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Original Article

Vitamin C is effective for the prevention and regression of endometriotic implants in an experimentally induced rat model of endometriosis

Ozlem Ulas Erten [a], Tuğba Altun Ensari [b, *], Berna Dilbaz [b], Huseyin Cakiroglu [c], Sadiman Kıykac Altinbas [b], Muzaffer Çaydere [d], Umit Goktolga [b]

[a] Silopi State Hospital, Department of Obstetrics and Gynecology, Sirnak, Turkey
[b] Etlik Zubeyde Hanim Women's Health Education and Research Hospital, Ankara, Turkey
[c] Republic of Turkey Ministry of Food, Agriculture and Livestock, Pendik Veterinary Control Institute, Istanbul, Turkey
[d] Department of Pathology, Ankara Education and Research Hospital, Ankara, Turkey

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ABSTRACT

Objective: Endometriosis is a chronic inflammatory disease pathologically defined as the presence of endometrial-like tissue outside the uterine cavity. It is one of the most important diseases affecting women of reproductive age. The process of endometriotic implant growth is mediated by many complex interactions of immunologic, hormonal, genetic, and environmental mediators. Vitamin C (ascorbic acid), besides playing a role in preventing invasion and metastasis, is an antioxidant having anti-inflammatory and -angiogenic effects. In this study, we aimed to investigate the effect of vitamin C on the prevention and regression of endometriotic implants in a rat model of endometriosis.

Materials and Methods: This was a prospective, comparative, experimental animal study. After endometriotic implants were induced simultaneously, rats were divided into three groups. Group A was given 500 mg/kg of intravenous vitamin C every 2 days, starting immediately after implantation ($n = 11$). All rats had a second operation 21 days after the initial one and had the lesion volumes measured. Group B was given 500 mg/kg of intravenous vitamin C every 2 days, starting 21 days after this operation ($n = 11$). All rats were sacrificed 21 days after the third operation. Implant volume, weight measurements, and histopathological evaluation of the lesions were carried out. Group A received vitamin C throughout the study, while Group C ($n = 11$) was not given any medication. The findings in the three groups were compared.

Results: At the second laparotomy after the induction, Group A had the smallest implant volume with a statistically significant difference compared to Group B ($p = 0.012$). The end-of-study volumes of endometriotic implants of group B were significantly smaller than the first volumes ($p < 0.05$).

Conclusion: Intravenous vitamin C treatment might have a suppressive effect on the prevention of endometriotic implant induction and regression of endometriotic implant volumes.

Introduction

Endometriosis is described as the presence of endometrial tissue (glandular epithelium and stroma) outside the uterine cavity. It is a benign chronic inflammatory disease, associated with pain (chronic pelvic pain, dysmenorrhea, dyspareunia) and infertility [1].

It is estimated to occur in 10% of all women [2] and is reported to affect up to 60% of reproductive aged women with pelvic or infertility symptoms [3,4]. This high prevalence in the infertile population underlines its complex and multifactorial role in infertility, having adverse effects on ovarian reserve, ovulation, tubal anatomy, embryo quality, and implantation [5].

The pathophysiology of endometriosis is still not entirely clear, and several theories have been proposed. During the past

* Corresponding author. Etlik Zubeyde Hanim Women's Health Education and Research Hospital, Emek Mahallesi 30, Sokak, Number: 34/4 Çankaya, Ankara, Turkey.
E-mail address: ensaritugba@gmail.com (T.A. Ensari).

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2 decades, the roles of immunologic factors, inflammatory mediators, various genes, and oxidative stress have been emphasized in the pathogenesis of endometriosis [6–12]. Recent studies imply a potential role of oxidative stress in the activation of nuclear factor-kappaB (NFκB), which stimulates inflammation and cell proliferation and inhibits apoptosis of endometriotic cells, having a major effect on the implantation and growth of endometriotic foci [12–14].

Vitamin C (ascorbic acid) seems to have a far more important role than being an antiscurvy vitamin alone. Besides playing a role in preventing invasion and metastasis, it is an antioxidant having anti-inflammatory and -angiogenic effects [15–18]. *In vitro* and *in vivo* studies have demonstrated the anticancer activity of vitamin C at pharmaceutical doses [19–21].

Vitamin C formulated as 2-3 endiol-L gulonic acid γ lactone having two enolic hydroxyl groups is a strong reducing agent. Vitamin C is a part of the antioxidant protective system, as are glutathione, α tocopherol, and nicotinamide [15–22]. Oxidants and endotoxins stimulate cytokine secretion during the early stages of the immune response via activation of NFκB. While vitamin C directly limits cytokine secretion by radical scavenger function, it also prevents cytokine secretion indirectly via intracellular suppression of NFκB [11–14].

