

Improved endometrial thickness and vascularity following vitamins E and C administration in infertile women undergoing controlled ovarian stimulation

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الخلاصة

يعتبر العقم احد اهم التحديات المهمة في الطب. ويعتبر عقار الكلوميفين سيترت من اكثر العقارات شيوعاً في معالجة العقم حيث يقوم بتحفيز المبايض لأننتاج البويض و زيادة حجمها من خلال تأثيره المثبط لهرمون الاستروجين لكنه يتصف بتأثيرات سلبية من خلال تقليله لسمك بطانة الرحم وبالتالي تأثيره على عملية انغراس الجنين المخصب في الرحم. وفي هذه الدراسة قمنا بأضافة فيتامين ج وفيتامين هـ لأربعين مريضة تعاني من العقم اثناء اخذهم لعقار الكلوميفين سيترت و عدم استعمال هذه الفيتامينات للمجموعة الاخرى من المريضات اللاتي يبلغ عددهن 40 فهم يستعملون فقط عقار الكلوميفين سيترت ومن ثم اخضاعهم لأجراء السونارلقياس عدد البويض التي تم تحفيزها , سمك بطانة الرحم و عامل المقاومة الوعائي لبطانة الرحم في ايام 2 و 12 من ايام الدورة الشهرية وقد تم استنتاج وجود زيادة في سمك بطانة الرحم و انخفاض عامل المقاومة الوعائي لبطانة الرحم لدى النساء اللواتي استعملن الفيتامينات المضادة للاكسدة وبالتالي هذا لديه تأثير ايجابي في انغراس الجنين في بطانة الرحم.

Abstract

Background: The most frequently used treatment to induce ovulation is clomiphene citrate which, unfortunately has the disadvantage of causing impaired endometrial growth. For successful conception to be achieved, optimal endometrial conditions are mandatory. The idea of giving vitamin C and E to infertile women on clomiphene citrate has been raised in published literature; however the subject is still controversial.

Aim of the study: To study the effect of adding vitamins C and E supplementation to infertile women undergoing controlled ovarian stimulation, on endometrial thickness and vascularity.

Patients and methods: The present case control study included 80 infertile women visiting infertility unit at Maternity and children hospital / Al-Dewaniyha city/ Iraq. From June 2014 to June 2016. Their age range was 20 to 35 years. They were classified into two groups: the first one included 40 patients and were treated using combine therapy of clomiphene citrate and vitamin E and C and the second group included 40 infertile women treated with clomiphene citrate only.

Results: Median endometrial thickness was significantly greater in group 1 than that of group 2, 4.5 mm versus 3.1 mm on day 2 and 8.3 mm versus 5.9 mm on day 12, respectively (P<0.001). Median endometrial vascular resistance was not significantly different in day 2; however it was significantly lower in group 1 compared to that of group 2 when assessed in day 12, 0.4 versus 0.6, respectively (P<0.001).

Conclusion: Administration of vitamins E and C to subfertile women undergoing controlled ovarian stimulation causes significant improvement in endometrial thickness and vascularity through anti-oxidant mechanism

Key words: Vitamins E, C, infertile women, endometrial thickness

Introduction

There is an observed trend for an increment in the proportion of infertile couples in the world; the majority being attributed to ovulation disorders which is responsible for infertility in approximately 15 % of couples and 40% of women (1). The most frequently used treatment to induce ovulation is clomiphene citrate which, unfortunately has the disadvantage of causing impaired endometrial growth. For successful conception to be achieved, optimal endometrial conditions are mandatory. The idea of giving vitamin C and E to infertile women on clomiphene citrate has been raised in published literature; however the subject is still controversial. Pregnancy outcome in infertile women undergoing assisted reproduction depends on several factors: oogenesis pathology, ovarian reserve and endometrial receptivity (2). The quality of endometrial receptivity is a function of several independent variables like endometrial thickness, pattern, volume and vascularity (1). The thickness to be regarded good needs to range from 7 up to 16 mm. The likelihood of pregnancy, when endometrial thickness is less than 7 mm, will be reduced to 29.4%, whereas the chance of pregnancy is estimated to be 35.8% when endometrial thickness is greater than 7 mm (3). Ultrasound may

