Doi:10.47572/muskutd.1095993

Evaluation of The Effects of Different Progesterone Treatment Methods on Fetal Aneuploidy Screening Tests

Farklı Progesteron Tedavi Yöntemlerinin Fetal Anöploidi Tarama Testleri Üzerine Etkilerinin Değerlendirilmesi

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Öz

Bu çalışmada abortus imminens nedeniyle progesteron kullanımının, anöploidi taramalarındaki biyobelirteçler üzerine etkisi araştırıldı. Bu retrospektif çalışmaya düşük tehdidi olan iki yüz yetmiş altı tekil gebe hasta dahil edildi. Tedavi grubu oral, vajinal veya intramüsküler progesteron (n=137) alan hastalardan, kontrol grubu ise progesteron almayan (n=139) hastalardan oluşturuldu. Çalışma gruplarının ikili ve üçlü tarama testlerinin sonuçları karşılaştırıldı. Nukal saydamlık (NT) ölçümleri ve gebelikle ilişkili plazma protein-A (PAPP-A), (MoM) değerleri progesteron tedavisi alan grupta kontrol grubuna göre anlamlı derecede düşüktü (sırasıyla p=0.009 ve p<0.001). Beta-insan koryonik gonadotropin (βhCG), (MoM) ve alfa-fetoprotein (MoM) oral progesteron tedavisi alan grupta diğer tür progesteron tedavisi alanlara göre istatistiksel olarak anlamlı derecede yüksek bulundu (p=0.032 ve p=0.001, sırasıyla). PAPP-A oral tedavi grubunda anlamlı olarak daha düşük bulundu (p=0.001). Hidroksiprogesteron kaproat tedavi grubunda da anlamlı olarak daha düşüktü (p=0.013). Vajinal progesteron tedavi grubunda βhCG, oral tedavi grubuna göre anlamlı derecede düşüktü (p=0.036). Çalışma, farklı progesteron uygulama yolları ile fetal anöploidi tarama belirteçleri arasında bir ilişki olduğunu göstermiştir.

Anahtar Kelimeler: Anoploidi Tarama Testleri, Düşük Tehdidi, Ense Saydamlığı Ölçümü, Progesterone Tedavisi

Abstract

In this study, the effect of progesterone use on biomarkers due to abortion complaints in aneuploidy screenings was investigated. Two hundred seventy-six singleton pregnant patients with threatened abortion were enrolled in this retrospective study. The treatment group consisted of patients who received oral, vaginal, or intramuscular progesterone (n=137) while the control group received no progesterone (n=139). The results of the double and triple screening tests of the study groups were compared. Nuchal translucency measurements and pregnancy-associated plasma protein-A (PAPP-A), (MoM) values were significantly lower in the group that received treatment with progesterone compared to the control group (p=0.009 and p<0.001, respectively). Beta-human chorionic gonadotropin (βhCG), (MoM) and alpha-fetoprotein (MoM) were found to be statistically significantly higher in the group receiving oral progesterone treatment compared to the group receiving other types of progesterone treatment (p=0.032 and p=0.001, respectively). The PAPP-A was found to be significantly lower in the oral treatment group (p=0.001). It was also significantly lower in the hydroxyprogesterone caproate treatment group (p=0.013). In the vaginal progesterone treatment group, βhCG was significantly lower (p=0.036) than that in oral treatment group. The study showed that there is a relationship between different progesterone administration routes and fetal aneuploidy screening markers.

Keywords: Nuchal Translucency, Prenatal Screening Tests, Progesterone Therapy, Threatened Miscarriage

Introduction

The risk of miscarriage is described as vaginal bleeding that occurs before the 20th week of pregnancy. Miscarriage is observed in 20% of pregnancies, and the risk of abortion increases by 2.6 times, and the possibility of developing complications during pregnancy is 17% (1). The risk of miscarriage is that the allogeneic embryo is accepted or rejected by the mother. In a normal, healthy pregnancy, progesterone receptors increase in maternal lymphocytes that encounter the embryo's antigen. Accordingly, there is a shift in the maternal immune system toward T helper (Th)2 lymphocytes. In addition, the levels of cytokines and interleukins (IL), i.e., IL -4, IL -6, and IL -10, increase, reducing natural killer (NK) cell activity (2). However, in the presence of impending miscarriage and preterm labor, the increase in progesterone receptors in lymphocytes is not observed and a shift toward Th1 occurs. Interferon (IFN) gamma, which is detrimental to pregnancy, may be elevated in this situation (3). Based on this information, previous studies have suggested that the use of progesterone may be effective in the treatment of miscarriage (4). Few data are available on the basis of which progesterone treatment has been evaluated in terms of maternal and fetal effects. Progesterone therapy can use progesterone, dydrogesterone, and hydroxyprogesterone caproate in various forms (e.g., capsule, gel, and ampoule). The use of exogenous oral micronized progesterone or vaginal micronized progesterone is allowed in the form of soft capsules (5). Each capsule contains 200

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Başvuru Tarihi / Received: 30.03.2022
Kabul Tarihi / Accepted : 23.07.2022

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milligrams (mg) of micronized progesterone. Hydroxyprogesterone caproate, on the other hand, is available in forms that can be administered intramuscularly and contains 250 mg of active ingredient (one milliliter).

