
OBSTETRICS

Association between Alpha-fetoprotein Levels during the Second Trimester of Pregnancy and the Incidence of Low Birth Weight Infants at Sunpasitthiprasong Hospital

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ABSTRACT

Objectives: To evaluate the association between maternal serum alpha-fetoprotein (MSAFP) levels during the second trimester and low birth weight (LBW) incidence, at Sunpasitthiprasong Hospital.

Materials and Methods: This retrospective cohort study included 1,937 pregnant women who underwent chromosomal abnormality screening between June 2021 and May 2025. Data from medical records included maternal baseline characteristics, antenatal history, quadruple test results, and delivery outcomes. Univariable and multivariable analyses were performed using logistic regression. Predictive performance of MSAFP for LBW was assessed by receiver operating characteristic curve analysis.

Results: The mean age of pregnant women was 28.5 ± 5.4 years. Birthweight less than 2,500 grams was observed in 10.9% of infants. The quadruple test was positive in 7.4% of cases. The median MSAFP levels were 34.36 ng/mL (interquartile range, IQR 26.94, 45.30), converting to 1.01 multiple of median (MoM) (IQR 0.83, 1.28). MSAFP levels were significantly higher in the LBW group compared to the non-LBW group (median 37.33 vs 34.12 ng/mL). In multivariable analysis, MSAFP in ng/mL, MoM, and > 2.0 MoM was significantly associated with LBW (adjusted odds ratios 1.01, 1.34, and 2.52 respectively). However, serum AFP demonstrated low predictive performance for LBW (area under the curve = 0.566).

Conclusion: MSAFP levels were significantly correlated with LBW, with higher levels observed in pregnancies with LBW. However, its predictive performance was low, limiting clinical utility. Elevated MSAFP may indicate risk to LBW but should be evaluated alongside other maternal and biochemical factors for effective risk assessment.

Keywords: serum Alpha-fetoprotein, Low birth weight, Second trimester pregnancy

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การศึกษาความสัมพันธ์ระหว่างค่า alpha-fetoprotein ในช่วงไตรมาสที่สองของการตั้งครรภ์กับการเกิดทารกน้ำหนักน้อย โรงพยาบาลสรรพสิทธิประสงค์

ธนาศักดิ์ อยู่ปูน, พงษ์สันต์ พันธะไชย

บทคัดย่อ

วัตถุประสงค์: เพื่อศึกษาความสัมพันธ์ระหว่างระดับแอลฟาฟิโตโปรตีนในเลือดของมารดา (MSAFP) ในไตรมาสที่สองกับการเกิดทารกน้ำหนักแรกเกิดน้อย ณ โรงพยาบาลโรงพยาบาลสรรพสิทธิประสงค์

วัสดุและวิธีการ: การศึกษานี้มีรูปแบบเก็บข้อมูลแบบย้อนหลังในกลุ่มสตรีตั้งครรภ์จำนวน 1,937 ราย ที่ได้รับการตรวจคัดกรองความผิดปกติของโครโมโซมระหว่างเดือนมิถุนายน พ.ศ. 2564 ถึง พฤษภาคม พ.ศ. 2568 โดยใช้ข้อมูลจากเวชระเบียน ได้แก่ ข้อมูลส่วนบุคคลของมารดา ประวัติการตั้งครรภ์ ผลการตรวจ quadruple test และข้อมูลการคลอด ทำการวิเคราะห์ข้อมูลแบบตัวแปรเดียวและพหุตัวแปรโดยใช้สถิติการถดถอยโลจิสติก

ผลการศึกษา: สตรีตั้งครรภ์มีอายุเฉลี่ยเท่ากับ 28.5 ± 5.4 ปี พบทารกแรกเกิดที่มีน้ำหนักน้อยกว่า $< 2,500$ กรัม ร้อยละ 10.9 การตรวจ Quadruple test พบผลบวกร้อยละ 7.4 ค่ามัธยฐานของ MSAFP level เท่ากับ 34.36 ng/mL (interquartile range, IQR 26.94, 45.30) คิดเป็น 1.01 multiple of the median (MoM) (IQR 0.83, 1.28) ระดับ MSAFP ในกลุ่มทารก LBW สูงกว่ากลุ่มปกติอย่างมีนัยสำคัญ (ค่ามัธยฐาน 37.33 เทียบกับ 34.12 ng/mL) การวิเคราะห์พหุตัวแปรพบว่า MSAFP ในหน่วย ng/mL, MoM และ > 2.0 MoM มีความสัมพันธ์กับ LBW อย่างมีนัยสำคัญ (adjusted odds ratios 1.01, 1.34 และ 2.52 ตามลำดับ) อย่างไรก็ตาม MSAFP มีประสิทธิภาพในการทำนาย LBW อยู่ในระดับค่อนข้างต่ำ (area under the curve = 0.566)

