

Gynaecology

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Delivery, Triple test, AFP, HCG, UE3, ROC curve.

**TO INVESTIGATE THE ASSOCIATION
BETWEEN TRIPLE TEST AND PRE-TERM
DELIVERY.**

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**ABSTRACT**

Background: Preterm labor is one of the main problems of health care and one of the main factors in neonatal mortality and morbidity. It's important that with efforts to predict and prevent premature delivery that can cause infant deaths.

Objective: To investigate the association between Triple test (HCG, MSAFP and UE3) and Pre-term delivery.

Methods: This is a historical Cohort Study that has been performed for 700 pregnant women, clients of Nilou Lab in the second trimester of pregnancy to take the Triple test between March and September 2012. The information of Mothers having required conditions to enter to the study has been registered and after delivery, they called again to be interviewed. These data sets using statistical tests: chi-square test and ROC Curve was analysis.

Results: There is a direct relationship between the increase AFP (P value =0.011) serum level and preterm delivery. Also, there is an inverse relationship between the UE3 (P value =0.002) serum level and preterm delivery. Moreover, there is not any relationship between the increase HCG (P value =0.68) serum level and preterm delivery.

Conclusion : The increase in the AFP and decrease in UE3 serum levels in the second trimester of pregnancy lead to enhance the probability of preterm delivery. Moreover, if the current study is done with higher samples and different sampling environment, may be having different results.

Introduction:

Preterm labor is one of the major health problems which is occurred and after major congenital malformations and mortality neonatal morbidity is operating. Due to the economic costs of mental death at community level to reduce, it is important that efforts to predict and prevent premature birth and infant mortality rates [1, 2 and 3]. Each year millions of children are born premature. Almost 75 percent of the prenatal morbidity and mortality is preterm delivery [4, 5 and 6]. Prevalence of preterm delivery in 2004 is about 12.5% in the United States [7]. Since stopping the processes of starting labor with preterm delivery has been associated with less success, today, researchers predict the possibility of preventing preterm labor and have more focus. The first step in preventing preterm delivery and its prediction in this early detection and treatment of women at risk during their prenatal care is part of the main objectives [8-11].

Increased incidence of preterm labor is a significant thread in the whole world. So that in Western countries in 2010 from 6 to 10 percent of all births were preterm, more than two-thirds of all deaths during prenatal period and 75 to 80 percent of all deaths in babies due to premature delivery occurs [11-13]. The incidence of preterm

delivery before 37 weeks is approximately 7 to 11 percent and before 34 weeks gestation are included 3 to 7 percent of all pregnancies [14]. Despite many advances in prenatal care, preterm birth rate has increased slightly in recent years. Increase in preterm deliveries could be due to increased prevalence of multiple deliveries following the use of ovulation drugs, increased obstetric intervention, increased stress and Progress in the discovery of preterm deliveries, the use of artificial reproductive techniques, and increased use of ultrasound to estimate gestational age.

Different methods such as biological and demographic factors, serum markers and cervical changes have been studied for screening to predict preterm delivery; also, during a study level of salivary Estradiol and its association with preterm delivery has been investigated [14-16]. Each of them has disadvantages, advantages, features, and its certain time to do. But, none of them has required sensitivity, specificity and positive predictive value

According to recent studies on maternal serum markers Biochemical changes in certain conditions, lead to face mother with premature delivery and preeclampsia during pregnancy, decided to evaluate the Possible Role of biochemical markers in the second trimester of pregnancy as a reliable test to predict preterm delivery [17-18].

The screening test is a Triple test assessed three biochemical markers (HCG, MSAFP, UE3) in maternal blood in the second trimester

This study is in fact the review of the value of triple test (HCG, MSAFP and UE3) in prediction of the incidence of second trimester of pregnancy for preterm labor.

Methods:

In a historical Cohort Study, asked of mothers about pregnancy and childbirth i.e. clients of Nilou Lab to perform screening for down in the second trimester of pregnancy between March 2012 to September 2012, Triple test has been determined to investigate the relationship between serum levels of biochemical markers and the incidence of preterm delivery.

These individuals are selected with consideration of the following conditions:

- Singleton pregnancy
- No maternal infection or chronic systemic diseases including liver disease, heart, kidney, diabetes, lupus and ...
- BMI >30
- Not to detect fetal abnormalities
- Not to detect intrauterine death
- Non maternal addiction to cigarettes, alcohol and drugs
- No diagnose placental abruption premature

-Not to Diagnosis of premature rupture of membranes

The mothers with higher risk of preterm delivery i.e. mothers who have one of specifications like fetus with Down syndrome, twin pregnancy, genetic or chromosomal disorder, history of preterm delivery, history of abortion and maternal diseases such as diabetes mellitus and chronic hypertension, addicted to cigarette, alcohol or opiate which was detectable were excluded.

Those who have entered into the study, have been selected and primary data set has been recorded and in the second trimester of pregnancy has been tested. Then, after delivery, study population has been taken by telephone. After obtaining verbal consent from them, all the mothers and neonates were completed and then with the help of appropriate statistical tests examined the relationship between the biomarker and the outcome of pregnancy.

