

Evaluating the Effects of Vitamin E Addition to Clomiphene Citrate on Endometrial Receptivity: A Prospective Controlled Study

Klomifen Sitrata E Vitamini İlavesinin Endometrial Duyarlılık Üzerine Olan Etkilerinin Değerlendirilmesi: Prospektif Kontrollü bir Çalışma

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ABSTRACT Objective: Vitamin E is a non-enzymatic antioxidant that protects the endothelium from oxidative stress. Oxidative stress is an important factor in the pathogenesis of female infertility. We aimed to investigate if vitamin E would improve the negative effects of clomiphene citrate (CC) on endometrium of women having ovulation induction, by using transvaginal color-pulsed Doppler ultrasonography. **Material and Methods:** In this study, 50 primary infertile normogonadotrophic patients with anovulatory cycles diagnosed by endocrine assays and ovarian transvaginal ultrasonographic scanning for follicle development, were recruited from patients attending to the Clinic of Obstetrics and Gynecology Erzurum Ataturk University. None of the subjects had male infertility or tubal occlusion. In 50 patients, CC (100 mg/day) was prescribed to be taken orally, twice daily for 5 days, starting on the 5th day of the menstrual period (CC group). Since four patients have already become pregnant in the CC group; remaining 46 patients received Vitamin E (300 mg/day) addition to CC, between first and thirteenth days of the following menstrual cycle (CC+vitamin E group). Follicular growth was followed by using transvaginal ultrasonography in all patients. When the mature follicle reached to 16-18 mm in diameter, endometrial thickness was measured. Flow velocity wave forms of spiral artery were recorded for calculation of the mean values pulsatility and resistance indexes (PI and RI) by using transvaginal color-pulsed Doppler ultrasonography. **Results:** The analysis of results showed that the mean PI and RI values of spiral artery blood flow were higher and endometrial thickness was lower in CC group (n= 50) when compared with CC + vitamin E group (n= 46) (p< 0.01, p< 0.05 and p< 0.01, respectively). The pregnancy rate in CC group (n= 4, 8%) was lower than in CC + vitamin E group (n= 13, 28%) (p< 0.01). **Conclusion:** The assessment of endometrial blood flow by transvaginal color-pulsed Doppler ultrasonography can be performed to detect endometrial receptivity in women using CC for the induction of ovulation. Spiral artery blood flow parameters may be helpful to demonstrate endometrial perfusion differences during the therapy. The addition of vitamin E to CC may have an improving effect on endometrial blood flow and pregnancy rate.

Key Words: Clomiphene; ultrasonography, doppler; infertility; tocopherols; endometrium; vitamin E

ÖZET Amaç: Vitamin E non-enzimatik bir antioksidant molekül olup endoteli oksidatif stresten korur. Oksidatif stres kadın infertilitesinde önemli bir faktördür. Bu çalışmanın amacı; ovulasyon indüksiyonu amacıyla klomifen sitrat (KS) kullanan hastalara ilave olarak E vitamini verildiğinde endometriumda oluşan değişiklikleri transvaginal renkli Doppler ultrasonografi ile değerlendirerek KS'in endometriumda oluşturduğu olumsuz etkisinin azaltılmasında E vitamininin etkinliğini araştırmaktır. **Gereç ve Yöntemler:** Bu çalışma, Erzurum Atatürk Üniversitesi Kadın Hastalıkları ve Doğum Kliniği'ne başvuran, endokrin ölçümler ve transvaginal ultrasonografi ile folliküler gelişim takip edilerek anovuluar siklus tanısı konulmuş olan 50 primer infertil hastada yapıldı. Vakaların hiçbirinde erkek infertilitesi ve tıkalı fallopian tüpler yoktu. Elli hastaya, KS (100 mg/gün) adet 5. gününde başlanarak, oral yolla 5 gün süre ile verildi (KS grup). Bu gruptaki 4'ü haric gebe kalamayan 46 hastaya, sonraki sıklusta KS'a ilave olarak adet 1-13. günleri arasında E vitamini (300 mg/gün) oral yolla verildi (KS+vitamin E grup). Transvaginal ultrasonografi yardımıyla folliküler büyüme takip edildi. Matür folikül çapı 16-18 milimetreye ulaştığında, endometrial kalınlık ve transvaginal renkli doppler ultrasonografi kullanılarak spiral arter kan akımı ölçülerek ortalama pulsatilite indeksi (PI) ve rezistans indeksi (RI) değerleri kaydedildi. **Bulgular:** KS + E vitamini grubu (n= 46) ile karşılaştırıldığında; KS (n= 50) alan hastalarda spiral kan akımının ortalama PI ve RI index değerlerinin daha yüksek ve endometrial kalınlığın azalmış olduğu görüldü (sırasıyla p< 0.01, p< 0.05, p< 0.01). KS grubundaki gebe kalma oranının (n= 4, %8), KS + E vitamini grubundaki gebe kalma oranına göre (n= 13, %28) daha düşük olduğu saptandı (p< 0.01). **Sonuç:** Ovulasyon indüksiyon ajanı olarak KS kullanan hastalarda, endometrial duyarlılığı tespit etmek için transvaginal renkli Doppler ultrasonografi ile endometrial kan akımı ölçümü yapılabilir. Tedavi sırasında endometriumda oluşan perfüzyon değişikliklerini göstermede, spiral arter kan akım parametrelerinin kullanılması faydalı olabilir. KS kullanan hastalara E vitamini desteği verilmesi; endometrium kan akımını ve gebelik oranını olumlu yönde etkileyebilir.

