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The effect of maternal polycystic ovary morphology on first-trimester maternal serum biochemical markers of aneuploidy and fetal nuchal translucency thickness

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Summary

Objective: To evaluate the effect of maternal polycystic ovary (PCO) morphology on maternal serum free beta-human chorionic gonadotropin (β-hCG), pregnancy associated plasma protein A (PAPP-A), and nuchal translucency (NT) thickness in the first-trimester. *Material and Methods:* A total of 92 pregnant women in the first-trimester were included in the study. Of them, 57 had PCO morphology, and 35 women constituted the control group, with apparently normal ovaries. Maternal serum free β-hCG, PAPP-A, and NT thickness were measured and compared in all patients. *Results:* The multiples of median (MoM) levels of serum free β-hCG were significantly higher in the PCO morphology group compared to the normal ovary group ($p = 0.024$). However, the MoM levels of PAPP-A were similar in both groups ($p = 0.947$). No difference was found between the groups in terms of fasting glucose levels and NT measurements ($p = 0.976$ and 0.565, respectively). *Conclusion:* In pregnancies with maternal PCO morphology, the presence of higher maternal serum free β-hCG levels may require correction in the calculation of risks related to first-trimester screening for chromosomal abnormalities. Larger studies are needed to confirm our preliminary data.

Key words: Maternal serum screening test; Nuchal translucency measurement; Polycystic ovary syndrome; Pregnancy-associated plasma protein-A.

Introduction

First-trimester screening using the combined test including maternal free beta-human chorionic gonadotropin (β-hCG), pregnancy-associated plasma protein-A (PAPP-A), and nuchal translucency (NT) thickness at 11 weeks to 13 weeks six days of gestation is now a well-established program of aneuploidy screening, possessing a detection rate of about 90% with a 5% false-positive rate (FPR) [1].

Prenatal screening programs first describe the concentrations of biochemical markers as multiples of median (MoM) of the expected normal median for a pregnancy with the same gestational age. After that the MoMs are adjusted for factors considered to influence their levels, such as maternal weight [2,3], ethnicity [4], smoking [5], number of fetuses [6], and conception method [7]. If the MoMs are not adjusted by the aforementioned factors, the FPR of the test may be adversely affected. For instance, high FPR of a screening test is always associated with a rise in the rate of chorionic villus sampling (CVS) or amniocentesis.

The polycystic ovary (PCO) morphology is a common finding in the reproductive age population with a prevalence of up to 30% [8-10] and the association of this finding with a risk of cardiovascular disease has been described by some investigations [11-13]. In a previous case-control study, it was calculated that the risk for myocardial infarction was seven-fold higher in women with PCO morphology [14]. Another cohort study found a marked increase in the prevalence of cardiovascular risk factors in patients with PCO morphology, including hypertension, diabetes, hypercholesterolemia, hypertriglyceridemia, and an elevated waist-to-hip ratio [15]. On the other hand, PAPP-A which is one of the two serum markers in first-trimester screening test for aneuploidy, has also been shown to be a marker of adverse outcome in both acute coronary syndrome and stable coronary disease patients [16]. It was reported that PAPP-A may play a role in the development of atherosclerotic lesions and also constitute a marker of atheromatous plaque instability and the extent of cardiovascular disease. In this sense, it can be postulated that PAPP-A, as a component of maternal serum markers of aneuploidy, may be increased in pregnant women with PCO morphology during the first-trimester.

Moreover, serum PAPP-A levels were found to be higher in non-pregnant polycystic ovary syndrome (PCOS) patients compared to control subjects [17]. However, no previous study evaluated the influence of maternal PCO morphology, which is a common finding in reproductive-

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aged women, on the first-trimester biochemical markers of aneuploidy and NT thickness.

The aim of the present study was therefore to evaluate the effect of maternal PCO morphology on maternal serum free β-hCG, PAPP-A and NT thickness from 11 weeks to 13 weeks six days of gestation in women screened prospectively for chromosomal anomalies.

Materials and Methods

In this prospective study, the relationship between PCO morphology and parameters of the combined test in the first-trimester of pregnancy was examined in women screened between October 2011 and September 2012 at a tertiary university hospital. Ethical approval was obtained from the institutional human research ethics committee. Informed consent was gained from all participants before entering the study.

A total of 92 pregnant women with a mean age of 27.3 ± 0.4 (17 to 37) years, at 11 + 0 to 13 + 6 weeks of gestation, were included in the study. All of the pregnancies were singleton. Of the 92 women, 57 women had PCO morphology at least in one ovary, and 35 age-, body mass index- (BMI), and gestational age-matched women with apparently normal ovaries constituted the control group. Women with systemic diseases, IVF pregnancy, pre-gestational diabetes mellitus, and ultrasound findings of fetal chromosomal abnormalities or structural defects during the period of the combined test were excluded from the study. Maternal age, BMI, and gestational age at the time of the ultrasound scan were recorded and fasting glucose levels and the combined test were studied for each case.

