unemployed	32 (4.8%)	113 (5.1%)	OR 1.15 (0.74 to 1.79)
Student	7 (1.0%)	11 (0.5%)	OR 2.58 (0.98 to 6.80)
Obstetric history			
Parity			
0	317 (47.3%)	968 (43.9%)	–
1	201 (30%)	716 (32.5%)	OR 0.86 (0.70 to 1.05)
>2	148 (22%)	517 (23.4%)	OR 0.87 (0.70 to 1.09)
Twin pregnancy	44 (6.2%)	27 (1.2%)	
Current pregnancy complications			
Preeclampsia	44 (6.6%)	n/a	N/a
Gestational diabetes	41 (6.1%)	n/a	N/a

*OR: odds ratios and 95% confidence intervals; MD: mean difference and standard deviation.

– = the reference group to which the comparisons are made.

n/a = data not available for comparison.

[†]Variable denominator numbers were a consequence of incomplete reporting and were as follows: BMI n = 645, parity n = 666 and occupational group n = 664.

[‡]Variable denominator numbers were a consequence of incomplete reporting and were as follows: BMI n = 2,009, parity n = 2,201, and occupational group n = 2,009.

to −2.27) (Table 4). There was a significant increase in the number of spontaneous and iatrogenic preterm deliveries. The majority of preterm deliveries were iatrogenic, with 17% (114 women) in the ICP population compared to 2.7% (60 women) in the controls (unadjusted OR 7.39; 95% CI 5.33 to 10.25) being

Table 2. Maternal Serum Biochemistry; All Results Are Given as Median (IQR)

	Level at time of diagnosis	Gestation (weeks+days) at measurement	Peak level	Gestation (weeks+days) at measurement	Level at time of delivery	Gestation (weeks+days) at measurement	Typical reference range for pregnancy*
Serum bile acids (μmol/L)	47 (27 to 75)	34+4 (31+4 to 36+4)	72.5 (53 to 109)	35+6 (34+0 to 37+1)	46 (21 to 75)	37+1 (36+2 to 38+0)	< 14 μmol/L
Alanine transaminase (IU/L)	92 (41 to 202)	34+4 (31+4 to 36+4)	151 (63 to 281)	35+4 (33+3 to 37+2)	80 (29 to 180)	37+2 (36+2 to 38+0)	6 - 32
Aspartate transaminase (IU/L)	82.5 (44 to 180)	34+4 (31+4 to 36+4)	110 (59 to 226)	35+5 (33+5 to 37+2)	79 (37 to 174)	37+2 (36+2 to 38+0)	11 - 30
Bilirubin (μmol/L)	10 (7 to 14)	34+4 (31+4 to 36+4)	12 (8 to 17)	35+5 (33+3 to 37+2)	9 (7 to 14)	37+2 (36+2 to 38+0)	3- 14
Gamma glutamyl transferase (IU/L)	26 (16 to 41)	34+4 (31+4 to 36+4)	28 (18 to 46)	35+1 (33+0 to 37+0)	22 (12 to 40)	37+1 (36+2 to 37+6)	3 - 41

*Please note that some hospitals use different reference ranges and it is recommended that the upper limit of normal for liver transaminases, bilirubin and gamma glutamyl transferase is reduced by 20% in pregnancy.

Table 3. Pharmacological and Antenatal Management of Severe ICP

Drug Therapy	n (%)
Ursodeoxycholic acid	481 (72%)
Ursodeoxycholic acid and rifampicin	7 (1%)
Vitamin K	368 (55%)
Cholestryamine	3 (0.5%)
S-adenosyl methionine	0 (0)
Guar gum	0 (0)
Dexamethasone / betamethasone	33 (4.9%)
Antihistamines	271 (41%)
Antenatal fetal monitoring	
Cardiotocography	581 (87%)
Fetal movement charts	29 (4.3%)
Ultrasound scan for fetal growth	437 (65%)
Doppler ultrasound scan	315 (47%)
Imaging	
Liver ultrasound scan	288 (43%)

induced or delivered electively (Fig. 2; Supporting Table 4). The differences in rates of preterm delivery remained significant following correction of the data for potential confounding factors (maternal age, BMI, occupation, ethnicity, and parity).