สรุป: ระดับ MSAFP มีความสัมพันธ์กับการเกิดทารกน้ำหนักแรกเกิดน้อยอย่างมีนัยสำคัญ โดยพบระดับที่สูงขึ้นในการตั้งครรภ์ที่ทารกน้ำหนักแรกเกิดน้อย อย่างไรก็ตามประสิทธิภาพในการทำนายของ MSAFP อยู่ในระดับต่ำ จึงมีข้อจำกัดในทางคลินิก ทั้งนี้การพบระดับ MSAFP สูงอาจเป็นข้อบ่งชี้ถึงความเสี่ยงต่อการเกิดทารกน้ำหนักแรกเกิดน้อย แต่ควรพิจารณาพร้อมกับปัจจัยทางชีวเคมีและปัจจัยอื่นๆ ที่เกี่ยวข้องกับมารดาเพื่อประเมินความเสี่ยงได้อย่างมีประสิทธิภาพ

คำสำคัญ: แอลฟาฟิโตโปรตีนในเลือด, น้ำหนักแรกเกิดน้อย, ไตรมาสที่สอง, การตั้งครรภ์ไตรมาสที่ 2

Introduction

Pregnancy and childbirth are significant events in a woman's life, as they directly impact the health of both the mother and the infant. Pregnancy is usually a normal physiological condition, but its present risks of various complications. Low birth weight (LBW) is one of the most common and clinically important issue. It is a major global public health concern. The World Health Organization (WHO) defines a low birth weight as a birth weight of less than 2,500 grams, regardless of gestational age⁽¹⁾. This condition is a leading cause of neonatal morbidity and mortality and are more likely to experience impaired physical growth and cognitive development later in life. A WHO report from 2019, approximately 20.5 million infants worldwide were born with low birth weight which is about 14.6% of all live births⁽²⁾. According to the Ministry of Public Health's Bureau of Policy and Strategy from 2020, 8.2% of live births in Thailand were low birth weight infants⁽³⁾. Although this number is lower than the global average, it is still a public health issue that needs to be monitoring because these infants face increased risks of both short-term and long-term complications.

Multiple maternal factors are associated with low birth weight, such as maternal age (either too young or too old), malnutrition, chronic illnesses, smoking, alcohol consumption, and infections while pregnancy⁽⁴⁾. Recent studies have investigated biochemical markers that could assist in predicting the risk of low birth weight. Among these, maternal serum alpha-fetoprotein (MSAFP) levels during the second trimester, have been examined for their possible association with low birth weight. It is commonly used to assess the risk of Down syndrome and other chromosomal abnormalities in pregnant women. Interpretation of the results is typically performed in conjunction with maternal serum free beta human chorionic

gonadotropin (β -hCG) levels and gestational dating by ultrasonography, in order to improve screening accuracy and reduce errors in risk estimation⁽⁵⁾.

Previous studies by Waller et al found that MSAFP levels above 2.0 multiple of the median (MoM) were associated with an increased risk of low birth weight⁽⁶⁾, whereas Krause et al (2001) observed a similar association when MSAFP levels were greater than 2.5 MoM⁽⁷⁾. Dugoff et al reported a significant correlation between elevated alpha-fetoprotein (AFP) alone and low birth weight, and when combined with other markers such as hCG, unconjugated oestriol (uE3), and inhibin A, the risk increased even more substantially⁽⁸⁾. In Thailand, Puntachai et al similarly found that maternal AFP levels above 2.0 MoM were significantly related to low birth weight outcomes⁽⁹⁾. These findings suggest that maternal serum biochemical markers, especially alpha-fetoprotein, which is part of the quadruple test, may be associated with various pregnancy outcomes, including low birth weight. However, international studies show inconsistent results, and data specific to the Thai population are still limited. The quadruple test is a simple, safe and widely accessible blood test routinely performed in antenatal care. If MSAFP levels obtained from this test could also help identify pregnancies at risk for low birth weight, it could enhance the clinical utility of prenatal screening.

Therefore, the present study aimed to examine the association between MSAFP levels during the second trimester (14–20 weeks of gestation), as measured by the quadruple test, and the occurrence of low birth weight at Sappasitthiprasong Hospital, a regional referral center in northeastern Thailand. In addition, the study evaluated the predictive performance of MSAFP levels in identifying low birth weight risk and explore their potential of MSAFP as an

auxiliary marker for screening pregnant women at risk. Other maternal factors influencing low birth weight will also be examined. The findings are expected to improve epidemiological understanding among Thai pregnant women and contribute to enhanced strategies for the prevention, early detection, and management of low birth weight in the future.

