EVALUATION OF THE ROLE OF MATERNAL SERUM ALPHA-PHYTOPROTEIN (MS-AFP) IN THE MANAGEMENT OF PERINATAL CARE

Sara Masihi[1], Maryam Ebrahimi-Lagha [2*], Mojgan Barati [3], Najmieh Saadati [4], Elham Farhadi[5]

1. Associate Professor of Obstetrics and Gynecology, Department of Obstetrics and Gynecology, Faculty of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.
2. Specialized Assistant, Department of Obstetrics and Gynecology, Faculty of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.
3. Associate Professor of Obstetrics and Gynecology, Department of Obstetrics and Gynecology, School of Medicine, Fertility and Infertility and Fetal Health Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.
4. Associate Professor of Obstetrics and Gynecology, Department of Obstetrics and Gynecology, School of Medicine, Fertility and Infertility and Fetal Health Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.
5. Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.

* Corresponding Author

Abstract

Introduction: Maternal serum alpha-phytoprotein (MS-AFP) is a valuable test used as an indicator to diagnose some clinical abnormalities during pregnancy. The aim of this study was to investigate the role of MS-AFP measurement during mid-pregnancy in the management of prenatal care.

Methods: This case-control study was conducted on two groups of singleton pregnant women, with MSAFP screening test (192 people) and without MSAFP screening test (192 people), in the 15-20th week of pregnancy referring to teaching hospitals in Ahvaz city in 2020. Based on the results of the MS-AFP test, mothers were divided into two groups with elevated levels (AFP≥2.5 MoM) and normal levels (AFP<2.5 MoM). Adverse pregnancy outcomes, including gestational diabetes, preeclampsia, intrauterine fetal death (IUFD), intrauterine growth restriction (IUGR), stillbirth, preterm birth, small for gestational age (SGA), and NICU admission, were investigated.

Results: There was no significant difference in the adverse outcomes of pregnancy between the two groups; with and without MS-AFP screening (P<0.05). However, the incidence of adverse pregnancy outcome in women with elevated MS-AFP was significantly higher than in the normal MS-AFP group (38.1% vs. 11.7%; P=0.004). The incidence of preeclampsia (P=0.021), preterm birth (P=0.030), SGA (P=0.003), and hospitalization of newborns in the NICU (P=0.021) were higher in women with elevated MS-AFP than in women with normal MS-AFP.

Conclusion: The results of this study showed that the increase in MS-AFP levels in mid-pregnancy was associated with increased risk of adverse pregnancy outcomes, including premature birth, pre-eclampsia, SGA, and NICU admission. The MS-AFP assay can help identify women at high risk of adverse outcomes early in the second trimester

Keywords: Maternal alpha-phytoprotein, Pregnancy, Negative pregnancy outcomes

DOI: 10.48047/ecb/2023.12.si4.1071

Introduction

Prenatal care is a type of preventive health care aiming at providing regular examinations in order to screen and prevent possible maternal and fetal problems during pregnancy (1, 2). Despite support measures in prenatal care, every year 2.6 million women around the world experience fetal death or stillbirth in the last trimester of pregnancy or during childbirth (3).

In case of appropriate interventions in early pregnancy, these negative consequences can be prevented (4). Therefore, in recent years, various markers and screening tests are used for prenatal screening, and abnormal values of many serum markers have been associated with various types of negative pregnancy complications (5-7).

Alpha-phytoprotein (AFP) is a glycoprotein produced by the yolk sac in early pregnancy and by the digestive system and liver of the fetus in late pregnancy (8, 9). Physiologically, the synthesis of AFP in the fetal liver increases until the 20th week of pregnancy and remains stable at that point until the 32nd week, and then decreases to lower levels until the end of pregnancy (10, 11). The fetus excretes AFP into the amniotic fluid through urine, where it is released into the maternal serum through the placenta (7).

The measurement of maternal serum alpha-phytoprotein (MS-AFP) is used as a non-invasive method to assess fetal growth and development. MSAFP is expressed as MoM, and its serum level changes based on gestational age, and values ≥ 2.5 - 0.2 MoM are considered abnormal (7, 12).