Between weeks 14 to 20 for Triple test, 3cc blood samples was taken for testing of maternal. The blood samples in the refrigerator for 24 hours at 2 to 8 degrees Celsius in order to separate plasma from serum was then maintained with the help of special devices and special way each level and each of these three biomarkers (HCG, AFP, UE3) was measured.

AFP and HCG Eleusis system and method using Electrochemiluminescence Cobase cholera kits were examined. But each of them was tested separately and UE3 is measured by ELISA. UE3 DEMEDITEC Free Esteriol ELISA kit was evaluated.

Statistical analysis:

To determine the cut-off point for the different biomarkers and first the false positive detection rate for the five-point 0.5, 0.75, 1, 1.25 and 1.5 and the percent detection rate and false positive percentage in the population studied have been determined.

The two graphs in terms of detection rates and cut the percentage rate of detection of false positive percentage was plotted. Percent of the charts in terms of percentage detection rate of false positive point is the curve with the minimum slope on the graph and the rate of diagnosis based on cut-off point to determine the appropriate cut-off point for the marker studied is how much. This procedure was repeated for all three markers and cut-off point for all of them was determined.

The most important thing in choosing a cut that MOM of choice with minimal false positive rate is the maximum sensitivity in this study, maximum 30% false positive was placed in the base.

This data set have been entered to useful computer format using SPSS 16.0 software and analyzed using the statistical and analytical tests like ROC Curve to obtain the cut-off point and Chi Square Test has been used to examine the relationship between preterm labor and Biomarkers, and each of the variables examined in this study.

Results:

This study was conducted 1,000 people who due to circumstances not having 300 of them were excluded for reasons such as twin pregnancy, preeclampsia, placental abruption, PROM and detect abnormalities in the fetus and risk of gestational diabetes and They follow up on stage and 100 were not available or were not willing to cooperate, at the end of the study population were 700 people.

In this study average age of mothers was 26.17 ± 4.72 years and mean duration of pregnancy were 38.4 ± 1.27 weeks. 7.3% of mothers have preterm labor and 93.7% have full term delivery.

Table 1 - Demographic characteristics of the studied mothers

	Preterm delivery (percent)	Term delivery (percent)		P value

Maternal age(year)	15-25	9(16.98)	104(16.07)		0.15
	26-30	22(41.50)	255(39.41)		
	31-35	22(41.50)	281(43.43)		
	>35	0(0)	7(1.08)		
First trimester Maternal BMI(kg/m2)	25>	15(47.16)	340(52.55)		0.38
	25-30	19(35.84)	237(36.63)		
	>30	9(16.96)	70(10.81)		
job	Housewife	37(69.81)	456(70.47)		0.91
	Employed	16(20.18)	191(29.52)		
Number of previous pregnancies	>2	49(92.46)	600(92.73)		0.96
	<2	4(7.54)	47(7.26)		
History of Abortion		11(20.76)	126(19.7)		0.80
History of chronic illness*		7(13.20)	60(9.27)		0.73
History of preterm delivery		13(24.52)	39(6.027)		0.90

* Maternal diseases such as diabetes mellitus and chronic hypertension

Between serum levels AFP ($p=0.011$) and preterm delivery direct relationship was seen. Relationship between serum levels of UE3 ($p=0.002$) and preterm labor was negative. Relationship between preterm delivery and HCG ($P=0.683$) was not observed.

Figure 1 - Relationship between the occurrence of preterm delivery and levels of serum markers in mothers in the second quarter of pregnancy

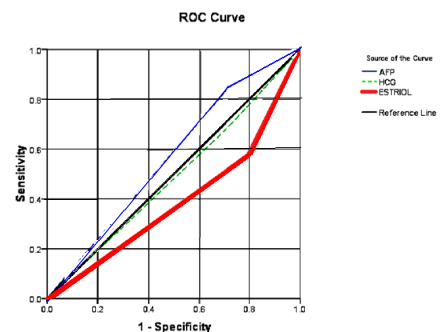


Table 2 - Relationship between the occurrence of preterm delivery and levels of serum markers in mothers in the second quarter of pregnancy

	Standard Error	P value	Confidence interval	Curve
HCG	0.41	0.74	0.47-0.567	0.4870
AFP	0.43	0.11	0.481-0.652	0.567
UE3	0.37	0.00	0.319-0.465	0.392

Table 3 - Frequency distribution of mothers according to study serum levels of mothers Triple test second trimester of pregnancy

Cut off(MOM)	Sensitivity	specificity	PPV 1	NPV2
UE3<0.8	20.8%	57.79%	40.7%	59.3%
HCG> 1.25	26.4	70.9	28.9	71.1
AFP>1.4	28.3	85	16	84

1-PPV: Positive Predictive value
2-NPV: Negative Predictive value

Table 4 - Frequency distribution of mothers according to study serum levels of mothers Triple test second trimester of pregnancy

Triple test	Preterm delivery		Delivery on time		Total	
	Number	Percent	Number	Percent	Number	Percent
Positive	31	58.49	426	65.84	457	65.3
Negative	22	41.50	221	34.11	243	34.7
P value =						
0.2						

Discussion, conclusion:

This study determined the level of maternal serum biochemical markers (AFP, HCG, UE3) in the second trimester of pregnancy and its association with preterm labor. 700 pregnant women according to inclusion criteria considered were the desired outcome (preterm delivery) were investigated.

