Elevated Serum Total Bile Acid Level at Second Trimester Increases the Risk of Gestational Diabetes Mellitus and Adverse Perinatal Outcomes in Chinese Pregnant Women

Yao Liu
Union Shenzhen Hospital of Huazhong University of Science and Technology
Yan Li
Southern Medical University
Hengying Chen
Shantou University Medical College
Ruifang Sun
Union Shenzhen Hospital of Huazhong University of Science and Technology
Yuanhuan Wei
Union Shenzhen Hospital of Huazhong University of Science and Technology
Nuoya Wang
Union Shenzhen Hospital of Huazhong University of Science and Technology
Guifang Deng (misyfly@163.com)
Union Shenzhen Hosipital of Huazhong University of Science and Technology https://orcid.org/0000-0002-9520-2793
Zheqing Zhang
Southern Medical University

Research article

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Abstract

Backgroud

Bile acid metabolism is reported to be closely involved in glycolipid metabolism. We investigated the association of the total bile acid (TBA) levels at the second trimester with the risk of gestational diabetes mellitus (GDM) and adverse perinatal outcomes.

Methods

We performed a retrospective cohort study in 2773 Chinese pregnant women. Serum TBA level was measured by a biochemistry automatic analyzer. Logistic regression models with or without restricted cubic splines were performed.

Results

652 of 2773 pregnant women developed GDM. Women with GDM had higher serum TBA levels at the second trimester than that of healthy women ($P < 0.001$). Elevated serum TBA levels at the second trimester were associated with an increased risk of GDM in a dose-response manner. After the adjustment of age, BMI, smoking and drinking status, education background, lipid profiles, blood pressure, liver function, uric acid, bilirubin, urea nitrogen and creatinine, the odds ratios (ORs) of GDM for the highest (vs. the lowest) quartile were 1.83 (95% CI 1.10–3.38) in women. The inclusion of TBA levels at the second trimester in the model increased the area under the curve from 0.68 to 0.72 (95% CI: 0.68–0.76) ($P < 0.05$). Additionally, a higher serum TBA level was associated with higher odds of preterm birth (the highest quartile vs. the lowest quartile, AOR 1.91, 95% CI 1.26–2.98, *P-trend* = 0.017) and preeclampsia (the highest quartile vs. the lowest quartile, AOR 2.07, 95% CI 1.19–3.63, *P-trend* = 0.002). Furthermore, the U-shaped relationship was observed between TBA levels and the risk of premature rupture of membranes, with the lowest risk in the TBA concentration of 3.14 μmol/L.

Conclusion

Women with higher fasting serum TBA levels at the second trimester have higher risk for development of GDM, preterm birth and preeclampsia.

Backgroud

The increasing prevalence of gestational diabetes mellitus (GDM) has aroused widespread concerns. According to WHO statistics in different regions during 2005–2015, the prevalence of GDM worldwide ranged from 1.8 to 25.1%. A newly published meta-analysis, which collected data from 25 studies, indicating that the total incidence of GDM in mainland China was 14.8% [95% confidence interval (CI) 12.8–16.7%] [1]. Moreover, a large number of studies abroad showed that the incidence of GDM in Asians was 3–7 times higher than that in other ethnic groups. GDM contributes to increasing the rate of pregnancy complications and adverse perinatal outcomes, including cesarean section, premature rupture of membranes (PROM), eclampsia, preterm delivery, macrosomia and deformity [2, 3]. Moreover, the

development of GDM greatly increases the risk of future type 2 diabetes (T2DM) and cardiovascular disease in pregnant women and their children [4, 5].

Bile acids (BAs) are products of cholesterol metabolism in the liver [6]. According to their structure, they are divided into free BAs and combined BAs, and also are divided into primary BAs and secondary BAs according to their source as well [6]. BAs could not only promote the absorption of fatty acids and fat-soluble vitamins as an emulsion, but also regulate glycolipid metabolism as a signal molecule [7]. Recently, evidence predominantly derived from animal studies in obesity, non-alcoholic fatty liver disease showed that BAs improved insulin resistance via the regulation of farnesoid-x-receptor (FXR) and the cell surface G protein coupled bile acid receptor (TGR5)-mediated signaling pathway, which hinted that quantification of serum BAs levels is of clinical significance to explore the effect of BAs on metabolic diseases in human [8, 9]. However, there are litter population evidence to discuss the association between TBA and GDM, and the conclusions were controversial. Gao *et al.* suggested that TBA concentrations of GDM subjects were decreased notably when compared with the control subjects [10]. Nevertheless, another study indicated that the GDM women have a higher TBA level at early pregnancy than that of healthy subjects, and higher first-trimester TBA levels might increase the risk of GDM [11]. A 1:1 nested case-control study also showed that Serum GUDCA and DCA nmol/mL at early pregnancy were positively correlated with an increased GDM risk [12]. Therefore, the association between TBA levels and GDM risk remains unclear, which requires further investigations.

Additionally, adverse perinatal outcomes are essential concerns in the field of GDM. Epidemiological studies reported that the increase of TBA levels caused by cholestasis during pregnancy would increase the incidence of premature delivery, eclampsia, meconium-stained fluid and stillbirth [13–15]. It was noteworthy that previous studies on TBA levels and adverse perinatal outcomes were only conducted in women with intrahepatic cholestasis of pregnancy (ICP). However, the association of fasting TBA levels within the normal range with adverse perinatal outcomes of pregnant women has not been explored.

In the present study, we aimed to investigate the association between fasting TBA levels at the second trimester and the risks of GDM and adverse perinatal outcomes in a retrospective cohort study.

Methods

Research design and participants

This retrospective cohort study was conducted in the Department of Gynecology and Obstetrics of Union Shenzhen Hospital of Huazhong University of Science and Technology from January 2015 and September 2019. A total of 3, 258 subjects aged 19–46 years old were recruited into this study. From the first prenatal examination to delivery, all clinical characteristics and biochemical indicators of the participants were recorded in the hospital information system. The study was approved by the Ethics Committee of the Union Shenzhen Hospital of Huazhong University of Science and Technology and was conducted ethically in accordance with the World Medical Association Declaration of Helsinki. All women recruited in this study were requested for written consent.