Although endometriosis occurs spontaneously only in humans and some nonhuman primates, animal models are introduced for further evaluation of pathophysiological mechanisms of this disease. Nonhuman primates may also be used for endometriosis research; however, a high cost of animal handling is the major concern that limits the use of baboons as an experimental model. As rodents do not menstruate, an endometriosis model in a rodent should necessarily be "induced." Despite differences between reproductive physiology of rodents and humans; these models of endometriosis are thought to be able to achieve a convenient understanding of immune and endocrine interactions that are altered during endometriosis [23].

There are two main types of experimental induction of endometriosis in animal models such as rats: the homologous model including autologous transplantation of uterine fragments into the peritoneal cavity and the heterologous model involving heterotransplantation of human endometrial or endometriotic tissue to immunodeficient mice [23]. Another type of animal model is the chicken chorioallantoic membrane assay model, which had been used to assess the invasive and angiogenic properties of endometriosis and neoplastic lesions as well [23,24]. This model was not attributable for investigating the immunological and inflammatory mechanisms of endometriosis [23]. Vernon and Wilson [25], after performing various interventions in their study in 1985, stated that autotransplantation of uterine tissues directly to the peritoneal wall was the only technique that achieved development of endometriotic implants made up of both glands and endometrial stroma.

There is only one study in the literature that has investigated the effect of oral administration of vitamin C on the growth of endometriotic cysts [26].

With the thought that it might be a cost-effective alternative with a fewer-side-effect profile to currently available hormonal medical treatment strategies for endometriosis, the aim of the present study was to investigate whether intravenously administrated vitamin C could have any prophylactic and therapeutic effects on the growth of endometriotic implants in a rat model of endometriosis.

Materials and methods

This study was carried out in the Laboratory of the Animal Research Center at the Ankara Education and Research Hospital, Ankara, Turkey. The Ankara Education and Research Hospital Committee on the Use and Care of Animals approved the experimental procedure on May 1, 2012, and all investigations were performed in compliance with the European Commission Directive 86/609/EEC guidelines on the ethical use of animals.

Animals

Thirty-three sexually mature, cycling, female Wistar Albino Rats (Laboratory of the Animal Research Center at the Ankara Education and Research Hospital, Ankara, Turkey) weighing between 209 g and 270 g were used in this study (Figure 1; flowchart of the study). The rats were checked for three consecutive regular estrous cycles for homogeneity. Temperature, humidity ratio, and light/dark time adjustment for room maintenance were 24 ± 1°C, 55 ± 5%, and from 14-hour to 10-hour (light to dark) photoperiod, respectively. Water and standard rat feed were provided *ad libitum*. The same team under supervision of a veterinarian carried out maintenance of the rats. All rats were allowed to acclimate in the vivarium for a 1-week period before the experimental research.

Surgical procedures

All rats were anesthetized by intraperitoneal injection of 50 mg/kg ketamine hydrochloride (Ketalar; Pfizer Pharmaceutical Co. Ltd, Istanbul, Turkey) and 7 mg/kg xylazine hydrochloride (Rompun; Bayer Turkish Chemical İndustry Trade Co. Ltd, İstanbul, Turkey). Anesthetized rats were immobilized in dorsal recumbence on a standard surgical rat platform, and abdominal surfaces were shaved and rinsed with 10% povidone iodine solution. All rats were weighed under anesthesia prior to the first operation, and this was recorded separately.

First operation

According to the modifications of Vernon and Wilson [25] and Uygur et al [27], surgical endometriosis was induced by transplanting autologous uterine tissue onto the peritoneal wall. A 3 cm vertical midline incision was made and a 1 cm segment was excised from the right uterine horn, the strip was cut longitudinally with microscissors, and the endometrial mucosa was exposed. This endometrial tissue was placed in a sterile saline solution. A tissue fraction with an approximate size of 0.5 cm × 0.3 cm was acquired for implantation. This endometrial tissue was sutured on both ends using a 4/0 polypropylene stitch to the inner surface of the peritoneum on the right side of the abdomen. The operation time was limited to 15 minutes, in order to minimize the effect of environment on the drying of the tissues.

Rats were randomly divided into three groups (A, B, and C). Simultaneously with the first operation, intravenous vitamin C (Redoxon ampoule, Bayer Turkish Chemical Industry Trade Co. Ltd., Istanbul, Turkey) 500 mg/kg was given every 2 days to the rats in Group A ($n = 11$) until the end of the study. The tail vein was used for intravenous injection starting from the distal to the proximal part. No drugs were given to the remaining 22 rats in Groups B and C after the first operation. Rats were caged separately and left for a 21-day recovery period. During this period, rats were weighed daily and none received estrogen or any other medications.

