Original Article

The Impact of Maternal Predisposing Factors on Level of Maternal Serum Pregnancy-Associated Plasma Protein A and Free Subunit Human Chorionic Gonadotropin and Nuchal Translucency

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Abstract

Background: This study aimed to investigate the relationship between maternal predisposing factors with the level of maternal serum pregnancy-associated plasma protein A and free subunit human chorionic gonadotropin and nuchal translucency.

Materials and Methods: We performed a cross-sectional-analytical study on 762 pregnant women who referred to the Gene Azma Medical Genetics Laboratory in Isfahan for amniocentesis. All pregnant women at high risk of screening in the first trimester of pregnancy for trisomy 21 and other aneuploidy were referred to a gynecologist for amniotic fluid sampling (amniocentesis). Multiple of the means (MoM) of PAPPA ≤0.5, 0.5 ≥ MoM free β-hCG >2.5, and NT ≥3.5 mm were considered abnormal. We used Chi-square method and Mann–Whitney U-test to compare data qualitative and quantitative, respectively.

Results: In individuals with less pregnancies and deliveries, the value of abnormal NT was higher ($P < 0.01$, $P < 0.001$, respectively). On the other hand, the highest abnormal rate of NT was observed in pregnant women under 35 years (21, 84%, $P < 0.012$). In addition, abnormal levels of free β-hCG are more common in women < 35 years of age (186, 66.9%, $P < 0.02$) and female fetuses (171, 58.8%) ($P < 0.006$).

Conclusion: According to the results of this study, it can be said that considering the underlying factors of pregnant mothers in performing tests related to screening in the first trimester of pregnancy can lead to a reduction in false positive rates.

Keywords: Beta subunit, human chorionic gonadotropin, maternal age, maternal weight, nuchal translucency, pregnancy-associated plasma protein A, prenatal screening

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Introduction

Currently, the optimal screening method in pregnancy to identify fetal chromosomal abnormalities is to measure the level of maternal serum pregnancy-associated plasma protein A (PAPPA) and free β-human chorionic gonadotropin (free β-hCG) combined with fetal nuchal translucency thickness (NT).[1]

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Numerous reports have shown an association between changes in chemical markers in the first trimester of pregnancy and chromosomal abnormalities such as Down syndrome Pregnancy-related plasma protein A (PAPPA) levels has also been shown to be significantly lower in cases with Down syndrome than in normal pregnancies.[2]

Furthermore, in many studies, a significant relationship has been reported between changes in the level of free β-hCG and PAPPA biomarkers and the occurrence of adverse pregnancy outcomes such as (preeclampsia, preterm delivery, fetal growth restriction and gestational hypertension).[2,3] Low PAPPA levels in the first trimester are also strongly associated with pathological changes in birth weight.[4,5] On the other hand, various factors such as ethnicity and maternal weight, smoking status and fetal sex are known to affect the level of biochemical markers in maternal serum and should be considered in calculating the final risk.[1]

Studies on markers in the first and second trimester of pregnancy show that the sex of the fetus changes the amount of biomarkers in the mother's serum. In female fetuses, alpha-fetoprotein (AFP) levels decrease and free *β*-hCG levels increase significantly compared to cases with male embryos.[1]

One of the main factors affecting biochemical markers is maternal smoking, which increases AFP and decreases free *β*-hCG levels; therefore, its effect should be considered in screening tests.[5]

The aim of this study was to determine whether the biochemical variables of first-trimester screening (PAPPA and free *β*-hCG) and NT level were influenced by maternal underlying factors such as maternal age, number of pregnancies and deliveries, gender, mother's occupation, and body mass index (BMI).

