Clinica Chimica Acta 489 (2019) 130–135

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/cca

Associations of serum markers screening for Down's syndrome with pregnancy outcomes: A Chinese retrospective cohort study

Xiaosong Yuan[a], Wei Long[a], Jianbing Liu[a], Bin Zhang[a], Wenbai Zhou[a], Jian Jiang[b], Bin Yu[a,*], Huiyan Wang[b,**]

[a] Department of Prenatal Diagnosis Laboratory, Changzhou Maternity and Child Health Care Hospital Affiliated to Nanjing Medical University, No. 16 Bo Ai Road, Changzhou, Jiangsu Province, China

[b] Department of Obstetrics and Gynecology, Changzhou Maternity and Child Health Care Hospital Affiliated to Nanjing Medical University, No. 16 Bo Ai Road, Changzhou, Jiangsu Province, China

ARTICLE INFO

Keywords:
Biomarker
Pre-eclampsia
Pre-term delivery
Intrahepatic cholestasis of pregnancy
Small for gestational age infants
Large for gestational age infants

ABSTRACT

Background: We examined the associations between Down's serum screening analytes and pregnancy outcomes in Chinese women.

Methods: A retrospective cohort study of 2470 pregnant women was conducted. Maternal serum triple tests (AFP, fβ-hCG, uE3), maternal characteristics and pregnancy outcomes were recorded from our prenatal screening and hospitalization information system, respectively.

Results: The elevated concentration of uE3 in the early-second trimester was associated with increased risk of LGA infants and macrosomia, decreased risk of PE and small SGA infants (for LGA: OR: 1.34, 95% CI: 1.09–1.65; for macrosomia: OR:1.39, 95% CI: 1.08–1.78; for PE: OR: 0.61, 95% CI: 0.40–0.95; for SGA: OR: 0.35, 95% CI: 0.25–0.49). The increased ratio of AFP/uE3 was associated with reduced risk of GDM in the study populations (BMI ≥ 25; OR: 0.96, 95% CI: 0.0.93–1.00). The higher ratio of AFP/fβ-hCG + uE3 associated with increased risk of SGA infants and ICP in these subjects (BMI ≥ 25) was also observed (for SGA: OR: 1.11, 95% CI: 1.03–1.18; for ICP: OR: 1.27, 95% CI: 1.06–1.53).

Conclusions: Down's serum screening analytes were associated with pregnancy outcomes in Chinese population and might provide an alternative tools for risk estimates on these unfavorable outcomes.

1. Introduction

Serum screening tests for Down's syndrome in the early second-trimester are routinely provided to pregnant women in western countries and China, including the double test [α-fetoprotein (AFP), free β-human chorionic gonadotropin (fβ-hCG)], the triple test with the addition of unconjugated estriol (uE3) and the quadruple test added by inhibin A [1,2].

These associations between maternal serum concentrations of these biochemical markers and abnormal pregnancy outcomes in addition to aneuploidy and open neural tube defects, such as pre-eclampsia (PE), fetal growth restriction (FGR), and preterm birth (PTB), have been reported by most previous studies [3–7]. However, there is still disagreement about their predictive values for the specific complications, and AFP and fβ-hCG are regarded as limited predictors of pregnancy complications [8,9]. Moreover, there is controversy about the association of abnormal concentrations of uE3 with unfavorable perinatal outcomes. For example, no correlation between elevated uE3 concentration and PTB was reported by Jelliffe-Pawlowski et al., however, Oslen et al. observed a direct linkage between high maternal serum uE3 concentrations and a higher incidence of PTB [10,11]. On the other hand, limited data have been published regarding the role of triple screen for Down syndrome in second-trimester as a predicting tool for pregnancy outcomes in Chinese pregnant women.

Abbreviations: AFP, α-fetoprotein; fβ-hCG, free β-human chorionic gonadotropin; uE3, unconjugated estriol; LGA, large for gestational age; SGA, small for gestational age; PE, pre-eclampsia; PTB, preterm birth; FTB, full term birth; GDM, gestational diabetes mellitus; ICP, intrahepatic cholestasis of pregnancy; PIH, pregnancy-induced hypertension; FGR, fetal growth restriction; NPC, non-pregnancy complication

* Corresponding author.

** Correspondence to: H. Wang, Department of Obstetrics and Gynecology, Changzhou Maternity and Child Health Care Hospital Affiliated to Nanjing Medical University, No. 16 Bo Ai Road, Changzhou 213003, Jiangsu Province, China.

E-mail addresses: ybcz0519@163.com (B. Yu), 1429249854@qq.com (H. Wang).

https://doi.org/10.1016/j.cca.2018.12.001
Received 25 October 2018; Received in revised form 22 November 2018; Accepted 5 December 2018
Available online 06 December 2018

In this observational study, we retrospectively investigated the potential associations between maternal concentrations of Down's serum screening analytes and detailed unfavorable obstetric outcomes including main pregnancy complications [gestational diabetes mellitus (GDM), intrahepatic cholestasis of pregnancy (ICP), PE, pregnancy-induced hypertension (PIH)] and perinatal outcomes [PTB, small for gestational age (SGA), and large for gestational age (LGA) infants, macrosomia].