Second operation

Eight rats died during the recovery period: two from Group A and three from Groups B and C. Three weeks after the first operation, at a second-look laparotomy, vitality of the endometriotic implants and the presence of cystic structures were evaluated in

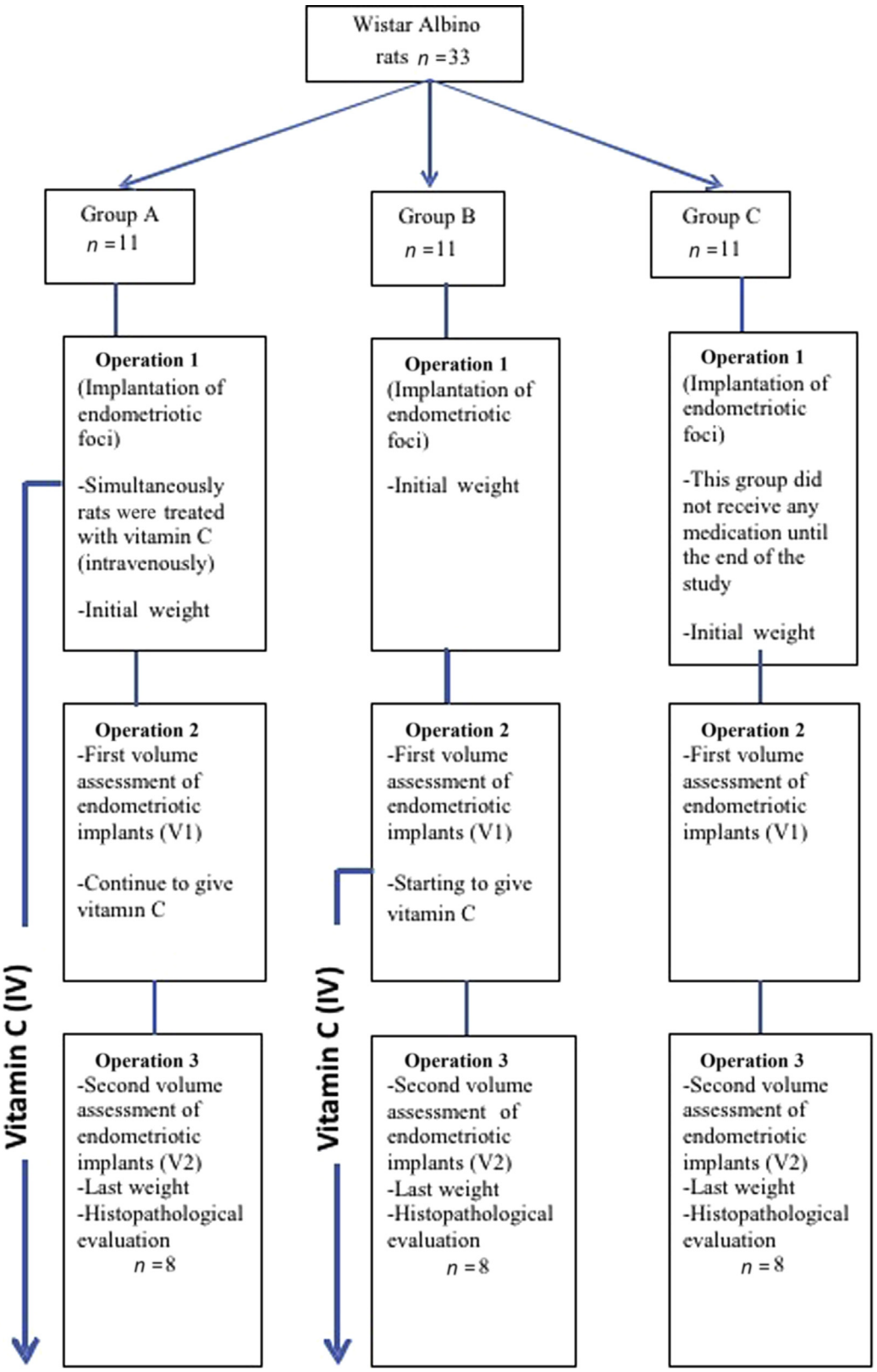

Figure 1. Flowchart of the study indicating operation times and interventions. IV = intravenous.

accordance with the criteria defined in previous studies [28]. Ectopic tissue was identified and three dimensions of the implant (length, width, and height) were measured using a digital millimetric caliper (Elite 6″ digital caliper-70416, Mercier İnc., Levis, Canada; Figure 2). The spherical volume of the ectopic uterine tissue was calculated using the ellipsoid formula:

$$[V\,(\mathrm{mm}^3) = 0.52 \text{ length (mm)} \times \text{width (mm)} \times \text{height (mm)}] \text{ and recorded } (V1). \quad (1)$$

