Re-evaluating diagnostic thresholds for intrahepatic cholestasis of pregnancy: case–control and cohort study

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Objective To determine the optimal total serum bile acid (TSBA) threshold and sampling time for accurate intrahepatic cholestasis of pregnancy (ICP) diagnosis.

Design Case–control, retrospective cohort studies.

Setting Antenatal clinics, clinical research facilities.

Population Women with ICP or uncomplicated pregnancies.

Methods Serial TSBA measurements were performed pre-/postprandially in 42 women with ICP or uncomplicated pregnancy. Third-trimester non-fasting TSBA reference ranges were calculated from 561 women of black, south Asian and white ethnicity. Rates of adverse perinatal outcomes for women with ICP but peak non-fasting TSBA below the upper reference range limit were compared with those in healthy populations.

Main outcome measures Sensitivity and specificity of common TSBA thresholds for ICP diagnosis, using fasting and postprandial TSBA. Calculation of normal reference ranges of non-fasting TSBA.

Results Concentrations of TSBA increased markedly postprandially in all groups, with overlap between healthy pregnancy and mild ICP (TSBA <40 μmol/l). The specificity of ICP diagnosis was higher when fasting, but corresponded to <30% sensitivity for diagnosis of mild disease. Using TSBA ≥40 μmol/l to define severe ICP, fasting measurements identified 9% (1/11), whereas non-fasting measurements detected over 91% with severe ICP. The highest upper limit of the non-fasting TSBA reference range was 18.3 μmol/l (95% confidence interval: 15.0–35.6 μmol/l). A re-evaluation of published ICP meta-analysis data demonstrated no increase in spontaneous preterm birth or stillbirth in women with TSBA <19 μmol/l.

Conclusions Postprandial TSBA levels are required to identify high-risk ICP pregnancies (TSBA ≥40 μmol/l). The postprandial rise in TSBA in normal pregnancy indicates that a non-fasting threshold of ≥19 μmol/l would improve diagnostic accuracy.

Keywords Cholestasis, clinical decision-making, liver diseases in pregnancy.

Tweetable abstract Non-fasting bile acids improve the diagnostic accuracy of intrahepatic cholestasis of pregnancy diagnosis.

Introduction

Intrahepatic cholestasis of pregnancy (ICP) is defined by gestational pruritus and elevated total serum bile acids (TSBA). The incidence of ICP varies between 0.2 and 5.6%

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of pregnancies,[1,2] which may be accounted for by differences in ethnic populations[3] as well as the diagnostic criteria used. Diagnostic thresholds for ICP range from ≥6 to >15 μmol/l, depending upon hospital and national guidelines,[4–6] and there is currently no consensus as to whether TSBA should be measured fasting or postprandially,[7] although it is well documented that TSBA concentrations rise after food.[8,9]

The reference interval for TSBA concentration has not been adequately established in pregnant women. Initial studies calculated the TSBA reference intervals based upon Gaussian data distribution, referencing mean ± 2× standard deviations.[10] However, analysis of TSBA levels from four studies during healthy pregnancy demonstrated that normal TSBA concentrations are not normally distributed,[11] with concentrations up to 16.7 μmol/l reported.[12]

Intrahepatic cholestasis of pregnancy is associated with an increased risk of adverse pregnancy outcomes, including meconium-stained amniotic fluid, neonatal unit admission, spontaneous preterm birth and stillbirth.[13–15] Glantz et al.[15] demonstrated an increased risk of fetal complications in 96 women with fasting TSBA >40 μmol/l, describing this as 'severe ICP'. An individual patient data meta-analysis, comprising fasting or postprandial samples from 5557 women with ICP, determined that only women with peak TSBA levels ≥100 μmol/l have an increased risk of stillbirth; those with TSBA ≥40 μmol/l had an increased risk of spontaneous preterm birth.[14]

No pharmacological treatment or monitoring strategy has been found to reduce stillbirth in ICP,[16] such that many women undergo iatrogenic preterm birth with the aim of reducing this risk.[4,14] However, preterm birth is associated with an increased risk for the neonate, including respiratory distress[17] and cognitive impairment in childhood.[18] As a result, correct diagnosis of the severity of ICP is necessary to enable clinicians to identify women with TSBA levels associated with increased risk of stillbirth to facilitate informed decision-making about timing of delivery.