The 75-g oral glucose tolerance test was performed for the diagnosis of GDM during 24-28th weeks of gestation. After the intake glucose, blood samples of subjects were collected at 0-and 2-h post-load. Based on the International Association of Diabetes and Pregnancy Study Group (IADPSG) criteria, GDM is diagnosed when fasting plasma glucose is greater than 5.1 mmol/L, and/or postprandial blood glucose at 1 hour is greater than 10.0 mmol/L, and/or postprandial blood glucose at 2 hours is greater than 8.5 mmol/L [16]. Serum glucose and insulin were measured enzymatically on a 7600–010 automated analyzer (Hitachi, Tokyo, Japan). The degree of insulin resistance was measured by homeostasis model assessment of insulin resistance index (HOMA-IR), calculated using the following formula: HOMA-IR = fasting glucose (mmol/L) × fasting insulin (μU/mL)/ 22.5. Glycosylated hemoglobin A1c (HbA1c) was determined by high-pressure liquid chromatography.

At the beginning of the study, 3258 women were involved in this prospective cohort study at the first trimester. 485 participants were excluded due to preconceptional diabetes, cholestasis before pregnancy, chronic or serious acute infections, cardiovascular diseases, hematological diseases, severely impaired liver or kidney function or if they have been tested for positive hepatitis C antibodies or HIV. Of the remaining 2773 subjects, 652 pregnant women ultimately developed GDM. The flow chart of selection of the pregnant women is shown in **Fig. 1**

Data collections

Weight, height and blood pressure of all subjects were measured by the same instrument. A face-to-face interview was performed to collect information about educational background, lifestyle and habits (e.g. smoking, drinking), the history of chronic diseases, and history of gestation. After delivery, adverse perinatal outcomes (e.g. preeclampsia and PROM) were confirmed according to the medical records and labor process records, and defined the newborn with a weight greater than 4000g as macrosomia.

Biochemical measurements

During 12-24th weeks of gestation, venous blood samples were collected for biochemical parameters measurement. After the preparation of serum, serum TBA concentrations were measured by a 7600–010 biochemistry automatic analyzer (Hitachi, Tokyo, Japan) with a linearity range of 0–180 μmol/L and normal range of 0–10 μmol/L. The coefficients of variation of TBA was 2.99%. Total bilirubin (TBIL), direct bilirubin (DBIL), glutamic-pyruvic transaminase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN), creatinine (Cr), uric acid (UA) and serum lipids including total cholesterol (TC), total triglycerides (TG), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) were determined by 7600–010 biochemistry automatic analyzer (Hitachi, Tokyo, Japan).

Statistical analyses

Data were displayed as mean ± standard deviation (SD) for continuous variables and percentages (%) for categorical variables. Differences between groups were evaluated with Student's *t* test, one-way ANOVA, Kruskal Wallis tests or Mann-Whitney U test for continuous variables and Chi-square test for categorical variables. Binary logistic regression analysis was conducted to evaluate the odds ratios (OR) and 95% CIs

for the risk of GDM, maternal pregnancy complications and neonatal-perinatal outcomes with increasing quartiles of serum TBA levels, using the lowest quartile as the reference group for all subjects. The adjusted OR was also analyzed by Binary logistic regression analysis after the adjustment of age, BMI, BUN, CRE, AST, ALT, TC, TG, HDL-C and LDL-C. In consideration of the hypothesis that the relationship between bile acid and risk of adverse perinatal outcomes was nonlinear, we further performed logistic regression models with restricted cubic splines (RCS) with five knots (the 10th, 25th, 50th, 75th and 90th percentiles) for age adjusting for all covariates above.

All the statistical analyses were performed by SPSS 24.0 (SPSS Inc., Chicago, IL, USA) and R software packages (V.4.0.0). A two-sided $P< 0.05$ was considered statistically significant.

Results

Characteristics of participants of subjects

In the present study, 2773 pregnant women without preconceptional diabetes were involved, with a mean age of 31.50±3.93 years and mean prepregnancy BMI of 21.00±3.21 kg/m^2. The mean value of fasting serum total bile acid concentrations at the second trimester was 2.57±1.58 (μmol/L). During this prospective study, 652 of 2773 (23.51%) women developed GDM. As shown in Table 1, compared with the Non-GDM group, subjects in the GDM group had older age, higher prepregnancy BMI levels, as well as higher levels of uric acid, total bilirubin, creatinine, TG, fasting insulin, 1-and 2-h post-load insulin, fasting plasma glucose, 1-and 2-h post-load glucose, HbA1c and HOMA-IR (all $P< 0.05$). Interestingly, serum total bile acid levels were increased in women in the GDM group compared with those in the Non-GDM group (2.98 ± 1.15 vs. 2.37 ± 1.33 μmol/L, $P< 0.001$) (Table 1).

Table 1
Baseline characteristics of participants with and without GDM: demographics and laboratory values

	Serum TBA level (μmol/L)			
	Total	Non-GDM	GDM	*P* for trend
N	2773	2121	652	
Age (years)	31.50±3.93	31.24 ± 3.89	32.37 ± 3.94	0.001
BMI, kg/ m2	21.00±3.21	20.67±3.18	22.07±3.06	0.001
< 24	2503	1990	513	0.001
≥ 24	270	139	131	
Education background (n, %)				0.028
High School and Below	629 (22.68%)	490 (23.10%)	139 (21.32%)	
Junior College	674 (24.31%)	490 (23.10%)	184 (28.22%)	
University	1470 (53.01%)	1141 (53.80%)	329 (50.46%)	
Smoking Status				0.997
Current smoker	68 (2.45%)	52 (2.45%)	16 (2.45%)	
Nonsmoker	2705 (97.55%)	2069 (97.55%)	636 (97.55%)	
Drinking Status				0.823
Current drinker	69 (2.49%)	52 (2.45%)	17 (2.61%)	
Nondrinker	2704 (97.51%)	2069 (97.55%)	635 (97.39%)	
Blood pressure (mmHg)				
SBP (mm Hg)	117.05±12.10	116.82 ± 12.04	117.76 ± 12.45	0.086
DBP (mm Hg)	66.84±9.14	66.72 ± 9.07	67.24 ± 9.33	0.199
Serum TBA level (μmol/L)	2.57±1.58	2.37 ± 1.33	2.98 ± 1.15	0.001
Glucose metabolism				
Fasting plasma insulin (μU/ml)	5.11±2.09	8.26±2.38	4.20±0.58	0.001

Data represent means ± SD or percentage (%).

Significant difference if $P < 0.05$

GDM, gestational diabetes mellitus; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TBA, total bile acid; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TC, total cholesterol; TG, total triglycerides; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; HbA1c, glycosylated hemoglobin; HOMA-IR, homeostasis model assessment of insulin resistance index.