In our unadjusted analysis, there were significantly higher rates of stillbirth in the ICP population (1.5%, 10/664 versus 0.5%, 11/2,205) (OR 3.05; 95% CI 1.29 to 7.21) (Table 4). There was also an increased risk of admission to the neonatal unit (OR 2.34; 95% CI 1.74 to 3.15). These differences remained significant following adjustment for potential confounding factors, and when compared to national data. The main reasons given for admission to the neonatal unit were preterm delivery (45%, 36 babies) and respiratory problems (30%, 24 babies). There were no cases of meconium aspiration syndrome. The median duration of stay on the neonatal unit was 7 days (IQR 2.25 to 13.75). Meconium-stained amniotic fluid was observed in 16% (106 women) of ICP cases, and occurred at lower gestational weeks than in the control population (Fig. 3). The odds ratio for meconium staining of the amniotic fluid in women with severe ICP compared to

Table 4. Maternal and Perinatal Outcomes of Severe ICP and Healthy Pregnancies

	ICP n (%)	UKOSS Controls n (%)					National ONS Controls n (%)		
	N = 669†	N = 2,205‡	Unadjusted Comparison*	*P* Value	Adjusted Comparison*	*P* Value	N = 668,195	Unadjusted Comparison*	*P* Value
Mean (SD) gestational age at delivery (weeks)	37.5 (1.6)	39.6 (1.9)	MD −2.12 (−1.98 to −2.27)	<0.001	MD −2.21 (−2.05 to −2.37)	<0.001	n/a	n/a	n/a
Preterm delivery	164 (25%)	144 (6.5%)	OR 4.68 (3.67 to 5.98)	<0.001	OR 5.39 (4.17 to 6.98)	<0.001	47657 (8.9%)	OR 3.39 (2.84 to 4.04)	<0.001
Spontaneous < 37 weeks	50 (7.5%)	84 (3.8%)	OR 2.05 (1.43 to 2.94)	<0.001	OR 2.25 (1.54 to 3.27)	<0.001	28444 (5.3%)	OR 1.46 (1.10 to 1.95)	0.009
Iatrogenic < 37 weeks	114 (17%)	60 (2.7%)	OR 7.39 (5.33 to 10.25)	<0.001	OR 8.75 (6.19 to 12.37)	<0.001	19213 (3.6%)	OR 5.38 (4.40 to 6.58)	<0.001
Mode of delivery									
Cesarean section	164 (25%)	508 (23%)	OR 1.09 (0.89 to 1.34)	0.39	n/a	n/a	162512 (24.3%)	1.09 (0.93 to 1.29)	0.29
Birthweight (g)	3049.5	3357.5	MD −308 (−262 to −353)	<0.001	MD −309 (−263 to −355)	<0.001	n/a	n/a	n/a
Mean (SD) customized birthweight centile	47.6 (28.8)	40.8 (28.3)	MD 6.7 (4.0 to 9.5)	<0.001	MD 6.2 (3.4 to 8.9)	<0.001	n/a	n/a	n/a
> 90th Centile	54 (8.5%)	82 (7.0%)	RR 1.21 (0.87 to 1.68)	0.26	RR 1.14 (0.82 to 1.59)	0.44	n/a	n/a	n/a
< 10th Centile	70 (11%)	193 (16%)	RR 0.67 (0.52 to 0.86)	0.002	RR 0.70 (0.54 to 0.91)	0.007	n/a	n/a	n/a
Adverse outcomes									
Stillbirth	10 (1.5%)	11 (0.5%)	OR 3.05 (1.29 to 7.21)	0.011	OR 2.58 (1.03 to 6.49)	0.044	2626 (0.44%)	OR 3.05 (1.65 to 5.63)	<0.001
5 min Apgar ≤ 7	18 (2.8%)	14 (1.6%)	OR 1.81 (0.89 to 3.66)	0.101	OR 1.92 (0.92 to 3.99)	0.081	n/a	n/a	n/a
Neonatal unit admission	80 (12%)	123 (5.6%)	OR 2.34 (1.74 to 3.15)	<0.001	OR 2.68 (1.97 to 3.65)	<0.001	n/a	n/a	n/a

*OR: odds ratios and 95% confidence intervals; MD: mean difference and standard deviation. Adjusted risk ratios are calculated with correction for potential confounding factors (maternal age, ethnicity, parity, body mass index and occupation).

n/a = data not available.