To address concerns regarding accuracy and thresholds for ICP diagnosis, we performed three interlinked studies. In the first, we measured fasting and serial postprandial TSBA levels in response to standardised meals in women with ICP and uncomplicated pregnancies. In the second study, we determined reference intervals for postprandial TSBA from a separate cohort of women in their third trimester of uncomplicated pregnancy. In the third study, data from a published individual patient data meta-analysis[14] were used to compare rates of adverse perinatal outcomes for women with ICP but peak TSBA below the upper reference limit for TSBA identified in Study 2 with matched normal populations, to determine the effect of altering the diagnostic threshold for ICP.
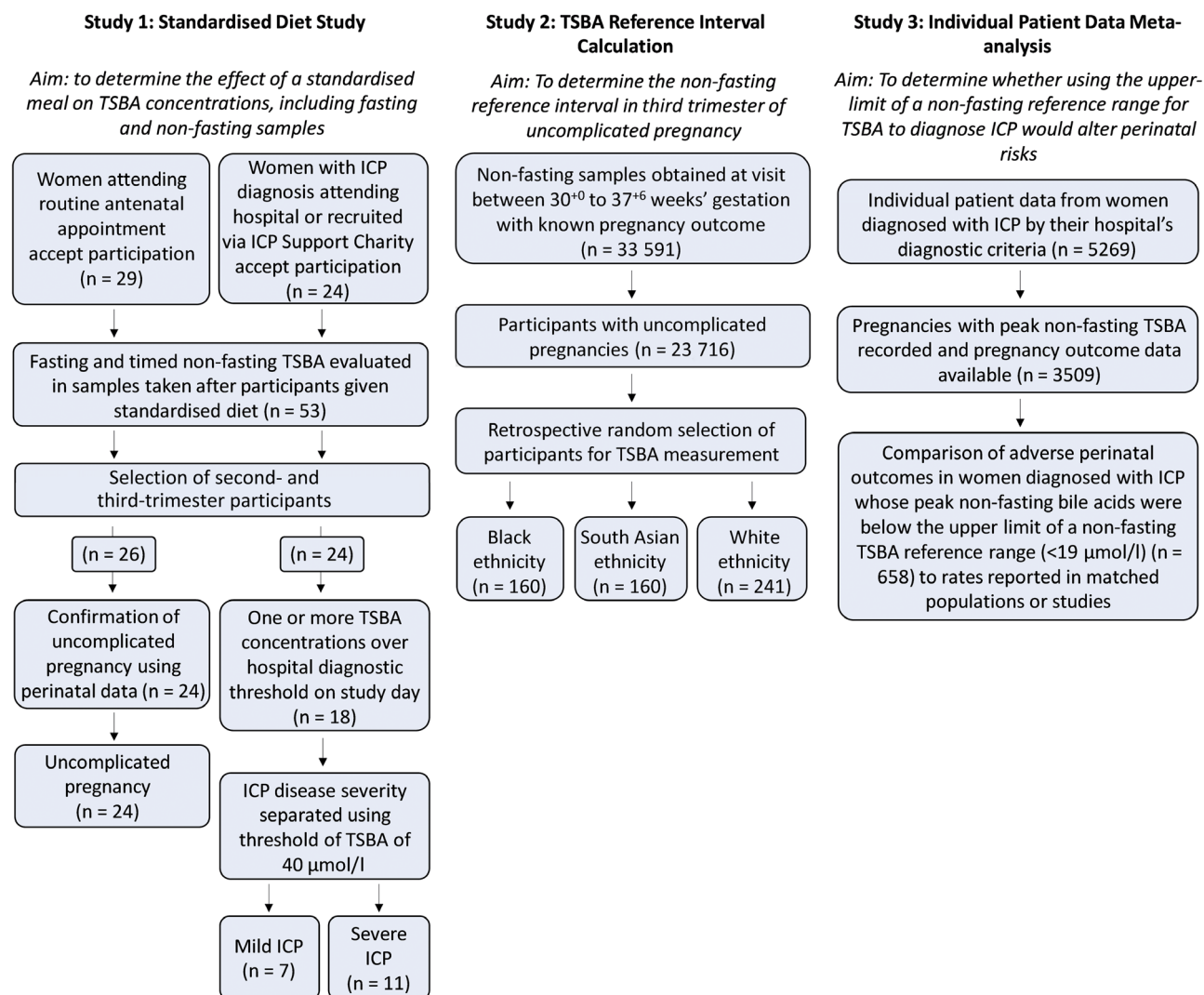
Methods

Patient and public involvement

This study was designed in consultation with the charity ICP Support (www.icpsupport.org), whose input was incorporated into the original study protocol, application for ethical approval, patient information leaflets and consent forms. The protocol for Study 1 was modified following feedback from participants to ensure that the breakfast meal was acceptable, and recruitment included women identified through the charity social media and website. A video produced by participants was available for subsequent women, which provided them with information on the timeline for the study (https://m.youtube.com/watch?v=D9Bv8YIUlZI). Similar channels will be used to disseminate the findings, including question and answer sessions, available on the charity's YouTube and Facebook pages, and website. For a study that was quite onerous to the participants, having patient and public involvement from the beginning enabled us to understand how much could be asked of women, and revealed how motivated women affected by ICP, in particular, are to participate in research. Similarly, ongoing feedback received through the study has enabled us to modify protocols for future studies. In future, formal collation of this feedback would be beneficial, particularly where undertaken at multiple sites and by multiple researchers.