MSAFP measurement in the second trimester of pregnancy is used for prenatal screening. The assessment of MS-AFP level is part of a triple (AFP, Estriol, and hCG) or quadruple (AFP, implies Estriol, hCG and Inhibin A) screening test to evaluate birth defects. High and low MS-AFP levels, respectively, indicate a high risk of fetal open neural tube defects (ONTDs) and chromosomal aneuploidy (13). Also, some studies showed that women with increased MSAFP levels in the second trimester of pregnancy are at high risk of adverse pregnancy outcomes, including premature birth, SGA, placental abruption, and fetal death (14-18).

Considering that the most common use of alpha-phytoprotein measurement is to screen for neural tube defects (NTD), and according to available sources, abnormal ultrasound is a part of routine prenatal care. There is a need to accurately check alpha-phytoprotein during prenatal care (19, 20). Therefore, this study was conducted to investigate the role of maternal alpha-phytoprotein in the management of prenatal care.

Methods

This was a retrospective analytical epidemiological study conducted with a case-control design on two groups of pregnant women with MSAFP screening test and without MSAFP screening test referring to teaching hospitals in Ahvaz city in 2022. This study was conducted after being approved by the Research Council of Ahvaz Jundishapur University of Medical Sciences and receiving an ethics code from the Medical Ethics Committee of Ahvaz Jundishapur University (IR.AJUMS.HGOLESTAN.REC.1401.045).
Also, in this study, all provisions of the Helsinki research ethics statement were observed, and the principles of confidentiality of patient information were followed.

Sampling was conducted in a simple random manner, and the sample size was estimated to be 384 people according to the Krechsi-Morgan formula. Considering a sampling error of 0.05 and a confidence level of 95%, 192 people were enrolled in two equal groups (21).

Women with spontaneous pregnancy (pregnancy without assisted reproductive methods) of singletons in the 15th to 20th week of pregnancy and without any underlying disease were included in the study. Gestational age was determined based on crown-rump length measured by ultrasound. Women with a history of autoimmune diseases or any specific underlying disease, and also those who had incomplete information in their medical files, were excluded from the study.

In one group, in addition to routine screenings, the MS-AFP test was performed in the 15th to 20th week of pregnancy, and in the other group, only routine pregnancy screenings were performed. The AFP test was performed using the fluorescence immunoassay method and standard kits. Based on the test results, people were divided into two groups: AFP≥2.5 and AFP<2.5. The MoM value of 2.5 was considered as a cutoff for evaluating adverse pregnancy outcomes (22).

At first, the basic characteristics of all participants, including gestational age, parity, number of pregnancies, abortion history, and medical records, were collected from the patients' medical records. All the participants in the study

were followed up until the end of pregnancy, and any special complications or problems were carefully examined by obstetricians and gynecologists (23). The results of ultrasound, laboratory examinations, and the presence of any abnormality and maternal, fetal, and neonatal complications, as well as the method of termination of pregnancy and the age of termination of pregnancy, were recorded.

The presence of intrauterine growth restriction (IUGR) was reported based on fetal weight assessment using ultrasound images and diagnosed when fetal weight was below the tenth percentile for gestational age (24). Intrauterine fetal death (IUFD) and placental abruption were diagnosed by evaluating the patient's symptoms, including bleeding and pain, and performing ultrasound and monitoring fetal symptoms. Stillbirth was diagnosed based on ultrasound results or stillbirth at 20 weeks of pregnancy. Gestational diabetes mellitus (GDM) was defined as any glucose intolerance first diagnosed during pregnancy. Preeclampsia was defined as systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg after 20 weeks of gestation, along with proteinuria (≥ 300 mg of protein in 24-hour urine, or a result of at least +1 on dipstick) in hypertensive women. Small for gestational age (SGA) was defined based on weight less than the 10th percentile based on the gestational age chart (25). Premature delivery (<34 weeks), oligohydramnios, CNS abnormalities, and neonatal hospitalization in the NICU were also recorded.

