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ORIGINAL ARTICLE

Short-term histopathological effects of dienogest therapy on ovarian endometriomas: *in vivo*, nonrandomized, controlled trial

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ABSTRACT

Ovarian endometriosis is a common gynecological disorder. To date, progestins are recommended as the first-line medical treatment for symptomatic ovarian endometriosis. The aim of this study was to evaluate the main histopathological effects of short-term dienogest therapy in patients with ovarian endometriomas scheduled for surgery. A prospective, nonrandomized controlled trial, including 70 symptomatic women with single ovarian endometriotic cyst (diameter between 30–50 mm) was conducted. Women scheduled for surgery were divided into two groups, depending on the treatment established at enrollment: 36 women received progestin therapy with dienogest (P group) and 34 women received no therapy (C group). At histopathological examination necrosis, inflammation, decidualization, glandular atrophy and angiogenesis were blindly evaluated. At tissue level, decidualization was significantly more frequent in P group compared to C group ($p = .001$). A nonsignificant tendency ($p = .29$) towards a slight decreased inflammation in P group was found. No significant differences were observed between the two groups in terms of necrosis, glandular atrophy and angiogenesis. The study suggests that high decidualization rate and the tendency to reduced inflammatory reaction in the short-term administration of dienogest might contribute to its therapeutic efficacy.

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Ovarian endometriosis; dienogest; progestins; histopathological features

Introduction

Endometriosis is a chronic and recurrent disease defined as the presence and proliferation of endometrial glands and stroma outside the uterine cavity. The ovary is the most common site involved, accounting for 80% of cases of endometriosis [1]. Symptoms usually associated with the disease are pelvic pain and infertility [2].

Most of the available international guidelines recommend progestins (P), with or without estrogens (estrogen–progestins, E/P), as first-line treatment for symptomatic, uncomplicated and nonsuspicious ovarian endometriosis [1,3,4]. The mechanisms of action of P and E/P therapies in the management of endometriosis consist in ovulation inhibition with subsequent induction of hypoestrogenic environment and endometrial decidualization, together with anti-inflammatory and antiangiogenetic effects [4,5]. The clinical role of long-term medical therapy in improving symptoms and reducing the rate of postoperative recurrence in endometriosis is today clearly ascertained [4,6,7].

Among the progestins used in endometriosis management, dienogest (DNG), a 19-nortestosterone derivative semisynthetic progestin [8], has been shown to be effective in relieving pain symptoms and reducing the size of endometriotic lesions, although its clinical benefits were basically detected after long-term treatment [9–11]. Being a chronic pathology, a possible explanation of the lacking of short-term clinical outcomes of this therapy could be the delayed changes that occur at tissue level. On the other hand, the histopathological effects of DNG treatment on endometriotic lesions has been poorly studied. Only one study reported an analysis of the effects of DNG treatment on some histopathological aspects of the endometriotic tissue 'in vivo' in a very limited sample of subjects [12].

The aim of the present study was to evaluate the short-term histopathological effects of DNG on endometriotic cyst wall of a larger cohort of women with ovarian endometriomas.

Materials and methods

Subjects

Out of 523 consecutive women, aged between 20 and 45 years, assessed for endometriosis at our tertiary center from October 2014 to January 2016, 70 women with ultrasonographic diagnosis of uncomplicated ovarian endometriosis and scheduled for conservative surgery were recruited in this prospective, nonrandomized, controlled trial with blinded outcome assessment.

The main inclusion criterion to participate in the study was to have an ultrasonographic diagnosis of single ovarian endometrioma with a mean diameter between 30 and 50 mm. Exclusion criteria were no hormonal treatment in the past six months, clinical and/or ultrasonographic signs of deep infiltrating endometriosis, ovarian endometriomas smaller than 30 mm or larger than 50 mm and no previous surgery for endometriosis.

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Study design

All patients were thoroughly counseled about the possibility to receive medical therapy or no therapy during the waiting time before surgery, and the choice was done according to the severity of pain symptoms or the fertility desire. Women were divided into two groups, depending on the treatment established at enrollment: 36 women received P therapy (P group) and 34 women received no medication (control – C Group) for six months until surgery. Progestin pill (dienogest, 17-hydroxy-3-oxo-19-nor-17 α-pregna-4,9-diene-21-nitrile, Visanne®, Bayer, Milan, Italy) was administered 2 mg/die in P group.

