

Relationship between Maternal Serum Biomarkers (HCG, AFP, UE3, and Inhibin A) in the Second Trimester of Pregnancy and Preterm Labor

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ABSTRACT

Aims Preterm labor (PTL) is one of the most important medical community problems and the main cause of infant morbidity and mortality after congenital anomalies. Several studies have shown a significant association between the abnormal levels of quadruple test with PTL. However, there is no general agreement on the value of quadruple tests for predicting complications among studies. This study aimed to determine the diagnostic accuracy of maternal serum biochemical markers (HCG, AFP, UE3, and Inhibin A) in the second trimester of pregnancy and their effect on PTL.

Methods & Methods This longitudinal study was performed on 240 pregnant women who underwent quad-marker testing in the second trimester of pregnancy in Yasuj in 2019. Pregnancy and labor information was followed up and recorded in a checklist. Also, ROC (Receiver Operative Characteristics) was used to determine the test efficiency in PTL and the optimum point with the highest sensitivity and specificity. All analyses were performed using SPSS 22 software.

Findings 19 pregnant mothers (7.9%) had PTL. The best amount of AFP to detect PTL with a sensitivity of 72% and a specificity of 68% was estimated at 1.515. The best values of Inhibin A and HCG were also estimated at 1.245 and 0.885, respectively.

Conclusion Of the quadruple tests, three AFP tests, HCG and inhibin, effectively diagnose PTL during pregnancy.

Keywords Preterm labor; Second trimester; Gonadotropin; AFP; Inhibin A

CITATION LINKS

[1] Investigating the correlation between c-reactive protein and preterm delivery [2] Serum zinc & iron in the first half of pregnancy and their relationship with preterm delivery: A prospective longitudinal study [3] Investigating association between second trimester maternal serum biomarkers and pre-term delivery [4] Studying the combination of cervical interleukins-6 and-8 with cervical dilatation in predicting preterm labor [5] Evaluate the prevalence of premature labor and its related factors in the Shahrood in 2012 [6] Histologic evaluation of subclinical chorioamnionitis associated with preterm labor at Mahdiah hospital in Tehran [7] The ability of the quadruple test to predict adverse perinatal outcomes in a high-risk obstetric population [8] Unjustified abnormalities of analytes in the second trimester [9] Triple test and pregnancy outcome association [10] The prediction of adverse pregnancy outcome using low unconjugated estriol in the second trimester of pregnancy without risk of Down's syndrome [11] Correlation of pregnancy outcome with quadruple screening test at second trimester [12] Second trimester total human chorionic gonadotropin, alpha-fetoprotein and unconjugated estriol in predicting pregnancy complications other than fetal aneuploidy [13] From down syndrome screening to noninvasive prenatal testing: 20 years' experience in Taiwan [14] The predictive value of quadruple tests in the second trimester of pregnancy in identifying pregnancy complications [15] Quad screen as a predictor of adverse pregnancy outcome [16] Second-trimester serum chorionic gonadotropin concentrations and complications and outcome of pregnancy [17] Second-trimester maternal serum marker screening: Maternal serum α -fetoprotein, β -human chorionic gonadotropin, estriol, and their various combinations as predictors of pregnancy outcome.

Introduction

Preterm labor (PTL) is referred to as delivery before 37 weeks of gestation [1] and is considered one of the main and important problems of the medical community [2]. PTL is the main cause of neonatal morbidity and mortality after congenital anomalies [3]. Despite profound advances in neonatal care, PTL is still a major cause of neonatal death and significant complications, such as cerebral palsy and disability in school-age [4]. Annually millions of premature neonates are born, accounting for approximately 75% of cases of perinatal mortality and morbidity [5]. The prevalence of PTL is 5-7% in developed countries, but it is estimated to be higher in developing countries [5]. According to studies on the prevalence of PTL in Iran, the prevalence of PTL in Iranian pregnant women is estimated at 8% [6].