2. Materials and methods

2.1. Study participants enrollment

A total of 2634 consecutive pregnant women who received routine screening for Down's syndrome and gave birth at Changzhou Maternity and Child Health Care Hospital was recruited in this retrospective observational cohort study from October 2015 to March 2017. The study protocol was approved by the ethics committee of Changzhou Maternity and Child Health Care Hospital. The written informed consent was obtained from all participants included in the study. The cohort was established based on following inclusion and exclusion criteria. Inclusion criteria included: 1) pregnancy at 16–18 gestational weeks; 2) integrated and clear medical records; 3) singleton pregnancy and live birth born without birth defects; 4) natural conception. Exclusion criteria were: 1) multiple pregnancy; 2) conception with assisted reproductive technology; 3) diabetes mellitus (type 1 or 2), chronic hypertension, thyroid diseases, chronic heart, liver and kidney diseases, immune rheumatic disease or thyroid diseases and syphilis before pregnancy; 4) cigarette smoking and alcohol drinking during pregnancy. Sixty-three of the 2634 observational subjects were excluded as they conceived with assisted reproductive technology. Also excluded were 101 cases for thyroid diseases (n = 45), chronic heart, liver and kidney diseases (n = 22), diabetes mellitus and chronic hypertension (n = 18), immune rheumatic disease (n = 10), and syphilis (n = 6). These diseases were regarded as potential confounders leading to adverse pregnancy outcomes and therefore were excluded. Finally, a total of 2470 eligible pregnant women was included in this retrospectively observational study. Data of maternal gestational age, height, weight at delivery, gravidity, parity, history of important pregestational diseases, history of cigarette smoking and alcohol consumption, pregnancy complications, delivery mode, perinatal outcomes including newborn birth weight and sex was reviewed and collected from our hospitalization information system. Information on maternal age, gestational age and BMI at Down's syndrome screening were recorded from the prenatal screening system. None of the observational subjects smoked and drank alcohol during gestation.

2.2. Laboratory measurements

For Down's syndrome screening, serum samples from were routinely collected at 16th–20th weeks of gestation, and prepared in according with the standard operating procedure. The maternal serum triple tests (AFP, uE3, fβ-hCG) were performed by an automatic time-resolved fluorescence immunoassay (Wallac) using commercial kits (for AFP and fβ-hCG: B067-101Z; for uE3: B083-301Z, Perkin-Elmer). The inter- and intra-assay CVs for AFP, fβ-hCG and uE3 were < 2%, < 3% and < 5%, respectively.

2.3. Diagnosis criteria of the outcomes

GDM was diagnosed using a 75 g oral glucose tolerance test (OGTT) at 24–28 weeks gestation with the International Association of Diabetic Pregnancy Study Group (IADPSG) criteria [12]. ICP is always found in the third trimester with characterization of jaundice and pruritus. The diagnosis of ICP was relied on abnormal tests of liver function and increased serum bile acids [13]. Previously normotensive pregnant women with a new-onset hypertension (systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg) developed after 20 weeks of gestation combined with or without significant proteinuria (≥300 mg/24 h) were diagnosed with PE and PIH, respectively [14]. PTB was diagnosed as having a birth happened at < 37 gestational weeks. According to a reference curve described in a recent Chinese cohort study, SGA and LGA referred to infants whose birth weights were respectively < 10th and > the 90th percentile by gestational age [15]. Macrosomia was defined as birth weight > 4000 g.

2.4. Statistical analysis

Continuous variables with skewed distribution and categorical variables were presented as median (interquartile range, IQR) and N (%), respectively. To compare differences of these non-normally distributed continuous variables in according with pregnancy complications and prenatal outcomes, the Mann-Whitney *U* test was performed. Comparison of these categorical variables was applied using the X^2 test. Logistic regression analysis adjusting for potential confounders was used to assess the associations between the concentrations of serum markers screening for Down's syndrome and pregnancy outcomes. GDM, ICP, PE, PIH and SGA, LGA, macrosomia, PTB were respectively considered as main pregnancy complications and abnormal perinatal outcomes. The odds ratios (ORs) and 95% confidence intervals (CIs) for these pregnancy complications were calculated controlling for maternal age, gravidity, parity, gestational age and BMI at Down's syndrome Screening etc. The parameters of PTB were additionally corrected for pregnancy complications, systolic and diastolic BP at delivery. Additionally, the values of SGA, LGA and macrosomia were corrected for gestational weight gain, gestational week and BMI at delivery and infant sex. General linear analysis were used to evaluate the association between fetal birth weight and the concentrations of maternal Down's serum screening analytes in the early-second trimester. The statistical analyses were applied with SPSS 22 statistical software. All $P < 0.05$ were defined statistically significant.

3. Results

3.1. Summary of the study population

The detailed characteristics of mother and their infants according to pregnancy complications and perinatal outcomes were described in Tables 1 and 2, respectively. The incidence of GDM, ICP, PE and PIH in our study were 7.37%, 5.18%, 4.13% and 2.02%, respectively. Forty-three cases had more than one kind of complications. Of the 2470 singleton live births, 209 (8.46%) and 365 (14.78%) were classified as SGA and LGA infants, respectively. 63.16% of SGA infants were female while 65.21% of LGA babies were male. Compared with non-pregnancy complications (NPC) group, BMI at Down's syndrome screening and at delivery, blood pressure at delivery were significantly higher in GDM, PE and PIH groups. GDM group had significantly increasing incidence of LGA infants and macrosomia as compared to the NPC group. On the contrary, higher incidence of SGA and low birthweight infants (birth weight < 2500 g) was observed in the PE group as compared to the NPC group.

3.2. Maternal serum uE3, AFP and fβ-hCG concentrations

The concentrations of serum markers screening for Down's syndrome in the early second trimester by pregnancy complications and perinatal outcomes were respectively presented in Tables 3 and 4. The concentrations of uE3 and AFP in maternal serum were significantly lower in the GDM than in the NPC group [median (IQR) for uE3: 1.77 (1.43–2.16) ng/ml vs. 1.89 (1.50–2.36) ng/ml, $P < 0.01$; for AFP: 43.99 (34.72–54.32) ng/ml, vs. 48.40 (38.64–61.46) ng/ml, $P < 0.01$, respectively]. Also, the PE group had statistically significantly lower

Table 1
Maternal and fetal characteristics in the study populations according to pregnancy complications.