†Variable denominator numbers were a consequence of incomplete reporting and were as follows: preterm delivery and stillbirth n = 664, caesarean section n = 665, Apgar score n = 643, neonatal unit admission n = 654.

‡Variable denominator numbers were a consequence of incomplete reporting and were as follows: preterm delivery n = 2190, caesarean section n = 2183, stillbirth n = 2,187, Apgar score n = 892, neonatal unit admission n = 2,185.

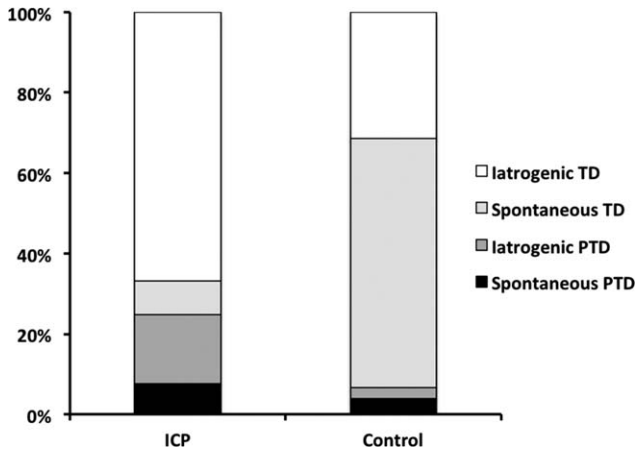

Fig. 2. Timing of spontaneous and iatrogenic deliveries of singleton pregnancies in women with severe ICP and controls. TD, term delivery (≥37 weeks gestation); PTD, preterm delivery (<37 weeks gestation).

published controls[14] was significantly greater at each week of gestational age up to and including 38 weeks' gestation. Of those who had a cord blood sample taken, cord artery pH ≤ 7.1 was present in 8.5% (24/283) and pH ≤ 7.0 in 3.5% (10/283). For the purposes of this study a reassuring cord artery pH was considered to be >7.1, as decreasing cord arterial pH levels below 7.1 and particularly below 7.0 are associated with worsening perinatal and long-term infant outcomes.[17] Cardiotocography abnormalities were recorded in 28% (163 cases). The most common abnormalities reported were variable decelerations/prelabor decelerations (80 cases, 49.1%), early decelerations (14 cases, 10.3%), bradycardia (<110 beats per minute; 14 cases, 8.6%), and tachycardia (>160 beats per minute; 11 cases, 6.7%) (Supporting Table 5).

Relationship Between Bile Acids and Fetal Complications. Significant relationships were found between the maternal serum bile acid level and preterm delivery, spontaneous preterm delivery, stillbirth, and meconium-stained amniotic fluid (Fig. 4; Supporting Fig. 2). Logistic regression analysis demonstrated that a doubling in the level of serum bile acids increased the risk of all preterm delivery by 68%, spontaneous preterm delivery by 66%, meconium staining of the amniotic fluid by 55%, and stillbirth by 200%. A weaker but still significant relationship was also found between serum ALT and preterm delivery, but not with any other adverse outcomes (Supporting Figs. 3, 4). None of the other biochemical parameters had a significant relationship with any perinatal complications.

Characteristics of Stillbirth Cases. There were 10 stillbirths in the ICP population and 11 in the control population, giving an incidence of 1.5% and 0.5%, respectively. The median gestational age at delivery in the ICP stillbirth cases was 36 ± 2 (IQR 35 ± 4 to 38 ± 1 days), compared with 30 ± 5 (IQR 28 ± 4 to 38 ± 0 days) in the controls with a stillbirth. Six of the 10 ICP stillbirths occurred before 37 weeks' gestation. Of the women with stillbirth, 50% of those with ICP were white, whereas the predominant ethnic group affected in the control population with stillbirths was Asian (55%). Seven of 10 ICP cases had coexistent pregnancy complications, including two cases with preeclampsia, three with gestational diabetes, and two with nonspecified complications. Spontaneous preterm delivery was observed in three ICP stillbirth cases and one control. There were no small for gestational age babies in the ICP stillbirth cases, but there were three large for gestational age babies; none of these mothers had coexistent gestational diabetes diagnosed prior or subsequent to the loss. Peak bile acid levels were significantly higher in the stillbirth cases (median 137 μmol/L; IQR 104 to 159) than in the live births from the ICP cases (median 72 μmol/L; IQR 53 to 107; $P = 0.0021$). There was one neonatal death among the women with ICP, after term delivery with meconium-stained amniotic fluid and normal birthweight.