Materials and Methods

This study utilized a retrospective cohort design, analyzing data collected from pregnant women who underwent blood testing for fetal chromosomal abnormalities by the quadruple test during the second trimester at the antenatal clinic of Sappasitthiprasong Hospital, Ubon Ratchathani Province, Thailand, between June 1, 2021, to May 31, 2025. The Human Research Ethics Committee of Sappasitthiprasong Hospital gave its approval for the study protocol (Approval No. 079/67R). The study population included pregnant women who met the following inclusion criteria: 1) singleton pregnancy, 2) viable fetus, and 3) complete follow-up data on birth outcomes. Exclusion criteria were as follows: 1) fetuses diagnosed with chromosomal abnormalities by amniocentesis, cordocentesis, or postnatal examination, 2) fetuses with congenital anomalies that led to pregnancy termination, and 3) mothers with medical conditions known to elevate maternal serum alpha-fetoprotein (MSAFP) levels, including hepatocellular carcinoma, embryonal cell carcinoma, gastrointestinal cancer, ovarian germ cell tumors, preeclampsia, fetal growth restriction, maternal hepatoma, or maternal teratoma.

The sample size was calculated using the study by Puntachai et al⁽⁹⁾, which indicated low birth weight rates of 15.8% in mothers with AFP > 2.0 MoM and 6.7% in those with AFP < 2.0 MoM. With $\alpha = 0.05$, $\beta = 0.20$ (80% power), and an exposed to unexposed ratio of 1:20, the cohort

study formula⁽¹⁰⁾ required minimum number of sample size was 1,743. To account for potential data loss or incomplete records, we increased the sample size by 20%, resulting in a total of 1,937 participants.

Data were retrospectively collected from the electronic medical records at Sappasitthiprasong Hospital. The collected data included maternal baseline characteristics such as age, pre-pregnancy weight, underlying diseases, history of drug or food allergies, alcohol consumption, and smoking habits. Delivery data was also extracted, including gestational age at delivery, infant sex, and birth weight. In addition, the quadruple test parameters were reviewed, which including the MSAFP (ng/mL and MoM), the serum unconjugated estriol level (in nmol/L and MoM), the total hCG (2T) level (mIU/mL and MoM), the serum inhibin A level (pg/mL and MoM), and the overall screening result. Pregnant women were then categorized into two groups based on birth weight: the low birth weight group (LBW), defined as infants weighing less than 2,500 grams at birth, and the normal birth weight group, defined as infants weighing 2,500 grams or more⁽¹¹⁾. The data selection and follow-up process are illustrated in Fig. 1.

Data analysis was performed using Stata version 17.0 (StataCorp, College Station, TX, USA). Descriptive statistics were used to presented maternal characteristics, delivery outcomes, and Quadruple test parameters. Quantitative variables with a normal distribution were represented as mean $\pm$ standard deviation (SD), while those with non-normally distributed variables were presented as median (interquartile range, IQR). Categorical variables were reported as frequencies and percentages. Independent t-test or Mann–Whitney U test for continuous data and the chi-square test for categorical data to compare the LBW and normal birth weight groups.

We conducted both univariable and multivariable logistic regression analysis were used to find the association between MSAFP levels and LBW. The results were shown as crude odds ratios (OR) and adjusted odds ratios (adjusted OR), along with 95% confidence intervals (CI). Receiver operating

characteristic (ROC) curve analysis was used to evaluate predictive performance of MSAFP levels for LBW, and the area under the curve (AUC) was reported. All statistical tests were two-tailed, and a significance level of 0.05 ($\alpha = 0.05$) considered statistically significant.