	Serum TBA level (μmol/L)			
1-h post-load insulin (μU/ml)	67.80±3.96	70.30±4.07	54.87±3.30	0.001
2-h post-load insulin (μU/ml)	46.18±3.87	34.87±2.84	49.89±2.93	0.001
Fasting plasma glucose (mmol/L)	4.62±0.38	4.53±0.29	4.91±0.47	0.001
1-h post-load glucose (mmol/L)	8.36±1.82	7.56±1.32	9.80±1.69	0.001
2-h post-load glucose (mmol/L)	7.08±1.51	6.48±0.98	8.58±1.55	0.001
HbA1c (%)	5.14±0.53	4.92±0.44	5.25±0.53	0.001
HOMA-IR	1.06±0.50	2.13±4.7	1.19±0.7	0.001
Lipid profile				
TC (mmol/L)	5.31±0.88	5.56 ± 0.68	5.23 ± 0.93	0.196
TG (mmol/L)	2.11±0.65	1.93 ± 0.67	2.17 ± 0.65	0.011
HDL-C (mmol/L)	1.67±0.30	1.78 ± 0.28	1.63 ± 0.31	0.022
LDL-C (mmol/L)	2.69±0.85	2.55 ± 0.83	3.06 ± 0.85	0.075
Liver function				
ALT (units/L)	17.80±3.87	17.54±3.45	18.60±5.05	0.164
AST (units/L)	18.14±4.69	18.21±7.53	17.94±8.17	0.527
Uric acid (μmol/L)	210.86±44.20	209.11±43.30	216.32±46.39	0.003
Total bilirubin (μmol/L)	7.44±2.78	7.14 ± 2.84	7.53 ± 2.75	0.009
Direct bilirubin (μmol/L)	2.39±1.42	2.47 ± 1.59	2.24 ± 0.98	0.106
Urea nitrogen (mmol/L)	2.60±0.63	2.60 ± 0.64	2.59 ± 0.58	0.607
Creatinine (μmol/L)	43.46±6.79	42.48 ± 6.56	43.77 ± 6.84	0.001
Maternal characteristics				
Gestational age at delivery (weeks)	38.68±1.61	38.73±1.63	38.02±1.52	0.003

Data represent means ± SD or percentage (%).

Significant difference if $P < 0.05$

GDM, gestational diabetes mellitus; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TBA, total bile acid; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TC, total cholesterol; TG, total triglycerides; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; HbA1c, glycosylated hemoglobin; HOMA-IR, homeostasis model assessment of insulin resistance index.

	Serum TBA level (μmol/L)			
Nulliparous (n,%)	1160	873 (41.16%)	287 (44.02%)	0.196
Abortion history (n,%)	1089	808 (38.10%)	281 (43.09%)	0.022
PROM (%)	607	471 (22.21%)	136 (20.86%)	0.467
Preeclampsia (n,%)	107	67 (3.16%)	40 (6.13%)	0.001
Premature birth (n,%)	177	131 (6.17%)	46 (7.06%)	0.002
Postpartum hemorrhage (mL)	211.60±129.82	212.58±140.71	208.42±85.19	0.475
Offspring				
Weight (g)	3287.80±481.92	3288.53±486.05	3285.44±468.60	0.886
Macrosomia (n,%)	199	159 (7.50%)	40 (6.13%)	0.239
Height (cm)	49.84±1.90	49.85±1.95	49.81±1.74	0.040
Head circumference (cm)	33.95±1.63	33.95±1.48	33.92±2.04	0.607
Chest circumference (cm)	33.20±2.08	33.22±1.97	33.12±2.43	0.262
Data represent means ± SD or percentage (%).				
Significant difference if $P < 0.05$				
GDM, gestational diabetes mellitus; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TBA, total bile acid; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TC, total cholesterol; TG, total triglycerides; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; HbA1c, glycosylated hemoglobin; HOMA-IR, homeostasis model assessment of insulin resistance index.				

Moreover, there were differences between GDM and Non-GDM groups in gestational age at delivery and abortion history, as well as the birth height of the fetus. GDM women tend to develop preeclampsia (6.13% vs. 3.16%, $P = 0.001$) and premature birth (7.06% vs. 6.17%, $P = 0.002$) (Table 1). However, significant differences were not found in the occurrence of premature birth, PROM and macrosomia between two groups (All $P > 0.05$).

Serum TBA Levels Were Associated with an Increased Risk of GDM

To further investigate the associations of serum TBA levels at the second trimester with GDM, the individuals were categorized according to quartiles of serum TBA levels. As shown in Table 2, the levels of fasting insulin, 1-and 2-h post-load insulin, fasting plasma glucose, 1-and 2-h post-load glucose, HbA1c and HOMA-IR and serum TG were elevated by increasing serum TBA quartiles (all $P < 0.05$), whereas the level of HDL-C reduced by increasing serum TBA quartiles ($P = 0.033$). Then, we explored the correlation between TBA levels and some metabolic risk factors. Serum TBA levels were correlated with age, prepregnancy BMI, fasting plasma glucose, 1-and 2-h post-load glucose levels, HbA1c, HOMA-IR, TG, BUN, CRE, AST and ALT

levels (All $P < 0.05$, **Additional File 1**). Additionally, elevated serum TBA levels at were associated with an increased occurrence of preeclampsia and premature birth in a dose-response manner (Table 2).

Table 2
Baseline characteristics of participants according to quartiles of serum TBA levels

	Serum TBA level (μmol/L)				
	Quartile 1	Quartile 2	Quartile 3	Quartile 4	*P* for trend
Serum TBA level (μmol/L)	< 1.60	1.60–2.20	2.20–3.11	≥ 3.11	
Participants, n	664	647	768	694	
Age (years)	31.53±3.89	31.46 ± 3.99	31.36 ± 3.77	31.67 ± 4.01	0.487
BMI, kg/ m2	20.68±2.50	20.95±3.95	21.05±3.02	21.29 ± 3.23	0.006
< 24	621	599	687	596	0.001
≥ 24	43	48	81	98	
Smoking Status					0.992
Current smoker	16 (2.41%)	16 (2.47%)	18 (2.34%)	18 (2.59%)	
Nonsmoker	648 (97.59%)	631 (97.53%)	750 (97.66%)	676 (97.41%)	
Drinking Status					0.932
Current drinker	15 (2.26%)	17 (2.63%)	18 (2.34%)	19 (2.77%)	
Nondrinker	649 (97.74%)	630 (97.37%)	750 (97.66%)	676 (97.41%)	
Blood pressure (mmHg)					
SBP (mm Hg)	117.26±12.14	116.90 ± 11.64	116.66 ± 11.99	117.40 ± 12.74	0.554
DBP (mm Hg)	66.72±8.92	66.87 ± 8.60	66.79 ± 9.06	66.99 ± 9.91	0.112
Glucose metabolism					

Data represent means ± SD or percentage (%).