Discussion

This is the largest prospective study of perinatal outcomes in women with severe ICP, demonstrating its association with significantly increased risks of spontaneous and iatrogenic preterm delivery, neonatal unit admission, and stillbirth. The incidence of severe ICP was ∼1 case per 1,000 deliveries. The overall incidence

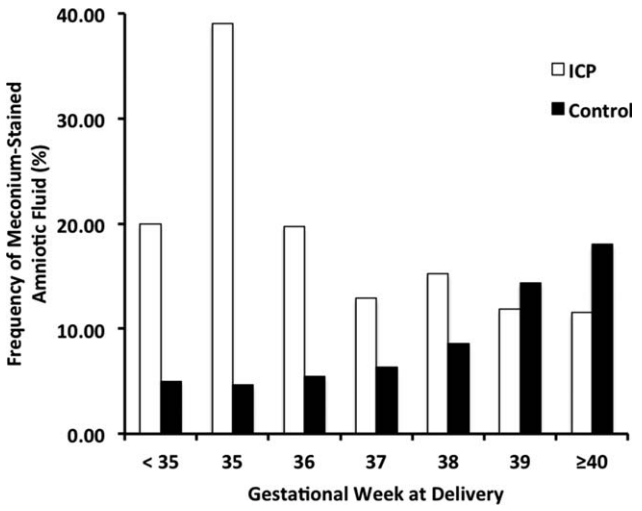

Fig. 3. The incidence of meconium staining of the amniotic fluid by gestational age in 669 women with severe ICP compared with 514,635 controls with a gestational age from 24 to 44 weeks gestation. Gestation was calculated as described.[14]