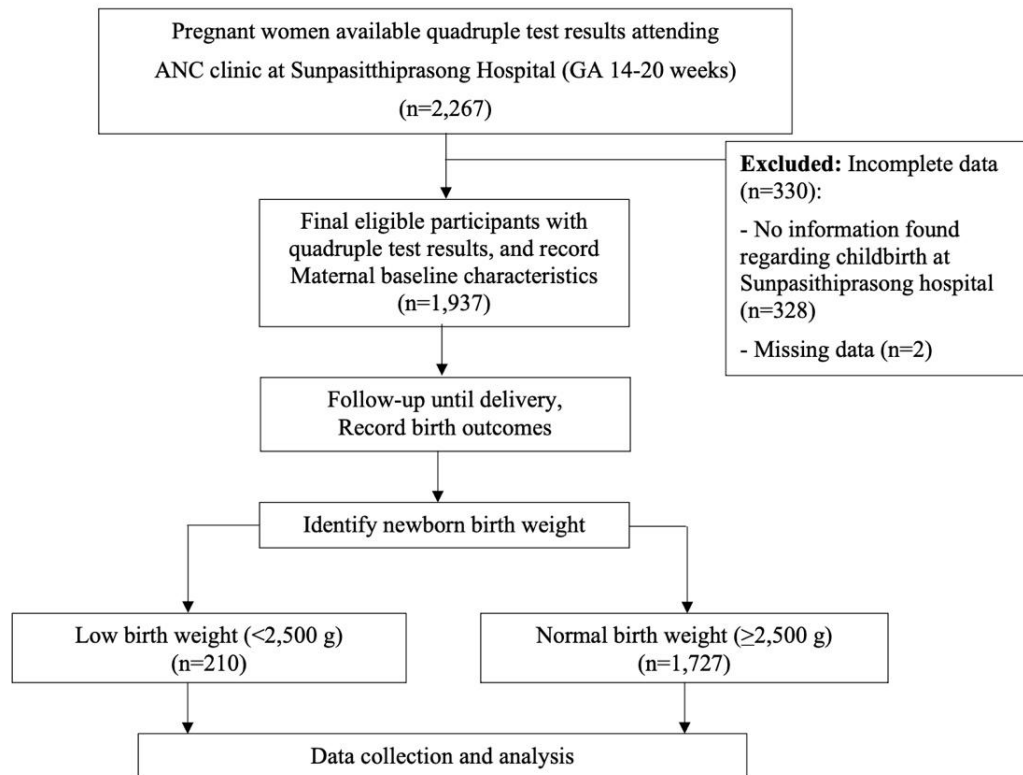


Fig. 1. Study flow chart

Results

This study included a total of 1,937 pregnant women. The mean maternal age was 28.51 ± 5.41 years, and the mean before pregnancy weight was 58.35 ± 13.34 kg. 9.7% of them had underlying diseases, 2.5% reported a history of drug allergy, and 0.2% reported food allergies. One woman continued smoking during pregnancy, and five women consumed

alcohol. The mean gestational age at delivery was 37.96 ± 2.64 weeks. When comparing maternal characteristics between the LBW and normal birth weight groups, mothers in the LBW group had significantly lower pre-pregnancy weights, a higher prevalence of underlying diseases and alcohol consumption, and shorter gestational age at delivery ($p < 0.05$) (Table 1).

Table 1. Maternal baseline characteristic between two groups, low birthweight (LBW) and normal birthweight (NBW).

Maternal baseline characteristics	Total n = 1,937 (100%)	LBW < 2,500 g n = 210 (10.8%)	NBW ≥ 2,500 g n = 1,727 (89.2%)	p value
Maternal age (years)	28.51 ± 5.41	28.53 ± 5.91	28.51 ± 5.35	0.957
Pre-pregnancy weight (kg)	58.35 ± 13.34	56.23 ± 12.69	58.60 ± 13.40	0.016
Height (cm)	158.37 ± 12.30	158.12 ± 6.31	158.39 ± 12.84	0.766
Pre-pregnancy BMI (kg/m ²)	23.07 ± 5.10	22.69 ± 5.13	23.11 ± 5.10	0.261
Underlying disease	188 (9.7)	39 (18.6)	149 (8.6)	< 0.001
Gestational diabetes mellitus	78 (4.0)	12 (5.7)	66 (3.8)	0.188
Hypertension in pregnancy	27 (1.4)	6 (2.9)	21 (1.2)	0.064 ^f
Anemia	11 (0.6)	4 (1.9)	7 (0.4)	0.024 ^f
Autoimmune disease	3 (0.2)	0 (0.0)	3 (0.2)	1.000 ^f
Liver disease	2 (0.1)	0 (0.0)	2 (0.1)	1.000 ^f
Chronic respiratory disease	1 (0.05)	0 (0.0)	1 (0.05)	1.000 ^f
Others	104 (5.4)	24 (11.4)	80 (4.6)	< 0.001
History of drug allergy	48 (2.5)	4 (1.9)	44 (2.5)	0.571
History of food allergy	4 (0.2)	0 (0.0)	4 (0.2)	1.000 ^f
Smoking history				0.885
Non-smoker	1,935 (99.9)	210 (100.0)	1,725 (99.9)	
Former smoker	1 (0.05)	0 (0.0)	1 (0.05)	
Current smoker	1 (0.05)	0 (0.0)	1 (0.05)	
Alcohol consumption history				0.002
Non-drinker	1,918 (99.0)	206 (98.1)	1,712 (99.1)	
Former drinker	14 (0.7)	1 (0.5)	13 (0.8)	
Current drinker	5 (0.3)	3 (1.4)	2 (0.1)	
Gestational age at delivery (weeks)	37.96 ± 2.64	35.72 ± 4.40	38.24 ± 2.16	< 0.001

Data were analyzed with independent t-test, Chi-square test, and Fisher exact test ^f.

Data are presented as mean ± standard deviation or n (%).

