

## Correlation of pregnancy outcome with quadruple screening test at second trimester

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Received: 24 January 2014

Accepted: 7 July 2015

Published: 19 October 2015

### Abstract

**Background:** Abnormal levels of the markers AFP, hCG, and uE3 could be useful in predicting adverse pregnancy outcomes. This study was designed to determine the correlation between second trimester maternal serum markers and adverse pregnancy outcome (APO).

**Methods:** In this historical cohort study, we randomly followed 231 obstetric patients with quadruple screening test in 14-18 weeks of gestation from March 2012 to March 2013 in a medical laboratory in Babol, Iran. We measured maternal serum levels of alphafetoprotein (AFP), human chorionic gonadotropin (hCG), unconjugated estriol (uE3), and inhibin-A. The risk of adverse pregnancy outcomes (APOs) were then compared between patients with negative and positive test results. We used Chi-square and Fisher-exact tests for qualitative variables and t-test for quantitative variables. Demographic differences between the two groups were minimized by applying logistic regression.

**Results:** The risk of having an APO such as pre-eclampsia ( $p=0.008$ ), fetal growth restriction ( $p=0.028$ ) and premature rupture of membrane ( $p=0.040$ ) increased significantly in patients with abnormal markers.

**Conclusion:** Abnormal results of quadruple screening test could be associated with APO in women with normal appearing fetus.

**Keywords:** Quadruples, Prenatal Screening, Pregnancy trimester.

*Cite this article as:* Yazdani Sh, Rouholahnejad R, Asnafi N, Sharbatdaran M, Zakershob M, Bouzari Z. Correlation of pregnancy outcome with quadruple screening test at second trimester. *Med J Islam Repub Iran* 2015 (19 October). Vol. 29:281.

### Introduction

Incidence of major abnormalities apparent at birth is 2 to 3 percent. These anomalies cause a significant portion of neonatal deaths; furthermore, more than a fourth of all pediatric hospital admissions are due to genetic disorders. Fetal chromosomal abnormalities screening is an essential part of antenatal care. In the past two decades, additional tests have been shown to increase the detection rate of chromosomal abnormalities while maintaining a low false-

positive rate. This gives pregnant women of all ages the opportunity to undergo screening or invasive diagnostic test before 20 weeks gestation (1-4). Triple test has been available since the late 1980s as a cost-effective serum pregnancy screening test for Down's syndrome and neural tube defects. Maternal serum alphafetoprotein (AFP), human chorionic gonadotropin (hCG), and unconjugated oestriol (uE<sub>3</sub>) are the three components of the second tri-

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mester triple screen. Researchers have shown that abnormal levels of the markers AFP, hCG, and uE<sub>3</sub> have been useful in predicting adverse pregnancy outcomes (5-10).

Despite the accessibility of such screening tests for Down's syndrome, Trisomy 18 and NTD, unfortunately no information is available on the rate of false-positive results and the invasive effect of such tests in Iran, particularly in Mazandaran region. This research aims to shed light on the statistics of such complications on fetus and mother's health by following up the health of the statistical population during pregnancy.

It is hoped that the results of this research help the health care authorities to take the necessary measures in planning special care services, and to find wherever the risk is high for these women and their fetus.

## Methods

### Data

Historical-cohort was planned on a population of 300 cases who were screened in 14–18 weeks of pregnancy (based on the last menstrual period or first trimester sonography) in a medical laboratory in Babol, Iran from March 2012 to March 2013. Sixty nine cases did not meet the initial criteria and were excluded from the study. From the remained 231 cases, 151 individuals were placed in the negative quadruple test and 80 were assigned in to the positive quadruple test control group. To find a correlation between each of these APOs with the measured markers, the two groups were compared with respect to the occurrence of APOs.

Two hundred individuals were randomly selected, considering 95% confidence level and 80% power. Written informed consent was obtained from all the individuals and the study protocol was approved by the Vice-Chancellor of Research's Ethics Committee of Babol University of Medical Sciences.