Materials and Methods

We performed a cross-sectional-analytical study on 762 pregnant women. This study was approved by the ethics committee of Tarbiat Modares University (Ethics code of the study: IR.MODARES.REC.1398.210). The cases were referred to the Gene Azma Medical Genetics Laboratory in Isfahan for amniocentesis testing and written informed consent was obtained from pregnant women. Collection of eligible samples began in December 2018 and ended in June 2020. Screening for fetal abnormalities in the first trimester is performed according to the national protocol of Iran in 11 weeks + 0 days to 13 weeks + 6 days. This type of screening which combines the study of chemical biomarkers and the measurement of fetal nuchal translucency thickness, is called combination screening. The concentration of biochemical screening markers in the first trimester of pregnancy were converted to multiple of the means (MoMs) and adjusted for gestational age, body mass index, delivery method, and history of Down syndrome in previous pregnancies. Fetal NT and crown-rump length were measured by an ultrasound specialist according to standard methods. The sex of the fetus is determined by karyotype used to diagnose fetal aneuploidies. All high risk pregnant women based on screening in the first trimester of pregnancy for trisomy 21 and other aneuploidy, were referred to a gynecologist for amniotic fluid sampling (amniocentesis). Inclusion criteria were included: screening in the first trimester of pregnancy, singleton pregnancy, Iranian citizenship, availability of information from pregnant women, and no suffering from chronic or systemic diseases according to medical records. The questionnaire was completed based on medical records and face-to-face interviews and included maternal age, number of pregnancies and deliveries, history of polycystic ovary syndrome, infant gender, maternal occupation, blood group, and maternal BMI. To investigate the relationship between changes in biochemical markers and the fetal nuchal translucency thickness with maternal underlying factors, MoM of PAPPA ≤0.5, 0.5 ≥ free *β*-hCG >2.5, and NT ≥3.5 mm were considered abnormal. Data were analyzed using the Statistical Package for the Social Sciences (SPSS) version 20(IBM. USA. Chicago). Furthermore, due to the nonnormal distribution of data, the Chi-square method and Mann–Whitney U-test were used to compare qualitative and quantitative data, respectively.

Results

Mean of age, BMI, number of pregnancies and deliveries, and NT of individuals were 33.56 ± 5.85, 25.64 ± 4.3, 2.49 ± 1.26, 1.10 ± 0.95, and 1.90 mm ± 0.75, respectively.

Table 1 summarizes some demographic/screening characteristics of pregnant women in this study and showed that there is no significant relationship between changes in PAPPA in pregnant women and normal or abnormal levels of free β-hCG ($P > 0.05$).

There was no significant difference between abnormal NT and maternal BMI ($P > 0.05$) [Table 1], and in individuals who had more pregnancies and deliveries, normal NT values were higher ($P < 0.01$, $P < 0.001$, respectively) [Table 2], while in pregnant women with abnormal NT levels, median PAPPA was higher (1.07 MoM) ($P < 0.001$) and median free β-hCG was lower (1.15 MoM) ($P < 0.001$) [Table 1]. In addition, abnormal PAPPA levels were higher in pregnant women with higher median BMI (25.71) ($P < 0.004$). Abnormal PAPPA levels were more common in pregnant women with lower median free *β*-hCG levels (1.62 MoM) ($P < 0.001$) [Table 1]. Unlike PAPPA, abnormal free β-hCG levels were observed in pregnant women with lower median BMI (24.86) ($P < 0.008$) [Table 1].

Table 2 is a collation of fertility characteristics of pregnant women and shows that the mean level of abnormal PAPPA was higher, but no significant relationship was observed ($P > 0.05$). On the other hand, in women over 35 years old, previous history of PCO and female infant, mean normal PAPPA was higher but no significant relationship was seen ($P > 0.05$).

Free *β*-hCG levels were not affected by maternal occupation and there was no significant relationship between its changes

with the number of pregnancies, previous delivery history, and PCO.

Table 3 describes the demographic characteristics of pregnant women understudy and shows that abnormal levels of free *β*-hCG are more common in women <35 years of age (186, 66.9%) ($P < 0.02$) and female fetuses (171, 58.8%) ($P < 0.006$).