Anahtar Kelimeler: Klomifen; ultrasonografi, doppler; kısırılık; tokoferoller; endometrium; vitamin E

Infertility should be investigated if a couple cannot conceive within a year despite regular coitus and without contraception. Both male (30-40%) and female (40-50%) factors are responsible from infertility and for the female infertility, ovulatory dysfunction is a major cause.¹ Transvaginal color-pulsed Doppler ultrasonography is useful method in the management of infertility. Various studies have been performed to evaluate the Doppler flow variations with different ovarian stimulation protocols in infertility.²⁻⁷

Clomiphene Citrate (CC) is used for treatment of women with ovulatory dysfunction. CC is usually given daily from day 5 to day 9 of menstrual cycle with a dosage of 50 mg. If ovulation did not occur, the CC may have been given in doses from 100 to 250 mg. Cheung et al⁸ compared the perfollicular vascularity and endometrial receptivity of ovulatory women who started CC on day 2 or day 5. They found no significant difference between the two groups in hormonal profile, follicular development, endometrial thickness and Doppler flow indices of uterine-subendometrial vessels.

CC binds the estrogen receptors in hypothalamus and activates secretion of gonadotropin-releasing hormone (GnRH). Indirectly, secretion of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) increase.⁹ CC has antiestrogenic effects in the uterus, cervix and vagina, therefore, it reduces the positive effect of estrogen in cervical mucus and endometrium in such patients. Delayed endometrial maturation, poor cervical mucus quality, thin endometrium and impaired uterine perfusion have been reported in patients using CC.¹⁰⁻¹³ This condition may affect implantation and pregnancy rates.

Vitamin E is a non-enzymatic antioxidant. Investigators have shown that vitamin E protects the endothelium from oxidative stress.^{14,15} Oxidative stress has been reported as an important factor in the pathogenesis of female infertility.^{16,17} Vitamin E improves the pregnancy rates in patients with a thin endometrium by increasing the endometrial thickness.¹⁸ Thus, we speculated that vitamin E addition to CC might reduce the negative effects of

CC on endometrium. In a recent study, Takasaki et al¹⁹ investigated the effect of vitamin E on the radial artery blood flow in the infertile patients with thin endometrium. They concluded that vitamin E improves endometrial growth by increasing radial artery blood flow. We aimed to investigate if vitamin E would lessen the negative effects of CC on endometrium of women having ovulation induction, by using transvaginal color Doppler ultrasonography. Moreover, the secondary purpose of the study was to determine pregnancy rate differences between the two groups.

MATERIAL AND METHODS

In this study, 50 normogonadotrophic patients as diagnosed by endocrine assays and transvaginal ultrasonographic ovarian scanning for follicle development, were recruited from patients attending to the Obstetrics and Gynecology Clinic of Research Hospital Ataturk University. The patients with confirmed polycystic ovarian syndrome were excluded. The women had regular menstrual cycles (28-30 days) and their partners had normal spermograms according to the World Health Organization criteria. None of the subjects were smoking or had a history of ovarian surgery including drilling, cystectomy or oophorectomy, chronic cardiovascular, otoimmun, hepatic, renal or pulmonary diseases. All subjects had patent fallopian tubes as determined by hysterosalpingography. The participants used no hormonal or nonhormonal medications during the last 3 months. Written informed consent was obtained from the patients after the ethical committee of the Ataturk University approval.