The trial protocol has been approved by our Local Ethics Committee and informed consent was obtained from all individual participants included in the study.

Surgical technique

Operated patients underwent laparoscopic excision of the ovarian endometrioma by stripping technique as previously described [13]. The technique briefly consists of the identification of the cyst capsule and of separating the cyst wall from health ovarian tissue by tractions exerted in opposite directions with atraumatic grasping forceps. Hemostasis was then obtained by suturing the health ovarian parenchyma with 2–0 suture thread (Vycril®) avoiding bipolar coagulation. In no case, laparotomic conversion was required.

Histological examination

Endometriotic cyst walls removed were placed in 4% formaldehyde-buffered solution. All surgical specimens were treated to obtain paraffin blocks, and then, sections were made on each slide and stained with haematoxylin/eosin. A blind analysis of all specimens was performed by the same experienced pathologist (G.C.), evaluating the following histopathological aspects: (a) necrosis; (b) inflammation; (c) decidualization; (d) glandular atrophy; (e) angiogenesis.

Necrosis was defined as the presence of ischemic and hemorrhagic areas and classified as follows: *absent* (no evidence of ectopic endometrial epithelial or stromal cell or fibroblast apoptosis or nuclear karyorrhexis in acute or chronic inflammatory context), *focal* (necrosis involvement up to 20% of the endometriotic tissue) and *diffuse* (necrosis involvement more than 20% of the endometriotic tissue).

Inflammation was defined as follows: *absent* (no evidence of inflammatory infiltration in the tissue), *acute* (the presence of polymorphonucleated granulocytes, such as neutrophils or eosinophils, and activated lymphocytes), *chronic* (the presence of activated plasma cells, non-activated macrophages, foam cells, hemosiderin and multinucleated giant cells).

Decidualization was evaluated as follows: *absent* (the absence of aspects of transformation of cytogenetic stroma) and *present* (the presence of aspects of transformation of cytogenetic stroma such as edema, eosinophilic cytoplasm transformation, increased intracytoplasmic granules and small and hyperchromic nucleus).

Atrophy of the ectopic endometrial glandular cells was defined as follows: *absent* (when the epithelial cells showed a large cytoplasmatic volume, the presence of mitotic and secretory activity) and *present* (when the epithelial cells showed a reduced cytoplasmatic volume, the absence of mitotic and secretory activity and flattened appearance).

Angiogenesis was assessed as the presence of newly formed vessels or individual endothelial cells, using the immunophenotypic marker CD 31 in an area of 10 mm^2 tissue adjacent to the focus of endometriosis, with the exclusion of evident granulation tissue. Its presence was evaluated as follows: *absent* (no evidence of vessels or endothelial cells), *focal* (presence of 1 to 5 vessels or endothelial cells) and *diffuse* (presence of more than 5 vessels or endothelial cells).

Statistical analyses

Statistical analyses were performed by using statistical program for social sciences (IBM SPSS, version 24.0, IBM Corp., Armonk, NY). Continuous data were expressed as mean ± SD. Categorical variables were expressed as numbers and percentages. Unpaired Student's *t*-test was used to compare continuous parametric data. Comparisons between categorical variables were made using the Yates' corrected chi-square test. A p values of $<.05$ was considered statistically significant.

Results

During treatment, three women discontinued therapy for intolerance and side effects in the P group, two women refused surgery and two women became pregnant in the C group. Therefore, 63 women concluded the study, 33 and 30 women in the P and C group, respectively. No significant differences among the two groups in terms of age, body mass, index, location and side of endometrioma were detected (Table 1).

Histological findings

Focal necrosis was detected in 50.0% of C group and 51.5% in P group, while *diffuse necrosis* was detected in 6.7% in C group and 12.1% in P group (Figure 1(a)). No differences between the two groups were observed.

