ORIGINAL PAPER

RJME
Romanian Journal of Morphology & Embryology
http://www.rjme.ro/

Endometriosis – clinical approach based on histological findings

C. CRISTESCU[1)], ANDREEA VELIŞCU[1,2)], B. MARINESCU[1,2)], ANCA PĂTRAŞCU[3)], E. T. TRAŞCĂ[4)], O. T. POP[5)]

[1)]*"Prof. Dr. Panait Sârbu" Clinical Hospital of Obstetrics and Gynecology, Bucharest*
[2)]*"Carol Davila" University of Medicine and Pharmacy, Bucharest*
[3)]*Department of Obstetrics and Gynecology*
[4)]*Department of Nursing*
[5)]*Research Center for Microscopic Morphology and Immunology*
University of Medicine and Pharmacy of Craiova

Abstract

Endometriosis is a benign disease defined by the presence of endometrial glands and stroma outside of the uterus and is associated with both pelvic pain and infertility. The most common sites of endometriosis, in decreasing order of frequency, are the ovaries, anterior and posterior cul-de-sac, posterior broad ligaments, uterosacral ligaments, uterus, fallopian tubes, sigmoid colon, appendix, and round ligaments. The main treatment is surgical, but often-microscopic islands of endometrial tissue remain, which proliferate and are responsible for relapses. We tested the efficacy of two drugs (Medroxyprogesterone and Triptorelinum), administered for six months to prevent recurrence after surgery. Treatment with Medroxyprogesterone was 100% effective in terms of relapse, while Triptorelinum could not prevent recurrence of endometriosis.

Keywords: endometriosis, progestative, estrogen, progesterone.

Introduction

Endometriosis is a benign disease defined by the presence of endometrial glands and stroma outside of the uterus and is associated with both pelvic pain and infertility; it is a hormonally dependent disease and as a result is chiefly found in women at reproductive age [1]. It should be iterated that the term endometriosis implies proliferating growth and function (usually bleeding) in an extrauterine site. An endometrioma may be defined as an area of endometriosis, usually in the ovary, that as enlarged sufficiently to be classified as a tumor. When an endometrioma is filled with old blood, resembling tar or chocolate syrup, it is commonly known as a chocolate cyst [2].

The most frequent sites of implantation are the pelvic viscera and the peritoneum. Endometriosis varies in appearance from a few minimal lesions on otherwise intact pelvic organs to massive ovarian endometriotic cysts that distort tubo-ovarian anatomy and extensive adhesions often involving the bowel, bladder, and ureter [3].

Considerable progress has been made in understanding the pathogenesis, spontaneous evolution, diagnosis, and treatment of endometriosis.

Several pathogenic mechanisms have been proposed, including retrograde menstruation and implantation, coelomic metaplasia, direct transplantation, and vascular dissemination. None of the mechanisms explain all cases of endometriosis and each probably contributes, at least to some extent [4].

The most widely accepted theory for the pathogenesis of endometriosis is the retrograde menstruation/ transplantation that claims the adhesion and growth of endometrial fragments deposited into the peritoneal cavity *via* retrograde menstruation [5]. Therefore, endometriosis would represent simply an auto-transplant, in which normal endometrial tissue is transplanted to an ectopic location in the organism. However, this theory fails to explain the presence of endometriosis in the areas outside the peritoneal cavity, as the lungs, skin, lymph nodes, breasts [2].

Moreover, the presence of the disease in early puberty and exceptionally also in newborns [6–8], as well as in women affected by the Mayer–Rokitansky–Küster–Hauser, a syndrome characterized by congenital aplasia of the uterus and the upper part of the vagina [9], and in males [2], further contrasts the validity of the theory.

The coelomic metaplasia theory states that endometriosis results from spontaneous metaplastic change in mesothelial cells derived from the coelomic epithelium (located in the peritoneum and the pleura); it also claims that formation of endometriomas in the ovary or recto-vaginal endometriosis is caused by metaplasia of the coelomic epithelium, perhaps induced by environmental factors [10]. This theory would explain why most women have some degree of retrograde menstruation but only a small percentage have endometriosis, and the presence of the disease in absence of menses. Although coelomic metaplasia might explain endometriosis in the pelvis,

the thoracic cavity, the urinary and digestive tracts, the inguinal canal and the umbilicus, evidence indicates that vascular or lymphatic dissemination of endometrial cells may also be involved [10, 11].

The theory of circulating stem cells originating from bone marrow or from basal layer of endometrium could differentiate into endometriotic tissue at different anatomical sites.

Direct transplantation of endometrial tissue during pelvic surgery or episiotomy repair might be a plausible explanation for endometriosis found in abdominal scars and the perineum.

