

Original Research Article

Association of false-positive quadruple test and adverse pregnancy outcomes

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ABSTRACT

Background: Aim of the study was to assess the relationship between false-positive quadruple test results and adverse pregnancy outcomes.

Methods: This retrospective case-control study was analysed data from 600 pregnant women who underwent second-trimester screening. The study compared 100 women with false-positive results (high risk group) to 500 women with true-negative results (low risk group). Odds ratios (OR) and adjusted OR (aOR) for various adverse pregnancy outcomes were calculated and compared between the groups.

Results: In this study, from 600 pregnant women in 2 groups. Some associations between high-risk group and adverse pregnancy outcomes can be found. The adjusted odds of developing gestational hypertension were significantly higher in the false-positive group (aOR 2.36, 95% CI: 1.07-5.18). Similarly, the risk of having a newborn with a low birth weight was also higher in this group (aOR 2.77, 95% CI: 1.06-7.24). No significant differences were observed between two groups regarding the rates of preeclampsia, gestational diabetes, fetal growth restriction and preterm labor.

Conclusions: Women with a false-positive quadruple test result have an increased risk of developing gestational hypertension and delivering a low-birth-weight newborn.

Keywords: Quadruple test, Adverse pregnancy outcome, Serum marker

INTRODUCTION

Down syndrome is trisomy 21, consisting of three copies of chromosome 21.¹⁻³ Leading to multiple anomalies such as heart anomalies, low IQ and need supervision throughout their lives.¹ Also, Prenatal screening has been recognized as crucial for the detection of chromosomal abnormalities.

We can perform combined screening using maternal serum markers and nuchal translucency measurement in the first trimester. On the other hand, the quadruple test still plays a role in pregnant women in the second trimester.³

Quadruple test offered a risk assessment by calculating maternal serum markers including AFP, B-hCG, inhibin-A and Estriol.^{4,5} In Thailand, pregnant women received care and coverage for Down's syndrome screening since

2023 by national health security office (NHSO). By using quadruple test, the cut off point for high risk Down syndrome is 1 to 250.⁶ The detection rate of Down's syndrome was approximately 80% with false positive rate was about 5%.^{6,7}

These abnormal levels are linked to placental pathology, for instance poor trophoblastic invasion or early placental dysfunction. This kind of placental abnormality is known to be associated with pre-eclampsia, fetal growth restriction and preterm birth. Therefore, a false-positive quadruple test might serve as an early indicator of placental problems, rather than just a screening error.⁸

Objective of our study was to assess the relationship between false positive quadruple test results and adverse pregnancy outcomes.

METHODS

A retrospective unicentric study was conducted in a Tertiary Care Hospital in Bangkok, Thailand between the period of January 2023 and September 2024. Patients’ data was collected and compiled from the hospital information system (HIS) in Rajavithi Hospital, Bangkok, Thailand. Ethics was approved by IRB of Rjavithi hospital number 67222.

Maternal serum marker screening results were taken from the database for the participants who had visited the lab for secondary level quadruple marker testing. Clinical data and postpartum data were collected from HIS to check any reported APOs at the time of patients’ discharge.⁶ Results were compared to find the correlation of biochemical markers with the APOs in the screened patients. Patient's data categorized into high-risk and low-risk groups vertically and in adverse outcomes horizontally.

Normality distribution was checked, and cross-tabulation was performed on STATA software between blood serum markers, their multiple of median (MoM’s) and selected adverse outcomes (dichotomous variable), other clinical signs helped in understanding the severity of diseases. MoM is a measure of deviation in test results.