Ultrasound examination

Two-dimensional pelvic ultrasound scans were performed using devices equipped with convex transducer. Before the examination, each woman was asked to empty her bladder. The ovary was examined when the plane gave the best image quality, which was optimized by aid of high magnification, appropriate frequency, and by use of the automatic optimization feature. Follicle size was found from the mean of two perpendicular diameters of the antral follicles. Follicles were counted by scanning from one margin of the ovary to the other.

Diagnosis of PCO morphology on ultrasound was made according to the Rotterdam criteria in which PCO was defined as 12 or more follicles, two to nine mm in diameter [18]. The patients with less than 12 follicles measuring two to nine mm in diameter in both apparently normal ovaries constituted the control group. Additionally, transabdominal ultrasound examination was performed to obtain the aneuploidy screening in the first-trimester. NT and crown-rump length (CRL) measurements as well as screening for structural anomalies were performed in accordance with the technique described by Fetal Maternal Medicine [19].

Biochemical measurements

Fasting blood, taken at the same visit as the ultrasound examination, was collected from the median cubital vein in order to evaluate serum levels of free β-hCG and PAPP-A, as well as the fasting glucose level. The serum glucose level was determined by an enzymatic-UV photometry method.

Free β-hCG and PAPP-A measurements were performed for all women with an analyzer as part of the routine maternal serum aneuploidy screening in the first-trimester of pregnancy. Concentrations of biochemical markers of the double test were entered into the database of the first-trimester prenatal screening program at the institutional laboratory of the Department of Clinical Biochemistry and automatically converted to MoMs by the laboratory information management system. The biochemistry and risk estimates were calculated using specific software.

Statistical analyses

Statistical analyses were performed using the SPSS software version 20. The variables were investigated using visual (histogram and probability plots) and analytical methods (Kolmogorov-Smirnov test) to evaluate normal distribution. Descriptive analyses were presented using means and standard errors of mean (SEM). The Student's t-test and the Mann-Whitney U test, where appropriate, were used to compare the continuous variables between the study and control groups. Statistical significance was defined as $p \leq .05$.

Results

The study included 92 pregnant women in the first-trimester of pregnancy. The participants consisted of 57 patients with PCO morphology in at least one ovary and 35 patients with apparently normal ovaries. Demographic and clinical characteristics of both groups are presented in Table 1. There was no difference between the groups in terms of variables such as age, BMI, CRL, and gestational weeks.

PCO morphology in maternal ovaries had a significant effect on plasma free β-hCG levels while no significant effect was seen on the plasma PAPP-A levels during 11 + 0 and 13 + 6 weeks of pregnancy. The PCO morphology group showed higher free β-hCG levels in maternal plasma than the control group ($p = 0.024$), whereas plasma PAPP-A levels were similar in both groups ($p = 0.947$) (Table 1).

No difference was found between the groups in terms of fasting glucose levels and NT measurements ($p = 0.976$ and 0.565, respectively).

Discussion

The results of this prospective study showed that the serum free β-hCG levels from pregnant women with PCO morphology in their ovaries were significantly higher compared with pregnant women with normal ovaries during the combined screening test of the first-trimester. However, PAPP-A levels, which is the other serum marker of the screening test, as well as the NT measurements and fasting glucose levels, were similar in both groups.

To the best of the authors' knowledge, a comparison in patterns of the markers from the combined screening test between pregnant women with PCO and normal ovaries has not been documented precisely in previous studies. The authors found that MoM levels of free β-hCG were significantly higher in the PCO morphology group (Table 1). Contrary to these results, a retrospective study conducted in patients who underwent assisted reproductive technology (ART) by Kosus *et al.* [20] found that free β-hCG MoMs were significantly decreased in infertile PCOS patients compared to infertile patients with male factor. The authors reported that factors such as hormonal disturbances, obe-

Table 1. — *Demographic and clinical features of the study and control groups.*

Characteristics	PCO (n=57)	Control (n=35)	*p*
Age (years)	27.2 ± 0.6	24.8 ± 1.0	0.822
BMI (kg/m^2)	25.1 ± 1.0	26.8 ± 1.7	0.352
CRL (mm)	56.8 ± 1.7	57.1 ± 4.2	0.762
Gestational age (weeks)	12.2 ± 0.1	12.2 ± 0.2	0.804
Fasting glucose (mg/dl)	90.2 ± 4.4	85.9 ± 3.2	0.976
NT (mm)	1.5 ± 0.1	1.5 ± 0.1	0.565
AFC in right ovary (n)	14.4 ± 1.3	7.2 ± 1.2	<0.001*
AFC in left ovary (n)	15.7 ± 1.1	6.1 ± 0.9	<0.001*
Right ovarian volume (cm^3)	12.2 ± 0.6	9.8 ± 0.8	0.017*
Left ovarian volume (cm^3)	11.9 ± 0.5	8.2 ± 0.9	<0.001*
Free β-hCG (MoM)	1.3 ± 0.1	0.8 ± 0.2	0.024*
PAPP-A (MoM)	1.1 ± 0.2	0.7 ± 0.1	0.947