Significant difference if $P < 0.05$

GDM, gestational diabetes mellitus; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TBA, total bile acid; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TC, total cholesterol; TG, total triglycerides; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; HbA1c, glycosylated hemoglobin; HOMA-IR, homeostasis model assessment of insulin resistance index.

	Serum TBA level (μmol/L)				
Fasting plasma insulin (μU/ml)	6.85±1.76	7.20±3.00	8.87±4.65	9.15±1.55	0.001
1-h post-load insulin (μU/ml)	55.03±2.90	58.52±1.43	66.52±3.93	67.67±3.35	0.001
2-h post-load insulin (μU/ml)	36.30±4.17	47.86±3.80	48.05±4.01	51.84±3.29	0.001
Fasting plasma glucose (mmol/L)	4.54±0.33	4.62±0.40	4.63±0.36	4.68±0.42	0.001
1-h post-load glucose (mmol/L)	7.94±1.68	8.18±1.79	8.28±1.59	8.99±2.00	0.001
2-h post-load glucose (mmol/L)	6.75±1.26	6.87±1.33	7.21±1.50	7.42±1.77	0.001
HbA1c (%)	5.01±0.40	5.14±0.81	5.18±0.40	5.20±0.52	0.005
HOMA-IR	1.06±0.50	2.13±4.7	1.19±0.7		0.001
Lipid profile					
TC (mmol/L)	6.29±0.30	5.27 ± 1.23	5.12 ± 0.83	5.26 ± 0.66	0.233
TG (mmol/L)	1.87±0.64	2.05 ± 0.74	2.32 ± 0.54	2.66 ± 0.08	0.024
HDL-C (mmol/L)	1.59±0.38	1.67 ± 0.30	1.71 ± 0.33	2.03 ± 0.22	0.033
LDL-C (mmol/L)	2.65±0.86	2.89 ± 1.13	2.59±0.82	2.62±0.70	0.825
Liver function					
ALT (units/L)	16.30±1.25	18.38±1.52	17.80±1.35	18.72 ± 1.41	0.053
AST (units/L)	17.39±6.81	18.48±8.08	18.09±7.74	18.64 ± 8.03	0.075
Data represent means ± SD or percentage (%).					
Significant difference if $P < 0.05$					
GDM, gestational diabetes mellitus; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TBA, total bile acid; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TC, total cholesterol; TG, total triglycerides; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; HbA1c, glycosylated hemoglobin; HOMA-IR, homeostasis model assessment of insulin resistance index.					

	Serum TBA level (μmol/L)				
Uric acid (mmol/L)	212.23±45.12	220.83±44.65	208.39±41.82	212.42±45.38	0.470
Total bilirubin (μmol/L)	7.42±2.59	7.40 ± 2.70	7.47 ± 2.89	7.45±2.90	0.975
Direct bilirubin (μmol/L)	2.53±1.20	2.28 ± 1.04	2.20 ± 1.06	2.57 ± 2.01	0.436
Urea nitrogen (mmol/L)	2.62±0.63	2.58 ± 0.64	2.59 ± 0.64	2.60 ± 0.62	0.854
Creatinine (μmol/L)	43.80±6.75	43.36 ± 6.86	43.31 ± 6.59	43.37 ± 7.01	0.676
Maternal characteristics					
Gestational age at delivery (weeks)	38.76±1.58	38.74±1.48	38.71±1.63	38.50±1.74	0.012
Nulliparous (n,%)	237 (35.69%)	309 (47.76%)	298 (38.80%)	315 (45.53%)	0.005
Abortion history (n,%)	263 (39.61%)	296 (45.75%)	279 (36.33%)	251 (36.17%)	0.213
PROM (%)	125 (18.83)	147 (22.72%)	158 (20.57%)	136 (25.50%)	0.177
Preeclampsia (n,%)	17 (2.56%)	18 (2.78%)	33 (4.30%)	39 (6.13%)	0.001
Premature birth (n,%)	27 (4.07%)	33 (5.10%)	60 (7.81%)	57 (8.21%)	0.003
Postpartum hemorrhage (mL)	214.44±173.16	207.50±106.04	210.22±103.46	214.25±128.22	0.721
Offspring					
Weight (g)	3247.15±502.40	3313.83±457.40	3178.99±488.25	3287.80±481.92	0.887
Macrosomia (n,%)	36 (5.42%)	48 (7.42%)	60 (7.81%)	55 (7.93%)	0.096

Data represent means ± SD or percentage (%).

Significant difference if $P < 0.05$

GDM, gestational diabetes mellitus; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TBA, total bile acid; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TC, total cholesterol; TG, total triglycerides; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; HbA1c, glycosylated hemoglobin; HOMA-IR, homeostasis model assessment of insulin resistance index.

	Serum TBA level (μmol/L)				
Height (cm)	49.66±2.20	49.93±1.71	49.84±1.96	49.84±1.90	0.021
Head circumference (cm)	33.84±1.59	34.06±1.53	33.93±1.48	33.95±1.90	0.133
Chest circumference (cm)	33.12±2.25	33.26±2.08	33.19±1.84	33.22±2.17	0.643
Data represent means ± SD or percentage (%).					
Significant difference if $P < 0.05$					
GDM, gestational diabetes mellitus; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TBA, total bile acid; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TC, total cholesterol; TG, total triglycerides; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; HbA1c, glycosylated hemoglobin; HOMA-IR, homeostasis model assessment of insulin resistance index.					

Then we performed binary logistic regression with the first quartile of serum TBA levels (< 1.60 μmol/L) as a reference to further assess the association between TBA levels and the risk of GDM in a series of adjusted models. Table 3 revealed that higher serum TBA quartile was associated with higher odds of GDM (the highest tertile vs. the lowest tertile, AOR 2.30, 95% CI 1.62–4.25, $P < 0.001$) after adjusting for age and BMI. Furthermore, these associations still maintained robustness after the adjustment for related factors in Multivariable model 2 and 3. After adjusting for the variables of age, BMI, smoking and drinking status, education background, systolic blood pressure, diastolic blood pressure, TC, TG, LDL-C, HDL-C, ALT, AST, uric acid, total bilirubin, direct bilirubin, urea nitrogen and creatinine, the ORs of GDM in Model 3 for the highest (vs. the lowest) quartile were 1.83 (95% CI 1.10–3.38) in women.