Adverse obstetric outcomes were examined by spontaneous fetal loss after 24 weeks, preterm delivery starting at 32 weeks of gestation or less, Baby weight was considered low if found <2.5 kg and marked as low birth weight (LBW). Gestational diabetes was declared by 50 gram glucose challenge test (GCT) following by 100 gm oral glucose tolerance test (OGTT) if 50 gm GCT ≥140 mg/dL during the second trimester of pregnancy. PE was defined as GHTN (if blood pressure was more than or equal to 140/90 in second trimester) in the setting of significant proteinuria (a minimum of 300 mg/24 h or urine protein creatine ratio (UPCR) ≥0.3).^{1,9}

Statistical analysis

Categorical variables are presented in number and percentage (%). Patient’s data were categorized into

high-risk and low-risk groups. To compare the continuous variables between low-risk and high-risk groups, Mann-Whitney U-test was used. To test the association of the risk groups with categorical variables, Chi-square test/Fisher’s exact test was used. Binary logistic regression analysis was used to assess the factors associated with the high-risk group.¹⁰

A p<0.05 was considered statistically significant. The statistical package for the STATA 15.0 (Stata Corp, TX, USA) Software was used for data analysis.

RESULTS

In the study one hundred were screened as high-risk pregnancy, whereas the remaining five hundred were reported as low risk of pregnancies using trisomy screening in the quadruple test.

Mean±SD, age, and gestational age at quadruple test of the patient in the high risk group were 34.1±5.9 years and 15.9±1.0 weeks, respectively which were statistically significant (each p<0.05, Table 1). All patient’s ethnicity were Asian and non-smoker.. The descriptive statistics of demographic and maternal variables were compared between high- and low-risk pregnancies (Table 1). There was no significant difference in patients' nation and BMI at blood test date. B-hCG, Inhibin-A and Estriol were significantly increased in the high-risk (each p<0.05, Table 2).

The adverse pregnancy outcomes between high-risk and low-risk groups were presented in Table 3. The result showed that gestational hypertension and low birth weight were higher in the high-risk group.

Adjusted odds ratio (AOR) with a 95% confidence interval was calculated for the independent predictors after mutual adjustment. Results showed that patients with the high-risk group had 2.36 times the chances of gestational hypertension (AOR: 2.36, p<0.05), 2.77 times the chances of low birth weight (AOR: 2.77, p<0.05), 4.86 times the chances of the pre-eclampsia (AOR: 4.86, p>0.05, Table 4).

Table 1: Baseline characteristic.

Variables	Low risk group, (n=500)	High risk group, (n=100)	P value
Nation (%)			
Thai	355 (71.0%)	66 (66.0%)	0.318
Non-Thai	145 (29.0%)	44 (44.0%)	
Age (in years), mean±SD	28.7±5.6	34.1±5.9	<0.001*
BMI, (kg/m²) mean±SD	23.4±5.0	24.7±5.2	0.016*
Parity, median (min-max)	1 (0-3)	1 (0-3)	0.118
Gestation age at quadruple test (weeks), mean±SD	16.3±1.5	15.9±1.0	0.004*

*Age, BMI and gestational age at test were significant in baseline characteristic.

Table 2: Serum markers.

Variables	Low risk group, (n=500)	High risk group, (n=100)	P value
AFP (MoMs), median (min-max)	1.1 (0.07-2.84)	1.0 (0.47-2.68)	0.024
β-hCG (MoMs), median (min-max)	0.9 (0.16-6.28)	1.9 (0.29-8.47)	<0.001
uE3 (MoMs), median (min-max)	1.1 (0.07-10.0)	0.5 (0.08-3.41)	<0.001
Inhibin-A (MoMs), median (min-max)	1.0 (0.12-4.62)	1.7 (0.61-9.16)	<0.001

*Maternal serum markers: β-hCG, uE3, Inhibin-A were significant

Table 3: Adverse pregnancy outcomes.