Anatomic abnormalities are also considered a possible precursor of endometriosis. It was concluded that the depth and volume of the cul-de-sac, differs in patients with endometriosis with or without deep lesions as compared to women with a healthy pelvis [12].

The eutopic and ectopic endometrium of women with endometriosis differs from normal endometrium in three distinct and important ways: high local estrogen production, high local PG production, and resistance to the action of progesterone.

In this ongoing study, which took place in "Prof. Dr. Panait Sârbu" Clinical Hospital of Obstetrics and Gynecology, Bucharest, Romania, we tried to evaluate and compare the effect of a progestative (Medroxyprogesterone) *vs.* a gonadotrophin-releasing hormone agonist (Triptorelinum) on endometriosis recurrence.

Patients and Methods

One of the objectives of this study is to diagnose the women affected by endometriosis (clinical symptoms: infertility, ultrasound, MRI, and laparoscopy) and to resolve it surgically keeping in mind the location [13].

Patients are enrolled as they present at the hospital for various complaints related to endometriosis (dysmenorrhea, dyspareunia, infertility); they are well informed regarding the pathology, treatment possibilities and its limitations. History, physical examination and laboratory findings determine which patients can enter the trial, considering the side effects of drugs.

According to the results, we have:

- Inclusion criteria: age between 18 and 45 years, non-pregnant state, laparoscopically diagnosed endometriosis, absence of associated pathologies that may be a contra-indication for treatment;
- Exclusion criteria: pregnancy, conditions that require immediate treatment, conditions that contraindicate treatment (coagulopathy, thrombosis, cerebrovascular condition, cardiovascular disease, cholestasis, neoplasia, liver tumors – benign or malignant, undiagnosed abnormal uterine bleeding) [13].

To participate in the study, patients gave their consent at first hospitalization.

All patients are examined clinically at study entry. We note: menarche, last menstrual period, previous hospitalization, complete history of the disease (endometriosis), family history, family history of endometriosis [13].

When including them in the trial, clinical examination of each patient is complete and includes general clinical examination, breast examination, gynecological examination, laboratory tests (CBC, glucose, urea, creatinine, uric acid, transaminases, coagulation, urinalysis exam). Patients appreciate the pain using a questionnaire and a scale from 1 to 10. Patients will note other medications that they used to improve comfort and quality of life, and the wellbeing regarding the treatment [13].

In our study, we included 20 women between 25 and 39 years, who presented in our hospital between 2010 and 2011 for chronic pelvic pain and especially infertility. The diagnosis consisted mainly in ultrasound findings correlated with symptoms and the value of CA125. The definitive diagnosis was made by laparoscopic findings and histopathological examination.

During laparoscopy all the cysts were removed, the endometriosis implants were electrocoagulated and the adhesions were resolved (totally or partially) [13].

The distribution is the following:

- First group (12 patients) receiving Triptorelinum 3.75 mg intramuscular every four weeks;
- The second group (eight patients) Medroxyprogesterone (depot 150 mg intramuscular, once a month).

After six months of drug treatment, patients in both groups underwent second-look laparoscopic surgery to be able to compare the efficacy of the two types of medication.

Patients with severe endometriosis, similar in location and degree of damage were analyzed and compared.

If there was a recurrence of endometriosis, the cysts were removed with subsequent histopathological examination, the implants were electrocoagulated and the adhesions removed [13].

The evaluation and treatment is based on current guidelines and recommendations. We used the *American Society for Reproductive Medicine* classification: each patient has a score. Depending on the staging, patients were divided into two groups. We compared the predominant involvement of specific anatomical sites, age, fertility. We were interested to have two corresponding final groups in terms of distribution depending on the degree of endometriosis, its location and age [13].

For the histological study, the collected material was fixed in 10% neutral formalin and processed using the classical paraffin embedding technique. Classical stains with Hematoxylin and Eosin (HE) and Goldner–Szekely trichrome were used in the Research Center for Microscopic Morphology and Immunology of the University of Medicine and Pharmacy of Craiova, Romania. For the positive and differential diagnosis, an immunohistochemical analysis was added with the following markers being assessed (Table 1).

For the immunohistochemical study, sections were cut using the same equipment, but with a thickness of 3-µm. Sections were collected on poly-L-Lysine coated slides, and dried in a thermostat at 37^0C for 24 hours.

After antigen retrieval, sections were cooled down to room temperature and were incubated for 30 minutes in a 1% hydrogen peroxide solution. Sections were next washed in PBS, followed by a blocking step of 30 minutes in 2% skim milk. Next, the slides were incubated with the primary antibodies overnight at 4^0C,

and the next day, the signal was amplified for 30 minutes using a peroxidase polymer-based secondary detection system (EnVision, Dako). The signal was detected with 3,3'-diaminobenzidine (DAB) (Dako) and the slides were coverslipped in DPX (Fluka) after Hematoxylin counterstaining. Sections were imaged with a Nikon Eclipse 55i microscope (Nikon, Apidrag, Romania) equipped with a 5-megapixel cooled CCD camera. Images were captured and archived using a Nikon frame grabber and the Image ProPlus 7 AMS software (Media Cybernetics Inc., Buckinghamshire, UK).