Acute or chronic inflammation (Figure 1(b)) was more frequent in C group (86.7%) compared to P group (72.7%), without reaching however the statistical significance ($p = .29$).

Table 1. Demographics and clinical characteristics of patients who concluded the study at baseline.

Characteristics	Control group ($n = 30$)	Progestin group ($n = 33$)	p value
Age, years	29.3 ± 4.8[a]	30.1 ± 4.8	NS[c]
Body Mass Index, kg/m^2	24.5 ± 4.6	23.3 ± 4.0	NS
Endometrioma location			
Left ovary	18 (60.0%)[b]	19 (57.6%)	NS[d]
Right ovary	12 (40.0%)	14 (42.4%)	NS

[a]Data are presented as mean with standard deviation.
[b]Data are presented as numbers and percentages.
[c]Unpaired Student's *t*-test.
[d]Yates' corrected chi-square test.

Decidualization was statistically more frequent in P group compared to C group (66.7% versus 23.3%, $p = .001$), as shown in Figure 1(c).

Glandular atrophy was detected in 43.3% in C group versus 30.3% in P group (Figure 1(d)). No differences between the two groups were detected.

Focal angiogenesis was detected in 46.7% in C group versus 51.5% in P group, while *diffuse angiogenesis* was detected in 6.7% in C group and no cases were reported in P group (Figure 1(e)). No differences between the two groups were observed.

The main histopatological features analyzed in the study are shown in Figure 2.

Discussion

Progestins are widely used to treat symptomatic endometriosis [1,4]. Several mechanisms of action may justify their use and partially explain their efficacy in patients with endometriosis: (a) reduction of prostaglandin secretion and inflammatory state [14–16]; (b) creation of a pseudo-pregnancy state inducing the atrophy of the endometriotic implants [17]; (c) influence on the complex regulation of cell proliferation and consequent induction of pro-apoptotic effects [18]. Among progestins, clinical trials showed the long-term effectiveness of DNG in the management of endometriosis [9,10,19]. Antiproliferative, anti-inflammatory and antiangiogenic effects of DNG have been also reported in both animal and human models *in vitro* [20–23].

Despite the wide clinical use of DNG in the medical treatment of endometriosis, very little is known about its *in vivo* effects at tissue level. Miyashita et al. [12] analyzed the action of DNG at histopathological level on endometriotic tissues in a small sample of 7 women compared with 11 controls. They detected a lower proportion of proliferating epithelial cells and a higher density of