Since predicting and preventing PTL has a significant impact on improving neonatal conditions, it seems that measuring the quadruple tests (HCG, AFP, UE3, Inhibin A) can be useful as an alternative approach to identifying women at risk for PTL [7]. Quadruple tests are performed on the 20th to the 15th week of gestation [8]. Several studies have shown a significant association between the abnormal levels of quadruple test with PTL, pre-eclampsia, intrauterine growth restriction (IUGR), and premature rupture of membrane (PROM) [7]. However, there is no general agreement on the value of quadruple tests for predicting complications among studies, and further studies have been suggested for clinical application of this test in predicting complications [3]. So that Feyzbakhsh *et al.* [9] and Kim *et al.* [10] study also showed that there was no relationship between PTL and maternal serum biomarkers in the second trimester of pregnancy [9, 10]. While Yazdani *et al.*, Duric *et al.*, and Shaw *et al.* also examined the predictive effect of maternal serum biomarkers on the second trimester of pregnancy in their study and concluded that these markers have a good predictive effect on various problems in a singleton pregnancy such as gestational toxemia, PTL and intrauterine death [11-13].

Therefore, this study aimed to determine the diagnostic accuracy of maternal serum biochemical markers including HCG (Human Chorionic Gonadotropin), AFP (Alpha-Fetoprotein), UE3 (Unconjugated Estriol), and Inhibin A in the second trimester of pregnancy and its effect on the pregnancy outcome in this area, in order to predict and prevent pregnancy complications and neonatal mortality for a step towards reducing the resulting economic and psychological cost at the community level.

Materials and Methods

This longitudinal study was conducted on pregnant mothers who underwent quad-marker testing in the second trimester of pregnancy to be screened for

Down syndrome in Yasuj in 2019. So, the 240 women who were willing to participate in the study were selected by simple random sampling from pregnant mothers referring to Imam Sajjad Hospital in Yasuj. The sample size required to diagnose pregnancy outcomes with a sensitivity of 66%, based on the results of the study conducted by Zahra Sehat *et al.*, with 95% confidence interval (CI) and maximum accuracy of 7% sensitivity and considering the sample loss of 5% was estimated to be 240 using the sample size formula [3].

The following women were excluded from the study because they were at risk of adverse pregnancy outcomes:

- Mothers over 35 years old
- Mothers with a positive history of PTL
- Mothers with a child suffering from Down's syndrome

After signing the informed consent, the pregnant mothers completed demographics and clinical data checklists. After taking the history and demographic characteristics of mothers and registering them on the checklist, the mothers were enrolled in the study. Pregnancy and delivery data of pregnant women tested by Quad Marker in the second trimesters of pregnancy during 2019 were evaluated to determine the relationship between serum levels of biochemical markers and the pregnancy outcome.

For the Quad Marker test, 3cc of the blood sample was taken between 14 and 20 weeks of gestation. This blood sample was stored in a refrigerator at a temperature of 27°C for one week to separate the serum's plasma. Then, the levels of each of these four biomarkers (HCG, AFP, UE3, Inhibin A) were measured.

A Microplate Reader device performed all measurements. UE3 was measured using UE3 AccuBind ELISA 300-5025 Kits (Monobind Inc.; USA). AFP was measured using AFP ELISA Kit0 (Ideal Company, Diagnostic Kit; Iran). Inhibin A was measured using Inhibin an ELISA AL-123SKU (AnshLabs; USA), and finally, HCG was measured using HCG ELISA Kit0 (Pishtaz Tabriz; Iran).

Contact information of mothers whose test results were clear was recorded. Then, these mothers were followed up by the researcher until childbirth. In the case of abortion in this period, the patient was visited by the researcher, and if the diagnosis was finalized, the result was measured and recorded.

To determine the sensitivity and specificity of each marker in determining the pregnancy outcome, two by two tables included true and false positive and negative cases were drawn, and sensitivity, specificity, positive and negative predictive values were calculated using the following formula:

Sensitivity = true positive / true patients

Specificity = true negative / true healthy individuals

Also, to determine the test efficiency in PTL and the optimum point with the highest sensitivity and

specificity, ROC (Receiver Operative Characteristics) was used. All analyses were performed using SPSS 22 software.

Findings

Two hundred forty pregnant women participated in the study. 70 (29.2%) of them were in the age group of 15 to 25 years old, 71 (29.6%) of them were in the 26 to 30, and 99 (41.3%) of them were in the age group of 31 to 35 years old. Sixty-four mothers were employed. In terms of pregnancy, 57.5% of mothers were in the second or more pregnancies. Also, 19 of them (7.9%) had PTL (Table 1).