Three interlinked studies were performed with the aim of establishing the optimal TSBA threshold and blood sampling time for accurate diagnosis of intrahepatic cholestasis of pregnancy (ICP) (Figure 1).

Study 1: Standardised diet case–control study

Pregnant women between 16[+0] and 39[+2] weeks of gestation with singleton or multifetal pregnancies were recruited prospectively between 2012 and 2017 from Queen Charlotte's and Chelsea Hospital, St Thomas' Hospital and West Middlesex University Hospital, UK, and through the charity ICP Support. Four women with uncomplicated pregnancies, one woman with mild ICP and two women with severe ICP (defined as peak TSBA ≥40 μmol/l) were in their second trimester at the time of study; all other women were in their third trimester at the time of participation. Women with ICP were diagnosed according to each hospital's diagnostic criteria, which included unexplained pruritus and elevated random TSBA greater than the laboratory upper limit of normal, and exclusion of pre-existing liver disorders and pregnancy-specific causes of liver dysfunction such as HELLP (haemolysis, elevated liver enzymes and low platelets) syndrome, pre-eclampsia and acute fatty liver of pregnancy. All women with ICP were diagnosed before study participation, and none with uncomplicated pregnancies later developed ICP.

Study 1: Standardised Diet Study

Aim: to determine the effect of a standardised meal on TSBA concentrations, including fasting and non-fasting samples

Women attending routine antenatal appointment accept participation (n = 29)

Women with ICP diagnosis attending hospital or recruited via ICP Support Charity accept participation (n = 24)

Fasting and timed non-fasting TSBA evaluated in samples taken after participants given standardised diet (n = 53)

Selection of second- and third-trimester participants

(n = 26)

(n = 24)

Confirmation of uncomplicated pregnancy using perinatal data (n = 24)

One or more TSBA concentrations over hospital diagnostic threshold on study day (n = 18)

Uncomplicated pregnancy (n = 24)

ICP disease severity separated using threshold of TSBA of 40 µmol/l

Mild ICP (n = 7)

Severe ICP (n = 11)

Study 2: TSBA Reference Interval Calculation

Aim: To determine the non-fasting reference interval in third trimester of uncomplicated pregnancy

Non-fasting samples obtained at visit between 30^{+0} to 37^{+6} weeks' gestation with known pregnancy outcome (n = 33 591)

Participants with uncomplicated pregnancies (n = 23 716)

Retrospective random selection of participants for TSBA measurement

Black ethnicity (n = 160)

South Asian ethnicity (n = 160)

White ethnicity (n = 241)

Study 3: Individual Patient Data Meta-analysis

Aim: To determine whether using the upper-limit of a non-fasting reference range for TSBA to diagnose ICP would alter perinatal risks

Individual patient data from women diagnosed with ICP by their hospital's diagnostic criteria (n = 5269)

Pregnancies with peak non-fasting TSBA recorded and pregnancy outcome data available (n = 3509)

Comparison of adverse perinatal outcomes in women diagnosed with ICP whose peak non-fasting bile acids were below the upper limit of a non-fasting TSBA reference range (<19 µmol/l) (n = 658) to rates reported in matched populations or studies

Figure 1. Participation flowchart of patient groups.

Serum samples for analysis of TSBA were taken from 42 women given a standardised diet from 18:00 the preceding day until 15:00 on the day of investigation, as previously described.[19] Participants were given fixed meals consisting of an evening meal the preceding day (50 g fat, 100 g carbohydrates, 1000 calories), breakfast (50 g fat, 75 g carbohydrates, 770 calories) and lunch (50 g fat, 100 g carbohydrates, 1000 calories). Blood samples were taken fasting and 3 hours post-lunch. A subset of 23 participants also underwent phlebotomy at 1 and 2 hours post-breakfast, immediately before lunch, and 20 minutes and 1 hour post-lunch; sample sizes for each blood sampling time can be found in the Supplementary material (Table S1). Samples where technical difficulties occurred were omitted from the analysis. Participants remained sedentary throughout the study. No adverse events were recorded. Blood samples were analysed blind using the Total Bile Acids Assay Kit (Diazyme, Diazyme Laboratories Inc, Poway, CA, USA) in validated clinical laboratories of the respective hospitals. Participants' maximum TSBA concentration during the study day was used to group women with ICP by disease severity according to previously suggested thresholds:[13,15] seven had mild ICP (all TSBA measurements <40 µmol/l), 11 had 'severe' ICP (one or more TSBA measurements ≥40 µmol/l) and 24 participants had uncomplicated pregnancies. Data were plotted using GraphPad Prism (version 8.4.1; GraphPad Software Inc., San Diego, CA, USA).