Table 1 – ***Antibodies used for the immunohistochemical study***

Antibody	Manufacturer	Clone	Host/Target/Clonality	Antigen retrieval	Dilution
Anti-CK7	Dako	OV-TL 12/30	Ms/Hu/Monoclonal	Sodium citrate, pH 6	1:50
Anti-CK18	Dako	DC 10	Ms/Hu/Monoclonal	EDTA, pH 9	1:25
Anti-PR	Dako	PgR 636	Ms/Hu/Monoclonal	EDTA, pH 9	1:50
Anti-ER	Dako	1D5	Ms/Hu/Monoclonal	EDTA, pH 9	1:50

This study has the consent of the Ethics Committee of the "Prof. Dr. Panait Sârbu" Clinical Hospital of Obstetrics and Gynecology, Bucharest.

Results

We wanted to conduct a comparative study between two types of pharmacological treatment of endometriosis, which could not be fully resolved surgically [13].

During the first laparoscopy, all ovarian cysts were surgically removed (cystectomy) and adhesions were partially or totally dissected. Peritoneal endometriosis was electrocoagulated. The two groups were matched in terms of degree of endometriosis (stage III) and age. All of the patients had ovarian endometriosis (one or both ovaries) and extensive adherents (Figure 1) [13].

In the first group, there were 11 patients who had bilateral ovarian endometriosis cysts and adhesions and one who had an ovarian cyst and adhesions. In the second group, all patients had bilateral cystic endometriosis and adhesions [13].

The "second look" laparoscopy revealed the following:

▪ Group 1, treated with Triptorelinum: all patients had a recurrence of endometriosis cyst on one ovary representing about 60% of the volume of one of the initial cyst [13];

▪ Group 2, treated with Medroxyprogesterone: in all eight cases that tolerated the treatment, at the "second look" laparoscopy we found no more endometriosis cysts present [13].

The histopathological study of tissues with endometriosis obtained at the beginning of the treatment showed the presence of microscopic structures similar with endometrial tissue, formed by endometrial glands with large lumina, filled with mucous secretions, lined by a layer of cubic or columnar cells (Figure 2).

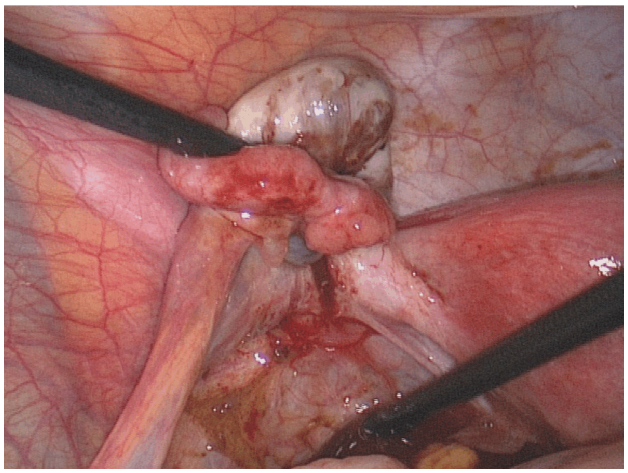

Figure 1 – ***Ovarian endometriosis: intraoperative gross appearance.***

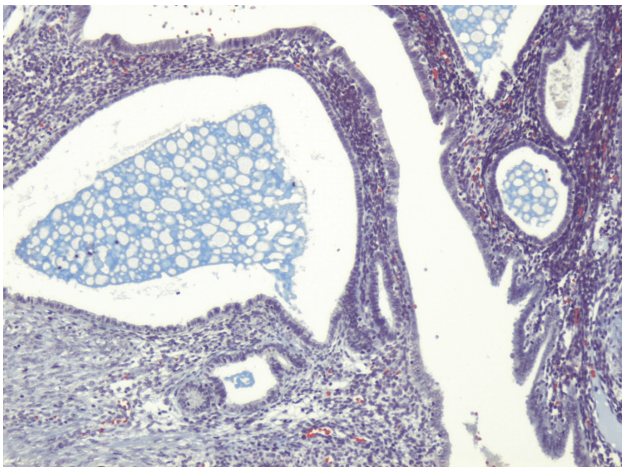

Figure 2 – ***Endometrial glands with large lumina, filled with mucous secretions (Goldner–Szekely's trichrome stain, ×40).***

The cytoplasm of glandular cells appeared in most cases basophilic, and the oval nuclei were arranged in the lower third part of the cells (Figure 3).