Data are presented as mean ± SEM
CRL: crown-rump length; NT: nuchal translucency; AFC: antral follicle count; β-hCG: β-human chorionic gonadotropin; PAPP-A: pregnancy associated plasma protein A.

sity, and insulin resistance in PCOS may have an effect on screening test results. This study differs from the current study as it was conducted in ART patients with PCOS or male factor rather than patients with PCO morphology. The difference in the results may be explained by the hormonal milieu of ART cycles and PCOS itself rather than a sole PCO morphology. Therefore, more studies are required to disclose the relationship between first-trimester serum markers and presence of maternal PCO morphology.

Moreover, some maternal characteristics such as maternal weight, smoking, ethnicity, parity, and mode of conception have previously been shown to alter serum markers of the double test [2-5, 7]. These altered serum markers have been shown to be related to false-negative or positive results in aneuploidy screening tests and this, in turn, may lead to increased invasive diagnostic tests or to missing a diseased fetus in utero. Therefore, the factors affecting serum levels of the screening markers should be identified and the serum analytes should be corrected accordingly. As a result of increased free β-hCG in the current study, pregnant women with PCO morphology are more likely to have a false-positive result for Down syndrome from the combined screening test and therefore are more likely to undergo CVS or amniocentesis. This is an important issue because it is well established that an increase in the rate of CVS or amniocentesis in healthy pregnancies will lead to an increase in maternal anxiety [21] and in procedure-related complications, even pregnancy loss [22]. As a consequence of higher false-positive test results related to higher free β-hCG in PCO morphology patients from the present study, some modifications regarding the screening protocols in such pregnancies may be necessary in order to decrease the FPR without changing the sensitivity of the screening test.

It is well known that PCO morphology may be present in up to 30% of asymptomatic women in the general population [8-10]. If such a prevalent condition is related to higher free β-hCG levels in pregnancy, resulting in higher false-positive results from the combined screening test, the condition should be identified during early pregnancy and the risk estimates for chromosomal abnormalities should be adjusted correspondingly in these women. Certainly it is difficult to recommend such a risk adjustment from the results of the present study which includes a relatively small sample size.

The association between the presence of PCO morphology and risk of cardiovascular disease has been shown in previous studies [11-13] and moreover, PAPP-A was demonstrated to be a marker of adverse outcome in both acute coronary syndrome and stable coronary disease patients [16]. For these reasons, one may expect to find higher serum PAPP-A levels in patients with PCO morphology. However, the present authors found serum PAPP-A levels were similar in these patients compared to the control group (Table 1). Contrary to the present findings, in a recent study conducted in PCOS patients, it was found that day 3 serum PAPP-A levels were significantly higher in PCOS patients compared to the normo-ovulatory subjects [17]. This study however included PCOS and non-pregnant women. Moreover, in another recent study including PCOS patients who underwent ART, it was found that PAPP-A levels were similar in patients with PCOS compared to infertile patients with male factor in the first-trimester serum screening test [20]. Therefore, the comparison of the levels of PAPP-A between these patients should be investigated in further studies.

The prime limitation of the present study is that it did not include perinatal outcomes of the pregnancies related to the presence of PCO morphology. The relatively small sample size is the other limitation in the study. A further issue is that if the maternal PCO morphology is associated with changes in the risk estimate for fetal aneuploidy, the odds ratio for this factor was not found by the present study to determine a corrected analyte MoM prior to the calculation of the patient's individual risk for a specific aneuploidy. However, the study has some strength, such as being prospective in design and giving the first preliminary data about the relation between maternal PCO morphology and the parameters of the combined test during pregnancy. Larger studies, with prospective design, evaluating the effect of maternal PCO morphology or polycystic ovarian syndrome on the first and second trimester aneuploidy screening tests and also on perinatal outcome would be invaluable.

Conclusion

This study provides preliminary data showing that first-trimester maternal serum levels of free β-hCG are increased in women with PCO morphology. This may result in a much higher FPR related to Down syndrome in the first-

trimester combined screening test. Serum PAPP-A, fasting glucose levels, and NT measurements were not affected by the presence of maternal PCO morphology. As changes in serum markers of the screening test may alter the screening test results, consequently it may be necessary to adjust the first-trimester risk calculation accordingly. Further prospective studies should be conducted to confirm this preliminary data.

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