We collected initial information of the patients from the existing files at Ayatollah

Rouhani hospital and from the personal inquiries before the screening test. All individuals, who met the inclusion criteria and consented to participate in the study, were selected. Those diagnosed with chronic hypertension, diabetes mellitus (DM), renal failure, heart disease, thalassemia, hyper or hypo thyroid, or those who had multiple pregnancy, congenital disorder in the father or mother, and positive amniocentesis were excluded from the study.

We collected data thorough records of the pregnancy conditions and APOs including anemia, VB in pregnancy, history of infertility and the type of the treatment in case, history of UTI in pregnancy, gestational diabetes mellitus (GDM), gestational hypertension (GHTN), preeclampsia, preterm labor (PTL), intrauterine growth restriction (IUGR), poly and oligohydramnios, premature rupture of membrane (PROM) and other disorders such as skin disorders or placental abruption after the labor. In addition, duration of the pregnancy, cause of pregnancy termination and infant weight were recorded in the check list.

Quadruple test including AFP, hCG, uE<sub>3</sub>, and inhibin-A were requested in this research for the pregnant women with 14 to 18 weeks of gestational age. Blood samples (5cc) were collected under sterile conditions and serum was separated for markers quantification. AFP and hCG were analyzed with electrochemiluminescence with cobus kit and Hitachi instrument, 2010. ELISA was used for the analysis of estriol and inhibin-A with uE<sub>3</sub> kit from Di Modtech, Germany and Bicmen kit, the US, respectively. Experimental data were then compared with the existing tables and were interpreted based on the age of the mother and pregnancy. Pregnancy age was estimated from the confident Imp or the sonography of the first trimester of pregnancy.

Then the correlation between each marker (component of the quadruple screening test) and the quadruple screening test with adverse pregnancy outcome were assessed.

### Statistical Analysis

Chi-square and Fisher-exact test were used for qualitative variables and t-test was utilized for quantitative variables. Logistic regression was used to eliminate the effect of relevant factors. P-value < 0.05 was considered as statistically significant.

### Results

From the 231 women in this study, 151 individuals were grouped as the negative-quadruple test control group and 80 individuals as the positive-quadruple test case group according to the samples available. The two groups had no significant differences in parity ( $p=0.060$ ), gravid ( $p=0.061$ ), BMI ( $p=0.070$ ), abortion history ( $p=0.392$ ), ectopic pregnancy history ( $p=0.158$ ), and education ( $p=0.059$ ). However, a significant difference was observed in the age of the two statistical populations; and we used logistic regression to eliminate its interfering influence (Table 1).

In the case group with positive screening, 18.8% ( $n=15$ ) were diagnosed with Pre-

eclampsia compared to 2.6% ( $n=10$ ) in negative screening group. The same trends were found in the increased risk of intrauterine growth restriction (IUGR), and premature rupture of membrane (PROM); of the participants, 12.5% ( $n=10$ ) vs. 1.3% ( $n=2$ ) had IUGR and 11.3% ( $n=9$ ) vs. 2% ( $n=3$ ) had PROM. These differences were statistically significant. The differences between the P-values for Gestational hypertension (GHTN), Preterm Labor (PTL), oligohydramnios, polyhydramnios, and Gestational Diabetes Mellitus were not statistically significant. Also, the mean of the two groups were significantly different in the pregnancy duration and infant weight, which were expected considering the adverse pregnancy outcome in the positive screening test group (Table 2).

Descriptive statistics (mean $\pm$ SD) of the serum markers in the case and control groups are demonstrated in Table 3.

The mean $\pm$ SD age of the total population was 28.3  $\pm$  6.48. Results from Table 4 revealed that only uE3 with a p-value of 0.007 had a significant correlation with age.