In this study the normal level AFP Maternal serum $MOM \leq 1.4$ and , HCG with $MOM \leq 1.25$ and UE3with $MOM \geq 0.8$ have been considered.

7.3% of mothers in this study have preterm labor. In other studies in the world, like Mehrabian in [12], Jacobson in [15] and Dug off in [17] and etc, similar prevalence has been reported [12-17].

In a study, that was conducted by Kim and colleagues [8], unlike the results obtained in this study, there was a relationship between the incidence of preterm delivery and lower maternal serum levels of UE3 in the second trimester of pregnancy. This study which was conducted in 2000, 1096 mothers in their second trimester pregnancy screening of AFP, HCG and UE3 had been performed, were studied. Individuals with levels of AFP and HCG $MOM > 2$ were excluded from the study were reduced to the exact role of the UE3 is anticipated consequences of pregnancy. Two groups of people with $MOM \leq 0.75$ levels and the level of UE3 $MOM > 0.75$ divided and adverse consequences of pregnancy were studied. But in terms of incidence of preterm delivery, there was no difference between the two groups.

Levels of UE3 in fetal and maternal serum depend on cooperation of placental steroids and availability of DHEA by the fetal adrenal. With the reduction in uterine blood flow, the pair is associated with reduced levels of UE3.

Thus, decreased levels of UE3 in the second trimester of the embryos or placental dysfunction can show, and in these cases fortunate pregnancy is not expected. In this study, people with abnormal levels of AFP and HCG have been eliminated. While studies have shown that reduced levels of HCG and UE3 with increased levels of AFP is associated with abnormal levels. But, in this study, UE3 levels has been measured without excluding individuals people with abnormal levels of AFP and HCG.

According Dug off and colleagues in 2005 a direct relationship has been seen between increasing maternal serum AFP in the second trimester of pregnancy and the incidence of preterm delivery ($p = 0.047$). Similarly, a direct relationship between increase of Inhibin A in maternal serum and second trimester of pregnancy and the incidence of preterm delivery has been observed ($p = 0.04$) [17].the same results for AFP have been obtained in the presented research. In this cohort study that 33,145 pregnant women take the Triple test in the second trimester of pregnancy, referred Triple test relationship between the three components with adverse pregnancy outcome was evaluated. Such a result was that people with abnormal levels of biomarker are in a modest but significant risk of undesirable consequences of pregnancy to be seen; But, if the risk of having abnormal levels of two or more biomarker is to be

increased. For example, the risk of preterm delivery in case of AFP levels alone is $MOM \geq 2$, OR: 1.76 but in case of AFP and InhibinA levels with $MOM \geq 2$, OR: 4.19 in case of AFP, InhibinA, and $MOM \geq 2$ with HCG and UE3 with $MOM < 0.5$, OR: 4.05. In this study, as well as sensitivity and positive predictive value test, the simultaneous use of multiple biomarkers reduced. But given the fact that screening tests along with the sensitivity and positive predictive value, negative predictive value rates and features can also be important to the exchange rate fairly high sensitivity of the feature was also tested.

Based on the study performed in 2003, Duric et al to assess the relationship between serum levels of three markers AFP, HCG and UE3 in 15 to 22 weeks of pregnancy with the consequences of pregnancy and the historical cohort method on 2384 pregnant women in the country Croatia was contrary to recent research results, increasing maternal serum HCG $MOM \geq 2.2$ in the second trimester of pregnancy is associated with the incidence of preterm delivery (Odds Ratio = 2.5, $p < 0.05$). This study compared with the recent study, higher levels of HCG Cut off placed the implementation of research results with a different environment and more likely with higher sample numbers. Also, obtained similar results in this study, between UE3 reducing maternal serum second trimester of pregnancy and preterm labor relations can be seen (Odds Ratio: 2.2, $p < 0.05$) [18].

In a study done by Lalooha and colleagues performed in 2004 , like the recent study of maternal serum AFP levels increased in mothers with preterm labor has more ($p < 0.05$) [19]. Therefore be concluded that the AFP as a valuable biochemical marker to predict preterm labor is used.

Although this study predicts AFP and UE3 in the incidence of preterm labor indicated, but due to the high cost of this screening test, usually not all are willing to do it. However, this point should be noted that these costs can spend a lot of psychological consequences and financial costs of preterm birth, fetal abnormalities and disease for individuals, families and communities to prevent. For this purpose, parental education about the benefits of screening tests during pregnancy is important.

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