	NPC	GDM	ICP	PE	PIH
Subjects [N(%)]	2051 (83.04)	182 (7.37)	128 (5.18)	102 (4.13)	50 (2.02)
Maternal age at delivery (years)	28 (26–30)	29 (27–31)**	27 (26–29)	27 (25–30)	28 (26–29)
< 20 [N(%)]	12 (0.59)	0	1 (0.78)	1 (0.98)	0
20–34 [N(%)]	2035 (98.63)	181 (99.45)	127 (98.22)	100 (98.04)	50 (100)
≥35 [N(%)]	16 (0.78)	1 (0.55)	0	1 (0.98)	0
BMI at delivery (kg/m2)	26.93 (25–29.17)	28.01 (25.39–30.36)**	26.31 (24.48–28.96)	29.36 (26.37–31.81)**	29.05 (27.63–32.37)**
BMI at Down's syndrome screening (kg/m^2)	21.67 (19.92–23.63)	23.48 (21.30–26.22)**	21.08 (19.64–23.22)*	23.78 (21.66–26.91)**	24.28 (21.80–27.34)**
< 25 [N(%)]	1735 (84.59)	117 (64.29)**	112 (87.5)	62 (60.78)**	30 (60)**
≥25 [N(%)]	316 (15.41)	65 (35.71)**	16 (12.5)	40 (39.22)**	20 (40)**
Gravidity	2 (1–2)	2 (1–3)	1 (1–2)	2 (1–2)	1 (1–2)**
< 3 [N(%)]	1572 (76.65)	124 (68.13)*	67 (52.34)	80 (78.43)	41 (82)
≥3 [N(%)]	479 (23.35)	58 (31.87)*	61 (47.66)	22 (21.57)	9 (18)
Parity	1 (1–2)	1 (1–3)	1 (1–2)	1 (1–2)	1 (1–1)**
No child [N(%)]	1316 (64.16)	112 (61.54)	87 (67.97)	75 (73.53)	43 (86)**
≥1 child [N(%)]	735 (35.84)	70 (38.56)	41 (32.03)	27 (26.47)	7 (14)**
Gestational weight gain (kg)[a]	13.1 (10.5–16)	10.8 (8–13)**	12.45 (10–16)	13 (9.5–16.6)	14.55 (11.7–18)
Gestational age at Down's syndrome screening (day)	122 (118–126)	121 (118–125)*	124 (119–128)**	122 (117–125)	122 (118–126)
Gestational age at delivery (week)	39 (38–40)	39 (38–40)**	39 (38–40)	38 (37–40)**	39 (38–40)
Systolic BP at delivery (mmHg)	118 (110–127)	121 (110 − 130)**	121 (110–130)**	145 (138–156)**	140 (134–145)**
Diastolic BP at delivery (mmHg)	70 (70–78)	73 (70–81)**	72 (70–80)	91 (84–100)**	87 (80–92)**
Delivery mode					
Vaginal delivery	1118 (54.51)	75 (41.21)**	68 (53.13)	35 (34.31)**	31 (62.00)
Cesarean section	933 (45.49)	107 (58.79)**	60 (46.87)	67 (65.69)**	19 (38.00)
PTB	63 (3.07)	7 (3.85)	9 (7.03)*	21 (20.59)**	1 (2.00)
FTB	1988 (96.93)	175 (96.15)	119 (92.97)*	81 (79.41)**	49 (98.00)
Infant's sex					
Female	977 (47.64)	84 (46.15)	62 (48.44)	49 (48.04)	21 (42)
Male	1074 (52.36)	98 (53.85)	66 (51.56)	53 (51.96)	29 (58)
Fetal birth weight (gram)	3400 (3130–3660)	3440 (3103–3823)	3355 (3055–3708)	3120 (2698–3500)**	3405 (3143–3713)
< 2500	54 (2.63%)	1 (0.55)	7 (5.47)	16 (15.69)**	2 (4.00)
2500–4000	1837 (89.57%)	155 (85.16)	111 (86.72)	82 (80.39)**	43 (86.00)
> 4000	160 (7.80%)	26 (14.29)**	10 (7.81)	4 (3.92)	5 (10.00)
Weight for gestational age					
SGA	171 (8.34%)	9 (4.95)	15 (11.72)	18 (17.65)**	2 (4.00)
AGA	1597 (77.86%)	126 (69.23)	91 (71.09)	70 (70.59)	43 (86.00)
LGA	283 (13.80%)	47 (25.82)**	22 (17.19)	12 (11.76)	5 (10.00)

Notes: Data was presented as median (IQR) or N(%). *$P < 0.05$, **$P < 0.01$, compared with NPC group.
Abbreviations: IQR, interquartile range; NPC, non-pregnancy complication; GDM, gestational diabetes mellitus; ICP, intrahepatic cholestasis of pregnancy; PE, Pre-eclampsia; PIH, pregnancy-induced hypertension; BMI, body mass index; BP, blood pressure; PTB, pre-term birth; FTB, full term birth; SGA/AGA/LGA small/appropriate/large for gestational age.
[a] Gestational weight gain in pregnancy from Down's syndrome Screening to delivery.

concentration of uE3 than the NPC group [median (IQR) for uE3: 1.71 (1.40–2.10) ng/ml vs. 1.89 (1.50–2.36) ng/ml, $P < 0.01$]. However, the concentrations of uE3 and AFP in the ICP group were significantly higher than those in the NPC group [median (IQR) for uE3: 2.03 (1.65–2.58) ng/ml vs. 1.89 (1.50–2.36) ng/ml, $P < 0.01$; for AFP: 53.75 (43.53–67.58) ng/ml, vs. 48.40 (38.64–61.46) ng/mL, $P < 0.01$, respectively]. The increased AFP/fβ-hCG and AFP/fβ-hCG + uE3 ratios were also presented in the ICP group. There was no significant difference in three biomarkers and their ratios between NPC group and PIH group. Table 4 showed that both AFP concentration and these ratios of AFP/fβ-hCG and AFP/fβ-hCG + uE3 in the PTB group were significantly higher than those in the FTB group [median (IQR) for AFP: 54.33 (38.64–65.36) ng/ml, vs. 48.04 (38.38–61.00) ng/ml, $P < 0.05$; for AFP/fβ-hCG: 4.80 (2.73–7.58) vs. 3.94 (2.50–5.98); for AFP/fβ-hCG + uE3: 4.04(2.43–5.62) vs.3.39(2.25–4.85), respectively]. Compared with the appropriate for gestational age (AGA) group, the concentration of uE3 in the SGA group decreased and the AFP/uE3 ratio increased, significantly. In addition, the higher uE3 concentration and the lower ratio of AFP/uE3 were found in the LGA group by comparison with AGA group. However, there was no significant difference between LGA group and AGA group.