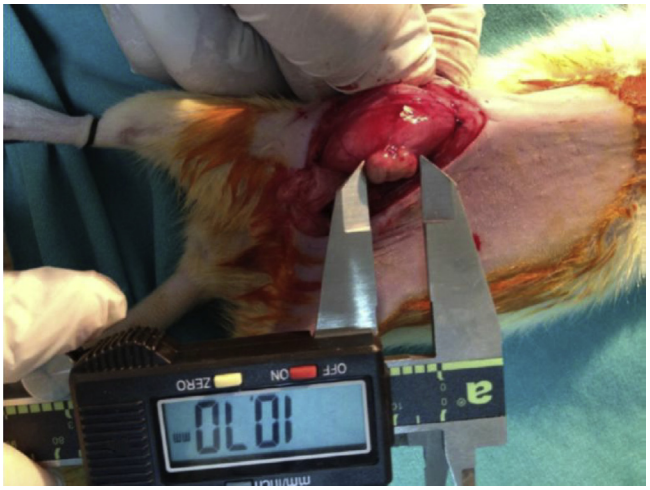

Figure 2. Measurement of ectopic tissue (length, width, and height) using a digital millimetric caliper at the second laparotomy.

After the second operation, 500 mg/kg intravenous vitamin C (Redoxon ampoule) was given every 2 days to the rats in Group B ($n = 8$) until the third operation. Rats in Group C ($n = 8$) did not receive vitamin C or any other medications throughout the study.

Third operation

The third laparotomy was carried out 21 days after the second operation, and the remaining 25 rats were sacrificed by ketamine anesthesia. Implant volumes were measured ($V2$). Two physicians blinded to the groups performed all surgeries and measurements. Endometrial foci were excised and subjected to histopathological examination after being fixed in a 10% formalin solution.

Histopathological examination

Endometriotic foci with surrounding normal tissue were extracted for the histopathologic evaluation, samples were embedded in paraffin blocks in the Pathology Laboratory (Pathology Laboratory of Ankara Education and Research Hospital, Ankara, Turkey), and 4–6 μm cross-sections were prepared and stained by hematoxylin–eosin (Figures 3A–3C). Specimens were examined under a light microscope. Persistence of epithelial cells in endometrial implants was evaluated semiquantitatively as follows: 3 = well-preserved epithelial layer; 2 = moderately preserved epithelium with leukocyte infiltrate; 1 = poorly preserved epithelium (occasional epithelial cells only); and 0 = no epithelium [29]. The stromal elements and fibrosis around the endometric foci were evaluated by Masson's trichrome staining: Grade 0, no fibrosis; Grade 1, minimal fibrous tissue growth; Grade 2, irregular fibrous

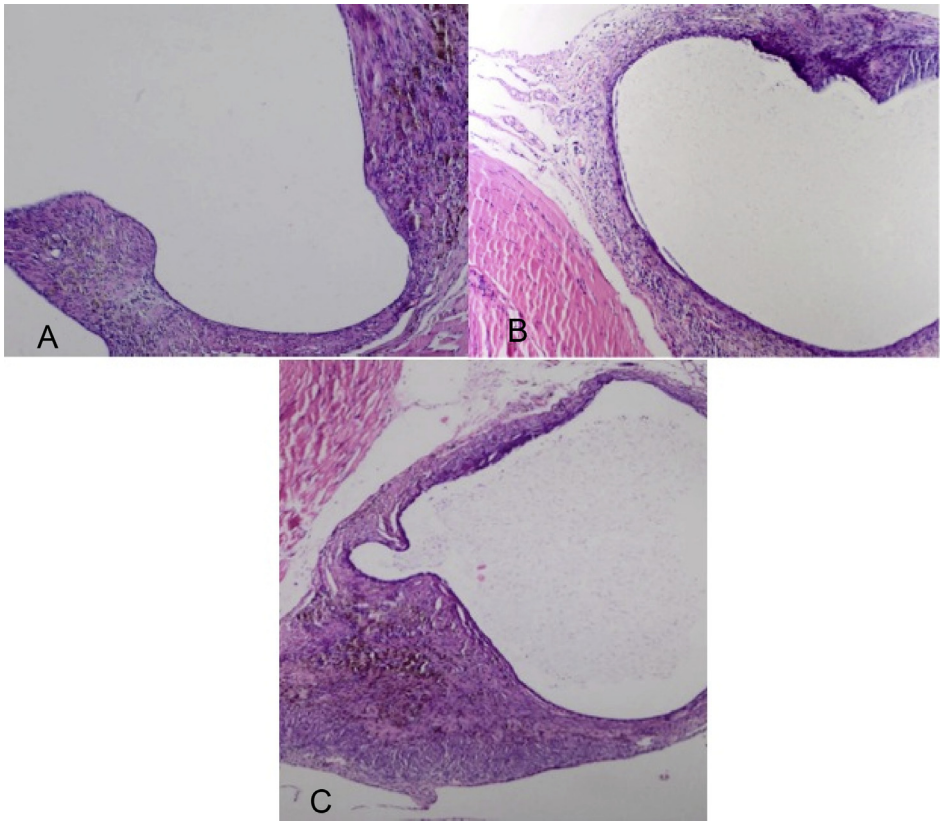

Figure 3. (A) Histopathological appearance of an implant in Group A (hematoxylin–eosin staining). Epithelial score is 1 (poorly preserved epithelium). (B) Histopathological appearance of an implant in Group B (hematoxylin–eosin staining). Epithelial score is 2 (moderately preserved epithelium with leukocyte infiltrate and detachments from the basal layer). (C) Histopathological appearance of an implant at Group C (control group). (hematoxylin–eosin staining). Epithelial score is 3 (well preserved epithelium).

tissue growth; and Grade 3, concentric fibrosis and hyalinization (Figures 4A–4D) [30,31]. The same pathologist who was blinded to the study groups examined all tissues.