On the other hand, the highest abnormal rate of NT was observed in pregnant women under 35 years (21, 84%) ($P < 0.012$) and there was no significant difference between its changes with sex of the fetus.

Discussion

The findings of this study show that factors such as maternal age, BMI, number of pregnancies, deliveries, and fetal sex are significantly associated with abnormal changes in biochemical markers and NT levels [Table 4]. In a study by Cowans NJ and *et al*., they evaluated the effect of fetal sex on the changes in biochemical markers used in prenatal screening and showed that in female fetuses, an average increase of 6.25% in median PAPPA and a decrease of 9.41% in delta NT are observed.[1] While in our study, no significant difference was observed between fetal sex and PAPPA changes or NT thickness. In another study by Spencer *et al*., they showed a 15% increase in median MoM free *β*-hCG in female pregnancies.[6] This increasing was consistent with our study. We found 58.8% abnormal changes of MoM free *β*-hCG (0.5 ≥ MoM free *β*-hCG > 2.5) when the embryo was female. Furthermore, in a study by Illescas *et al*., they reported that in pregnancies with female fetus, MoM levels of PAPPA and free β-hCG were significantly higher.[7] In a study by Yaron *et al*., they also reported that the MoM level of free *β*-hCG is higher in female fetuses.[8] Since changes in the level of biochemical markers can cause a slight increase in false-positive values among women with female fetuses, the effect of fetal sex on changes in biomarkers should be considered.

In study by Arfi *et al*. sex was correctly recognized for 89% of the females and 87% of the males from 11 to 14 weeks.[9] Whereas Efrat *et al*. were able to determine the sex of the fetus with 100% accuracy, from as early as (12 weeks + 0 day). Female fetuses were correctly distinguished (100%) from (11 weeks + 2 days to 11 weeks + 6 days), while at this particular time of pregnancy only 46% of men were identified.[10] In addition Colmant *et al*. (2013) to determination of fetal sex, they found a sensitivity and specificity approximately 100% from 8 weeks gestation through cffDNA and at 13 weeks gestation ultrasound respectively.[11] In a study by Fahimeh

Table 1: Comparison of demographic characteristics/screening of pregnant women understudy in two groups with normal and abnormal levels of biochemical markers and nuchal translucency

Groups characteristics	PAPPA, median±IQR		*P*	Free β-hCG, median±IQR		*P*	NT, median±IQR		*P*
	≤0.5	>0.5		0.5≥ ≥2.5	0.5< <2.5		≥3.5	≥3.5	
BMI	25.71±5.83	24.7±4.91	0.004	24.86±5.86	25.47±5.20	0.008	24.38±5.37	25.20±4.33	0.28
MoM PAPPA	-	-	-	0.68±0.58	0.63±0.50	0.15	1.07±1.43	0.64±0.49	0.000
MoM free β-hCG	1.62±1.36	1.75±0.89	0.000	-	-	-	1.15±1.24	1.87±1.71	0.000

PAPPA: Pregnancy-associated plasma protein A, BMI: Body mass index, NT: Nuchal translucency, hCG: Human chorionic gonadotropin, IQR: Interquartile range, MoM: Multiple of the mean

Table 2: Comparison of demographic/fertility characteristics of pregnant women in two groups with normal and abnormal levels pregnancy-associated plasma protein A and nuchal translucency

Groups characteristics	PAPPA, χ^2		*P*	NT, median±IQR		*P*
	≤0.5	<0.5		≥3.5	<3.5	
Number of pregnancy	206 (80)	394 (78)	0.5	1±2	2±1	0.01
Number of delivery	192 (74.7)	371 (73.5)	0.7	0±1	1±2	0.001

PAPPA: Pregnancy-associated plasma protein A, NT: Nuchal translucency, IQR: Interquartile range

Table 3: Comparison of demographic/fertility characteristics of pregnant women in two groups with normal and abnormal levels free β-human chorionic gonadotropin and nuchal translucency