Table 1) Frequency distribution of demographic characteristics of the studied mothers with preterm labor (n=19) and timed delivery (n=221)

Variables	Preterm labor	Timed delivery	P. value
Age group (Years)			
15-25	5 (7.1)	65 (92.9)	0.1
26-30	9 (12.7)	62 (87.3)	
31-35	5 (5.1)	94 (94.9)	
BMI (Kg/m ²)			
<25	7 (10.4)	60 (89.6)	0.6
25-30	9 (6.5)	129 (93.5)	
>30	3 (8.6)	32 (91.4)	
Occupation			
Employed	9 (14.1)	55 (85.9)	0.05
Housewife	10 (5.7)	166 (94.3)	
Number of pregnancies			
<2	7 (6.9)	95 (93.1)	0.1
≥2	12 (8.7)	126 (91.3)	
A positive history of chronic diseases in the mother*			
-	3 (6.7)	42 (93.3)	1

*Diseases included diabetes mellitus, lung and digestive diseases, UTI, and chronic hypertension

Diagram 1 compares the ROCs of quadruple diagnostic tests, AFP, HCG, UE3, and Inhibin, in detecting PTL. The AUC (Area under the ROC Curve) for Inhibin and AFP was greater than the other curves, and the AUC of Inhibin, AFP, and HCG curves were statistically significant, while the AUC of UE3 was not statistically significant (Table 2).

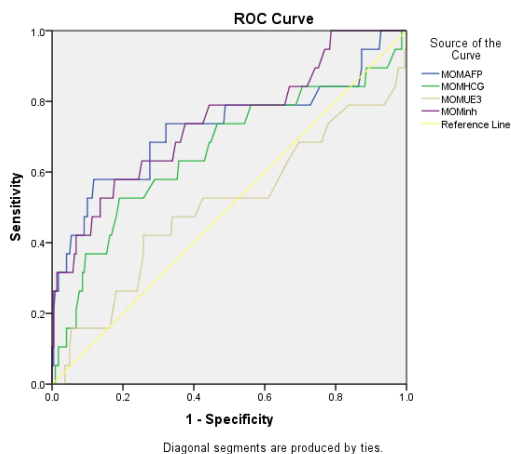


Diagram 1) ROC curve for determining the efficacy of quadruple tests in detecting PTL

Table 2) AUC of the ROC curve for determining the efficacy of quadruple tests in detecting PTL

Quadruple tests	AUC	P. value	95% CI	
			Lower bound	Upper bound
MOM* AFP	0.711	0.003	0.556	0.865
MOM HCG	0.696	0.006	0.557	0.835
MOM UE3	0.521	0.770	0.361	0.680
MOM Inhibin A	0.747	0.001	0.609	0.884

*Multiple of Median

The best AFP for detecting PTL with a sensitivity of 72% and a specificity of 68% was 1.515. Also, INH and HCG's best values were 1.245 and 0.885, respectively (Table 3).

Table 3) Optimum values for each test in the detection of PTL

Test	Optimum point	Sensitivity*	Specificity**
AFP	1.515	72%	68%
HCG	0.885	58%	72%
UE3	0.585	47%	66%
Inhibin A	1.245	63%	75%

*Sensitivity= true positive / true patients; **Specificity= true negative / true healthy individuals

Discussion

Despite the many advances made in newborns' care, preterm delivery is still a major cause of death in infants and causes important complications such as cerebral palsy and disability during school hours. Since stopping the process of starting labor with preterm delivery has been associated with less success, today, researchers predict the possibility of preventing preterm labor. The first step to prevent preterm delivery and predict it in this early detection and treatment of women at risk during prenatal care is part of the main objectives.

Multiple tests can be used to predict pregnancy effects on pregnancy at different times. Meanwhile, several markers have been introduced. Nevertheless, the test used to predict should have a high positive sensitivity, feature, and value and give the physician enough time to intervene. However, there is no general agreement on the value of quadruple tests for predicting complications among studies. Extensive studies have been recommended for the clinical application of this test in predicting complications. This study determined the level of maternal serum biochemical markers (AFP, HCG, Inhibin A, UE3) in the second trimester of pregnancy and its association with preterm labor.