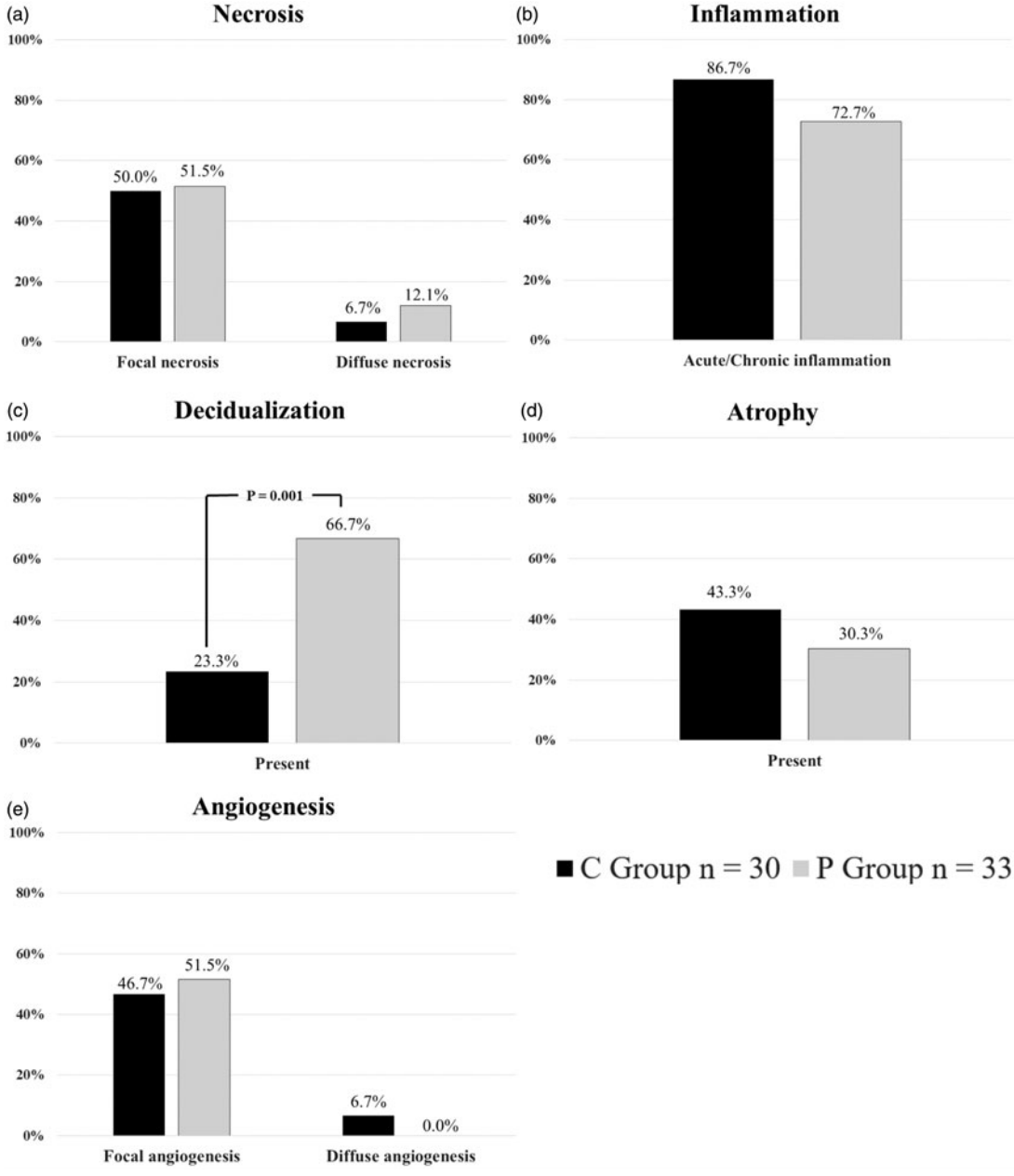

Figure 1. Detection of necrosis (a), inflammation (b), decidualization (c), atrophy (d) and angiogenesis (e) in the two groups of women studied. Only significant results are listed (Yates'corrected chi-square test).