be used efficiently to assess endometrial vascularity with ease and is regarded as non-invasive technique (4). Clomiphene citrate ranks the first for ovulation induction in women with an-ovulatory cycles (5). The anti-estrogen clomiphene citrate works through binding to estrogen receptors that are distributed throughout the reproductive organs (1). It is well established that rate of pregnancy induce by clomiphene citrate is highly variable and this has been attributed to several factors among which is the prolonged antiestrogenic effect of this drug on endometrial receptivity and uterine blood flow (6). Several studies reported significantly thinner endometrium in women receiving clomiphene citrate when compared to that of normal healthy women, and this probably has negative impact on rate of pregnancy through impaired implantation (1). On the contrary, other authors raised the opinion that endometrial thickness is not significantly reduced in women taking clomiphene citrate (7,8). Estrogen plays a vital role in determining the thickness of endometrium through induction of angiogenesis following activation of vascular endothelial growth factor (VEGF) (9). There is a balance between antioxidants and reactive oxygen species (ROS) in normal physiologic conditions; however, disruption may occur to this balance favoring excess

ROS (10) and it has been shown to have a relationship with cytokine receptor expression in vascular smooth muscle cells and endometrial cells (11). It was suggested by some authors that steroid hormones are associated with increased production of ROS; it was shown that estradiol can cause a 200-fold increase in peroxidase activity due to eosinophils infiltration mediated by this hormone and it was proven that hydrogen peroxide (H₂O₂) production increases significantly in vitro and in vivo in the presence of Estradiol. The accumulation of ROS is involved in the endometrium decay. The existence of antiestrogen such as clomiphene citrate will lead to a decrease in cell survival and induces apoptosis giving clomiphene citrate will initiate oxidative stress in the endometrium that negatively affects the growth and angiogenesis of the endometrium. Antioxidants can inhibit and reduce the damaging effects of oxidative stress (1). So the aim of the present study was to investigate the role of administration of vitamin C and E to infertile women subjected to clomiphene citrate.

Patients, Materials and Methods

The present case control study included 80 infertile women visiting infertility unit at Maternity and children teaching hospital/ Al-Dewaniyha city/ Iraq from June 2014 to June 2016. Their age range was 20 to 35 years. They were classified into two groups: the first one included 40 patients and were treated using combined therapy of clomiphene citrate (from day 2-6 of the cycle) and vitamin E(400IU) and C(500 mg) –from day 1-14 days of the cycle-

and the second group included 40 infertile women treated with clomiphene citrate only. Height and weight was measured in order to calculate body mass index (BMI) for all women enrolled in the present study. Ultrasound examination was done at day 2 and day 12 to estimate number of follicles, endometrial vascular radial resistance and endometrial thickness. Statistical analysis was carried out using (IBM SPSS version 22.0). Data were presented as mean, standard deviation, median and interquartile range. The level of significance was considered at P-value of 0.05.

Results

Comparison of characteristics of infertile women, who were classified into two groups, is shown in table 1. Median age of group 1, who received in addition to clomiphene citrate, vitamins E and C supplementation, was not significantly different from that of the second group, women who received neither vitamin E nor vitamin C supplementation, 28 years versus 29 years, respectively (P>0.05). This result ensured matching of the two group regarding age. On the other hand, no significant difference was reported regarding median BMI between the two groups, 22 kg/m² versus 22 kg/m², respectively (P>0.05). It was also noticed that the number of mature follicles, assessed by ultrasound examination, was nearly the same in both groups with no reported statistical difference, 12.5 versus 12 (P>0.05). Median endometrial thickness was significantly greater in group 1 than that of group 2, 4.5 mm versus 3.1 mm on

day 2 and 8.3 mm versus 5.9 mm on day 12, respectively ($P < 0.001$). Median endometrial vascular resistance was not significantly different in day 2;

however it was significantly lower in group 1 compared to that of group 2 when assessed in day 12, 0.4 versus 0.6, respectively ($P < 0.001$).

Table 1: Characteristic of the study groups

Characteristic	Group 1		Group 2		P*
	Median (IQR)	Mean \pm SD	Median (IQR)	Mean \pm SD	
Age	28.00 (9.75)	28.18 \pm 5.06	29.00 (7.00)	28.78 \pm 4.51	0.639
BMI	22.00 (2.00)	21.88 \pm 1.70	22.00 (2.00)	22.00 \pm 2.12	0.781
Number of follicles	12.50 (7.50)	12.88 \pm 4.70	12.00 (4.75)	11.80 \pm 3.76	0.313
ET day 2	4.50 (0.80)	4.59 \pm 0.62	3.10 (1.00)	3.33 \pm 0.72	<0.001
ET day 12	8.30 (2.32)	8.43 \pm 1.39	5.90 (1.65)	5.77 \pm 1.37	<0.001
RI day 2	1.15 (0.68)	1.42 \pm 1.23	1.40 (0.70)	1.32 \pm 0.37	0.156
RI day 12	0.40 (0.20)	0.41 \pm 0.10	0.60 (0.10)	0.54 \pm 0.08	<0.001