Statistical analysis

Data are given as median (range) and mean ± standard deviation. Statistical analyses were performed using SPSS version 15.0 (SPSS Inc., Chicago, IL, USA). Non-normally distributed metric variables were analyzed by Kruskal–Wallis test and Mann–Whitney *U* test. Implant volumes within the groups were compared statistically using Wilcoxon signed rank test, while the implant volumes of the groups and differences between *V*1 and *V*2 were compared using the Kruskal–Wallis test and Mann–Whitney *U* test, respectively. A p value < 0.05 was considered as statistically significant.

Results

Three parameters were evaluated during the study: implant volumes, weight changes, and histopathological scores.

No statistically significant difference between the groups was determined in terms of weight change (Table 1) and histological scores (Tables 2 and 3; epithelial cell score and Masson's trichrome score; $p > 0.05$). Although ectopic endometrial glands and stroma seemed to be more preserved in specimens from the control group, there was no statistical significance between the histological scores of the groups.

Significant differences were determined in endometriotic implant volumes. Both Groups A and B were significantly different from the control group when the differences between volumes (*V*1 − *V*2) were compared (Table 4).

Group A had the lowest implant volume during the second and third laparotomies, and the difference between Groups A and B was statistically significant ($p = 0.012$). A significant regression was detected in Group B (109.93 ± 104.70 mm^3 vs. 42.45 ± 60.35 mm^3, $p = 0.025$) after the treatment with vitamin C, while an increase in the volume was found in the control group that received no medication (34.71 ± 2.81 vs. 59.22 ± 3.61; Table 4).

Discussion

Exact pathogenic processes that underlie the development and provide maintenance of endometriosis are still unclear [1,5]. As known to date about the pathophysiology of endometriosis, induction and progression of the disease require a proinflammatory environment, increased angiogenesis, resistance to apoptosis,

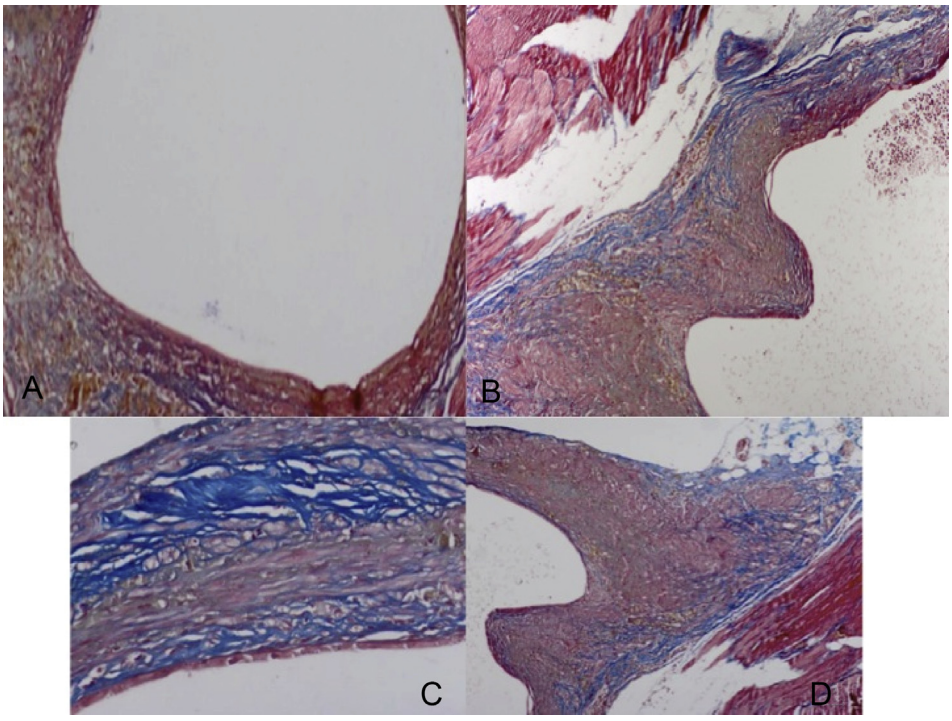

Figure 4. (A) Histopathological appearance of an implant in Group A (Masson's trichrome staining). Epithelial score is 1 and trichrome staining score is considered as 2 reflecting fibrosis and collagen formation area (blue areas) in the viewed section. (B) Histopathological appearance of an implant in Group B (Masson's trichrome staining). Epithelial score 2 and trichrome staining score is considered as 3 reflecting fibrosis and collagen formation area. (C and D) Histopathological appearance of an implant in Group C (control group) (Masson's trichrome staining). Epithelial score is 2 and trichrome staining score is considered as 3 refllecting the common area of fibrosis and collagen formation (blue areas). Two preparations from the same rat of the control group are viewed.