The prevalence of PTL was estimated at 7.9% in this study. Other studies in Iran and other parts of the world have also reported similar rates [3].

We observed that among the quadruple tests conducted during pregnancy, three AFP tests, HCG, and Inhibin were effective in diagnosing PTL during pregnancy. Although these tests' predictive value was not excellent, they can be considered appropriate

predictors for pregnancy outcomes. Therefore, this test is consistent with Yazdani *et al.* study, which examined the relationship between the Quadri tests with PTL, so that there was also a significant relationship between AFP, hCG, and inhibin-A with pr PTL in this study, but there was no significant relationship between UE3 and PTL [14].

In Zahra Sehat and colleagues' study, 700 pregnant women were investigated in the second trimester of pregnancy; 53 mothers (7.6%) had PTL. The sensitivity and specificity of the quadruple tests for PTL were 20.8% and 32%, respectively, which did not have a significant relationship with PTL. UE3 with Multiple of Median (MoM) <0.8 showed a significant statistical relationship with PTL. The results showed that the decrease in serum level of UE3 in the second trimester of pregnancy was related to the incidence of PTL, which was contrary to our study results, which showed that UE3 was the only marker that was not a significant predictor for PTL. Nevertheless, it has been mentioned in their study that the clinical application of this test in predicting PTL has to be further investigated [3].

According to Dugoff *et al.*, if only one of the markers is abnormal, there would be a small but significant association between pregnancy problems and abnormal levels of markers. Also, if two or more of the markers are abnormal, the risk will increase. One of the pregnancy problems investigated in this study was PTL, and among the markers studied in this study, the highest correlation was observed with increased inhibin A ($p=0.04$) [15]. However, in our study, although the AUC of Inhibin was greater than other markers, the overlap between confidence intervals of AUC of three markers of Inhibin, AFP, and HCG showed no significant difference in AUC of these markers.

In the cohort study by Duric *et al.*, 2384 pregnant mothers were assessed in Croatia. The results indicated that increased serum level of HCG in mothers in the second trimester was associated with PTL and intrauterine death, and decreased UE3 in the second trimester of pregnancy was associated with PTL IUGR [9]. There was also a significant relationship between hCG and PTL in the present study, but in contrast to the above study, there was no significant relationship between UE3 and PTL.

In Walton *et al.*, high HCG levels in the mother's serum were associated with an increased incidence of placental anomalies, stillbirth, and PTL. Finally, they concluded that maternal serum HCG measurement had a small predictive value for pregnancy problems and outcomes [16]. Therefore, the present study showed that UE3 was the only marker that was not a significant predictor for PTL.

In Yaron *et al.*, the results indicated that AFP increase, greater than MOM =2.5 as the cut-off point, was significantly associated with increased risk of PTL, oligohydramnios, and early placental detachment. The increase in HCG serum levels greater than MOM

=2.5 was significantly associated with hypertension, abortion, PTL, and intrauterine death. Finally, they concluded that the Multiple-Marker screening test is useful to screen Down's syndrome and determine high-risk pregnancies [17].

In the present study, the best AFP for detecting PTL with a sensitivity of 72% and a specificity of 68% was 1.515. Furthermore, AFP greater than 1.515 was significantly associated with an increase in PTL.

One of the limitations of the present study was that all mothers were investigated for Down's syndrome screening. Thus, perhaps, they could not represent all pregnant women and, notably, mothers at risk of PTL. Therefore, studying mothers with a high risk of PTL can be helpful. It is necessary to reach a general agreement on the appropriate cutting point before introducing these tests as a screening test and using them in the patient's bed.

Conclusion

Of the quadruple tests, three AFP tests, HCG and Inhibin, effectively diagnose PTL during pregnancy.

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Ethical Permissions: The Ethics Committee approved this study of Yasuj University of Medical Sciences with IR's code number.YUMS.REC. 1398.066. Informed consent was obtained from pregnant women before their inclusion in the research.

Conflicts of Interests: The authors declared no conflict of interest.

Authors' Contribution: Pouzesh M. (First author), Introduction author/Discussion author (20%); Nakhaee Moghaddam M. (Second author), Methodologist/Assistant (20%); Davoodi M. (Third author), Introduction author/Methodologist/Original researcher/Statistical analyst/Discussion author (60%).

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