BMI: body mass index

Delivery and neonatal data revealed that most births were by cesarean section (52.3%), and 52.6% of the newborns were male. The APGAR scores at 1, 5, and 10 minutes were

normal range (7–10), with rates of 98.6%, 99.4%, and 99.7%, respectively. A Comparison between LBW infants and normal birth weight found a significantly difference in the mode of delivery

between groups. Moreover, their APGAR scores at 1, 5, and 10 minutes were significantly lower than those in the normal birth weight group ($p < 0.05$). LBW infants accounted for 10.8% of all

births, with a median birth weight of 2,312 g (IQR 2,020, 2,435). Infants with normal birth weight was 89.2%, with a median birth weight of 3,100 g (IQR 2,860, 3,350) (Table 2).

Table 2. Delivery outcomes and newborn between two groups, low birthweight (LBW) and normal birthweight (NBW).

Delivery outcomes and newborn	Total n = 1,937 (100%)	LBW < 2,500 g n = 210 (10.8%)	NBW ≥ 2,500 g n = 1,727 (89.2%)	p value
Mode of delivery	n = 1,936	n = 210	n = 1,726	0.009
Normal	801 (41.4)	91 (43.3)	710 (41.1)	
Cesarean section	1,013 (52.3)	116 (55.2)	897 (52.0)	
Vacuum/ Forceps extraction	122 (6.3)	3 (1.4)	119 (6.9)	
Sex of newborn	n = 1,934	n = 210	n = 1,724	0.710
Male	1,018 (52.6)	108 (51.4)	910 (52.8)	
Female	916 (47.4)	102 (48.6)	814 (47.2)	
APGAR score at 1 min	n = 1,936	n = 209	n = 1,727	< 0.001
0-3	14 (0.7)	9 (4.3)	5 (0.3)	
4-6	14 (0.7)	7 (3.3)	7 (0.4)	
7-10	1,908 (98.6)	193 (92.3)	1,715 (99.3)	
APGAR score at 5 min	n = 1,933	n = 206	n = 1,727	< 0.001
0-3	4 (0.2)	3 (1.5)	1 (0.1)	
4-6	7 (0.4)	3 (1.5)	4 (0.2)	
7-10	1,922 (99.4)	200 (97.1)	1,722 (99.7)	
APGAR score at 10 min	n = 1,927	n = 202	n = 1,725	< 0.001
0-3	4 (0.2)	3 (1.5)	1 (0.1)	
4-6	1 (0.05)	0 (0.0)	1 (0.1)	
7-10	1,922 (99.7)	199 (98.5)	1,723 (99.9)	
Birth weight (g)	3,045 (2,770, 3,315)	2,312 (2,020, 2,435)	3,100 (2,860, 3,350)	< 0.001

Data were analyzed with chi-square test.

Data are presented as n (%) or median (interquartile range).

Results from the quadruple test indicated that mothers in the LBW group had a median MSAFP level of 37.33 ng/mL (IQR 27.81, 52.01), equivalent to 1.10 MoM (IQR 0.85, 1.46), with 7.1% having AFP > 2.0 MoM. In contrast, mothers in the normal birth weight group had a median MSAFP of 34.12 ng/mL (IQR 26.77, 44.48) or 1.01 MoM (IQR 0.83, 1.27), with 2.8% above 2.0 MoM. These differences were statistically significant ($p < 0.05$). Additionally,

mothers in the LBW group also showed significantly higher levels of hCG (mIU/mL), inhibin A (pg/mL and MoM), and a greater proportion of positive quadruple test results than those in the normal birth weight group ($p < 0.05$) (Table 3).

The predictive performance of MSAFP for LBW showed an AUC of 0.566 [95%CI 0.523, 0.610] in ng/mL and 0.563 [95%CI 0.518, 0.608] in MoM, indicating limited predictive (Fig. 2).

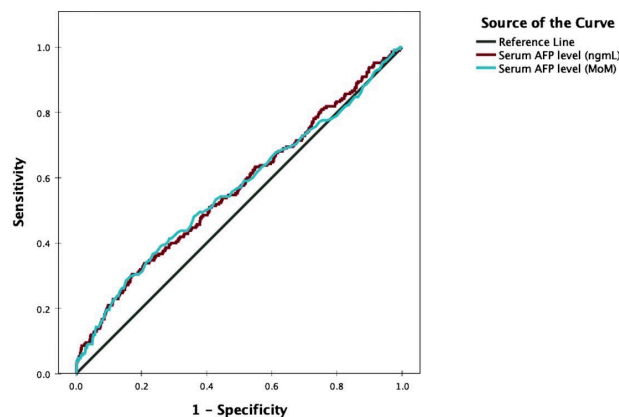
Table 3. Quadruple test results between two groups, low birthweight (LBW) and normal birthweight (NBW).