The correlation between the APO and

Table 1. Demographic specification of the two controls and case group

	Group 1 (-QT) (mean $\pm$ SD)	Group 2 (+QT) (mean $\pm$ SD)	p
Age	28.7 $\pm$ 5.83	31.7 $\pm$ 6.45	<0.001
BMI	25.7 $\pm$ 4.65	26.7 $\pm$ 3.64	0.070
Gravid	1.7 $\pm$ 0.89	2 $\pm$ 0.80	0.061
Parity	0.5 $\pm$ 0.63	0.6 $\pm$ 0.58	0.060
History of abortion	0.2 $\pm$ 0.54	0.3 $\pm$ 0.59	0.392
History of ectopic pregnancy	0.1 $\pm$ 0.11	0	0.158
Infant weight	3295.3 $\pm$ 440.39	2840 $\pm$ 798.08	0.000
Mean duration of pregnancy (week)	39 $\pm$ 1.414	36.4 $\pm$ 4.60	0.000

Table 2. Frequency of adverse pregnancy outcomes (APO), infant weight and gestational age in the case and control groups

	Group 1 (-QT) %(n)	Group 2 (+QT) %(n)	p
Pre-eclampsia	2.6 (4)	18.8 (15)	0.008
IUGR	1.3 (2)	12.5 (10)	0.027
PROM	2 (3)	11.3 (9)	0.040
GHTN	6.6 (10)	20 (16)	0.110
PTL	6.6 (10)	15 (12)	0.436
Oligohydramnios	2 (3)	8.8 (7)	0.437
Polyhydramnios	3.3 (5)	3.8 (3)	0.322
GDM	17.9 (27)	31.3 (25)	0.294

IUGR: Intrauterine Growth Restriction, PROM: Premature Rupture of Membrane, GHTN: Gestational hypertension, PTL: Preterm Labor, GDM: Gestational Diabetes Mellitus

Table 3. Descriptive statistics of the serum markers in the case and control groups

Criteria	Group 1 (-QT) (mean±SD)	Group 2 (+QT) (mean±SD)	p
MSAFP level	32.2±18.40	42.4±37.23	0.024
AFP (MoM)	1.1±0.52	1.5±1.35	0.013
UE3 level	2.2±2.32	1.7±1.98	0.115
UE3(MoM)	1.1±0.58	0.7±0.41	0.000
hCG level	30381.5±18079.06	45438.3±29801.64	0.000
hCG (MoM)	1±0.54	1.7±1.03	0.000
Inhibin-A level	265.6±135.09	389.8±216.39	0.000
Inhibin-A(MoM)	1.1±0.59	1.7±0.95	0.000

markers were as follows: preeclampsia was associated with the higher level of Inhibin-A ( $p < 0.001$ ). The mean was 418.684pg/ml (2.037MoM) in preeclampsia and 297.072pg/ml (1.318MoM) in normal pregnancy.

IUGR was associated with the higher level of Inhibin-A ( $p = 0.020$ ) and AFP ( $p = 0.015$ ). The mean of Inhibin-A was 559.909pg/ml (2.652MoM) in IUGR vs. 294.384pg/ml (1.313MoM) in normal pregnancy and the mean of AFP was 62.041 ng/ml (2.364MoM) vs. 34.370ng/ml (1.197MoM) in normal pregnancy.

PROM was associated with the higher level of AFP ( $p = 0.020$ ) and hCG ( $p = 0.024$ ). The mean of AFP was 51.280ng/ml (1.855MoM) in PROM vs. 34.960ng/ml (1.224MoM) in normal pregnancy and the mean of hCG was 45740.000iu/ml (1.807 MoM) in PROM vs. 45040.175 iu/ml (1.254MoM) in normal pregnancy.

PTL was associated with the higher level of AFP ( $p = 0.030$ ), hCG ( $p < 0.001$ ) and Inhibin-A ( $p = 0.001$ ). The mean of AFP was 45.873 ng/ml (1.661MoM) in PTL vs. 34.757 ng/ml (1.215MoM) in others; the mean of hCG was 50981.409iu/ml (1.900 MoM) in PTL vs. 33976.495iu/ml (1.219MoM) in others, and the mean of Inhibin-A was 417.500pg/ml (1.890MoM) in PTL vs. 295.419pg/ml (1.321MoM) in oth-

ers.

## Discussion

In this research, we assessed the correlation between APO with serum markers, which are used as anuploidy screening, especially for diagnosing Down's syndrome.