3.3. Associations between serum markers screening for Down's syndrome and abnormal pregnancy outcomes

Table 5 presented the associations between serum markers screening for Down's syndrome in the early second trimester and pregnancy complications. The results showed that the elevated concentration of uE3 in the early-second trimester was associated with increased risk of LGA and macrosomia, decreased risk of PE and SGA (for LGA: OR: 1.34, 95% CI: 1.09–1.65; for macrosomia: OR: 1.39, 95% CI: 1.08–1.78; for PE: OR: 0.61, 95% CI: 0.40–0.95; for SGA: OR: 0.35, 95% CI: 0.25–0.49). Also, SGA was significantly associated with the increased ratios of AFP/fβ-hCG, AFP/uE3 and AFP/fβ-hCG + uE3 ((OR: 1.06, 95% CI: 1.01–1.10; OR: 1.03, 95% CI: 1.02–1.05; OR: 1.11, 95% CI: 1.03–1.18), respectively). The increased ratio of AFP/uE3 was also significantly associated with an elevated risk of PE and PTB (all OR: 1.01, 95% CI: 1.00–1.02), a reduced risk of GDM in the study populations who had a parameter of BMI ≥25 at Down's syndrome screening (OR: 0.96, 95% CI: 0.93–1.00). Additionally, the high ratio of AFP/fβ-hCG + uE3 associated with increased risk of ICP was found in these study objects whose BMI of Down's syndrome screening was ≥25 (OR: 1.27, 95% CI: 1.06–1.53). In contrast, these significant associations between PIH and the concentrations of serum biomarkers screening for Down's syndrome was not observed.

Table 6 showed regression coefficients for maternal Down's serum screening analytes concentrations with neonatal birth weight. No significantly statistical associations of maternal serum AFP concentrations with fetal birth weight was found in our study. The neonatal birth weight was significantly positive correlated with maternal uE3 and fβ-hCG concentrations at Down's syndrome Screening (for uE3: β: 88.95, 95% CI: 63.80–114.10; for fβ-hCG: β: 2.14, 95% CI: 0.89–3.39).

Table 2
Maternal and neonatal characteristics in the study populations in according to perinatal outcomes.

	FTB	PTB[b]	AGA	SGA[c]	LGA[c]
Subjects [N(%)]	2376 (96.19)	94 (3.81)	1896 (76.76)	209 (8.46)	365 (14.78)
Maternal age at delivery (y)	28 (26–30)	27 (25–29)	27 (26–30)	27 (25–29)	28 (26–31)**
< 20 [N(%)]	14 (0.59)	0	9 (0.47)	3 (1.44)	2 (0.55)
20–34 [N(%)]	2345(98.70)	93 (98.94)	1875 (98.90)	204 (97.61)	359 (98.36)
≥35 [N(%)]	17 (0.71)	1 (1.06)	12 (0.63)	2 (0.95)	4 (1.09)
BMI at delivery (kg/m^2)	27.05 (25.12–29.34)	26.35 (24.18–29.81)	26.9 (25.00–29.07)	25.65 (23.63–28.09)**	28.56 (26.64–31.18)**
BMI at Down's syndrome screening (kg/m2)	21.83 (20.04–23.95)	21.92 (19.94–25.71)	21.72 (19.97–23.83)	20.76 (19.27–22.53)**	23.13 (21.30–25.55)**
< 25 [N(%)]	1966 (82.74)	68 (72.34)*	1585 (83.60)	192 (91.87)**	257 (70.41)**
≥25 [N(%)]	410 (17.36)	26 (27.66)*	311 (16.40)	17 (8.13)**	108 (29.59)**
Gravidity	2 (1–2)	2 (1–2)	2 (1–2)	1 (1–2)	2 (1–3)**
< 3 [N(%)]	1815 (76.39)	71 (75.53)	1487 (78.43)	165 (78.95)	234 (64.11)**
≥3 [N(%)]	561 (23.61)	23 (24.47)	409 (21.57)	44 (21.05)	131 (35.89)**
Parity	1 (1–2)	1 (1–2)	1 (1–2)	1 (1–2)*	2 (1–2)**
No child [N(%)]	1538 (64.73)	64 (68.09)	1267 (66.82)	154 (73.68)	181 (49.59)**
≥1 child [N(%)]	838 (35.27)	30 (31.91)	629 (33.18)	55 (26.32)	184 (50.41)**
Gestational weight gain (kg)[a]	13.0 (8.1–13.8)	10.5 (8.1–13.8)**	13.0 (10.0–16.0)	12.0 (9.4–15.0)**	14.0 (11.0–17.5)**
Gestational age at Down's syndrome Screening (day)	122 (118–126)	124 (119–128)*	122 (118–126)	122 (117–126)	122 (118–125)
Gestational age at delivery (week)	39 (38–40)	35 (33–36)**	39 (38–40)	39 (38–40)	39 (38–39)**
Systolic BP at delivery (mmHg)	120 (110–128)	128 (115–136)**	120 (110–129)	119 (110–130)	118 (110–128)
Diastolic BP at delivery (mmHg)	71 (70–79)	75 (70–85)**	72 (70–79)	73 (70–80)	70 (70–77)*
Delivery mode					
Vaginal delivery	1267 (53.32)	50 (42.55)*	1048 (55.27)	133 (63.64)*	126 (34.52)**
Cesarean section	1109 (46.68)	54 (57.45)*	848 (44.73)	76 (36.37)*	239 (65.48)**
Infant's sex					
Female	1136 (47.81)	40 (42.55)	917 (48.36)	132 (63.16)**	127 (34.79)**
Male	1240 (52.19)	54 (57.45)	976 (51.64)	77 (36.84)**	238 (65.21)**
Fetal birth weight (gram)	3410 (3150–3680)	2385 (1953–2745)**	3360 (3150–3560)	2800 (2570–2915)**	4030 (3810–4225)**
GDM	175 (7.37)	7 (7.45)	126 (6.65)	9 (4.31)	47 (12.88)**
ICP	119 (5.00)	9 (9.57)*	91 (4.80)	15 (7.18)	22 (6.03)
PE	81 (3.41)	21 (22.34)**	72 (3.80)	18(8.61)**	12 (3.29)
PIH	49 (2.06)	1 (1.06)	43 (2.27)	2 (0.96)	5 (1.37)