During the first trimester of each pregnancy, several combined tests are performed to assess the health of the fetus. These include sonographic measurement of nuchal translucency (NT), a fetal aneuploidy screening test combined with crown-rump length (CRL), pregnancy-associated plasma protein-A (PAPP-A), and beta-human chorionic gonadotropin (βhCG) levels (6, 7). The first trimester screening test is performed between weeks 11 and 14 of gestation (8, 9). The triple test is performed in the second trimester, usually between weeks 15 and 18 of gestation, and is based on the measurement of alpha-fetoprotein (AFP), unconjugated estriol (uE3), and βhCG as serum markers in maternal serum no later than 22 weeks of gestation (6, 10). Biochemical marker variables include maternal age, weight, smoking, multiple pregnancies, and whether the pregnancy was achieved by assisted reproductive techniques. Screening test risk ratios are calculated using the multiple of the median (MoM), which allows control for these variables (11, 12).

The effect of progesterone, used to support the luteal phase, particularly after assisted reproductive technology, on screening markers in pregnancy has been studied previously and suggests an increase in nuchal translucency through an increase in uterine blood flow (13). However, it is unclear whether the change in serum markers and nuchal translucency is due to the effect of progesterone treatment or to other variables (14). The aim of this study was to compare the results of aneuploidy screening markers in patients who underwent double and triple screening and were taking progesterone for impending miscarriage.

Material and Method

Based on the records available at Etlik Zübeyde Hanım's Health Training and Research Hospital, single pregnant women admitted for screening between March 1, 2019, and March 1, 2020, were eligible for the study if they had an impending miscarriage in the presence of painful or painless bleeding before 20 weeks of gestation. This study was conducted in accordance with the Declaration of Helsinki on Research Involving Human Subjects and was approved by the institutional review board of Etlik Zübeyde Hanım's Health Training and Research Hospital on August 28, 2020, number 21.

Pregnant women enrolled in this study underwent a double screening test between 11 + 0 and 13 + 6 weeks of gestation. CRL was 45-84 mm and a triple screening test between 16 + 0 and 19 + 6 weeks of gestation. Patients with significant bleeding on ultrasonography with threatened abortion were excluded from the study. Women with certain medical conditions, including (1) pregnancy by assisted reproductive technology, (2) multiple pregnancy, (3) pregnancy with fetal anomaly, (4) pre-existing chronic disease (e.g., diabetes, hypertension, thyroid dysfunction, uncontrolled endocrine disease, renal dysfunction, or autoimmune disease), (5) < 18 or > 40 years of age, (6) had a body mass index (BMI) > 30 kg/m^2, and (7) had a history of smoking were not included in this study. The treatment group consisted of patients who were treated with progesterone between 6 + 0 and 8 + 0 weeks of gestation because of threatened miscarriage (Figure 1). Progesterone treatment was administered in one of three regimens: oral micronized progesterone 200 mg three times daily for seven days, vaginal micronized progesterone 200 mg soft capsules once daily for 14 days, or intramuscular hydroxyprogesterone caproate 500 mg once weekly for two weeks. The control group received no progesterone treatment. The results of double and triple screening tests of patients in the treatment and control groups were compared.

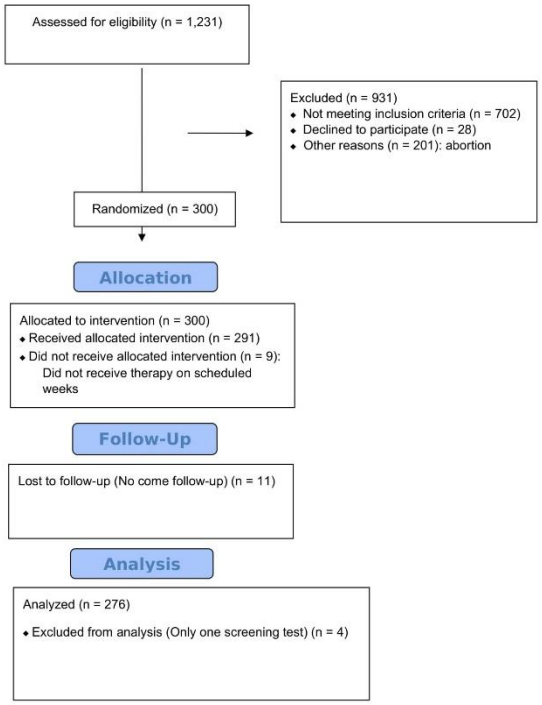

Figure 1. A flow chart of the study groups showing all the required steps for including a patient in the sampling universe

Immulite One VR system kits (Siemens Medical Solutions Diagnostics Limited, United Kingdom) were used for serum marker results. All NT thickness measurements were performed transabdominal using a Samsung HS70A. Screening test risk ratios were determined using PRISCA 4.0 and recorded as MoM (Prenatal Risk Calculator,

TYPOLOGY Software, GmBH, Hamburg, Germany).