* Mann Whitney test; IQR: interquartile range; SD: standard deviation; Group 1: on vitamin C and E; Group 2: No vitamins; BMI: body mass index; ET: Endometrial thickness; RI: endometrial vascular resistance index

Discussion

The results of the present study pointed out to the role of vitamins E and C in improving two uterine characteristics, endometrial thickness and endometrial blood flow, in subfertile women subjected to controlled ovarian stimulation. We agree with the result of Takasaki *et al* (12) who observed improvement in endometrial thickness and uterine radial artery resistance after treatment with either vitamin E. Similar results were also described by Cicek *et al.* in 2012 and Al-Katib *et al.* 2013(13,14). Kurniawan *et al.* in 2014 (1) described marked improvement in endometrial thickness but no change in vascularity following vitamins E and C administration.

The proposed role for vitamins E and C in improving endometrial thickness and blood flow is related to the antioxidant effect of these vitamins. In

support for such hypothesis, the observation of Lédée-Bataille *et al.* in 2002 (15) in that women who were exposed to irradiation had significantly thinner endometrial than comparable healthy women and that their endometrium showed marked improvement in thickness following administration of vitamin E with pentoxifylline. Similar observation has also been made by Letur-Konirsch *et al.* in 2003(16), in that the addition of vitamin E caused remarkable improvement in women endometrial thickness. The conclusion of the above mentioned studies was based on the remarkable improvement in endometrial stromal tissue that directly determines the ultimate endometrial thickness. Another remarkable observation was made by Paszkowski *et al.* in 1995(17) who stated that follicular fluid selenium, a marker of

anti-oxidant activity, was significantly lower in subfertile women in comparison with women of normal fertility and hence they proposed that the pathophysiology of infertility in those women might be explained by defect of anti-oxidant enzymes that uses selenium as a co-enzyme. The improved endometrial thickness and vascularity following vitamins E and C administration, made evident in the current study, supports the philosophy of helpful anti-oxidant role in improving endometrial growth. Apart from anti-oxidative ability of Vitamin E, another proposed mechanism may help explaining the improved endometrial growth in women with infertility. This mechanism may be due to the anti-coagulant effect exerted by vitamin E, so that improvement in ovarian circulation, due to anti-coagulant activity, may increase proliferation of granulosa cell layer with subsequent increment in estrogen production that caused improved uterine circulation and hence endometrial thickness, beside direct improvement of endometrial blood flow due to vitamin E anti-coagulant effect; this mechanism has been proposed by several other authors Cicek *et al.* 2012(13) and Tovar *et al.*, 2006 (18). It is well documented that clomiphene citrate has an anti-estrogenic role and may cause thinning of endometrial when taken by women undergoing controlled ovarian stimulation (19,20). In the present study, it seems that this anti-estrogenic effect has been markedly reduced following the use of vitamin E and vitamin C. The anti-oxidative role

subjected by these two vitamins on endometrial micro-environment may cause in improvement in the response of endometrial cells to low estrogen level. By conclusion, the administration of vitamin E made endometrial thickness and vascularity better through improvement in anti-oxidant mechanism and modulation of anti-estrogenic effect exerted by clomiphene citrate administration.

Conclusion

The antioxidant and anticoagulant effects of vitamins C and E play a role in improving fertility outcomes by increasing the success rate of clomiphene citrate action through increasing the endometrial thickness and vascularity thus leading to increase in the endometrial receptivity and finally the conception rate

References

- 1-Kurniawan E, Djuwantono T, Sabarudin U, Krisnadi SR, Permadi W, Madjid TH. Difference of endometrial thickness and vascularity in women stimulated by clomiphene citrate with and without vitamin C and E. American Journal of Research Communication, 2014, 2(10): 1-10
- 2-Kupesic S, Kurjak A. Predictors of IVF outcome by three dimensional ultrasound. Hum Reprod. 2002;17(4):950-5.
- 3-Al-Ghamdi A, Coskun S, Al-Hassan S, Al-Rejjal R, Awartani K. The correlation between endometrial thickness and outcome of in vitro fertilization and embryo transfer (IVF-ET) outcome. Reprod Biol Endocrinol. 2008;6(1):37.
- 4-Wang L, Qiao J, Li R, Zhen X, Liu ZH. Role of endometrial blood flow assessment with color Doppler energy in predicting pregnancy outcome of IVF-ET cycles. Reprod Biol Endocrinol. 2010;18(8):122.
- 5-Miywally MFM and Caper RF. Use of an aromatase inhibitor for induction of ovulation in patients with an inadequate response to