Notes: Data was presented as median (IQR) or N(%).
Abbreviations: IQR, interquartile range; FTB, full term birth; PTB, pre-term birth; SGA/AGA/LGA, small/appropriate/large for gestational age; BMI, body mass index; BP, blood pressure; GDM, gestational diabetes mellitus; ICP, intrahepatic cholestasis of pregnancy; PE, Pre-eclampsia; PIH, pregnancy-induced hypertension.
[a] Gestational weight gain in pregnancy from Down's syndrome screening to pre-delivery.
[b] compared with FTB group.
[c] compared with AGA group.
* $P < 0.05$.
** $P < 0.01$.

Furthermore, birth weight was negatively correlated with the ratios of AFP/uE3, AFP/fβ-hCG and AFP/fβ-hCG + uE3 (for AFP/uE3: β: -3.28, 95% CI: -4.42 to -2.13; for AFP/fβ-hCG: β: -5.81, 95% CI: -10.08 to -1.55; for AFP/fβ-hCG + uE3: β: -10.08, 95% CI: -17.10 to -3.07).

4. Discussion

The fβ-hCG and uE3 are secreted by the placenta and the transfer of AFP into the maternal peripheral blood is also tightly regulated by the placenta. Disorders of placenta in function can contribute to both abnormal concentrations of these biochemical analytes and unfavorable pregnancy outcomes. In our retrospective study, we not only investigated these associations of pregnancy outcomes with abnormalities of AFP and fβ-hCG, which have been widely reported, but also determined the correlation between maternal uE3 concentrations and adverse obstetric outcomes described by few published data [16–18]. This study verified maternal uE3 concentrations in the early second trimester were negatively associated with the risk of PE and SGA infants in Chinese population, which was consistent with previous reports in other ethnicities/races [19]. Furthermore, our study suggested maternal high uE3 concentrations were significantly associated with increased risk for LGA babies and macrosomia. As we know, this is the earlier retrospective study correlated increased uE3 concentrations in the second trimester with LGA infants and macrosomia. Also, we report earlier the relationship between the AFP/uE3 ratio and adverse pregnancy outcomes, including GDM, PE, PTB and SGA.

Table 3
Maternal serum uE3, AFP, fβ-hCG concentrations and their combination by pregnancy complications.

	NPC (N = 2051)	GDM (N = 182)	ICP (N = 128)	PE (N = 102)	PIH (N = 50)
uE3 (ng/ml)	1.89 (1.50–2.36)	1.77 (1.43–2.16)**	2.03 (1.65–2.58)**	1.71 (1.40–2.10)**	1.74 (1.47–2.12)
AFP (ng/ml)	48.40 (38.64–61.46)	43.99 (34.72–54.32)**	53.75 (43.53–67.58)**	46.42 (36.00–60.31)	44.59 (36.07–59.14)
fβ-hCG (ng/ml)	12.20 (8.44–18.72)	12.55 (7.69–19.82)	12.70 (8.56–17.23)	13.71 (9.22–18.66)	12.19 (6.68–18.74)
AFP/fβ-hCG	3.97 (2.56–6.08)	3.61 (2.25–5.55)	4.61 (2.96–6.42)*	3.51 (2.06–5.63)	4.14 (2.50–6.16)
AFP/uE3	25.42 (19.98–33.37)	24.38 (19.30–32.81)	26.50 (20.36–31.99)	27.22 (21.30–35.86)	24.37 (19.51–33.33)
AFP/fβ-hCG + uE3	3.39 (2.28–4.90)	3.17 (2.04–4.62)	3.66 (2.59–5.31)*	3.28 (1.92–4.63)	3.41 (2.30–4.93)

Notes: Data was presented as median (IQR). *$P < 0.05$, **$P < 0.01$ compared with NPC group.
Abbreviations: IQR, interquartile range; NPC, non-pregnancy complication; GDM, gestational diabetes mellitus; ICP intrahepatic cholestasis of pregnancy; PE, Pre-eclampsia; PIH, pregnancy-induced hypertension;

Table 4
Maternal uE3, AFP, fβ-hCG concentrations and their combination by pregnancy perinatal outcomes.

	FTB (N = 2376)	PTB[a] (N = 94)	AGA (N = 1896)	SGA[b] (N = 209)	LGA[b] (N = 365)
uE3 (ng/ml)	1.87 (1.50–2.34)	1.87 (1.50–2.42)	1.88 (1.51–2.35)	1.67 (1.37–2.13)**	1.95 (1.56–2.40)
AFP (ng/ml)	48.04 (38.38–61.00)	54.33 (38.64–65.36)*	48.16 (38.13–61.11)	49.25 (39.21–68.18)	47.43 (38.90–60.38)
fβ-hCG (ng/ml)	12.26 (8.42–18.60)	11.74 (7.24–19.45)	12.22 (8.52–18.50)	12.90 (8.39–21.48)	11.99 (7.83–17.58)
AFP/fβ-hCG	3.94 (2.50–5.98)	4.80 (2.73–7.58)*	3.94 (2.48–5.97)	3.88 (2.33–6.35)	4.08 (2.74–6.29)
AFP/uE3	25.42 (19.96–33.05)	26.39 (20.32–35.60)	25.27 (19.88–32.85)	29.27 (23.30–39.35)**	24.90 (19.66–30.96)
AFP/fβ-hCG + uE3	3.39 (2.25–4.85)	4.04 (2.43–5.62)*	3.39 (2.24–4.86)	3.37 (2.15–5.16)	3.46 (2.46–4.95)

Notes: Data was presented as median (IQR).
Abbreviations: IQR, interquartile range; FTB, full term birth; PTB, pre-term birth; SGA/AGA/LGA small/appropriate/large for gestational age.
* $P < 0.05$.
** $P < 0.01$.
[a] compared with FTB group.
[b] compared with AGA group.