Quadruple test	Total n = 1,937 (100%)	LBW < 2,500 g n = 210 (10.8%)	NBW ≥ 2,500 g n = 1,727 (89.2%)	p value
Serum AFP level				
ng/mL	34.36 (26.94, 45.30)	37.33 (27.81, 52.01)	34.12 (26.77, 44.48)	0.002
MoM	1.01 (0.83, 1.28)	1.10 (0.85, 1.46)	1.01 (0.83, 1.27)	0.003
Serum AFP level				< 0.001
< 2 MoM	1,873 (96.7)	195 (92.9)	1,678 (97.2)	
> 2 MoM	64 (3.3)	15 (7.1)	49 (2.8)	
Serum unconjugated estriol level				
nmol/L	3.34 (2.52, 4.49)	3.45 (2.37, 4.44)	3.34 (2.53, 4.50)	0.681
MoM	1.08 (0.87, 1.34)	1.06 (0.86, 1.31)	1.08 (0.87, 1.35)	0.494
Total hCG (2T) level				
mIU/mL	49,651 (33,952, 69,482)	53,620 (35,653, 76,406)	49,270 (33,768, 68,716)	0.027
MoM	1.01 (0.72, 1.39)	1.09 (0.75, 1.56)	1.01 (0.72, 1.37)	0.094
Serum Inhibin A level				
pg/mL	178.70 (135.15, 236.55)	200.60 (151.03, 265.15)	177.0 (133.0, 232.4)	< 0.001
MoM	1.04 (0.79, 1.37)	1.13 (0.86, 1.56)	1.03 (0.79, 1.35)	< 0.001
Screening result				< 0.001
Positive	143 (7.4)	28 (13.3)	115 (6.7)	
Negative	1,792 (92.6)	182 (86.7)	1,610 (93.3)	

Data were analyzed with chi-square test, Mann-Whitney U test.

Data are presented as n (%) or median (interquartile range).

AFP: alpha-fetoprotein, MoM: multiple of the median, hCG: human chorionic gonadotropin

**Fig. 2.** Receiver operating characteristic curve analysis of serum alpha-fetoprotein levels for predicting low birth weight of area under the curve 0.566 in ng/mL and 0.563 in multiple of the median.

Further multivariable analysis of maternal factors associated with LBW the following significant predictors ($p < 0.05$), including maternal body weight (adjusted OR 0.98 [95% CI 0.97,

0.99]), underlying diseases (adjusted OR 2.67 [95%CI 1.73, 4.13]), and gestational age at delivery (adjusted OR 0.74 [95%CI 0.69, 0.80]) (Table 4).

Table 4. The association between serum AFP with low birth weight.

Serum AFP level	Crude OR (95%CI)	p value	Adjusted OR (95%CI) [#]	p value
ng/mL	1.01 (1.01, 1.02)	< 0.001	1.01 (1.00, 1.02)	0.001
MoM	1.48 (1.19, 1.82)	< 0.001	1.35 (1.09, 1.69)	0.007
> 2 MoM	2.63 (1.45, 4.79)	0.001	2.52 (1.31, 4.34)	0.006

Adjusted for potential confounders, including maternal age, maternal underlying disease, alcohol consumption, gestational age.

Data were analyzed with binary logistic regression.

AFP: alpha-fetoprotein, OR: odds ratio, CI: confidence interval, MoM: multiple of the median

Table 5. Univariable and multivariable analysis of factors associated with low birth weight.

Variables	Crude OR (95%CI)	p value	Adjusted OR (95%CI)	p value
Maternal age (years)	1.00 (0.98, 1.03)	0.957	0.99 (0.97, 1.03)	0.879
Weight (kg)	0.98 (0.98, 0.99)	0.016	0.98 (0.97, 0.99)	< 0.001
Underlying disease	2.42 (1.64, 3.55)	< 0.001	2.67 (1.73, 4.13)	< 0.001
Alcohol consumption history				
Non-drinker	Reference		Reference	
Former/current drinker	2.22 (0.73, 6.74)	0.161	2.81 (0.90, 8.75)	0.075
Gestational age at delivery	0.74 (0.69, 0.79)	< 0.001	0.74 (0.69, 0.80)	< 0.001

Data were analyzed with binary logistic regression.

OR: odds ratio, CI: confidence interval

Discussion

Findings from this study revealed that elevated MSAFP levels during the second trimester were significantly associated with an increased risk of LBW infant. Specifically, each 1 ng/mL and 1 MoM increase in MSAFP, the likelihood of LBW rised by 1.01 and 1.35 times, respectively. This result suggested that AFP could serve as a useful biochemical marker for predicting adverse pregnancy complication related to fetal growth. The association between high AFP levels and LBW is probably due to placental dysfunction or ischemic placental disease (IPD), both of which are known to be major causes of intrauterine

growth restriction (IUGR), which often leads in LBW⁽¹¹⁾.