Variables	Low risk group, (n=500)	High risk group, (n=100)	P value
Gestation age at delivery (days), mean±SD	270.0±10.3	270.6±78.7	0.623
Gestational hypertension (%)	22 (4.4%)	11 (11%)	0.008*
Pre-eclampsia (%)	3 (0.6%)	3 (3.0%)	0.028*
Gestational diabetes (%)			
Type A1	42 (8.4 %)	4 (4%)	0.153
Type A2	28 (5.6 %)	9 (9%)	0.197
FGR (%)	9 (1.8 %)	2 (2%)	1.000
Placenta previa (%)	1 (0.2 %)	0 (0.0%)	1.000
Preterm labor (%)	35 (7 %)	3 (3%)	0.177
Birth weight (gm), mean±SD	3067.3±423.1	3065.6±449.3	0.972
Admission to NICU (%)	15 (3 %)	4 (4%)	0.540
Low birth weight (<2500 g) (%)	23 (4.6 %)	7 (7%)	0.315
APGAR score, mean±SD			
1-min	8.7±0.7	8.6±0.9	0.121
5-min	9.7±0.6	9.7±0.6	0.712
10-min	9.9±0.4	9.8±0.4	0.348

*Adverse pregnancy outcomes: Gestational hypertension and pre-eclampsia were significant.

Table 4: Association between false positive quadruple test and APOs.

Variables	Crude OR (95% CI)	P value	Adjusted OR (95% CI)	P value
Gestational hypertension	2.69 (1.26, 5.73)	0.011*	2.36 (1.07, 5.18)	0.033*
Preeclampsia	5.12 (1.02, 25.76)	0.047	4.86 (0.96, 24.59)	0.056
GDMA1	0.45 (0.16, 1.30)	0.140	0.41 (0.14, 1.18)	0.099
GDMA2	1.67 (0.76, 3.65)	0.201	0.97 (0.40, 2.35)	0.941
Preterm labor	0.41 (0.12, 1.36)	0.146	0.67 (0.16, 2.70)	0.570
FGR	1.11 (0.24, 5.23)	0.892	0.96 (0.20, 4.59)	0.958
Admission to NICU	1.34 (0.44, 4.13)	0.608	1.96 (0.60, 6.42)	0.266
Low birth weight	1.56 (0.65, 3.74)	0.318	2.77 (1.06, 7.24)	0.037*

*By adjusting odd ratio: Gestational hypertension and low birth weight were significant, and pre-eclampsia nearly significant.

DISCUSSION

The quadruple test screening as an alternative tool is being tried to be established in the past three decades as an alarming tool to predict adverse outcomes other than just screening trisomy in pregnancies.^{7,12} In our study, we found gestational hypertension and low birth weight had a significant correlation (p<0.05), and pre-eclampsia was also close to the significance value (p>0.056) might be due to small group size.

The odd of having a low birthweight infant in the high-risk group was observed to be almost three times increased in comparison to the low-risk group. In study from Thailand, over 578 women with SGA among 10115 total pregnant women were in agreement up to an extent with our study

and found a higher level of AFP (area under the curve [AUC] 0.724) and b-HCG (AUC 0.655) and lower level of uE3 (0.597) was significantly associated to the SGA in the fetal aneuploidy testing results.⁸

Inhibin A is a glycoprotein hormone that has a fetoplacental origin during pregnancy and it is important in the growth of the placenta’s angiogenesis and impacts direct/indirect mothers’ blood pressure levels.^{5,13} The study from Yazdani et al on 80 quadruple positive samples among 231 total pregnancies found adverse outcomes were increased in the quadruple positive group by a significant amount.¹⁴ They observed PE (p=0.008) was associated with inhibin A (p<0.001), IUGR was (p=0.028) was associated with inhibin (p=0.020) and AFP (p=0.015) with PROM was also (p=0.040) present in trisomy

high-risk group, but they could not find the association with the low birth weight with any marker in their study and the age difference between the study groups were significant in contrast of our analysis.

From our study, we could perform some hypothesis that placental injury can elevate maternal serum AFP and is linked to low-birth-weight infants.^{9,15} Failure of normal placental vessel formation raises Inhibin A and decreases blood supply to the fetus, contributing to low birth weight.

Limitations

Limitation for this study was a single site study and retrospective design may lead to some bias and confounding factors, also, the sample size was small.

CONCLUSION

Women with false-positive quadruple test results are associated with increased risks of gestational hypertension and low birth weight.

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Ethical approval: The study was approved by the Institutional Ethics Committee

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