All patients underwent a transvaginal ultrasound scan on day 2 of their menstrual cycle to rule out any ovarian cysts before starting CC. In 50 patients, CC (100 mg/day, Klomen[®]; Bayer, Istanbul, Turkey or Gonophene[®]; Organon, Istanbul, Turkey) was prescribed to be taken orally, two times per day for 5 days, starting on the 5th day of the period (CC group). Since four patients have already become pregnant in the CC group; remaining 46 patients received Vitamin E (300mg/day Ephyval[®]; Bayer, Istanbul, Turkey) addition to CC, between

first and thirteenth days of the following menstrual cycle (CC + vitamin E group). All ultrasonographic scans were performed at a lithotomy position, using the equipment pulse Doppler 7.5 MHz transvaginal probe (Shimadzu SDU-2200; Tokyo, Japan) for imaging and determining the blood flow rate. Doppler measurements were performed by the same investigator (A.A.) between 8a.m-11a.m. hours to prevent the bias due to circadian changes in spiral artery blood flow rate.

Follicular growth was followed by using transvaginal ultrasonography in all patients. When the mature follicle reached to 16-18 mm in diameter, endometrial thickness was measured at the maximum thickness between the highly reflective interfaces of the endometrial-myometrial junction. Flow velocity wave forms of spiral artery were recorded (the insonation angle at 0°) at the highest color intensity within innermost endometrial-subendometrial region by using transvaginal color-pulsed Doppler ultrasonography. An average of three to five cardiac cycles was selected for calculation of the mean pulsatility index ([mean PI= peak systolic velocities-end diastolic velocities]/mean velocities) and the mean resistance index ([mean RI=peak systolic velocities-end diastolic velocities]/peak systolic velocities). After recording mean PI and RI values, 10.000 IU human chorionic gonadotropin (hCG) (Pregnyl®; Scherring Plough, Istanbul, Turkey) was administered to induce the ovulation and purposed the coitus after 36 hours. Plasma hCG levels were measured at day 14 after administration of hCG. Pregnancy was defined when hCG values reach to > 100 IU. The statistical software package SPSS for Windows, version 10.5 (SPSS, Chicago, IL, USA) was used for data analysis. Comparisons among the groups were made by “Independent-t test, X² test, Mann-Whitney U test” where appropriate.

RESULTS

The characteristics of the patients are summarized in Table 1. Mean PI value of the spiral artery blood flow in CC + vitamin E group was found to be significantly decreased compared to CC group (1.78 ± 0.16, 2.00 ± 0.52, p< 0.01). Mean RI value was high-

TABLE 1: Clinical characteristics of all patients (n= 50).

Age (years)	31.68 ± 2.25
Duration of infertility (years)	2.92 ± 0.80
The prevalence of menstrual cycle (days)	29.80 ± 1.33
BMI (kg/m ²)	26.26 ± 1.27

Values are mean ± SD, by Mann-Whitney U test.

her in CC group than in CC+vitamin E group (0.75 ± 0.12, 0.69 ± 0.10, p< 0.05). Mean endometrial thickness in CC + vitamin E group was significantly increased when compared with CC group (8.80 ± 0.98, 7.90 ± 1.40, p< 0.01) (Figure 1).

Pregnancy was achieved in four of the patients in CC group. Mean PI and RI values of pregnant and non pregnant patients in CC group were not statistically significant (PI, 2.00 ± 0.52, 1.91 ± 0.55; RI, 0.75 ± 0.12, 0.73 ± 0.12). However, in CC group, increased endometrial thickness was found in pregnant cases when compared with nonpregnants (9.50 ± 1.00, 7.83 ± 1.37, p< 0.05) (Figure 2).

Pregnancy was achieved in thirteen of the patients in CC + vitamin E group. Mean PI and RI values of pregnant and nonpregnant patients in CC + vitamin E group were not statistically significant (PI, 1.75 ± 0.10, 1.84 ± 0.25; RI, 0.70 ± 0.09, 0.68 ± 0.12). However, in CC + vitamin E group, increased endometrial thickness was found in pregnant cases when compared with nonpregnants (9.30 ± 0.85, 8.60 ± 0.90 p< 0.05) (Figure 3).

X² test was used to compare pregnancy rate differences between two groups (X²= 10.43, p= 0.001). The pregnancy rate in CC group (8%) was lower in CC + vitamin E group (28%) (p< 0.01) [OR= 6.5, 95 % CI (1.91-22.18)] .