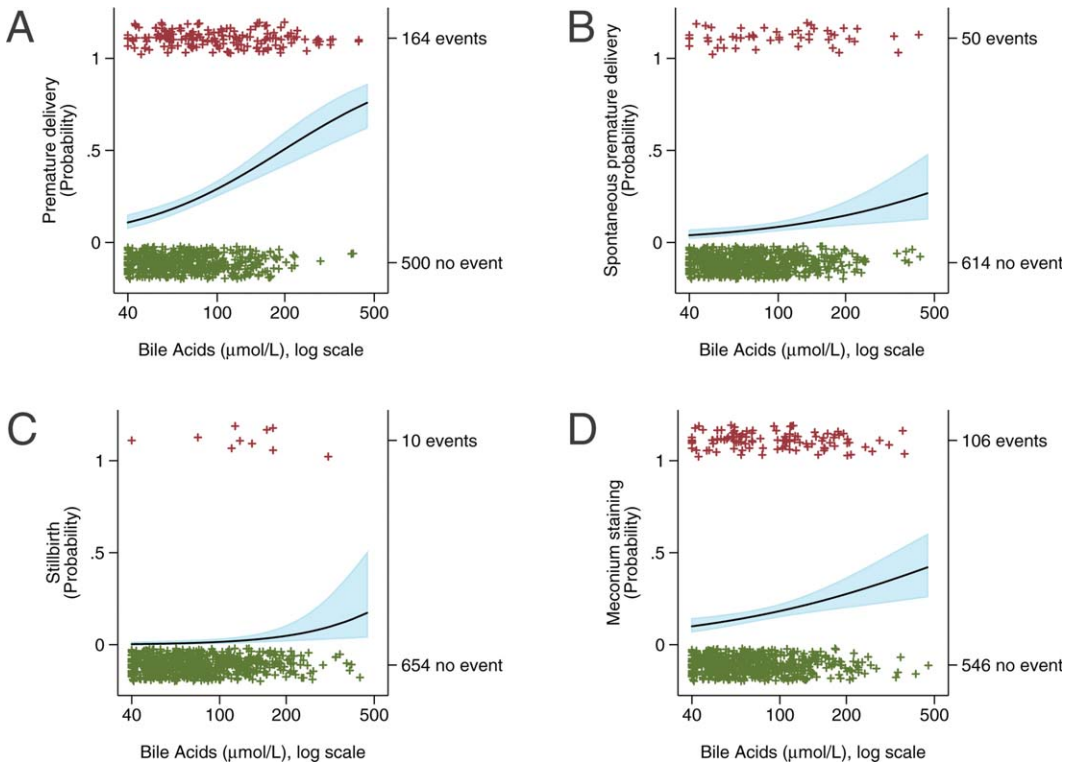

Fig. 4. The estimated probability and 95% CIs of preterm delivery (A), spontaneous preterm delivery (B), stillbirth (C), and meconium-stained amniotic fluid (D) in relation to the maternal serum bile acid level, based on simple logistic regression.

of ICP in the UK has been estimated at 0.7%,[1,18] and severe ICP accounts for ~15% of all ICP in the UK. Significant positive correlations between maternal serum bile acid levels and adverse fetal outcomes, including preterm delivery, spontaneous preterm delivery, meconium staining of the amniotic fluid, and stillbirth were demonstrated. ALT also has a significant, albeit weaker, positive correlation with preterm delivery. A relatively large proportion (17%) of women with severe ICP were delivered prior to 37 weeks' gestation. This may reflect concerns with regard to the risk of fetal death or adverse outcome in pregnancies complicated by high maternal serum bile acids, and therefore the clinicians managing these pregnancies may have preferred a management strategy of induction of labor prior to 37 weeks despite emerging concerns about special education needs[19] and poorer school performance[20] in babies born late preterm. This is the first study of severe ICP to show a significant association with stillbirth. It should be noted that a large proportion (7 of 10) of the women with stillbirth also had other pregnancy complications. Importantly, this may indicate that women with severe ICP and other coexisting conditions require closer monitoring than those with ICP alone, as the etiology of fetal death in these cases may be multifactorial.

Use of the UKOSS national data collection system adds considerable weight to the findings, given the extensive coverage of UK maternity units by this surveillance method (https://www.npeu.ox.ac.uk/ukoss/annual-reports), which has been informative previously for similar cohort studies.[21,22] This also enabled recruitment of a prospective cohort of ICP cases with serum bile acids $\geq$40 μmol/L that is more than seven times larger than the number of severe ICP cases in the only previous cohort[3] that aimed to establish the relationship between maternal serum bile acid levels and adverse pregnancy outcome. Furthermore, the wide coverage of maternity units provided by UKOSS obviates the publication bias associated with previous case series in which selected ascertainment and reporting has led to uncertainty over the estimation of adverse perinatal outcomes. It is therefore likely that these findings are generalizable to maternity units in the UK, and to other similar populations.

A further strength of our study is examination of the relationship between nonfasting maternal serum bile acid levels and perinatal complications, demonstrating that the previously described association observed with fasting bile acid levels[3] remains when postprandial samples are used. As most women are not fasting when they attend for antenatal care, this means

that the results presented here are directly applicable to all UK maternity units and to other populations in which nonfasted serum bile acid levels are used for monitoring ICP. A limitation is the lack of directly comparable control data for some secondary outcomes, such as meconium-stained amniotic fluid. Although it is likely that this would not change the conclusions of the study, future research should aim to capture complete data on cases and controls.

A limitation of using the UKOSS system to acquire the data for this study was the inability to obtain prospective data from ICP pregnancies with bile acid levels of 10-39 μmol/L. The criteria for inclusion of a study in the UKOSS program state that the condition is an uncommon disorder of pregnancy affecting no more than 1 in 2,000 births per year in the UK (https://www.npeu.ox.ac.uk/ukoss/survey-applications). When we initiated the study we anticipated that ICP affected 1 in 2,000 pregnant women in the UK. Therefore, we were unable to use this system to study ICP with lower bile acid levels as the incidence in the UK is 0.7%.[18] However, the published literature regarding perinatal outcomes in women with ICP and lower levels of bile acids are generally reassuring,[3] but further studies are required to fully establish the risk in this subpopulation.