Table 1
Weights of rats in the control and treatment groups and statistical analysis of weight changes during the experiments.

Weight (g)	Group A ($n = 9$)	Group B ($n = 8$)	Group C ($n = 8$)	Inter- & intragroup assessments	Statistical analysis
Weight 1	241.11 ± 21.23	235.25 ± 13.63	238.25 ± 13.99	0.777	$p > 0.05$
Weight 2	238.44 ± 18.50	237.50 ± 13.92	236.25 ± 15.62	0.962	$p > 0.05$

Data are presented as mean ± standard deviation.

Table 2
Histological cell scores among groups.[a]

Histological scores	Group A	Group B	Group C
Mean	1.0	1.62	2.0
Median	1.0	1.5	2.0
Min–max	0.0–3.0	0.0–3.0	1.0–3.0
Standard deviation	0.86	1.06	0.92

[a] Kruskal–Wallis test and histological scoring showed no statistically significant difference between groups ($p = 0.096$).

Table 3
Trichrome fibrosis scores between groups.[a]

Trichrome scores	Group A	Group B	Group C
Mean	2.22	1.62	2.12
Median	2.0	2.0	2.0
Min–max	1.0–3.0	1.0–2.0	1.0–3.0
Standard deviation	0.83	0.51	0.83

[a] The difference in the Kruskal–Wallis test trichrome score between groups was not statistically significant ($p = 0.234$).

changes in the structural and epigenetic elements, as well as oxidative stress [5,7–9]. A novel medical treatment while having an impact on many of these steps, but also should not adversely affect the fecundity and also should have minimal adverse effect profile that makes the long-term use possible for endometriosis.

Most vitamin C studies in the literature are cancer studies based on antiangiogenic and -tumoral effects [20,29]. There are also studies that mention anti-inflammatory and -angiogenic effects of vitamin C. Knowing these features of vitamin C, our study was designed with the goal of investigating the effect of vitamin C on endometriotic implants.

The results of the experiments of this study demonstrate that vitamin C effectively causes a significant decrease in the volume of endometriotic tissue and may support the hypothetical value of antioxidant therapy in endometriosis. Group A, which received vitamin C at the induction of endometriosis and continued during the study, had the lowest implant volumes. This group may be considered as the "prophylaxis" group. The implant volume measured during the third laparotomy (42 days after the induction) decreased significantly in Group B between the second and third operations—the group that received vitamin C starting after the second operation and may be considered as the "treatment" group. In the control group that had no treatment, implant volumes continued to increase during the study (42 days). The histopathological structure of the disease was not found to be any different in the two treatment groups. This might be related to the short duration of the treatment.

Santanam et al [14] used a combination of vitamin C and vitamin E as antioxidants, and found that chronic pain improved significantly in the group that received antioxidant supplementation in comparison with the placebo group. Furthermore, there was a decrease in peritoneal inflammatory markers in this group. Other studies have demonstrated that NFκB inhibitors prevent proinflammatory signal transmission in the endometriosis of nude mice models by suppressing the development of endometriosis [32], and all medications that are currently used for treatment of endometriosis act through suppression of NFκB activation [31]. In endometriotic cells, NFκB appears to be constitutively activated, and suppression by NFκB inhibitors or proteasome inhibitors suppresses proliferation *in vitro*. Furthermore, it has also been shown that antioxidant intake provides a protective effect from NFκB activation and that it treats the symptoms caused by the disease [11,14]. Ascorbic acid with a concentration higher than 199 mg/dL demonstrates a noncytotoxic anticancer activity by preventing cell migration and angiogenesis [18]. All these studies imply that vitamin C may play a protective or therapeutic role in endometriosis.

Studies have shown that the diets of women with endometriosis include less antioxidant, and antioxidant intake is beneficial for decreasing pelvic pain [14,33]. Hence, the use of vitamin C is encouraging in terms of its side effects, cost, and ease of access, at least for symptomatic relief. Durak et al [26] investigated the effect of different doses of vitamin C supplementation via oral gavage, beginning the day after implantation, on endometriotic cyst volume in a rat model. They reported a significant dose-related reduction in the weight and volume of the cysts in the study group compared with that of the control group. Similar to our study, they failed to demonstrate a significant difference in the stromal and glandular tissue contents of the groups. It is already known that due to the strict control of intestinal absorption of vitamin C, oral intake is not sufficient for reaching therapeutic plasma levels [15]. Therefore, in the present study, we gave vitamin C via intravenous administration, and also the timing of the treatment was different as we aimed to investigate both prophylactic and therapeutic effects of vitamin C on endometriosis.