Table 3
Odds ratios (95% confidence intervals) of baseline serum TBA levels on the risk of GDM.

	Serum TBA Level (μmol/L)				
	Quartile 1 (< 1.60)	Quartile 2 (1.60–2.20)	Quartile 3 (2.20–3.11)	Quartile 4 (≥3.11)	*P* for trend
Median, mg/mL	1.18	1.86	2.46	4.00	
Cases, n	115	140	191	206	
Incident rate	17.32	21.64	24.87	29.68	
OR (95% CI)					
Multivariable model 1	Ref.	1.38 (1.13, 2.79)	1.63 (1.35, 3.38)	2.30 (1.62, 4.25)	0.001
Multivariable model 2	Ref.	1.16 (1.01, 1.59)	1.43 (1.07, 2.56)	1.91 (1.09, 3.35)	0.002
Multivariable model 3	Ref.	1.09 (1.04, 1.15)	1.34 (1.06, 2.65)	1.83 (1.10, 3.38)	0.015
Multivariable model 1 adjusted for age and BMI; Multivariable model 2 adjusted for the variables in Model 1 plus smoking and drinking status, education background, systolic blood pressure, diastolic blood pressure, TC, TGs, LDL-C and HDL-C. Multivariable model 3 adjusted for the variables in Model 2 plus ALT, AST, uric acid, total bilirubin, direct bilirubin, urea nitrogen and creatinine. PY, person-year; Ref., referent.					
Significant difference if $P < 0.05$					

Next, we assessed the predictive values of bile acids for GDM. As displayed in Fig. 2, the similar Area under the curve (AUC) existed in the TBA model (AUC: 0.66, 95% CI: 0.63–0.68) and the traditional risk factor model (AUC: 0.68, 95% CI: 0.66–0.70). After including TBA in the traditional risk factor model, the AUC markedly increased (AUC: 0.72, 95%CI: 0.68–0.76) ($P < 0.001$).

Table 4
Adjusted odds ratios (95% confidence intervals) of adverse outcomes according serum TBA quartiles.

Outcomes		TBA (μmol/L)				
		Quartile 1	Quartile 2	Quartile 3	Quartile 4	*P* for trend
		(< 1.60)	(1.60–2.20)	(2.20–3.11)	(≥3.11)	
Preterm birth	Model 1	Ref.	1.16 (0.70, 1.77)	1.43 (0.80, 2.56)	1.94 (1.09, 3.35)	0.005
	Model 2	Ref.	1.09 (0.75, 1.28)	1.14 (0.71, 1.84)	1.91 (1.26, 2.98)	0.017
PROM	Model 1	Ref.	0.98 (0.63, 1.41)	1.15 (0.38, 1.84)	1.71 (0.41, 3.78)	0.248
	Model 2	Ref.	0.89 (0.69, 1.14)	1.02 (0.46, 1.78)	1.65 (0.51, 2.83)	0.347
Preeclampsia	Model 1	Ref.	1.17 (0.33, 2.63)	1.48 (0.55, 3.27)	2.28 (0.87, 4.91)	□0.001
	Model 2	Ref.	1.10 (0.54, 1.67)	1.34 (0.83, 2.63)	2.07 (1.19, 3.63)	0.002
Macrosomia	Model 1	Ref.	1.28 (0.81, 1.92)	1.19 (0.79, 1.79)	1.15 (0.75, 1.76)	0.781
	Model 2	Ref.	1.22 (0.80, 1.88)	1.15 (0.76, 1.74)	1.09 (0.71, 1.67)	0.822
Model without adjustment.						
Model 2 adjusted for the variables of age, BMI, smoking and drinking status, education background, systolic blood pressure, diastolic blood pressure, TC, TGs, LDL-C, HDL-C, ALT, AST, uric acid, total bilirubin, direct bilirubin, urea nitrogen and creatinine. Ref., referent.						
GDM, gestational diabetes mellitus; TBA, total bile acid; PROM, premature rupture of membranes.						
Significant difference if $P < 0.05$						

Association between serum TBA levels and adverse perinatal outcomes in women

We next further identified whether TBA levels responsible for the adverse perinatal outcomes in women. We performed binary logistic regression with the first quartile of serum TBA levels (< 1.60 μmol/L) as a reference. We found that a higher serum TBA level was associated with higher odds of preterm birth (the highest quartile vs. the lowest quartile, AOR 1.91, 95% CI 1.26–2.98, *P-trend* = 0.017) and preeclampsia (the highest quartile vs. the lowest quartile, AOR 2.07, 95% CI 1.19–3.63, *P-trend* = 0.002). No linear association was detected between TBA and PROM or macrosomia ($P > 0.05$) (Table 4). In Fig. 3, we used restricted cubic splines to flexibly model and visualize the non-linear relation of TBA with the risk of PROM and macrosomia. Regarding the U-shaped relation between TBA and PROM, the plot showed a substantial

increased risk within the low range of TBA, which reached the lowest risk for PROM around 3.14 μmol/L, and then increased thereafter (All *P-nonlinear* < 0.01).

Discussion

In this study, we quantified serum fasting TBA level at the second trimester of pregnant women. Increased fasting serum TBA concentrations were associated with an elevated risk of GDM in Chinese pregnant women. Inclusion of TBA levels in the model increased the area under the curve of GDM. Additionally, we found that a higher serum TBA level was associated with a higher risk of preeclampsia and preterm birth. To our knowledge, this present study evaluated for the first time the association between serum TBA levels and the risk of GDM and adverse perinatal outcomes.

Bile acids, a kind of steroidal C24 carboxylic acids, are produced from cholesterol metabolism, which are important components of bile and has effects on lipid metabolism [17]. Increasing epidemiologic evidence have reported that bile acid metabolism was involved in the development of metabolic diseases, such as obesity, fatty liver and type 2 diabetes [18–21]. Haeusler R *et al.* indicated that a nearly 2-folds increase of TBA levels was observed in T2DM participants [19]. Compared with healthy people, subjects with obese and T2D have higher fasting TBA levels, and both plasma CDCA, CA and DCA concentrations were negatively associated with insulin sensitivity [20]. In Jiao's study, serum levels of TBA, primary and secondary bile acids were both elevated in patients with nonalcoholic fatty liver disease (NAFLD) [21]. Therefore, higher TBA levels potentially increase the risk of several metabolic diseases, included T2DM. Both GDM and T2DM showed insulin resistance, which had some similarities in pathogenesis. However, as far as we know, the association of TBA levels with GDM has not fully explored. In the current study, we indicated that there was a longitudinal link between fasting serum TBA concentrations at the second trimester and GDM in pregnant women.