In both of CC and CC + vitamin E groups, there were no significant differences between pregnant and nonpregnant patients with regard to age, duration of infertility, duration of menstrual cycle and body mass index (BMI) (Table 2).

DISCUSSION

Transvaginal color-pulsed Doppler ultrasonography is a non-invasive method to detect endometrial receptivity in infertility treatment.

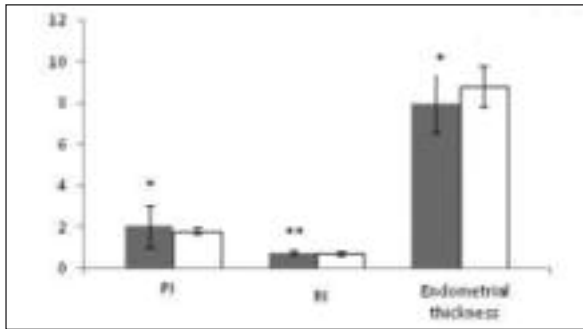


FIGURE 1: The mean values of PI, RI and endometrial thickness in two groups. ■; clomiphene citrate, □; clomiphene citrat+Vitamin E, * $p < 0.01$, ** $p < 0.05$ by Independent-t test, $n = 50$ for clomiphene citrate group and $n = 46$ for clomiphene citrate+vitamin E group.

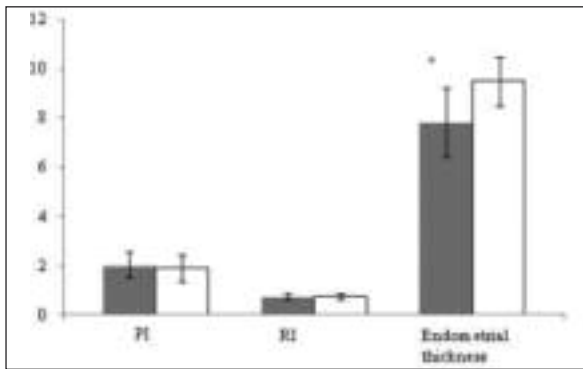


FIGURE 2: The mean values of PI, RI, endometrial thickness of pregnant and nonpregnant patients in CC group. □: pregnant, ■: nonpregnants, * $p < 0.05$, by Mann-Whitney U test, $n = 4$ for pregnant group and $n = 46$ for nonpregnant group.

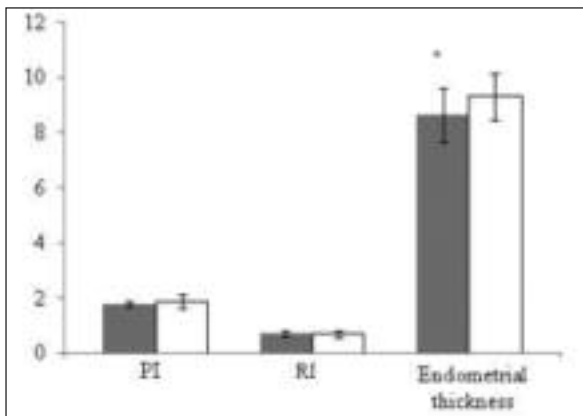


FIGURE 3: The mean values of PI, RI and endometrial thickness of pregnant and nonpregnant patients in CC+vitamin E group. □: pregnant, ■: nonpregnants, * $p < 0.05$, by Mann-Whitney U test, $n = 13$ for pregnant group and $n = 33$ for nonpregnant group.

Decreased endometrial thickness and anties-trogenic effects on cervical mucus have been documented in patients using CC.²⁰ CC is given

generally for 5 days period starting on the 5th day of the menstrual cycle as we did. Biljan et al²¹ investigated the effect of administration time of CC on pregnancy rates and found that period of 5 days starting on day 1 of menstrual cycle is more useful due to more follicular growth and higher pregnancy rates obtained. In the current study, uterine artery PI was found significantly lower in CC started on day 1 compared to on day 5. In another study, Hosnie et al²² demonstrated that endometrial abnormalities were related CC dosage.