The specificity and sensitivity of ICP diagnosis were calculated using a spread of commonly used diagnostic thresholds (≥6, ≥11 and >15 µmol/l), and ≥40 µmol/l as the threshold for severe ICP. Specificity and sensitivity were

calculated for TSBA levels when fasting, and 20 minutes, 1 hour and 3 hours post-lunch.

Study 2: Reference interval serum samples

Non-fasting serum samples were retrospectively analysed from samples collected for a prospective observational cohort study for early prediction of pregnancy complications at King's College Hospital, London, UK. Women attending this visit, held at 30^{+0} to 34^{+6} weeks of gestation between 2011 and 2014 and at 35^{+0} to 37^{+6} weeks of gestation between 2014 and 2016, were invited to participate in the study, and serum samples were stored at $-80°C$ from those who provided informed written consent. Maternal characteristics and medical history were recorded. Data on pregnancy outcome were obtained from the maternity computerised records or the general medical practitioners of the women.

Five hundred and sixty-one samples were randomly selected from our database of stored samples with uncomplicated pregnancies resulting in live birth after 38 weeks of gestation of phenotypically normal neonates with birthweight between the 10th and 90th centiles for gestational age. The downloaded database file was sorted at random, and cases were selected according to gestation at sampling to ensure a comparable distribution of samples across the gestational weeks of the third trimester of pregnancy. Samples were analysed retrospectively using Total Bile Acid reagent (Randox) run on a Siemens Advia 1800 by Affinity Biomarker Labs (London, UK). Samples were analysed blind. Five samples produced TSBA values below the limit of detection at <0.3 μmol/l; these data were included at an estimated value of 0.2 μmol/l to retain all data. No adverse events were recorded from performing the venepuncture.

Non-fasting TSBA reference intervals were calculated for the third trimester of pregnancy according to black ($n = 160$), south Asian ($n = 160$) or white ($n = 241$) ancestry, exceeding the minimum suggested 120 samples for determining reference intervals and confidence intervals according to the Clinical Laboratory and Standards Institute.[20] Analyses were performed in STATA software (version 15.1; StataCorp, College Station, TX, USA). TSBA values were log-transformed and differences between ancestral groups were compared using a one-way analysis of variance with post-hoc Bonferroni correction, with multiple linear regression with robust standard errors used to determine the size of the differences in non-fasting TSBA values.[21] Correlations between the log(TSBA) and maternal age, maternal body mass index or gestational age were assessed using Pearson's correlation coefficient. All TSBA values were included for reference interval calculation, according to the International Federation of Clinical Chemistry and Clinical and Laboratory Standards Institute C28-A3 recommendations. Calculation of the lower and upper limits of reference intervals was performed using the nonparametric method[22] with 95% CI.

Study 3: Comparison of adverse perinatal outcome rates for women with ICP with peak bile acids below a suggested diagnostic threshold with background population rates

Rates of adverse perinatal outcomes for women with peak TSBA concentrations below or equal to the highest upper limit of the calculated reference interval were obtained from participants in a published individual patient data meta-analysis of women with ICP,[14] selecting women with non-fasted peak TSBA concentrations only. These were compared with published rates of adverse perinatal outcomes in uncomplicated pregnancies or at population levels, matching comparators as closely as possible, and compared using the chi-square test with Yates' correction and WOOLFLOGIT to calculate the 95% confidence interval (CI) in GRAPHPAD PRISM.

De-identified original and summary data, and the study protocol are available upon reasonable request of the authors. There are no core outcome sets relevant to this study.

Results

To assess the diurnal TSBA variation, women with uncomplicated pregnancies and women diagnosed with ICP were given standardised meals with known calorie and fat content, and TSBA was measured fasting and postprandially over the course of the day (Table 1). Women with ICP were separated into those with TSBA <40 μmol/l throughout the day (mild ICP), and those with at least one sample ≥40 μmol/l (severe ICP) (Figure 1).

In all participants, TSBA concentrations increased markedly after a meal, particularly in women with severe ICP (Figure 2A, and see Supplementary material, Table S1). We sought to determine the optimal time after starting the meal to measure TSBA in women with mild or severe ICP, calculating the sensitivity and specificity for fasting and postprandial time-points using different mild (≥6, ≥11 and >15 μmol/l) and severe (≥40 μmol/l) TSBA thresholds currently used[4–6,13] (Figure 2B–D, and see Supplementary material, Table S2).