Only a few population studies have explored the relationship between TBA levels and GDM. A prospective cohort study in Wuhan China suggested that pregnant women with GDM have a higher TBA level in early mid-pregnancy than that of healthy women [22]. *Hou et al.* also reported that a higher TBA levels were observed in pregnant women with GDM at early pregnancy [11]. However, another population-based study has reached inconsistent conclusions, in which the researchers indicated that both the levels of TBA, TBIL and DBIL in the GDM group were decreased significantly when compared with control subjects [10]. *Shaham O et al.* also reported that the elevation of plasma BAs in prediabetic patients after oral glucose tolerance test was less than that in healthy subjects [23]. In our study, after adjusting the confounders extensively, we found higher TBA concentrations in pregnant women with GDM at the second trimester. Moreover, we discovered that subjects in the highest quartile of the TBA group had a significantly higher occurrence of GDM when compared with those in the lowest quartile of the TBA group. The composition and the size of the BAs pool changed with the development of GDM, which might be related to the pathogenesis of GDM. Therefore, further studies with larger samples and animal experiments are needed to investigate the exact relationship and mechanism between circular TBA during different pregnancy and GDM.

Two main bile acid receptors involved in the potential mechanisms might explain the association between TBA and GDM risks [6]. First, farnesoid-x-receptor (FXR), which involved in the regulation of BAs synthesis, could regulate glucose metabolism by mediating gluconeogenesis and glycolysis of liver, and peripheral insulin sensitivity of striated muscle and adipose tissues [6]. Li *et al.* found that taurine-β-mouse bile acid (T-β-MCA), as an antagonist of FXR, could eventually improve obesity, as well as obesity-induced insulin resistance and NAFLD [24]. An *in vivo* study reported that theabrownin in Pu'er tea reduced cholesterol and lipid contents, which is mainly through inhibiting the FXR-Fibroblast Growth Factor (FGF15) signaling pathway [25]. Inhibition of FXR activation also promoted the expression of glucagon like peptide-1 (GLP-1), which could not only reduce food intake but also accelerate glucose decomposition by promoting insulin secretion and inhibiting glucagon secretion [26, 27]. Interestingly, in NAFLD patients with T2DM, oral OCA (25 or 50 mg) for 6 weeks increased insulin sensitivity, which was related to FXR activation mediated FGF19 production [28]. It was also found in animal studies that intervention of GW4064, an agonist of FXR, reduced insulin resistance of ob/ob and db/ db diabetic mice by enhancing insulin sensitivity [29]. Second, the cell surface G protein coupled bile acid receptor (TGR5) is expressed in various tissues related to metabolic diseases, including liver, intestine and brown adipose tissue [30]. BAs could promote islet cells to release insulin by directly stimulating TGR5 [31]. After binding with endogenous ligand bile acid, TGR5 promoted the synthesis of cyclic adenosine monophosphate (cAMP) and activated protein kinase A (APK) pathway [32]. Moreover, LCA and DCA promoted the expression of GLP-1 by activating TGR5 on the surface of intestinal endocrine L cells [33]. Therefore, the detailed mechanisms of the inhibition of GDM by third-trimester TBA levels reduction require more further investigations.

During pregnancy, the serum TBA of pregnant women showed a physiological increase due to the disturbance of bile acid transport in the liver caused by hormone changes [14]. Combined with clinical manifestations, ICP can be diagnosed when the TBA levels are higher than or equal to 10 μmol/L [14, 34]. Studies have reported that ICP, which mainly manifested the increase of TBA levels in the liver and systemic circulation, would increase the risk of premature birth [13]. An aggregate meta-analysis of 23 studies compared the perinatal outcomes of intrahepatic cholestasis in pregnancy women (n = 5,557) with healthy controls (n = 165,136), finding that ICP was associated with increased risks of spontaneous preterm birth (OR 3.47 95% CI [3.06–3.95]) and iatrogenic preterm birth (OR 3.65 95% CI [1.94–6.85]) when compared with healthy controls [13]. A prospective population-based case-control study indicated that women with severe ICP and a singleton pregnancy (n = 5,669) had increased risks of preterm delivery (164/664; 25% vs. 144/2200; 6.5%; AOR 5.39, 95% CI 4.17 to 6.98) [35]. Besides, it has been found that the composition of BAs in the meconium of preterm and term infants was also significantly different [36]. Similarly, Raz y *et al*. indicated that severe ICP could significantly increase the risk of preeclampsia in singleton and twin pregnancies [37]. A study detected serum samples from preeclampsia and normal pregnant women and found remarkable changes in the metabolic group of preeclampsia pregnant women, which including Bas [38]. However, so far as we know, no studies have reported the association between TBA levels within the normal range and the risk of adverse perinatal outcomes. In this study, after excluding the subjects with ICP, the level of TBA, even within the normal rage, was still positively associated with preeclampsia in pregnant women and preterm birth. A significant increase of maternal TBA concentrations would affect the placental clearance of fetal BAs, thus affecting fetal liver metabolism [39]. In addition, animal experiments showed

that BAs stimulated the secretion of the prostate, increase the contractility of uterine muscle and the sensitivity of uterine muscle to oxytocin [40]. The combination of the above factors might induce the occurrence of preterm birth. However, the mechanism by which elevated bile acids increase the risk of preeclampsia remains unclear.

We conducted a well-characterized retrospective cohort study with small selection bias. To the best of our knowledge, this is the first study to explore the association between TBA levels within the normal range with the risk of GDM and adverse perinatal outcomes. However, there are several limitations to the present study. First, the diagnosis of GDM was based on IADPSG criteria, which was still controversial even though it significantly increased identify the rate of GDM and contributed to the management of GDM. Additionally, whether IADPSG criteria is completely suitable for pregnant women in China still needs a large-scale and multicenter study. Second, because of limited data, we could not analyze the association between first trimester TBA levels, as well as the change levels of TBA during pregnancy and the risks of GDM and adverse perinatal outcomes. Further prospective studies are required to determine how changes in TBA levels would affect the GDM development.

Conclusions

In summary, elevated serum TBA level at second trimester increases the risk of GDM and adverse perinatal outcomes in Chinese pregnant women. Although causality cannot be determined, our results demonstrated that serum second-trimester TBA levels might be used as a potential novel biomarker which helps to identify the risk of GDM and adverse perinatal outcomes in pregnant women.