- clomiphene citrate. *Fertil Steril.* 2001;75(2):305–9.
- 6- Gerli S, Gholami H, Manna A, Scotto Di Prega A, Vitiello C, Unfer V. Use of ethinyl estradiol to reverse the antiestrogenic effects of clomiphene citrate in patients undergoing intrauterine insemination: a comparative randomized study. *Fertil Steril.* 2000;73(1):85–9.
- 7- Sereepapong W, Suwajanakorn S, Trivatanachat S, Sampatanakul P, Pruksananonda R, Boonk Asemsanti W, et al. Effect of clomiphene citrate on the endometrium of regularly cycling women. *Fertil Steril.* 2000;73(2):287–91.
- 8- Dehbashi S, Parsanezhad ME, Alborzi S, Zarei A. Effect of clomiphene citrate on endometrium thickness and echogenic patterns. *Int J Gynecol Obstet.* 2003;80(1):49–53.
- 9- Albrecht ED, Babischkin JS, Lidor Y, Anderson LD, Udoff LC, Pepe GJ. Effect of estrogen on angiogenesis in co-cultures of human endometrial cells and microvascular endothelial cells. *Hum Reprod.* 2003;18(10):2039–47.
- 10- Agarwal A, Gupta S, Sharma RK. Role of oxidative stress in female reproduction. *Reprod Biol Endocrinol.* 2005;3:28.
- 11- Banerjee S, Smallwood A, Moorhead J, Chambers AE, Papageorghiou A, Campbell S, et al. Placental expression of interferon-gamma (IFN-gamma) and its receptor (IFN-gamma) R2 fail to switch from early hypoxic to late normotensive development in pre-eclampsia. *J Clin Endocrinol Metab.* 2005;90(2):944–52.
- 12- Takasaki A, Tamura H, Miwa I, Taketani T, Shimamura K, Sugino N. Endometrial growth and uterine blood flow: a pilot study for improving endometrial thickness in the patients with a thin endometrium. *Fertil Steril.* 2010; 93:1851–1858.
- 13- Cicek N, Eryilmaz OG, Sarikaya E, Gulerman C, Genc Y. Vitamin E effect on controlled ovarian stimulation of unexplained infertile women. *Journal of Assisted Reproduction and Genetics.* 2012; 29(4):325-328.
- 14- Al-Katib S R, Al-Kaabi M H and Al-Jashamy K A. Effects of Vitamin C on the Endometrial Thickness and Ovarian Hormones of Progesterone and Estrogen in Married and Unmarried Women. 2013; 1(8): 24-31.
- 15- Lédée-Bataille N, Olivennes F, Lefaix JL, et al. Combined treatment by pentoxifylline and tocopherol for recipient women with a thin endometrium enrolled in an oocyte donation programme. *Hum Reprod.* 2002;17(5):1249–1453.
- 16- Letur-Konirsch H, Delanian S. Successful pregnancies after combined pentoxifylline-tocopherol treatment in women with premature ovarian failure who are resistant to hormone replacement therapy. *Fertil Steril.* 2003;79(2):430–441.
- 17- Paszkowski T, Traub AI, Robinson SY, McMaster D. Selenium dependant glutathione peroxidase activity in human follicular fluid. *Clin Chim Acta.* 1995;236:173–180.
- 18- Tovar A, Ameho CK, Blumberg JB, et al. Extrahepatic tissue concentrations of vitamin K are lower in rats fed a high vitamin E diet. *Nutr Metab (Lond)* 2006;20(3):29.
- 19- Bromer JG, Aldad TS, Taylor HS. Defining the proliferative phase endometrial defect. *Fertil Steril.* 2009; 91:698–704.
- 20- Siebert TI, Kruger TF, Lombard C. Evaluating the equivalence of clomiphene citrate with and without metformin in ovulation induction in PCOS patients. *J Assist Reprod Genet.* 2009;26(4):165–171.