This is a preliminary study investigating a novel therapeutic approach that seems to promise a favorable outcome. Several limitations to this study need to be acknowledged; the first limitation, common with all studies on rats, is that rats are different from women regarding reproductive biology and anatomy, as they do not menstruate and do not have endometriosis spontaneously [23]. Vernon and Wilson [25], after performing various interventions in their study in 1985, stated that autotransplantation of uterine tissues directly to the peritoneal wall was the only technique that achieved developing endometriotic implants made up of both glands and endometrial stroma. Endometriosis in our model was induced according to these findings.

The second limitation is that the current study does not include a comparison of the effects of a well-known antioxidant agent or a Gonadotropin-Releasing Hormone agonist (GnRH agonist), which are proven therapies used in humans. [34]. As all new therapeutic agents, further investigations are needed in order to assess the

Table 4
Differences of implant volumes among groups.

Variable	Group A	Group B	Control group	p[a]
No. of rats	9	8	8	
Mean volume of the implants at the 2nd laparotomy (mm^3)	18.7539 ± 1.23013[b]	109.9367 ± 104.70553	34.7153 ± 2.81311	0.025
Mean volume of the implants at the 3rd laparotomy (mm^3)	11.4073 ± 8.49556	42.4597 ± 60.35632	59.2247 ± 3.61833	0.672
Differences between volumes	7.34 ± 24.29[c]	67.47 ± 91.85[d]	−24.50 ± 48.90	0.022
p[e]	0.953	0.025	0.401	

[a] Comparison of groups done by Kruskal–Wallis test and Mann–Whitney *U* tests.
[b] Statistically significant difference from Group B ($p = 0.012$).
[c] Statistically significant difference from Group B ($p = 0.043$).
[d] Statistically significant difference from the control group ($p = 0.09$).
[e] Comparison within a group done by Wilcoxon signed rank test.

exact effect of the interested agent on a particular issue of disease. As we mentioned before, in this preliminary study with small size of animal samples, according to the ethical limitations of the animal studies, we speculated the preventive and regressive effects of "high dose" intravenous vitamin C in an endometriosis model. Different dose regimens and additional settings including GnRH agonists will be our future direction for investigating the effects of vitamin C properly.

In conclusion, we may speculate that vitamin C has a remarkable effect on the reduction of endometrial implant induction and growth, and further research is required to improve targeted interventions aimed at the prophylaxis and treatment of human endometriosis. The use of systemic high-dose vitamin C as a potential therapeutic agent in the treatment of endometriosis is at an experimental stage. These findings can be useful if they are supported by subsequent research carried out with a greater number of cases and various different experimental designs.

Conflicts of interest

The authors have no conflicts of interest relevant to this article.