Based on fewer published data, the association of high uE3 concentrations with PTB has been conflicting. Jelliffe-Pawlowski et al. observed that elevated concentrations of uE3 did not remarkably increase a PTB risk [10]. Conversely, Olsen et al. investigated the relationship between high uE3 and PTB and noted that uE3 concentration had a direct correlation with gestational age at delivery [11]. They got a conclusion that elevated maternal serum concentrations of uE3 in the second trimester is an independent factor that causes increasing of spontaneous PTB. More interestingly, the strongest association between low uE3 and PTB (< 32 weeks) had been reported by Huang et al. [18]. It still remains controversial. Yaron et al. and Settiyanan et al. found there were no statistical difference in the incidence of PTB between the groups of normal and low uE3 concentrations [16,17]. Our results also showed there was almost no difference in uE3 concentration between PTB group and FTB group; and no significant association between PTB and the concentration of uE3 with or without controlling for possible confounders. Increased concentrations of AFP and HCG were correlated with functional disturbance of placenta resulting in abnormal obstetric outcomes [6]. Up till to now, these reported outcomes include hypertension, miscarriage, FGR, fetal death, placental abruption, PE, and PTB [19–24]. Our results showed that PTB group had statistically significantly higher concentration of AFP than the full term birth (FTB) group; maternal high AFP concentrations significantly increased the risk of PTB and SGA infants supporting previous studies. Also, our study demonstrated clearly significant difference in AFP concentration between NPC group and GDM or ICP group. However, AFP was not independent risk factors of GDM and ICP when examined by logistic regression analysis controlling for possible confounders. Furthermore, we observed no difference in fβ-hCG concentration between groups either by complication outcomes or by perinatal outcomes. The inconsistency of these results could be related to racial factors, strict adjustment for confounders. Another possible explanation was that the nature of the retrospective study and the size of the study population lead to inconsistent results. It is necessary to investigate the potential

Table 6
Regression coefficients for fetal birth weight associated with Down's serum screening analytes in pregnant women in the early-second trimester.

Variables	β (95%CI)	*P* value
uE3	88.95 (63.80–114.10)	< 0.001
hAFP	0.09 (−0.69–0.87)	0.8282
fβ-hCG	2.14 (0.89–3.39)	0.0008
AFP/fβ-hCG	-5.81 (−10.08 to −1.55)	0.0076
AFP/uE3	−3.28 (−4.42 to −2.13)	< 0.001
AFP/fβ-hCG + uE3	−10.08 (−17.10 to −3.07)	0.0049

Notes: The β value were adjusted for available variables in this study including maternal age, gravidity, parity, gestational age and BMI at Down's syndrome screening, gestational weight gain, GDM, ICP, PE, PIH, systolic and diastolic BP, gestational week and BMI at delivery and infant sex.
Abbreviations: GDM, gestational diabetes mellitus; ICP, intrahepatic cholestasis of pregnancy; PE, Pre-eclampsia; PIH, pregnancy-induced hypertension; BP, blood pressure.

Table 5
Significant influence of variables on pregnancy complications and perinatal outcomes.

Outcomes	N	uE3	AFP	fβ-hCG	AFP/fβ-hCG	AFP/uE3	AFP/fβ-hCG + uE3
		OR (95%CI)	OR (95%CI)	OR (95%CI)	OR (95%CI)	OR (95%CI)	OR (95%CI)
GDM	182	0.93 (0.70–1.25)	0.99 (0.98–1.00)	1.00 (0.99–1.01)	0.99 (0.94–1.03)	0.99 (0.98–1.01)	0.98 (0.91–1.06)
GDM (BMI ≥ 25[a])	65	1.08 (0.61–1.91)	0.98 (0.96–1.00)	0.98 (0.95–1.01)	0.99 (0.91–1.07)	0.96 (0.93–1.00)*	0.97 (0.85–1.11)
ICP	128	1.13 (0.86–1.48)	1.01 (1.00–1.01)	1.00 (0.99–1.02)	1.02 (0.98–1.07)	1.01 (1.00–1.02)	1.06 (0.98–1.14)
ICP (BMI ≥ 25[a])	16	0.93 (0.39–2.20)	1.02 (1.00–1.05)	0.92 (0.83–1.03)	1.08 (0.99–1.18)	1.02 (0.98–1.05)	1.27 (1.06–1.53)**
PE	102	0.61 (0.40–0.95)*	1.01 (1.00–1.01)	1.01 (1.00–1.02)	0.99 (0.93–1.05)	1.01 (1.00–1.02)*	0.99 (0.90–1.09)
PIH	50	0.83 (0.48–1.42)	1.00 (0.99–1.02)	1.00 (0.98–1.03)	1.02 (0.95–1.10)	1.00 (0.98–1.02)	1.03 (0.91–1.17)
PTB	94	1.04 (0.73–1.49)	1.01 (1.00–1.02)*	1.01 (0.99–1.02)	1.04 (0.99–1.09)	1.01 (1.00–1.02)*	1.08 (0.98–1.18)
SGA	209	0.35 (0.25–0.49)**	1.01 (1.00–1.01)	1.00 (0.99–1.01)	1.06 (1.01–1.10)**	1.03 (1.02–1.05)**	1.11 (1.03–1.18)**
LGA	365	1.34 (1.09–1.65)**	1.00 (1.00–1.01)	1.01 (1.00–1.02)**	0.98 (0.94–1.01)	0.99 (0.98–1.00)	0.96 (0.90–1.02)
Macrosomia	203	1.39 (1.08–1.78)*	1.00 (0.99–1.01)	1.02 (1.00–1.03)**	0.96 (0.91–1.01)	0.98 (0.97–1.00)*	0.93 (0.86–1.01)