Fasting TSBA concentration was 100% specific for the diagnosis of ICP using thresholds ≥11 μmol/l and 91% specific at ≥6 μmol/l (Figure 2B). For women with mild ICP, all fasting TSBA were <11 μmol/l (0% sensitive), and a threshold of ≥6 μmol/l gave only 29% sensitivity (2/7 women). Optimal sensitivity was observed 20 minutes post-lunch (Figure 2C), but only a threshold of ≥6 μmol/l achieved >90% sensitivity, which corresponded to just 10% specificity for mild ICP. Hence, no time-point or currently

Table 1. Clinical and demographic characteristics of women with uncomplicated pregnancies, and women with mild or severe ICP

	Uncomplicated pregnancy	Mild ICP	Severe ICP
Number of participants	24	7	11
Characteristic			
Age (years), median (range)	34 (28–40)	35 (27–38)	36 (27–43)
Gestational age (weeks) at study participation, median (range)	33^{+1} (16^{+0} to 39^{+2})	35^{+5} (29^{+1} to 38^{+4})	35^{+2} (16^{+2} to 37^{+3})
Ethnicity, *n* (%)			
White	15 (63)	6 (86)	8 (72)
Black	1 (4)	0 (0)	0 (0)
Asian	5 (21)	1 (14)	3 (27)
Other	1 (4)	0 (0)	0 (0)
Not recorded	2 (8)	0 (0)	0 (0)
Pregnancy details			
Previous pregnancies ≥24 weeks, *n* (%)			
0	11 (44)	1 (14)	7 (64)
1	8 (32)	6 (86)	1 (9)
≥2	3 (12)	0 (0)	3 (27)
Not recorded	3 (11)	0 (0)	0 (0)
Singleton pregnancy, *n* (%)	24 (100)	6 (86)	9 (82)
Gestational diabetes mellitus, *n* (%)	0 (0)	0 (0)	3 (27)
Liver function*			
Maternal cholelithiasis, *n* (%)	0 (0)	0 (0)	2(18)**
Receiving UDCA treatment at time of study, *n* (%)	0 (0)	1 (14)	10 (91)
Highest gestational total bile acids (μmol/l), median (range)	8 (1–20)	31.5 (22–52)	103.5 (63–444)
Highest gestational AST (IU/l), median (range)	28.5 (16–42)	71.5 (24–327)	190 (25–403)
Highest gestational ALT (IU/l), median (range)	16 (8–46)	77 (9–306)	366 (22–729)
Highest gestational ALP (IU/l), median (range)	98 (47–219)	249.5 (160–335)	264 (172–383)
Highest gestational Bilirubin (μmol/l), median (range)	5 (3–11)	7.5 (4–31)	9 (8–48)
Highest gestational GGT (IU/l), median (range)	12 (7–21)	35 (7–37)	23 (15–152)

ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, γ-glutamyltransferase; UDCA, ursodeoxycholic acid.

Values presented are median (range), or number (%). Participants in the second and third trimester were included; of the participants in the second trimester, five women had uncomplicated pregnancies, one woman had mild ICP, and two women had severe ICP.

*Reference ranges for liver function during pregnancy are detailed in ref. 23.

**Both patients had a history of gallstones before pregnancy.

used TSBA threshold achieved both good sensitivity and specificity for the diagnosis of mild ICP. When considering a diagnosis of severe ICP, the fasting TSBA concentrations were only 9% (1/11) sensitive, whereas all postprandial measurements were >90% sensitive (Figure 2D) with 27% (3/11) of women with severe ICP having postprandial TSBA ≥100 μmol/l. As a result, the majority of women with severe ICP would not be diagnosed as having severe disease using a fasting sample.

Postprandial TSBA concentrations for women with uncomplicated pregnancies were greater for many women than currently used diagnostic thresholds for ICP (Figure 2A, and see Supplementary material, Table S1). We calculated reference intervals for TSBA in uncomplicated pregnancies using non-fasting samples (a sensible time for phlebotomy during pregnancy, when fasting before sampling is particularly unacceptable[24]). We measured non-fasting TSBA concentrations from 561 women in the third trimester of uncomplicated pregnancies of black, south Asian or white ethnicity (Study 2, Figure 1, Table 2).