Statistical analysis was performed with SPSS (Statistical Package for the Social Sciences) 24 (SPSS Inc, Chicago, IL). Descriptive statistics were presented as mean ± standard and median (smallest-largest value) for variables. The distribution of parameters was assessed by Shapiro-Wilk normality tests. The independent-samples t test was used for normally distributed data, and the Mann Whitney U test was used for variables that were not normally distributed. The one-way test ANOVA was used to compare three groups according to treatment type. Bonferroni correction was used for the significance threshold of the two-tailed p-level test. In the study by Karaca et al. (14), with a total number of 210 patients (70 patients in each group), a statistical significance level of 0.05, and an effect size f ¼ 0.25 (median value) for each marker, the power of the study was rated as three. Group comparisons were calculated at 90.7%. On this basis, we assume that significance is greater than this value because our sample size is larger in each group. P values <0.05 were considered statistically significant.

Results

The mean age in the study group (29.6 ± 6.3 years) was higher than that in the control group (26.8 ± 5.4 years). There was no significant difference between groups in gravidity, parity numbers, and maternal weight (Table 1). Progesterone treatment was administered orally in 125 patients, vaginally in seven patients, and intramuscularly in five patients. NT Measurements and MoM values of PAPP-A markers were significantly lower in the study group than in the control group (p=0.009 and p<0.001, respectively). The groups did not differ in the MoM values of the serum markers uE3, βhCG, and AFP (Table 2). The βhCG (MoM) and AFP (MoM) levels were significantly higher in the group receiving oral treatment than in the group receiving progesterone by other routes (p=0.032 and p= 0.001, respectively). The PAPP-A (MoM) value was significantly lower in the group receiving oral treatment (p = 0.001). The PAPP-A (MoM) value was significantly lower in the group of patients receiving intramuscular progesterone than in the group treated by other routes (p= 0.013, Table 3).

Table 1. Baseline characteristics of the study groups

	Control Group (n=139)	Study Group (n=137)	*P-value*
Age (years)	26.8±5.4	29.6±6.3	**<0.001[a]**
Maternal weight (kg)	72.9±11.9	77.4±13.4	0.146[a]
Gravidity	2 (1-5)	2 (1-5)	0.294[a]
Parity	1 (0-3)	1 (0-3)	0.114[a]

Data are expressed as mean ± standard deviation, median (min-max). [a]Independent Sample T test

Table 2. Comparison of the study groups in terms of NT, BhCG, uE3, AFP, PAPP-A

	Control Group (n=139)	Study Group (n=137)	*p-value*
NT (mm)	1.3±0.4	1.2±0.3	***0.009[a]***
uE3 (MoM)	1.0±0.3	1.0±0.3	*0.689[a]*
βhCG (MoM)	1.1±0.5	1.2±0.7	*0.650[a]*
AFP (MoM)	1.2±0.4	1.4±0.7	*0.059[a]*
PAPP-A (MoM)	1.2±0.7	0.9±0.5	***<0.001[a]***

Data are expressed as mean ± standard deviation. [a]Independent Sample T test

Table 3. Evaluation of serum markers according to the type of progesterone treatment

	Oral progesterone			Intramuscular progesterone			Vaginal progesterone		
	Yes n=125	No n=151	*p*	Yes n=5	No n=271	*P*	Yes n=7	No n=269	*p*
uE3 (MoM)	1.04±0.33	1.02±0.30	*0.711[a]*	1.01±1.03	0.32±0.32	*0.895[a]*	1.03±0.14	1.03±0.32	*0.993[a]*
βhCG MoM)	1.19±0.69	1.03±0.49	***0.032[a]***	1.00±0.22	1.11±0.60	*0.627[a]*	0.64±0.32	1.12±0.59	***0.036[a]***
AFP (MoM)	1.39±0.72	1.16±0.43	***0.001[a]***	1.19±0.41	1.27±0.60	*0.723[a]*	1.00±0.30	1.27±0.60	*0.229[a]*
PAPP-A (MoM)	0.92±0.51	1.18±0.70	***0.001[a]***	0.79±0.22	1.07±0.64	***0.013[a]***	0.83±0.88	1.07±0.63	*0.321[a]*