Notes: Odds ratios were adjusted for maternal age, gravidity, parity, gestational age and BMI at Down's syndrome Screening. Parameters of PTB were additionally corrected for GDM, ICP, PE, PIH, systolic and diastolic BP at delivery. The values of SGA, LGA and macrosomia were additionally corrected for gestational weight gain, gestational week and BMI at delivery and infant sex.
Abbreviations: GDM, gestational diabetes mellitus; BMI, body mass index; ICP intrahepatic cholestasis of pregnancy; PE, Pre-eclampsia; PIH, pregnancy-induced hypertension; PTB, pre-term birth; SGA/LGA small/large for gestational age.
[a] Study populations who had a parameter of BMI ≥ 25 at Down's syndrome screening.
* $P < 0.05$.
** $P < 0.01$.

mechanisms of this inconsistency by further parallel studies.

The serum analytes assessed in conventional aneuploidy screening have independently been found to have a statistically significant association with adverse obstetric outcome. Furthermore, the combination of biomarkers may obtain the best predictive performance in predicting complications of late pregnancy, such as PE, spontaneous PTB, GDM, and FGR [25]. For example, the ratio of soluble fms-like tyrosine kinase 1 to placental growth factor have recently been acknowledged as a biomarker of PE [26]. We hypothesized that using these markers in combination (their ratios, etc.) might improve their predictive value and ultimately produced a useful screening tool. Among the study population (BMI ≥25), we unexpectedly found that the ratio of AFP/uE3 was significantly negatively associated with risk of GDM and that the ratio of AFP/fβ-hCG + uE3 was significantly positively associated with the risk of ICP.

We purposed on analyzing the association of maternal serum screening analytes for Down's syndrome in the second trimester and the risks of unfavorable pregnant outcomes. The strengths of this study are: 1) sufficient sample size of the pregnant women with detailed pregnant and perinatal outcomes including GDM, PE, ICP, SGA and LGA, etc. 2) All laboratory analysis were carried out by the same personal in the same environment using the same automatic analyzer. 3) Our hospital network including rural and urban residents, reflected the general population rather than a referral group with high-risk. However, our study has 2 limitations. Firstly, our study population lacked diversity. Considering that China is a country with large population and unbalanced district economic development, a large sample of multicenter study is more representative. Secondly, the data source did not provide information on other maternal characteristics that have been shown to be independent impact factors of these unfavorable outcomes, such as maternal education concentration and family economics.

5. Conclusion

We used maternal serum screening analytes for Down's syndrome in the second trimester as biomarkers to investigate associations between the concentrations of analytes and pregnancy outcomes. We found that abnormal concentrations of second trimester AFP, fβ-hCG, uE3 and their ratios were associated with unfavorable pregnancy outcomes. The serum biomarkers investigated in our study prove to have additional value apart from Down syndrome screening and may provide an alternative tool for risk estimates on other adverse pregnancy outcomes.

Acknowledgments

We thank all of the project participants for their contributions. This study was funded by Changzhou science and technology support project (Social Development CE20175021) and key project of science and technology development fund of Nanjing Medical University (2017NJMUZD044). Funders did not play a role in study design, sample collection and analysis, date statistics or manuscript preparation.

References

[1] F.D. Malone, J.A. Canick, R.H. Ball, D.A. Nyberg, C.H. Comstock, R. Bukowski, R.L. Berkowitz, S.J. Gross, L. Dugoff, S.D. Craigo, I.E. Timor-Tritsch, S.R. Carr, H.M. Wolfe, K. Dukes, D.W. Bianchi, A.R. Rudnicka, A.K. Hackshaw, G. Lambert-Messerlian, N.J. Wald, M.E. D'Alton, First-trimester or second-trimester screening, or both, for Down's syndrome, N. Engl. J. Med. 353 (2005) 2001–2011.

[2] Z.Y. Miao, X. Liu, T.K. Shi, Y. Xu, Q.H. Song, S.H. Tang, First trimester, second trimester, and integrated screening for Down's syndrome in China, J. Med. Screen. 19 (2012) 68–71.

[3] J.L. Cohen, K.E. Smilen, A.T. Bianco, E.L. Moshier, L.A. Ferrara, J. L. Stone, Predictive value of combined serum biomarkers for adverse pregnancy outcomes, Eur. J. Obstet. Gynecol. Reprod. Biol. 181 (2014) 89–94.

[4] Y. Zhong, F. Zhu, Y. Ding, Serum screening in first trimester to predict pre-eclampsia, small for gestational age and preterm delivery: systematic review and meta-analysis, BMC Pregnancy Childbirth 15 (2015) 191.

[5] P. Puntachai, C. Wanapirak, S. Sirichotiyakul, F. Tongprasert, K. Srisupundit, S. Luewan, K. Traisrisilp, T. Tongsong, Associations between pregnancy outcomes and unexplained high and low maternal serum alpha-fetoprotein concentrations, Arch. Gynecol. Obstet. 292 (2015) 81–85.

[6] S. Tancrède, E. Bujold, Y. Giguère, M.H. Renald, J. Girouard, J.C. Forest, Mid-trimester maternal serum AFP and hCG as markers of preterm and term adverse pregnancy outcomes, J. Obstet. Gynaecol. Can. 37 (2015) 111–116.

[7] E. Yefet, O. Kuzmin, N. Schwartz, F. Basson, Z. Nachum, Predictive value of second-trimester biomarkers and maternal features for adverse pregnancy outcomes, Fetal Diagn. Ther. 42 (2017) 285–293.

[8] W. Yuan, L. Chen, A.L. Bernal, Is elevated maternal serum alpha-fetoprotein in the second trimester of pregnancy associated with increased preterm birth risk? A systematic review and meta-analysis, Eur. J. Obstet. Gynecol. Reprod. Biol. 145 (2009) 57–64.