Abbreviations

BAs: Bile acids; cAMP:Cyclic adenosine monophosphate; FGF15:FXR-Fibroblast Growth Factor; FXR:Farnesoid-x-receptor; GDM:Gestational diabetes mellitus; GLP-1:Glucagon like peptide-1; IADPSG:International Association of Diabetes and Pregnancy Study Group; ICP:Intrahepatic cholestasis of pregnancy; NAFLD:Nonalcoholic fatty liver disease; ORs:Odds ratios; PROM:Premature rupture of membranes; T2DM:Type 2 diabetes; TBA:Total bile acid; TGR5:The cell surface G protein coupled bile acid receptor; T-β-MCA:Taurine-β-mouse bile acid.

Declarations

Competing Interest

The authors have no conflicts of interest to declare.

Consent of publication

Not applicable

Ethics approval and consent to participate

The study was approved by the Ethics Committee of the Union Shenzhen Hospital of Huazhong University of Science and Technology. All women recruited in this study were requested for written consent

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Authors' contributions

Guifang Deng and Zheqing Zhang developed the overall research plan and oversaw the study. Yao Liu designed and conducted of the study, and completed the writing of the manuscript. Ruifang Sun and Yuanhuan Wei helped the data collection and processing. Yan Li and Hengying Chen were contributed to the data analysis. The manuscript is the original work of all authors and the final manuscript has been read and approved by all authors. The authors accept full responsibility for the design and conduct of the study, have access to the data, and controlled the decision to publish.

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Figures

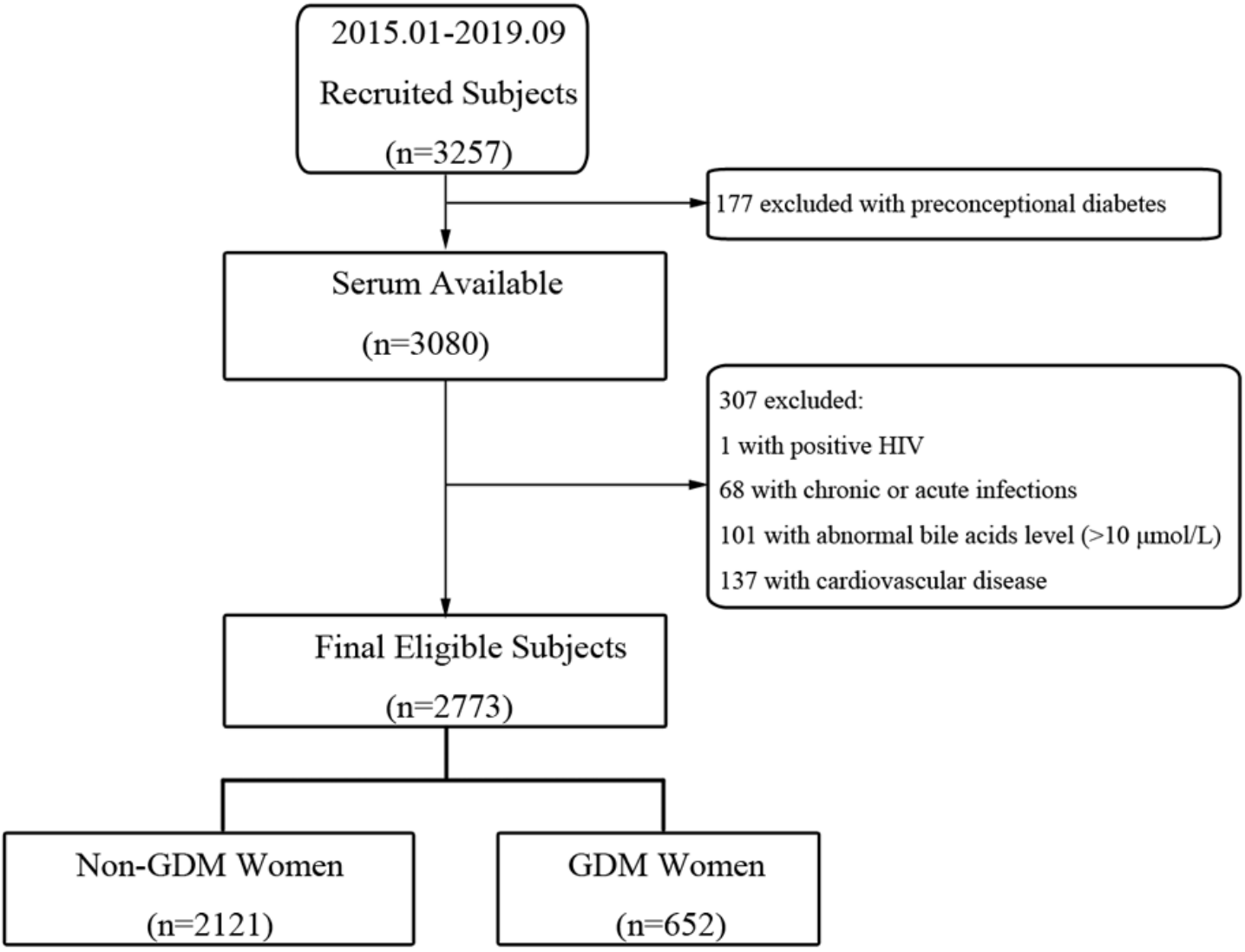

Figure 1

The flowchart of the selection of the study women in the retrospective study.