Data are expressed as mean ± standard deviation. [a]One-way test ANOVA, Bonferroni correction

Discussion

In our study, we observed significantly lower NT thickness and PAPP-A levels in the group treated with progesterone. Increased βhCG and AFP levels were observed, while uE3 levels did not change, although this information could not be statistically verified. The reason for this information, which differs from other studies, is probably that the routes of progesterone administration were not compared in previous studies. Levels of βhCG and AFP were higher in the oral micronized progesterone group

than in the control group; PAPP-A levels were low, and these differences were statistically significant. PAPP-A levels were lower in the group receiving intramuscular hydroxyprogesterone caproate than in the orally administered group, but the change in βhCG and AFP levels could not be statistically demonstrated. The βhCG levels were significantly lower in the group receiving vaginal treatment.

In some studies, low PAPP-A and uE3 levels were associated with poor perinatal outcomes (15, 16). It is known that pregnancy complications are predicted to some extent by serum parameters used in screening tests. However, it cannot be said that such parameters can be safely used to predict negative perinatal outcomes (17).

Progesterone is thought to be essential for the establishment and maintenance of pregnancy, which is why it is used to treat threatened abortion (18). In some studies, exogenous progesterone was not effective in the treatment of abortion (19). However, oral progesterone treatment has been found to be effective in patients with a history of repeated miscarriages (20). There are few studies in the literature investigating the effect of exogenous progesterone on fetal aneuploidy screening tests.

A study by Giorlandino et al. suggested that progesterone in the first trimester may affect fetal development by altering uterine blood flow, causing an increase in NT (13). Another study reported increased βhCG levels with progesterone treatment (21). However, these studies did not clearly clarify whether these changes were due to progesterone treatment or early bleeding.

It is known that maternal serum aneuploidy screening results are influenced by many factors. These include maternal age, smoking status, assisted reproductive techniques, week of gestation, multiple pregnancies, and maternal weight. A risk algorithm that takes these factors into account could lead to a reduction in the false-positive rate (22). It is controversial whether one of these factors is hemorrhage in the early weeks of pregnancy. In a study by Spencer, first-trimester screening was shown not to be affected by bleeding in the early weeks of pregnancy (23). Some studies have found that the maternal-fetal barrier is disrupted by bleeding in the early weeks of pregnancy, and therefore serum markers, especially βhCG, are found in higher concentrations in the blood (24, 25). It would not be surprising if other serum markers should also be high. Nevertheless, two different studies by Di Biasio et al. and Karaca et al. reported that high βhCG levels did not significantly alter PAPP-A levels (14, 26).

Two studies examining the effect of progesterone treatment on serum markers in the first trimester, independent of bleeding, showed an increase in βhCG levels and NT thickness when progesterone was used (21, 27). However, the first of these studies examined only the effect of oral progesterone use (21). The second study examined the effect of vaginal progesterone treatment on screening tests in the group of women who became pregnant using assisted reproductive technology (27). In the study by Karadağ et al. there was no statistical difference in NT thickness, PAPP-A, and βhCG levels between the groups that received progesterone and those that did not (28).

Changes in blood flow and growth factors and consequently fetal blood flow are caused by exogenous progesterone in the first trimester, which could increase NT thickness (13). The presence of progesterone receptors in the fetoplacental barrier and their vasoactive effects are well known (29). In addition, progesterone receptors are thought to play a similar physiological role in the fetus at 11 to 21 weeks as in the fetomaternal barrier (30, 31).

The limitation of this study is that it was retrospectively evaluated. Future studies may prospectively evaluate screening test results and perinatal outcomes with progesterone by different routes of administration.

In our retrospective study, women with similar age groups, no systemic diseases, no smoking history, no multiple pregnancies, no pregnancies with assisted reproductive techniques, and those who had received progesterone treatment at similar weeks were evaluated to control for factors thought to influence serum aneuploidy screening. Thus, in our study, we aimed to determine the relationship between different routes of progesterone administration and fetal aneuploidy screening markers. Because there is no consensus on the effective dose and delivery methods of progesterone treatment for hemorrhage in early pregnancy, the dose levels and delivery methods commonly used in our hospital were used as the standard.

In conclusion, significantly lower NT measurements and MoM values of PAPP-A markers were measured in the study group compared with the control group. The groups did not differ in terms of MoM values of serum markers uE3, βhCG, AFP. This is the first study to investigate the effect of different routes of progesterone treatment on dual and triple screening biomarkers. However, the results may be controversial due to the small sample size in some groups. Therefore, this result is an indication that the conclusions need to be verified by further well-designed studies with larger samples.

Ethics Committee Approval: Approval was obtained by the institutional review board from Ankara Etlik Zubeyde Hanım Women's Health Training and Research Hospital on August 28th, 2020, # 2020/21.

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