Finally, adverse pregnancy outcomes were compared between two groups with and without MS-AFP elevation (AFP≥2.5 and AFP<2.5 groups).

Statistical Analysis

In order to perform statistical analysis, SPSS version 22 software was used. For quantitative variables, mean and/or median was used to describe the data, and standard deviation and/or interquartile range (IQR) were used to describe data dispersion. For qualitative variables, frequency and percentage were used to describe the data. The normality of the data was checked by the Kolmogorov-Smirnov test and Q-Q diagram. The independent t-test (or Mann-Whitney non-parametric test) was used to analyze the data and compare quantitative variables between the two groups, and Fisher's exact test was used for qualitative variables. The significant level in the tests was considered 0.05.

Results

In this study, 384 pregnant women at 15-20 weeks of pregnancy were studied in two groups: with and without MS-AFP screening. The basic characteristics of the participants in the two groups are presented in Table 1. The results showed that pregnant women in the two groups (with and without MS-AFP screening) had no significant differences in terms of age, gravidity, parity, and abortion history (P<0.05).

Table 1- Basic characteristics of pregnant women in two groups with MS-AFP screening and without MS-AFP screening

Variable	MS-AFP screening (192 people)	No screening for MS-AFP (192 people)	P-value
Mother's age (years), mean±S.D	31.40 ± 5.24	31.23 ± 4.18	0.541*
Gravid, mean±S.D	2.05 ± 1.00	1.96 ± 1.07	0.239*
Parity, mean±S.D	0.86 ± 0.81	0.83 ± 0.94	0.387*
History of abortion, n (%)	38 (19/8)	33 (17.2)	0.599**

* Independent t test

**Fisher's exact test

MS-AFP: maternal serum alpha-fetoprotein

Totally, adverse outcomes (maternal, fetal, and neonatal) were observed in 14.1% of the women studied. The frequency of women with negative outcomes was 28 (14.6%) in the MS-AFP screening group and 26 (13.5%) in the non-screening group.

The comparison of negative pregnancy outcomes between the two groups (with MS-AFP screening and without MS-AFP screening) is presented in Table 2, including pre-eclampsia, IUGR, oligohydramnios, gestational diabetes, cesarean delivery, preterm birth, stillbirth, SGA, and birth weight. There was no significant difference in the admission of newborns to the NICU. Abnormalities of the CNS and postnatal abnormalities were not observed in any of the two groups (P<0.05).

In this study, the level of maternal serum alpha-phytoprotein was increased (MoM AFP ≥2.5) in 21 people (10.9%), and normal values (MoM AFP<2.5) were detected in 171 women in the MS-AFP screening group. The comparison of adverse pregnancy outcomes based on normal and elevated MS-AFP levels has been presented in Table 3. In general, negative outcomes were observed in eight cases (38.1%) in women with MS-AFP levels ≥2.5 and 20 (11.7%) of women with MS-AFP levels <2.5 (P=0.004). Also, the incidence of pre-eclampsia (P=0.021), preterm birth (P=0.030), SGA (P=0.003) and admission to the NICU (P=0.021) in women with MS-AFP ≥ 2.5 was significantly higher than in the MS-AFP<2.5 group.

Table 2- Comparison of negative pregnancy outcomes in the groups with and without MS-AFP screening

Variables	MS-AFP screening (192 people)	No screening for MS-AFP (192 people)	P-value
Preeclampsia, n (%)	11 (5.7)	14 (3/7)	* 0.680
IUGR, n (%)	5 (2.6)	4 (1/2)	* 1/000
Oligohydramnios, n (%)	0 (0)	1 (0/5)	* 1/000
Gestational diabetes, n (%)	16 (8.3)	14 (3/7)	* 0.850
Cesarean delivery, n (%)	89 (46.4)	84 (8/43)	* 0.682
Preterm birth, n (%)	12 (6.3)	14 (3/7)	* 0.839
stillbirth, n (%)	0 (0)	1 (0/5)	* 1/000
Baby's birth weight (grams), mean±SD	485.82 ± 2995.23	505.94 ± 2908.72	** 0.246
SGA, n (%)	16 (8.3)	18 (4/9)	* 0.858
Newborn admission in NICU, n (%)	11 (5.7)	13 (8/6)	* 0.834
Percentage of negative outcome, n (%)	28 (14.6)	26 (5/13)	* 0.883