The distribution of TSBA concentrations was skewed for all ethnicities, which best transformed to an approximately Gaussian distribution using log transformation (see Supplementary material, Figure S1). Black women had non-fasting TSBA 25.8% higher (95% CI 9.6–44.4%, $P = 0.001$) than white women, and 24.3% higher (95% CI 5.7–46.1%, $P = 0.008$) than south Asian women. Non-fasting TSBA levels from south Asian women were similar to those of white women (1.22% higher, 95% CI −12.1 to 16.5%, $P = 0.866$) (see Supplementary material, Figure S2). No correlation was observed between TSBA and gestational age within the third trimester, maternal age or body mass index in any ethnic group (see Supplementary material, Table S3).
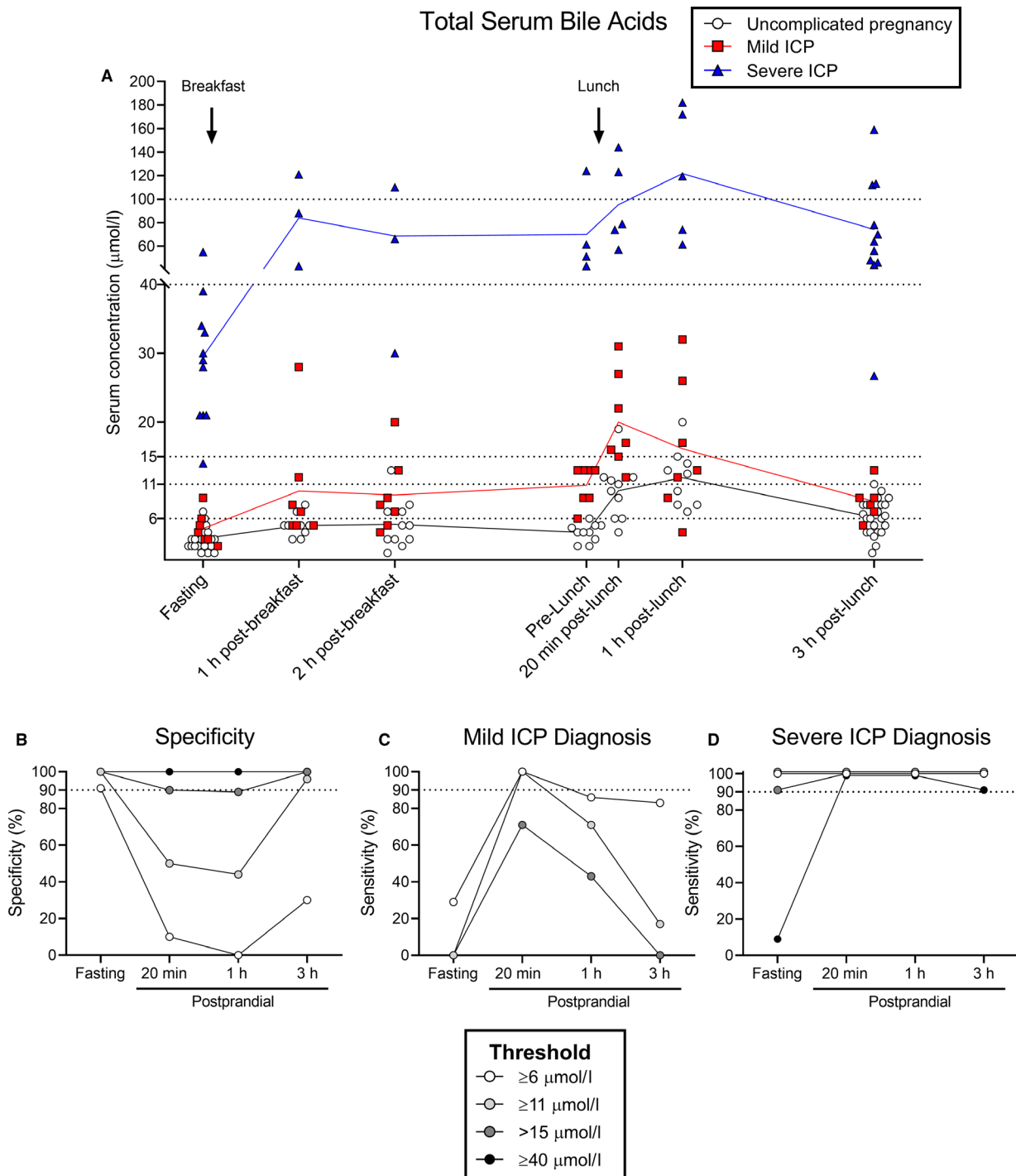
Figure 2. Total serum bile acids are elevated postprandially. (A) Bile acids were measured before and after standardised meals (arrows) in women with uncomplicated pregnancy (n = 24, white circles), or women diagnosed with ICP, who were separated by disease severity during the study day: mild ICP (n = 7, all TSBA measurements <40 μmol/l; red squares) or severe ICP (n = 11, one or more TSBA measurements ≥40 μmol/l; blue triangles); mean values are indicated by the corresponding coloured line. Dotted lines indicate different current diagnostic thresholds for ICP, with ≥40 μmol/l indicating severe ICP, and ≥100 μmol/l indicating increased risk of stillbirth. Specificity and sensitivity analyses were calculated using 6, 11 and 15 μmol/l thresholds as a diagnosis for mild ICP, and 40 μmol/l threshold for diagnosis of severe disease at different times of day (fasting, and 20 minutes, 1 hour and 3 hours post-lunch). (B) Specificity for correct identification of women without either mild or severe ICP, determined as the percentage of correct identification of women without ICP. Sensitivity to detection of women with (C) mild ICP or (D) severe ICP, determined as the percentage of women correctly identified as having ICP.