In our study, APOs including oligohydramnios, PTL, PROM, Preeclampsia, IUGR, polyhydramnios, and GDM were investigated; and PROM, Preeclampsia and IUGR had a meaningful increase compared to the control group. Our results were in agreement with existing reports on elevation of these disorders (PROM, Preeclampsia and IUGR) in individuals with abnormal markers (9, 11-13).

In the study conducted by Feizbakhsh et al., no significant correlation was found between the intrauterine fetal death (IUFD), Pre-eclampsia, Placental Abruption and PTL with the screening test and any of the markers (14). We did not find any correlation between Placental Abruption and PTL; however, our results revealed significant differences between the two groups in Preeclampsia.

In contrast to some recent studies (15), we did not find an increase in the occurrence of oligohydramnios in cases with abnormal markers.

We discovered a high level of AFP and Inhibin-A with IUGR. Nevertheless, Mc Pherson suggest that IUGR was correlated with the increase in AFP and hCG and the decrease in uE3; and Dugoff found it to be correlated with a higher AFP, hCG and Inhibin-A and the decrease in uE3 (9, 16). The discrepancy between our results with the previous reports might have been due to our smaller sample size (231 persons in our study compared to 692 in Mc Pherson's and

Table 4. The relationship mean of the four markers with the patients' age in the case and control groups

Criteria	MOM	p
MSAFP	1.2±0.91	0.437
uE3	1±0.55	0.007
hCG	1.2±0.82	0.058
Inhibin-A	1.3±0.790	0.172

33145 in Dugoff's reports). Because of our limited sample size, we found a significant difference in the age of the case and control groups. To check if this could affect our results, we analyzed the correlation of each of the markers with age. Among these markers only uE3 had a significant correlation and this could explain why we could not find any correlation.

We found preeclampsia to be correlated with higher level of inhibin-A. Our results showed no correlation between AFP and preeclampsia, the same as Wald's results on this subject (17). He reported a statistical correlation with increased hCG and decreased uE3, but we did not observe such a dependency. In other studies, Preeclampsia was correlated with the higher level of AFP (9, 12). Our results were more similar to those of Kang's findings. Kang et al. reported a meaningful correlation of Preeclampsia with inhibin-A, and found no correlation with AFP and uE3 (18).

Our results on the correlation of PROM with markers were similar to the studies by Karsidag (13). He also reported correlations with AFP and hCG. Also, Deghani Firuzabadi found a correlation between PROM and higher level of AFP (12).

Compared to the PTL and markers, there was a correlation with AFP, hCG and inhibin-A, but no correlation was found with uE3. Dugoff reported the same trend (9). Sehat also reported a direct correlation of PTL with AFP and inhibin-A and an inverse correlation with uE3 (19). He found no correlation with hCG.

Infant weight had a negative linear correlation with three markers of AFP, hCG and inhibin-A so that the infant weighed less when these markers had higher levels. In most studies, statistically significant correlations were found between higher AFP and lower fetus weight (SGA or LBW) (12, 13, 20-22). Our results were in good agreement with the mentioned results.

AFP, hCG and inhibin-A had linear positive correlations with each other in our research.

Michael and Dugoff discussed the com-

bination of markers and their correlation with pregnancy disorders. They suggested that simultaneous increase of these markers may increase the risk of occurrence of the adverse consequences of pregnancy. Low sensitivity and low positive predictability of the analytical methods prevent yet any decisive conclusion whether the screening tests cause adverse pregnancy outcome (9, 23).

### Conclusion

We found a statistically significant association (correlation) between APO and quadruple test and could also determine correlation of APO with a few markers. Since there was a significant difference in the age of the two groups, correlation of each of the markers with age was analyzed. Among these markers only uE3 had a significant correlation and this could explain why we could not find any correlation between uE3 and APO.

We found a correlation between inhibin-A with most disorders. Based on the results, we suggest conducting further studies on this marker and assessing its usefulness in diagnosis and management of high risk pregnancies. Application of the screening test results for predicting APO requires further investigation.

### Acknowledgments

The Authors would like to thank Pastor Medical Laboratory and Polyclinic and Center for Development of Clinical Research of Rouhani Hospital for their cooperation.

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