* Fisher's exact test

** Mann-Whitney test

MS-AFP: maternal serum alpha-fetoprotein; IUGR: Intrauterine growth restriction; SGA: Small for gestational age; NICU: Neonatal Intensive Care Unit

Table 3- Comparison of the frequency (percentage) of negative pregnancy outcomes based on MS-AFP level

Negative consequences of pregnancy	2.5 <MS-AFP (21 people)	2.5 >MS-AFP (171 people)	* P-value
Preeclampsia	4 (19/0)	7 (4/1)	0.021
IUGR	1 (4/8)	4 (2/3)	0.443
Gestational Diabetes	2 (9/5)	14 (8/2)	0.689
Cesarean delivery	10 (47/6)	79 (46/2)	0.902
Pre-term birthday	4 (19/0)	8 (4/7)	0.030
SGA	6 (28/6)	10 (5/8)	0.003
NICU admission	4 (19/0)	7 (4/1)	0.021
Negative outcome percentage	8 (38/1)	20 (11/7)	0.004

* Fisher's exact test

MS-AFP: maternal serum alpha-fetoprotein; IUGR: Intrauterine growth restriction; SGA: Small for gestational age; NICU: Neonatal Intensive Care Unit

Discussion

The results of this study showed that there was no significant difference in the frequency of negative pregnancy outcomes between pregnant women with and without MS-AFP screening, which indicated that MS-AFP screening had no effect on prenatal management and the incidence of complications and negative pregnancy outcomes. Although some studies have shown that the MS-AFP assay can help identify women at high risk of negative pregnancy outcomes in the early second trimester of pregnancy (6, 19). Based on these results, MS-AFP screening can be used to improve prenatal counseling and management. However, in the present study, MS-AFP screening did not have a significant effect in this regard.

On the other hand, the results of the present study showed that the incidence of negative pregnancy outcomes in the elevated MS-AFP group increased by 38.1% compared to 11.7% in the normal MS-AFP group, showing a statistically significant difference between the two groups. The incidence of preeclampsia, preterm birth, gestational age at delivery, SGA, and NICU admission in women with increased MS-AFP levels was significantly higher than in those with normal MS-AFP levels. However, the incidence of gestational diabetes, IUGR, and cesarean delivery was not significantly different between the two groups. These results show that MSAFP can be helpful in determining which group of mothers need more care and more diagnostic tests during the perinatal period. Although these findings are not new, and some past studies have reported similar results. MS-AFP assessment is still not used as a routine screening test to identify women at risk of adverse pregnancy outcomes.

In line with the findings of the present study, the results of the study by Hu and his colleagues who examined pregnancy outcomes in women with increased levels of maternal serum alpha-phytoprotein in the second trimester of pregnancy showed that negative pregnancy outcomes such as preterm birth, stillbirth, spontaneous abortion, preeclampsia, low birth weight, oligohydramnios, and placental abruption were higher in the group with increased levels of MS-AFP (≥2.5 MOM) than in the group with normal MS-AFP levels (33.80% vs. 6.04%). However, the incidence of gestational diabetes and cesarean delivery was not

significantly different between the two groups (6). In a study by Bartkute and his colleagues, pregnancy outcomes and maternal serum alpha-phytoprotein levels were investigated in the second trimester of pregnancy, and the results showed that the weight and height of the baby, as well as the gestational age at the time of delivery, were significantly lower in mothers with elevated levels of MS-AFP (>2.5 MoM) compared to others. Also, the prevalence of negative pregnancy outcomes such as SGA and IUFD was 26.1% in the group with elevated MS-AFP levels, which was significantly higher than in those with normal (5.6%) and reduced (7.3%) MSAFP levels. As a result, the level of MSAFP in the second trimester of pregnancy is an important indicator of negative pregnancy outcomes (19).