Table 2. Maternal demographics of women with uncomplicated pregnancies and reference intervals calculations of total serum bile acids from non-fasting serum samples

	Black	South Asian	White
Number of women	160	160	241
Gestation (weeks[+days]), median (range)	33^{+6} (30^{+0} to 37^{+6})	33^{+6} (30^{+0} to 37^{+6})	34^{+0} (30^{+0} to 37^{+6})
Age (years), median (range)	31 (18–49)	32 (20–43)	34 (19–43)
BMI (kg/m^2), median (range)	29.4 (20.1–45.0)	27.6 (20.4–44.5)	27 (21.3–43.9)
Nulliparous, %	40.3	47.5	55.2
TSBA (μmol/l), median (range)	4.2 (0.2–35.7)	3.3 (0.2–51.1)	3.9 (0.2–11.7)
Reference interval for TSBA (μmol/l)			
Lower limit (95% CI)	1.0 (0.2–1.5)	0.7 (0.2–1.2)	0.9 (0.2–1.5)
Upper limit (95% CI)	18.3 (15.0–35.6)	15.5 (11.5–47.6)	10.4 (8.7–11.5)

BMI, body mass index.

Reference intervals were calculated using a nonparametric method to account for non-normally distributed results. The lower (2.5%) and upper (97.5%) reference limits are noted with 95% confidence intervals (CI) indicated in brackets.

Due to the non-normal distribution of TSBA values, we determined the normal reference range for the different ancestral groups using the nonparametric method.[22] The lower reference limit for TSBA was between 0.7 and 1.0 μmol/l (95% CI 0.2–1.5 μmol/l) for all groups; however, the upper reference limit varied by ethnicity, at 10.4 μmol/l (95% CI 8.7–11.5 μmol/l), 15.5 μmol/l (95% CI 11.5–47.6 μmol/l) and 18.3 μmol/l (95% CI 15.0–35.6 μmol/l) for white, south Asian and black women, respectively (Table 2).

Applying the highest upper TSBA reference limit of 18.3 μmol/l, two of seven women with mild ICP who participated in the standardised diet study (Study 1) did not reach this threshold (Figure 2A). Applying a TSBA threshold of ≤19 μmol/l, 0.9% (5/561) of women with uncomplicated pregnancy from Study 2 would have been falsely diagnosed, compared with 2.3% (13/561) of women using the >15 μmol/l non-fasting threshold (used by the South Australia Maternal and Neonatal Community of Practice[5]) or 6.8% (38/561) using a ≥10 μmol/l non-fasting threshold (commonly used in hospitals in the UK[25]).

We therefore considered the implications for using ≥19 μmol/l as the threshold for the diagnosis of ICP for women who had previously been diagnosed with ICP but whose non-fasting TSBA values remained <19 μmol/l during pregnancy. Using individual patient data previously collected for a meta-analysis of perinatal outcomes in ICP from an international cohort (Study 3, Figure 1),[14] we found that 18.5% (658/3509) of women would not have been diagnosed with ICP using this threshold. To determine whether this would alter the risk of adverse outcomes were this group of women not diagnosed as having ICP, we compared rates of adverse perinatal outcomes of these women with studies in women with uncomplicated pregnancies or at the population level, selecting studies that most closely represented the ICP cohort.

Consistent with previous reports,[14,15] women with ICP whose peak non-fasting TSBA was <19 μmol/l had no increased risk of stillbirth compared with the background population (and see Supplementary material, Table S4). Although these women with ICP had higher rates of preterm birth, this probably resulted from iatrogenic preterm birth as the rate of spontaneous preterm birth was lower than in the background population. It is also probable that the higher preterm birth rate in this cohort of women with ICP contributed to higher rates of neonatal unit admission and low Apgar scores.[26]

Discussion

Main findings

This study has revealed the diurnal variation in TSBA in both cholestatic and uncomplicated pregnancies, demonstrating the difficulties in determining clinically appropriate diagnostic thresholds to identify affected women with acceptable sensitivity and specificity. Considering that the adverse perinatal outcomes of ICP are associated with peak TSBA concentration, measuring non-fasting rather than fasting TSBA has greater clinical relevance for decisions about patient management according to severity of hypercholanaemia. Use of this time-point for diagnosis necessitates definition of the non-fasting normal range for TSBA in uncomplicated pregnancies. The women assessed did not receive a standardised diet; however, they represent a real-world cohort whose TSBA measurements are likely to represent typical levels based upon their normal diets. As a woman's typical TSBA concentrations are probably more relevant to the fetal bile acid exposure and stillbirth risk

than TSBA results after a fixed meal stimulant (akin to the oral glucose load in gestational diabetes mellitus), we suggest that this non-fasting reference range is of greater clinical relevance.