[9] L. Dugoff, J.C. Hobbins, F.D. Malone, J. Vidaver, L. Sullivan, J.A. Canick, G.M. Lambert-Messerlian, T.F. Porter, D.A. Luthy, C.H. Comstock, G. Saade, K. Eddleman, I.R. Merkatz, S.D. Craigo, I.E. Timor-Tritsch, S.R. Carr, H.M. Wolfe, M.E. D'Alton, Quad screen as a predictor of adverse pregnancy outcome, Obstet. Gynecol. 106 (2005) 260–267.

[10] L.L. Jelliffe-Pawlowski, G.M. Shaw, R.J. Currier, D.K. Stevenson, R.J. Baer, H.M. O'Brodovich, J.B. Gould, Association of early-preterm birth with abnormal concentrations of routinely collected first and second-trimester biomarkers, Am. J. Obstet. Gynecol. 208 (2013) e1–11.

[11] R.N. Olsen, R. Dunsmoor-Su, D. Capurro, K. McMahon, M.G. Gravett, Correlation between spontaneous preterm birth and mid-trimester maternal serum estriol, J. Matern. Fetal Neonatal Med. 27 (2014) 376–380.

[12] B.E. Metzger, S.G. Gabbe, B. Persson, T.A. Buchanan, P.A. Catalano, P. Damm, A.R. Dyer, Ad. Leiva, M. Hod, J.L. Kitzmiler, L.P. Lowe, H.D. McIntyre, J.J. Oats, Y. Omori, M.I. Schmidt, International association of diabetes and pregnancy study groups recommendations on the diagnosis and classification of hyperglycemia in pregnancy, Diabetes Care 33 (2010) 676–682.

[13] C. Williamson, V. Geenes, Intrahepatic cholestasis of pregnancy, Obstet. Gynecol. 124 (2014) 120–133.

[14] E. Eiland, C. Nzerue, M. Faulkner, Preeclampsia 2012, J. Pregnancy 2012 (2012) 586578.

[15] H. Wang, L. Liu, Y.F. Hu, J.H. Hao, Y.H. Chen, P.Y. Su, L. Fu, Z. Yu, G.B. Zhang, L. Wang, F.B. Tao, D.X. Xu, Maternal serum cadmium concentration during pregnancy and its association with small for gestational age infants: a population-based birth cohort study, Sci. Rep. 6 (2016) 22631.

[16] Y. Yaron, M. Cherry, R.L. Kramer, J.E. O'Brien, M. Hallak, M.P. Johnson, M.I. Evans, Second-trimester maternal serum marker screening: maternal serum alpha-fetoprotein, beta-human chorionic gonadotropin, estriol, and their various combinations as predictors of pregnancy outcome, Am. J. Obstet. Gynecol. 181 (1999) 968–974.

[17] T. Settiyanan, C. Wanapirak, S. Sirichotiyakul, F. Tongprasert, K. Srisupundit, S. Luewan, K. Traisrisilp, T. Tongsong, Association between isolated abnormal concentrations of maternal serum unconjugated estriol in the second trimester and adverse pregnancy outcomes, J. Matern. Fetal Neonatal Med. 29 (2016) 2093–2097.

[18] T. Huang, B. Hoffman, W. Meschino, J. Kingdom, N. Okun, Prediction of adverse pregnancy outcomes by combinations of first and second trimester biochemistry markers used in the routine prenatal screening of Down syndrome, Prenat. Diagn. 30 (2010) 471–477.

[19] P.A. Benn, D. Horne, S. Briganti, J.F. Rodis, J.M. Clive, Elevated second-trimester maternal serum hCG alone or in combination with elevated alpha-fetoprotein, Obstet. Gynecol. 87 (1996) 217–222.

[20] K. Spencer, Second-trimester prenatal screening for Down syndrome and the relationship of maternal serum biochemical markers to pregnancy complications with adverse outcome, Prenat. Diagn. 20 (2000) 652–656.

[21] T.G. Krause, P. Christens, J. Wohlfahrt, U. Lei, T. Westergaard, B. Nørgaard-Pedersen, M. Melbye, Second-trimester maternal serum alpha-fetoprotein and risk of adverse pregnancy outcome, Obstet. Gynecol. 97 (2001) 277–282.

[22] S. Chandra, H. Scott, L. Dodds, C. Watts, C. Blight, M. Van Den Hof, Unexplained elevated maternal serum alpha-fetoprotein and/or human chorionic gonadotropin and the risk of adverse outcomes, Am. J. Obstet. Gynecol. 189 (2003) 775–781.

[23] K. Duric, S. Skrablin, J. Lesin, D. Kalafatic, I. Kuvacic, E. Suchanek, Second trimester total human chorionic gonadotropin, alpha-fetoprotein and unconjugated estriol in predicting pregnancy complications other than fetal aneuploidy, Eur. J. Obstet. Gynecol. Reprod. Biol. 110 (2003) 12–15.

[24] L.L. Jelliffe-Pawlowski, R.J. Baer, Y.J. Blumenfeld, K.K. Ryckman, H.M. O'Brodovich, J.B. Gould, M.L. Druzin, Y.Y. El-Sayed, D.J. Lyell, D.K. Stevenson, G.M. Shaw, R.J. Currier, Maternal characteristics and mid-pregnancy serum biomarkers as risk factors for subtypes of preterm birth, BJOG 122 (2015) 1484–1493.

[25] S.C. Kane, Fda.S. Costa, S. Brennecke, First trimester biomarkers in the prediction of later pregnancy complications, Biomed. Res. Int. (2014) (2014) 807196.

[26] S. Verlohren, I. Herraiz, O. Lapaire, D. Schlembach, H. Zeisler, P. Calda, J. Sabria, F. Markfeld-Erol, A. Galindo, K. Schoofs, B. Denk, H. Stepan, New gestational phase-specific cutoff values for the use of the soluble fms-like tyrosine kinase-1/placental growth factor ratio as a diagnostic test for preeclampsia, Hypertension 63 (2014) 346–352.