A positive association between increased MSAFP levels at 19-24 weeks of gestation and SGA has been reported in past studies, including the study by Poon et al. (21) and Lesmes et al. (27). It was also reported in Barta et al.'s study that MS-AFP could be used as a marker to determine fetal status and SGA (28). In another study, Sharony et al. reported that MS-AFP could be a predictor of SGA and gestational age at delivery, and that increased levels of alpha-phytoprotein were associated with low birth weight and lower gestational age (29). These results are consistent with the findings of the present study.

In a prospective study by Kaur and colleagues, the effects of MSAFP screening in high-risk pregnancies were investigated, and it was shown that increased levels of maternal serum MSAFP were associated with NTD, gut atresia, intrauterine fetal death, premature delivery, and neonatal complications (30). Also, Lalooh et al. reported in a case-control study that the level of alpha-phytoprotein in the mother's serum predicted the occurrence of premature birth (31).

Most negative pregnancy outcomes have placental origins in terms of pathogenesis, and in recent years, these disorders are considered placenta-mediated diseases (4, 32). At the same time, an increase in MS-AFP is said to be caused by placental dysfunction (15). Therefore, placental dysfunction can be a potential factor associated with increased MS-AFP levels and negative pregnancy outcomes (6).

Although a significant correlation between MS-AFP level and many negative pregnancy outcomes has been observed in the second trimester of pregnancy, the sensitivity and positive predictive value of this method as a routine clinical screening test is very low. Therefore, ultrasonography is still superior as a screening method, and women with increased MSAFP levels and failure to detect fetal malformations in ultrasound should undergo ultrasound again in the third trimester to check other possible complications of pregnancy (19).

Finally, MS-AFP screening for NTDs is optional in many countries such as the United States, Canada, Switzerland, and the United Kingdom. It is also quite evident that ultrasound technology is a suitable and effective screening method (19, 33). However, according to the results of the present study regarding the relationship between increased levels of MS-AFP and some negative outcomes of pregnancy, performing MS-AFP along with ultrasound for prenatal care screening and management can be valuable and useful. Elevated MS-AFP levels among other screening tests can show an increased risk of negative pregnancy outcomes. Therefore, it is recommended that pregnant women with increased MS-AFP levels in the second trimester of pregnancy, even without any fetal malformations in the second trimester ultrasound, undergo ultrasound evaluation again in the third trimester of pregnancy to check for IUGR and other negative pregnancy outcomes and be managed if necessary.

This study also faced some limitations such as the small number of women with increased MS-AFP levels. Other maternal factors, such as PIGF, MS-hCG and UtA-PI, that can predict some negative outcomes, including SGA, were not investigated in this study. Some factors related to the etiology of negative pregnancy outcomes were also not assessed, including socioeconomic status, heavy smoking, and maternal exposure to environmental pollutants (6). Also, the relationship between the increased levels of MS-AFP and negative pregnancy outcomes is unclear, and more studies in this field are necessary.

Conclusion

The results of this study showed that there was no significant difference in the incidence of adverse pregnancy outcomes between the two study groups (i.e., women with and without MS-AFP screening at 15-20 weeks of pregnancy), which indicates that MS-AFP screening in this study did

not have a significant effect on the incidence of negative pregnancy outcomes. Also, the results showed that the increased levels of MS-AFP were related to the increased risk of negative pregnancy outcomes. Therefore, MS-AFP assessment in the second trimester of pregnancy can help identify women at high risk of negative pregnancy outcomes in the early second trimester, and as a result, better management of prenatal care and appropriate measures to prevent subsequent problems in mothers.

Acknowledgments

This article is taken from the student thesis of the specialized assistant course in obstetrics and gynecology with the research project number FIRC-0111 in the Faculty of Medicine of Jundishapur University of Medical Sciences, Ahvaz, and this research was financially supported by the research vice-chancellor of Ahvaz Jundishapur University.

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