Groups characteristics	Free β-hCG, χ^2		*P*	NT, χ^2		*P*
	0.5≥ ≥2.5	0.5< <2.5		≥3.5	<3.5	
Maternal age <35	186 (66.9)	262 (55.6)	0.02	21 (84)	427 (57.9)	0.012
Maternal age ≥35	105 (36.1)	209 (44.4)		4 (16)	310 (42.1)	
Fetal gender (female fetuses)	171 (58.8)	228 (48.4)	0.006	11 (44)	388 (52.6)	0.4
Male fetuses	120 (41.2)	243 (51.6)		14 (56)	349 (47.4)	

hCG: Human chorionic gonadotropin, NT: Nuchal translucency

Table 4: Relationship between underlying maternal factors and screening markers in the first trimester of pregnancy

Variables	Maternal age		Delivery	Pregnancy	BMI >25	PAPPA	Free	Fetal sex
	35>	≥35	<1	<1			β-hCG	female
NT ≥3.5 (mm)	++		++	++		++	–	–
MOM PAPPA					–			++
MOM free β-hCG		+++				++		+++

BMI: Body mass index, +: Increasing, -: Decreasing, PAPPA: Pregnancy-associated plasma protein A, hCG: Human chorionic gonadotropin, NT: Nuchal translucency, MoM: Multiple of the mean

Rezaei Tonekaboni *et al*. (2020) fetal sex with good accuracy through cffDNA analysis has revealed 100% specificity in all duration of pregnancy.[12] Due to the dependence of the accuracy of ultrasound on the type of machine and the skill of the operator, it is recommended that studies performed to examine the criteria and determine their accuracy in relation to sex determination.

In a study by Huang *et al*., their data showed that weight differences had a different effect on each of the serum markers. Meanwhile, PAPPA is more sensitive than other markers so that with increasing BMI, PAPPA levels decreased.[13] We also found a significant relationship between maternal weight and changes in PAPPA. As with increasing BMI the level of PAPPA decreased. Since the biochemical variables such as PAPPA and free β-hCG are influenced by a number of maternal and pregnancy variables such as gestational age and BMI these variables should be corrected when calculating the MoM for use in the risk algorithm.[14]

Interestingly, there was a significant relationship between changes in PAPPA and free β-hCG in all cases, which can be seen by increasing PAPPA levels increased free β-hCG. We do not know whether the opposite is true, and further investigation is needed. On the other hand, it is important to pay attention to the value of NT and the amount of biochemical biomarkers (PAPPA, free β-hCG), so that in cases with NT ≥3.5 mm, the median PAPPA was higher and the median free β-hCG was lower. Nevertheless, both the markers were in the normal range. This suggests that perhaps the value of NT is more valuable than the PAPPA and free β-hCG levels for making decision to determine diagnostic tests. If the value of NT is ≥3.5 mm, diagnostic methods must be considered.

In our study, a significant association was observed between the number of pregnancies and deliveries with abnormal NT values. The highest rate of abnormal NT was seen in individuals with less pregnancies and deliveries. Furthermore, individuals in this group were under the age of 35 years. This may be because individuals over the age of 35 years, in addition to being at risk for Down syndrome due to their age, are less likely to become pregnant at this age. Unfortunately, we did not find any studies in this area.

We also found a significant association between free β-hCG levels and maternal age. Pregnant women under the age of 35 years had the highest amount of abnormally free β-hCG. The reason is not clear, but it is probably because more individuals get pregnant in this age. As a result, more pregnancies with Down syndrome are expected.

Conclusion

According to the results of this study, it can be said that considering the underlying factors of pregnant mothers in performing tests related to screening in the first trimester of pregnancy can lead to a reduction in false positive rates. For example, due to the effect of fetal sex as one of the important underlying factors on changes in screening markers, fetal sex determination by ultrasound in early pregnancy was recommended.

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Conflicts of interest

There are no conflicts of interest.

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