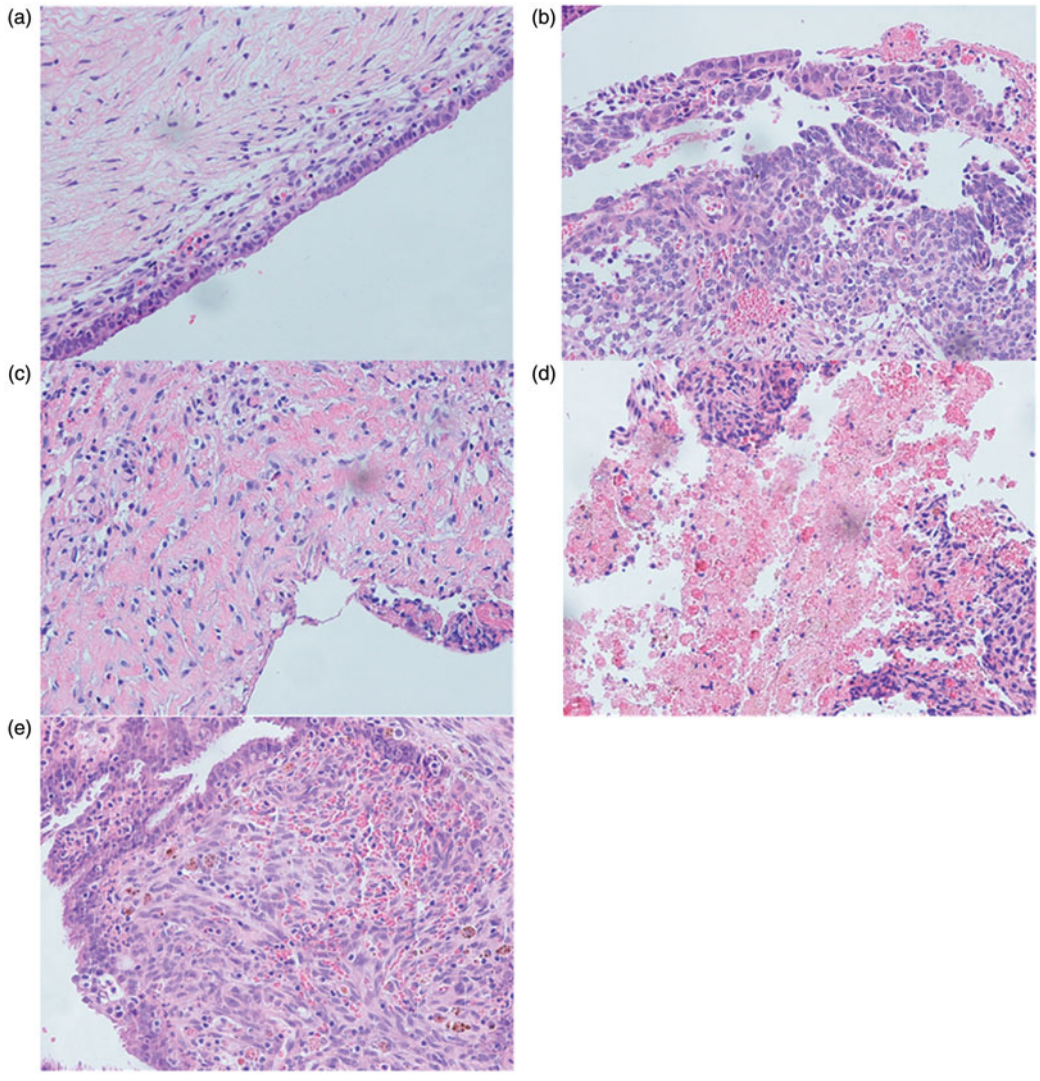

Figure 2. Histopathological features analyzed: (a) atrophy in endometriotic nodule, (b) flogosis in endometriotic nodule, (c) pseudo-decidualization of the stroma in endometriotic nodule, (d) necrosis with ghost cells and nuclear debris in a cyst, (e) neovascularization of the decidual stroma in endometriotic nodule. Staining with hematoxylin/eosin. Magnification 10×.

apoptotic stromal cells, as well as no difference in the concentration of blood vessels and angiogenesis.

In the present study, we analyzed the histological findings on endometriotic cyst wall in a larger cohort of women with strict selection criteria. We compared different histological aspects on ovarian endometriosis with short-term DNG treatment compared with no treatment. We observed that the cyst walls obtained from women treated with DNG for six months tend to have a slightly, nonsignificant lower inflammation rate than controls. Moreover, a significantly higher decidualization rate was observed in the study patients compared to controls. No differences between groups were detected regarding the other histopathological aspects examined: necrosis, glandular atrophy and angiogenesis.

Since inflammation and estrogen responsiveness are common findings in endometriotic tissues [24], it is likely that the high decidualization rate and the tendency to a reduced inflammatory reaction due to DNG effect, contribute to its therapeutic efficacy. However, the potential delay in the short-term clinical response to treatment might be explained by the absence of significant difference in the anti-inflammatory effect, together with the lack of histological impact in terms of necrosis, glandular atrophy and angiogenesis. A further prospective study on long-term histopathological effects of DNG in the treatment of endometriosis is ongoing at our institution to assess if long-term treatment might produce different results.

Main limitations of the study are the lack of randomization and the small sample size of the study group, which does not allow to reach definitive conclusions. Despite these limitations, the prospective design, the strict selection criteria for the enrolled patients, the standardization of surgical technique and blind histological analysis can represent strengths of this study.

Short-term therapy with DNG seems to have efficacy on inducing decidualization of the endometriotic cyst wall, while the slight decrease in inflammation suggests that a long-.term administration might be needed to observe other significant variations at histopathological level.

Disclosure statement

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