Our findings of increased risks of adverse perinatal outcomes in ICP are consistent with previous smaller studies that reported preterm delivery, fetal asphyxia, and meconium staining in white-European and Latina populations.[4-8,23] Previous work has led to uncertainty over the risks of stillbirth; early studies suggested an increased risk but were considered subject to case ascertainment bias, while later reports suggested lower rates of stillbirth, largely attributed to policies of active management, with increased antenatal fetal monitoring and elective delivery at around 37 weeks' gestation. Interestingly, the most recent study of fetal outcomes in ICP showed no significant increase in the number of stillbirths in an actively managed ICP population compared to controls during the study period (1997-2009).[24] However, this epidemiological study based on registry data, in common with many others, was not able to consider the extent of the rise in serum bile acids when evaluating the stillbirth risk in ICP. Furthermore, the data presented here demonstrate that despite high rates of iatrogenic preterm delivery (17%), suggesting the use of policies of active management, severe ICP remains associated with a significantly increased risk of stillbirth in the UK.

The mechanisms underlying the fetal complications in ICP are unclear, but appear to relate to the effect of high levels of bile acids in the fetal compartment. Spontaneous preterm labor may be explained by a dose-dependent bile acid effect on myometrial contractility, as has been demonstrated in rodents.[25] Furthermore, myometrial cells from women with ICP are more responsive to oxytocin, and cells from normal women demonstrate an increased response to oxytocin in the presence of bile acids.[26,27] Meconium-stained amniotic fluid may be explained by an increase in colonic motility secondary to bile acids; 100% of pregnant sheep infused with cholic acid have meconium-stained amniotic fluid but no other signs of fetal distress.[28] Evidence for the involvement of bile acids in the etiology of neonatal respiratory distress comes from studies of rabbits undergoing intratracheal injection of bile acids,[29] which results in atelectasis, eosinophilic infiltration, and the formation of hyaline membrane, all of which can be reversed by the administration of surfactant. Interestingly, a recent series of infants with unexpected respiratory distress in association with ICP reported an improvement in condition following treatment with intratracheal surfactant therapy.[30] The mechanisms causing stillbirth in ICP are poorly understood. At autopsy the babies have no signs of chronic uteroplacental insufficiency, but do have evidence of acute anoxia.[31] Histological changes described in the placentas of women with ICP support the hypothesis of a sudden acute event leading to fetal death.[32] A possible mechanism would be fetal cardiac arrhythmia and there are case reports of this in the literature.[33] Further evidence for this hypothesis comes from studies of cultured rat neonatal cardiomyocytes, which have a decreased rate of contraction when exposed to bile acids and also develop arrhythmogenic activity.[34,35]

The implications of this study are that women with severe ICP warrant increased surveillance for adverse perinatal outcomes. Clinicians need to make a difficult and individualized judgment as to whether the risks of early delivery are greater than the risks associated with the disease. A previous feasibility trial compared early delivery against expectant management in women with ICP,[18] but the study did not have sufficient power to provide a definitive answer on best management. The finding that the infants of women with severe ICP had normal birthweight centiles and were not growth restricted suggests that management strategies involving ultrasound assessment of fetal growth for identification of placental dysfunction will not be of use in identifying babies at risk. However, the risk of adverse perinatal outcome is associated with increased serum bile acids, suggesting that bile acids should be routinely

used for surveillance, contrary to the most recent national guidelines on management of the disease.[2] Our finding of increased stillbirth in women with ICP and serum bile acids $\geq$40 μmol/L provides the first evidence base on which to offer delivery from 37 weeks' gestation, as the benefits of intervention are likely to outweigh the risks of preterm delivery for the fetus.

Future research now needs to focus on improving prediction and treatment of severe ICP. Women with genetic variants in biliary transporters[36,37] and receptors[38] have increased susceptibility to ICP. New biomarkers may also be useful in identifying women with ICP at greatest risk of subsequent adverse outcomes. Specific compounds of interest are sulfated progesterone metabolites.[39-41] Ursodeoxycholic acid has been shown to improve pruritus in women with ICP[9,18,40] but definitive proof of its protective effect on the fetus remains elusive until larger trials are undertaken.

In summary, severe ICP in the UK affects 0.1% of pregnant women and is associated with an increased risk of preterm delivery, neonatal unit admission, and stillbirth. The risk of these perinatal complications increases with increasing levels of maternal serum bile acids. These findings support the current practice of close antenatal monitoring and indicate the need for a randomized controlled trial to assess the benefit of treatment at reducing these risks.

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