Kupesic et al⁶ measured the flow velocities of uterine, spiral, radial and ovarian arteries and endometrial thickness during the periovulatory period in spontaneous and induced CC cycles. In their study, mean PI value was 3.16 in day 2 before ovulation and decreased at the day before ovulation in spontaneous cycles. However these changes were not shown in stimulated cycles and mean PI value remained at 3.06 level during the periovulatory period. In Kupesic et al's study,⁶ endometrial thickness was found to be significantly decreased in CC group compared to control group. Nakai et al⁷ concluded that the patients induced with CC cycles had lower endometrial perfusion during the periovulatory period compared to subjects with spontaneous menstrual cycles. The results of our study are consistent with research findings that were reported by Kupesic et al⁶ and Nakai et al.⁷

In another study, Schild et al²³ administered the patients a standard regime of the GnRH agonist triptorelin (Decapeptyl^R) in an in vitro fertilization programme (IVF) followed by standart dose of FSH (Puregon^R). On the day of oocyte retrieval, authors measured blood flow parameters of spiral and uterine artery as well as thickness and volume of endometrium. They found no significant correlations between endometrial flow parameters or endometrial measurements on pregnancy rate. Schild et al²⁴ evaluated subendometrial blood flow by three-dimensional-ultrasound. In their study, the pregnant and nonpregnant groups had no significant difference with regard to serum estradiol concentrations, endometrial thickness and volume. Chien et al²⁵ also indicated that Doppler indices of subendometrial blood flow velocity were not correlated

TABLE 2: Clinical characteristics of pregnant and non pregnant patients in CC and CC+vitamin E groups.

	Pregnant groups		Nonpregnant groups	
	CC (n= 4)	CC + vitamin E (n= 13)	CC (n= 46)	CC + vitamin E (n= 33)
Age (years)	33.75 ± 4.19	32.15 ± 2.08	31.50 ± 1.99	31.24 ± 1.92
Duration of infertility (years)	2.75 ± 0.96	2.77 ± 0.93	2.93 ± 0.80	3.00 ± 0.75
The prevalence of menstrual cycle (day)	29.50 ± 1.29	30.46 ± 1.33	29.83 ± 1.34	29.58 ± 1.28
BMI (kg/m ²)	26.00 ± 0.82	26.69 ± 1.03	26.28 ± 1.31	26.12 ± 1.39

Values are mean ± SD, by Mann-Whitney U test.

with pregnancy rates. They concluded that Doppler indices of subendometrial vessels may not be suitable for assessing endometrial receptivity. Cacciatore et al²⁶ suggested that in stimulated IVF cycles, uterine receptivity was poor when uterine PI and RI values were > 3.3 and > 0.95. Steer et al² detected that the day 14 PI value was significantly lower in patients who achieved pregnancy as compared with those who did not. On the other hand, we found no significant differences in the mean PI and RI values between pregnant and nonpregnant subjects in CC and CC + vitamin E groups. In our study, endometrial thickness was higher in pregnant of both groups when compared to nonpregnant ones.

Zhang et al²⁷ concluded that increased endometrial thickness is associated with improved pregnancy rates in IVF cycles. Weissman et al²⁸ found that an endometrial thickness above 14 mm is detrimental for successful pregnancy after IVF. In another study,²⁹ endometrial thickness on the day of ovulation for a possible pregnancy was calculated to be above 8 mm. On the contrary, Yuval et al³⁰ and Zaidi et al³¹ found no difference in endometrial thickness between conception and nonconception cycles. In our study endometrial thickness was found to be 9.5 mm (mean) in pregnant cases in CC and CC + vitamin E group.

Oxidative stress plays an important role in the pathogenesis of the infertility.³² Vitamin E is an antioxidant molecule. Konirsch et al³³ reported that adding pentoxifylline and vitamin E to hormonal

replacement therapy in women with premature ovarian failure and uterine resistance increases endometrial thickness and improves uterine parameters. Takasaki et al¹⁹ concluded that vitamin E may have been useful for the patients with thin endometrium by increasing uterine radial artery blood flow. We also have found that the spiral artery blood flow, mean PI and RI values in CC+vitamin E group were significantly decreased and endometrial thickness was increased compared with CC group. The pregnancy rate was lower in CC group than in CC+vitamin E group. Therefore, we suggest that adding vitamin E (as a antioxidant agent) to CC protocol may reduce antiestrogenic effects of CC.

In conclusion, the assessment of endometrial blood flow with transvaginal color Doppler ultrasonography can be performed to detect endometrial receptivity in women using CC for the induction of ovulation. Spiral artery blood flow parameters may be helpful to demonstrate endometrial perfusion differences during the therapy. Adding vitamin E to CC may improve the pregnancy rate by increasing endometrial thickness, angiogenesis and endometrial blood flow. The need for a randomized controlled trial, to assess the effects of vitamin E with CC combination for ovulation induction, is still warranted.

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