References

[1] Matarese G, De Placido G, Nikas Y, Alviggi C. Pathogenesis of endometriosis: natural immunity dysfunction or autoimmune disease? Trends Mol Med 2003;9:223–8.
[2] Crosignani P, Olive D, Bergqvist A, Luciano A. Advances in the management of endometriosis: an update for clinicians. Hum Reprod Update 2006;12:179–89.
[3] Amsterdam LL, Gentry W, Jobanputra S, Wolf M, Rubin SD, Bulun SE. Anastrazole and oral contraceptives: a novel treatment for endometriosis. Fertil Steril 2005;84:300–4.
[4] Giudice LC, Kao LC. Endometriosis. Lancet 2004;364:1789–99.
[5] Gauché-Cazalis C, Koskas M, Cohen Scali S, Luton D, Yazbeck C. Endometriosis and implantation: myths and facts. Middle East Fertil Soc J 2012;17:79–81.
[6] Paul Dmowski W, Braun DP. Immunology of endometriosis. Best Pract Res Clin Obstet Gynaecol 2004;18:245–63.
[7] Seli E, Berkkanoglu M, Arici A. Pathogenesis of endometriosis. Obstet Gynecol Clin North Am 2003;30:41–61.
[8] Prieto L, Quesada JF, Cambero O, Pacheco A, Pellicer A, Codoceo R, et al. Analysis of follicular fluid and serum markers of oxidative stress in women with infertility related to endometriosis. Fertil Steril 2012;98:126–30.
[9] Ruder EH, Hartman TJ, Blumberg J, Goldman MB. Oxidative stress and antioxidants: exposure and impact on female fertility. Hum Reprod Update 2008;14:345–57.
[10] Peltomaki P, Butzow R. Endometriosis as an epigenetic disease. Epigenomics 2011;3:690–1.
[11] Van Langendonckt A, Casanas-Roux F, Donnez J. Oxidative stress and peritoneal endometriosis. Fertil Steril 2002;77:861–70.
[12] Gonzalez-Ramos R, Defrere S, Devoto L. Nuclear factor-kappaB: a main regulator of inflammation and cell survival in endometriosis pathophysiology. Fertil Steril 2012;98:520–8.
[13] Gonzalez-Ramos R, Rocco J, Rojas C, Sovino H, Poch A, Kohen P, et al. Physiologic activation of nuclear factor kappa-B in the endometrium during the menstrual cycle is altered in endometriosis patients. Fertil Steril 2012;97:645–51.
[14] Santanam N, Kavtaradze N, Murphy A, Dominguez C, Parthasarathy S. Antioxidant supplementation reduces endometriosis-related pelvic pain in humans. Transl Res 2013;161:189–95.
[15] Du J, Cullen JJ, Buettner GR. Ascorbic acid: chemistry, biology and the treatment of cancer. Biochim Biophys Acta 2012;1826:443–57.
[16] Lane DJ, Lawen A. Ascorbate and plasma membrane electron transport—enzymes vs efflux. Free Radic Biol Med 2009;47:485–95.
[17] Berger TM, Polidori MC, Dabbagh A, Evans PJ, Halliwell B, Morrow JD, et al. Antioxidant activity of vitamin C in iron-overloaded human plasma. J Biol Chem 1997;272:15656–60.
[18] Cameron E, Pauling L, Leibovitz B. Ascorbic acid and cancer: a review. Cancer Res 1979;39:663–81.
[19] Mikirova NA, Ichim TE, Riordan NH. Anti-angiogenic effect of high doses of ascorbic acid. J Transl Med 2008;6:50.
[20] Yeom CH, Lee G, Park JH, Yu J, Park S, Yi SY, et al. High dose concentration administration of ascorbic acid inhibits tumor growth in BALB/C mice implanted with sarcoma 180 cancer cells via the restriction of angiogenesis. J Transl Med 2009;7:70.
[21] Verrax J, Calderon PB. Pharmacologic concentrations of ascorbate are achieved by parenteral administration and exhibit antitumoral effects. Free Radic Biol Med 2009;47:32–40.
[22] Chen P, Stone J, Sullivan G, Drisko JA, Chen Q. Anti-cancer effect of pharmacologic ascorbate and its interaction with supplementary parenteral glutathione in preclinical cancer models. Free Radic Biol Med 2011;51:681–7.
[23] Grümmer R. Animal models in endometriosis research. Hum Reprod Update 2006;12:641–9.
[24] Malik E, Meyhöfer-Malik A, Berg C, Böhm W, Kunzi-Rapp K, Diedrich K, et al. Fluorescence diagnosis of endometriosis on the chorioallantoic membrane using 5-aminolaevulinic acid. Hum Reprod 2000;15:584–8.
[25] Vernon MW, Wilson EA. Studies on the surgical induction of endometriosis in the rat. Fertil Steril 1985;44:684–94.
[26] Durak Y, Kokcu A, Kefeli M, Bildircin D, Celik H, Alper T. Effect of vitamin C on the growth of experimentally induced endometriotic cysts. J Obstet Gynaecol Res 2013;39:1253–8.
[27] Uygur D, Aytan H, Zergeroglu S, Batioglu S. Leflunomide—an immunomodulator—induces regression of endometrial explants in a rat model of endometriosis. J Soc Gynecol Investig 2006;13:378–83.
[28] Guney M, Nasir S, Oral B, Karahan N, Mungan T. Effect of caffeic acid phenethyl ester on the regression of endometrial explants in an experimental rat model. Reprod Sci 2007;14:270–9.
[29] Keenan JA, Williams-Boyce PK, Massey PJ, Chen TT, Caudle MR, Bukovsky A. Regression of endometrial explants in a rat model of endometriosis treated with the immune modulators loxoribine and levamisole. Fertil Steril 1999;72:135–41.
[30] Masson PJ. Trichrome stainings and their preliminary techniques. J Tech Met 1929;12:75.
[31] Anaf V, Simom PH, El Nakadi I. Relationship between endometriotic foci and nerves in rectovaginal endometriotic nodules. Hum Reprod 2000;15:1744–50.
[32] Fedele L, Somigliana E, Frontino G, Benaglia L, Vigano P. New drugs in development for the treatment of endometriosis. Expert Opin Investig Drugs 2008;17:1187–202.
[33] Mier-Cabrera J, Aburto-Soto T, Burrola-Mendez S, Jimenez-Zamuido L, Tolentino MC, Casanueva E, et al. Women with endometriosis improved their peripheral antioxidant markers after the application of a high antioxidant diet. Reprod Biol Endocrinol 2009;7:54.
[34] Brown J, Pan A, Hart RJ. Gonadotrophin-releasing hormone analogues for pain associated with endometriosis. Cochrane Database Syst Rev 2010;12:CD008475.