Under normal physiological conditions, AFP is initially produced by the yolk sac and later by the fetal liver. It enters the amniotic fluid through fetal urine and then diffuses in a regulated manner across the placenta into the maternal circulation⁽¹²⁾. But this balance is upset when placental complications stop blood from flowing between the mother and the fetus, especially in the intervillous space. This causes MSAFP to leak abnormally from fetal circulation or amniotic fluid into the maternal bloodstream^(13,14).

Pathophysiological evidence supports that inadequate remodeling of the spiral arteries can leads

to placenta infarction, placenta necrosis, and increased placental permeability. These changes in structural and functional make it impair for nutrients and oxygen between the mother and fetus, which is a direct cause of IUGR⁽¹⁵⁾. Damage allows fetal proteins like MSAFP to leak into the maternal bloodstream^(11,16).

Consequently, increased MSAFP levels in the absence of structural fetal anomalies may indicate placental injury and related pathological processes associated with to fetal growth restriction (FGR) or small-for-gestational-age (SGA) outcomes. Ultimately, these conditions manifest as LBW, either from intrauterine growth restriction or preterm delivery⁽¹⁷⁾. These findings indicate that MSAFP may be utilized as a screening biomarker for predicting pregnancy complications, especially among populations at risk for placental insufficiency⁽¹⁸⁾.

Previous studies focusing on second-trimester pregnancies have found a strong association between elevated MSAFP levels (commonly defined as above 2.0 MoM) and various adverse pregnancy outcomes, including FGR or SGA infants, preterm birth, placental abruption, and preeclampsia⁽¹⁷⁻¹⁹⁾. Each of these conditions is a well-established direct or indirect contributor to LBW. Importantly, this association remains statistically significant even after excluding cases involving fetal structural abnormalities such as neural tube defects. This further supports hypothesis that unexplained elevations in MSAFP levels may reflect placental pathology directly⁽¹⁸⁾.

In the present study, it is noteworthy that the adjusted odds ratio per 1 MoM increase in MSAFP (1.35) was significantly higher than that per 1 ng/mL increase (1.01). This discrepancy can be explained by the nature of the MoM unit, which is standardized for gestational age and maternal characteristics⁽²⁰⁾, thereby offering a more accurate and sensitive measure of abnormality compared to absolute expressed in ng/mL.

These findings are consistent with those of Dai et al⁽¹⁵⁾, who analyzed data from 22,574 pregnant women and reported a 2.55-fold increased risk of

LBW in cases with high MSAFP levels. The study also found that high MSAFP was associated to several other factors, such as placenta previa, maternal hepatitis B carrier status, premature rupture of membranes (PROM), advanced maternal age, increased free β -hCG MoM levels, and female fetal sex. Basbug et al⁽²¹⁾ similarly discovered that elevated MSAFP levels were significantly associated with various obstetric complications, including preterm birth, preterm premature rupture of membranes (PPROM), oligohydramnios, and IUGR. However, a study by Ozdemir et al⁽²²⁾ presented slightly different findings: elevated AFP in ng/mL was significantly associated with LBW, this relationship was not observed when AFP levels were analyzed in MoM units. This indicates that different measurement methods may yield varying predictive accuracies depending on population characteristics and study design.

With a cutoff value of 2.0 MoM, mothers with MSAFP levels above this threshold were 2.52 times increased risk of LBW infant than mother with MSAFP levels below 2.0 MoM. The incidence of LBW in the high-MSAFP group was 23.4%, significantly higher than the 10.4% observed in the low-MSAFP group. These results are consistent with several previous studies utilizing the 2.0 MoM threshold. Williams et al⁽¹⁷⁾ indicated an adjusted relative risk (RR) of 3.7, Puntachai et al⁽⁹⁾ indicated an adjusted RR of 2.35, and Allen et al⁽²³⁾ indicated an OR of 2.7. Additionally, Buckland et al⁽²⁴⁾ also found that 8.5% of mothers with LBW infants had AFP levels above 2.0 MoM, a significantly higher proportion than for mothers with normal birth weight infants.

Some studies have used even higher cutoffs than the 2.0 MoM threshold and found stronger associations. For example, Krause et al⁽⁷⁾ used a 2.5 MoM threshold and found an RR of 5.8. In similarly, Bartkute et al⁽²⁵⁾ found that infants born to mothers with AFP levels exceeding 2.5 MoM had significantly lower birth weight and body length compared to those with AFP below 2.5 MoM. These finding highlight the influence of placental dysfunction on overall fetal

growth. In addition to its association with LBW, elevated MSAFP have been linked to a broad spectrum of placental-mediated complications. Karya et al⁽²⁶⁾ found that AFP levels above 2.0 MoM were significantly associated to preterm delivery (RR 3.2), preeclampsia (RR 2.48), oligohydramnios (RR 1.97), IUGR (RR 2.48), placental abruption (RR 7.08), and PPROM (RR 3.54). This extensive array of associations underscores the role of elevated MSAFP as a general marker of placental dysfunction, which may manifest through multiple pathways leading to adverse pregnancy outcomes, including LBW.