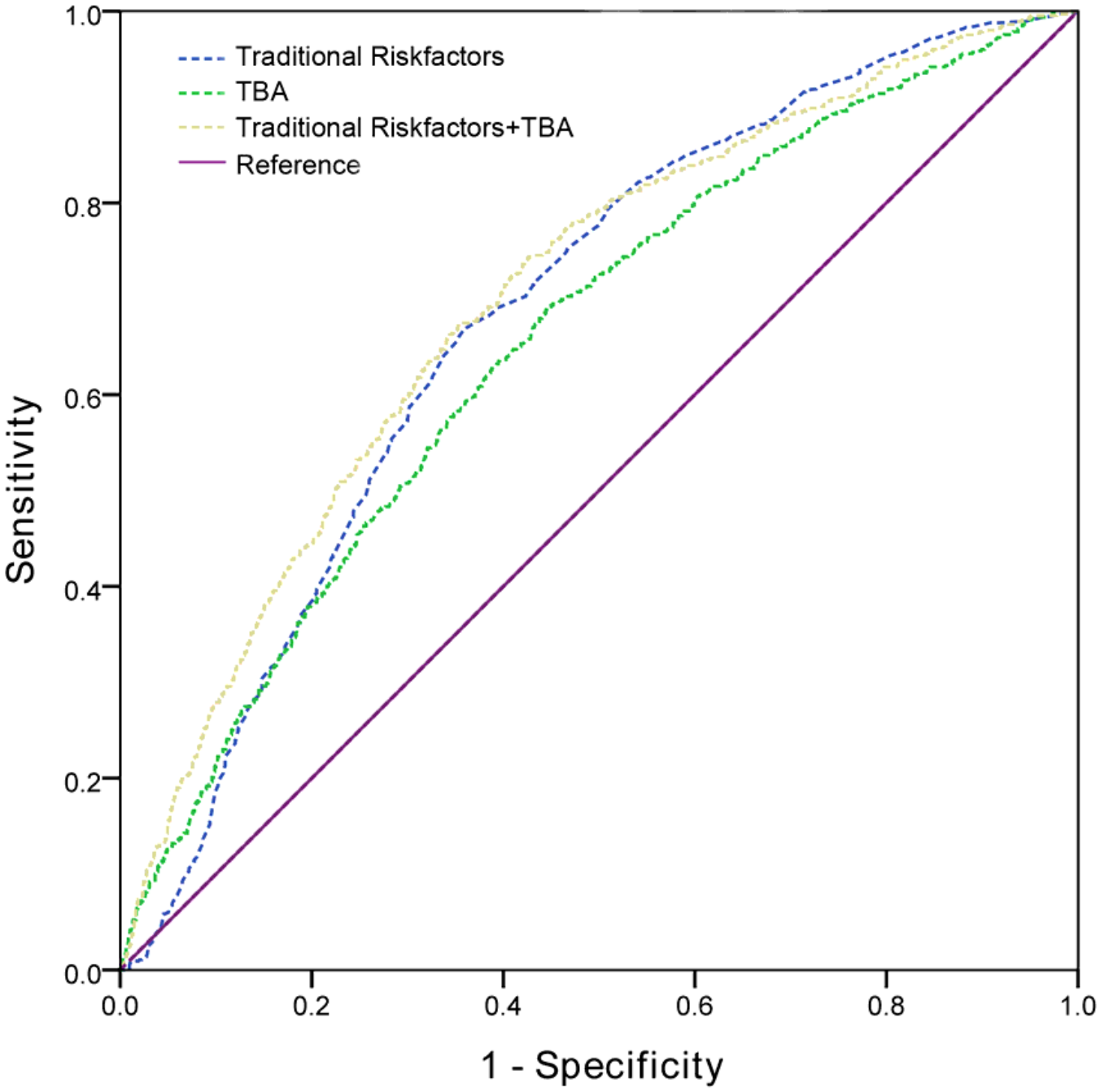

Figure 2

Receiver operating characteristic curves of TBA plus traditional risk factors for gestational diabetes mellitus. Abbreviations: TBA, total bile acid; ROC: receiver operating characteristic curve. Legends: The blue (solid) curve stands for the traditional risk factor model (Multivariable Model 1 in Table 4 for the list of variables); the green (dash-dot) curve for the TBA model, the yellow (dashed) curve for the traditional risk factor plus TBA model. The area under the operating characteristic curve (AUC) was 0.68 (95% CI: 0.66–0.70) for the traditional risk factors model, 0.66 (95% CI: 0.63–0.68) for the traditional risk factors model and 0.70 (95%

CI: 0.68–0.72) for the traditional risk factor plus TBA model (P <0.001 for comparison of the traditional risk factor plus TBA model with either of the other two models).

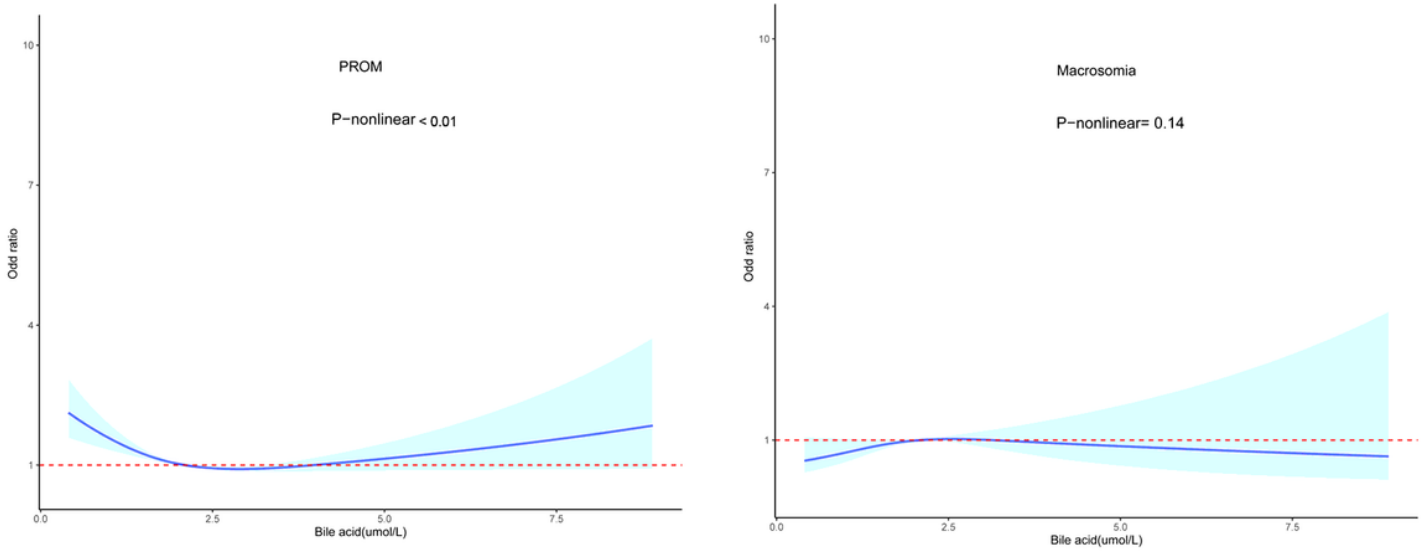

Figure 3

Non-linear relation of TBA and adverse outcomes, derived from a multivariable-adjusted spline logistic regression model. The solid line indicates OR for bile acid, with corresponding 95% CIs indicated by the shaded area. Abbreviations: TBA, total bile acid; PROM, premature rupture of membranes; OR, odds ratio.

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