Fasting TSBA concentrations of women with mild ICP from our standardised diet study were generally below the current diagnostic thresholds, and most women who had severe ICP (TSBA ≥40 μmol/l[13]) would not have been diagnosed as such using fasting samples. The European Association for the Study of Liver threshold currently suggests that fasting TSBA ≥11 μmol/l is diagnostic.[6] However, this may miss correct risk stratification, as TSBA levels in women whose fasting TSBA level is <40 μmol/l can rise postprandially to ≥40 μmol/l, when risk of perinatal complications is increased,[13] or even higher to ≥100 μmol/l, when the risk of stillbirth is elevated.[14]

Strengths and limitations

A limitation to this study is the relatively small numbers of women included with ICP for the standardised diet study, and that TSBA concentrations were not assessed at all time-points during the study in all women because of technical issues or patient consent. However, with the data stratified into mild and severe disease, differences between fasting and postprandial elevation between these groups were still evident.

Another limitation of the standardised diet study was that most women with severe ICP were treated with ursodeoxycholic acid (UDCA), whereas only one woman with mild ICP took the drug. It is possible that UDCA treatment influenced the TSBA concentration and caused the proportion of UDCA in the TSBA assay to increase, as was reported in a study where most women had TSBA <40 μmol/l.[27] However, a recent study of longitudinal changes in individual bile acids in ICP did not show a significant increase in TSBA concentration in UDCA-treated women.[28]

A strength of this study is the large cohort of women analysed for non-fasting TSBA in the third trimester, which demonstrated the variation in the normal range for TSBA between different ethnic groups. Samples were obtained alongside routine clinical appointments, and so reflect a pragmatic time-point upon which to base real-world clinical decisions. Rates of adverse perinatal outcomes assessed using individual patient data provided for meta-analysis were selected from one of the largest international studies of women with ICP yet performed, and comparisons were made with the best matched data obtainable for each comparison – the selection of which was performed before analysis in order to reduce potential bias.

Interpretation

Our re-evaluation of the reference limits for TSBA based on non-fasting samples holds implications for ICP diagnosis. We demonstrated no definitive evidence of markedly adverse spontaneous perinatal outcomes for women with ICP with non-fasting peak TSBA <19 μmol/l compared with uncomplicated pregnancies, with iatrogenic preterm birth probably responsible for the higher rates of overall preterm birth, neonatal unit admission and low Apgar score,[26] which could potentially be avoided by redefining the diagnostic threshold for ICP. Reassuringly, our findings are similar to previous studies, which reported that fetal complications increased in ICP when TSBA levels were >40 μmol/l,[13–15] and another study that did not find any differences in perinatal outcomes between women with ICP when stratified into groups with TSBA <13 μmol/l or between 13 and 40 μmol/l.[29]

Conclusion

Based upon the results of this study, we suggest that a non-fasting TSBA value of ≥19 μmol/l is used as a clinical diagnostic threshold for ICP, as an alternative to fasting thresholds. It is important that pregnant woman with otherwise unexplained pruritus whose non-fasting TSBA levels are <19 μmol/l are monitored regularly for changes in TSBA, given that pruritus often predates hypercholanaemia.[30] Use of appropriate diagnostic levels is likely to reduce the number of women diagnosed with ICP, thereby reducing maternal anxiety, avoiding unnecessary antenatal consultations and potential complications associated with iatrogenic preterm birth. Furthermore, the use of postprandial TSBA measurements is more likely to identify women with TSBA ≥100 μmol/l and at increased risk of stillbirth, and will empower clinicians to individualise interventions aimed at reducing the risk of adverse outcomes in this small but important group of women.

processed serum samples from the standardised diet study. Regarding the samples for the reference range of TSBA in uncomplicated pregnancies, KN selected the appropriate samples, AS and KS performed the sample collection and data acquisition. PTS advised on appropriate data and statistical analysis for reference interval calculation and correlations. ALM analysed the TSBA results for the standardised diet, and the reference intervals. CO produced the meta-analysis comparison. All authors edited, revised and contributed to the intellectual content of the manuscript.

Details of ethics approval

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Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. Power transformations of non-fasting total serum bile acid values.

Figure S2. Total serum bile acid concentrations of women in their third trimester of uncomplicated pregnancy, separated according to ethnicity.

Table S1. Total serum bile acid concentrations before and after standardised meals in women in the third trimester of uncomplicated pregnancy, or women diagnosed with intrahepatic cholestasis of pregnancy.

Table S2. Sensitivity and specificity of intrahepatic cholestasis of pregnancy diagnosis using different diagnostic thresholds for fasting and postprandial samples.

Table S3. Relationships between total serum bile acid values and maternal age, body mass index or gestational age.

Table S4. Comparison of adverse perinatal outcomes in an intrahepatic cholestasis of pregnancy cohort with highest recorded total serum bile acids <19 µmol/l compared with uncomplicated pregnancies or background population.

Video S1. Author insights. ■

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