Current clinical guidelines acknowledge the link between elevated MSAFP levels and complications mediated by placental dysfunction and recommend increased fetal surveillance for high-risk pregnancies⁽²⁷⁾. However, in this study, the predictive power of MSAFP for LBW was poor classification, with an AUC of just 0.566. In contrast, Boonpiam et al⁽²⁸⁾ reported an AUC of 0.724 when using MSAFP levels to predict SGA fetuses, suggesting that MSAFP alone may not be a highly accurate predictor. Nevertheless, when high MSAFP levels are detected in the second trimester in the absence of any identifiable fetal structural abnormalities, close monitoring of fetal growth is warranted. This should include regular assessment of maternal blood pressure and careful observation for clinical indicators of placental dysfunction. Such clinical measures are justified as they may help in the early detection and management of FGR, SGA, or LBW outcomes.

Potential confounding variables that may influence MSAFP levels include fetal abnormalities known to elevate MSAFP (e.g., neural tube defects and abdominal wall defects), multiple gestation, fetomaternal hemorrhage, and maternal liver disease. These factors addressed these issues methodically through clear inclusion and exclusion criteria that excluded pregnancies with medical or fetal conditions could independently increasing MSAFP levels. Furthermore, multivariable analyses were conducted to adjust for confounding effects. The continued presence of a significant association between

elevated MSAFP and adverse outcomes such as FGR and SGA (even after adjustments) further supports the hypothesis that placental dysfunction is the primary biological mechanism linking elevated MSAFP and the increased risk of LBW.

Finding of additional factors significantly associated with LBW revealed that maternal baseline characteristics play a crucial impact, including maternal body weight, underlying diseases, and gestational age at delivery. Maternal weight gain was associated with a 2.0% reduction in the likelihood of LBW, reflecting the role of maternal nutrition and stored energy in supporting fetal growth. Mothers with appropriate weight and nutrition tend to have adequate blood flow and nutrient transport to the placenta, resulting in infants having a better chance of meeting growth standards⁽²⁹⁾. Clinically, therefore, emphasis should be placed on pre-pregnancy nutritional assessment and weight gain during pregnancy to prevent IUGR, a major cause of LBW⁽³⁰⁾. These results are consistent with the research by Ruan X et al, which reported that mothers who gained less than 10 kg and had imbalanced diet had a 1.46 and 1.15 times increased risk of LBW, respectively⁽³¹⁾.

Furthermore, maternal underlying diseases increased the likelihood of LBW by 2.67 times. This association may be explained by the fact that many chronic maternal diseases contribute to uteroplacental insufficiency, or impaired placental function, leading to reduced oxygen and nutrient delivery to the fetus and an increased risk of IUGR and/or preterm birth. Furthermore, certain medical conditions are associated with complications during pregnancy, such as preeclampsia, which is a direct risk factor for LBW⁽³²⁾. The findings of this study consistent with systematic review study that found a significant association between maternal medical conditions and LBW, particularly hypertension (3.9-fold), hypothyroidism (1.4-fold), anemia (1.32-fold), depression (1.84-fold), and periodontal disease (2.41-fold)⁽³³⁾.

Regarding gestational age at delivery, higher gestational age was associated with a 26.0%

decrease in the risk of LBW, indicating that premature birth is a significant risk factor. This finding with the physiological principles of fetal growth, as fetal weight increases significantly during the third trimester, especially between 32–40 weeks of gestation⁽³⁴⁾. Preterm delivery limits the duration of fetal mass accumulation, resulting in a higher likelihood of LBW. These findings are consistent with the study by Shaohua Y et al, which reported that mothers who delivered between 37–42 weeks of gestation and after 42 weeks were associated with an 85.0% and 81.0% reduced risk of LBW, respectively, compared with delivered before 37 weeks of gestation⁽³⁵⁾.

Conclusion

This study demonstrated that elevated MSAFP levels during the second trimester were significantly associated with an increased risk of LBW infant, whether expressed in ng/mL, MoM, and above 2.0 MoM threshold. Although, the predictive performance of MSAFP for LBW in this cohort was relatively limited, with limited overall classification accuracy. These findings indicate the potential usefulness as a biochemical marker of placental dysfunction. Clinically, detection of unexplained high MSAFP levels should induce closer fetal growth surveillance and maternal monitoring to early diagnosis of placental-mediated complications. Moreover, MSAFP levels should be interpreted conjunction with other maternal and biochemical factors for effective risk assessment.

Potential conflicts of interest

The authors declare no conflicts of interest.

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