The endometrial stroma was scarce, heterogeneous, containing fibroblast-like spindle cells (Figure 4) and sometimes round and oval cells with spongy appearance similar to decidual cells. Quite frequently macrophage- and lymphocyte-like cells have been seen (Figure 5). In the stroma, we identified blood vessels similar to spiral arterioles (Figure 6) and also numerous stromal microhemorrhages (Figure 7). Endometrial stroma was lined by fibroblast-like connective cells or by cells that are similar to those seen in smooth muscle. All the selected cases showed a microscopic appearance of typical endometriosis without cellular atypia. In order to differentiate the endometrial glandular cells from other structures, we used four types of immunohistochemical markers: cytokeratin 7 (CK7), cytokeratin 18 (CK18), estrogen and progesterone receptors.

The cells of the glandular epithelium showed intense

positivity for CK7 (Figure 8) and CK18 (Figure 9). Also, these cells were intensely positive for progesterone (Figure 10) and moderately positive for estrogen (Figure 11).

Histopathological examination of the biological material endoscopically collected from patients treated with Triptorelinum for six months showed the presence of local recurrence, identifying isolated islands of endometrial mucosa. Compared with the initial appearance of the lesion, we noted that the treatment with Triptorelinum reduced the endometrial stroma, particularly the presence of inflammatory cells (Figure 12).

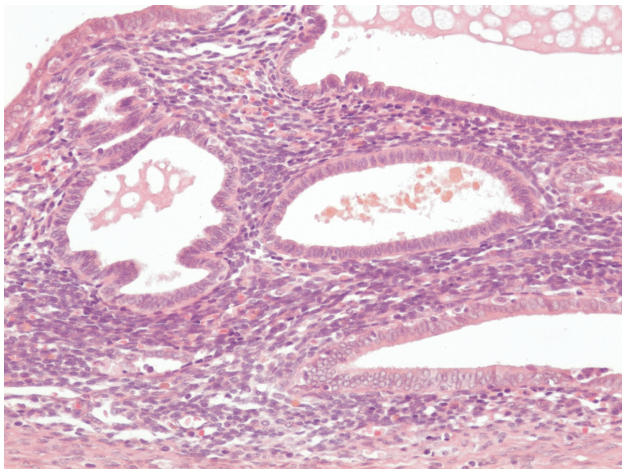

Figure 3 – ***Microscopic image of typical endometriosis with numerous dilated glands without atypia (HE stain, ×100).***

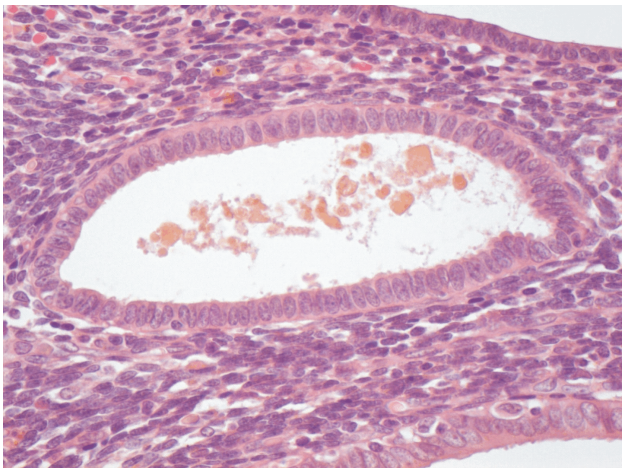

Figure 4 – ***Endometrial gland with wide lumen lined by a single layer of columnar cells, surrounded by a stroma rich in fibroblastic cells: detail from Figure 2 (HE stain, ×200).***

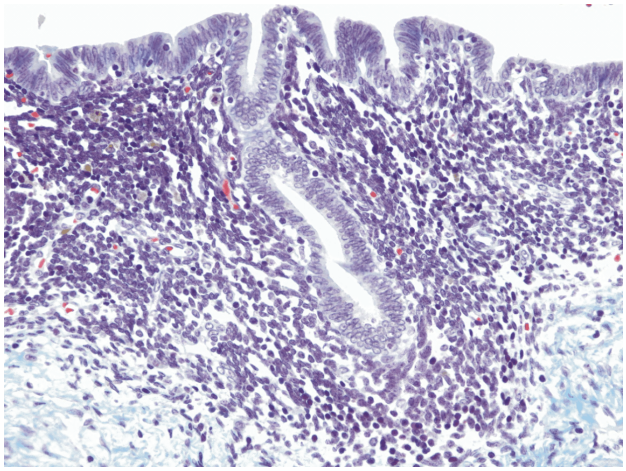

Figure 5 – ***Endometrial tissue infiltrated with macrophage- and lymphocyte-like cells (Goldner–Szekely's trichrome stain, ×200).***

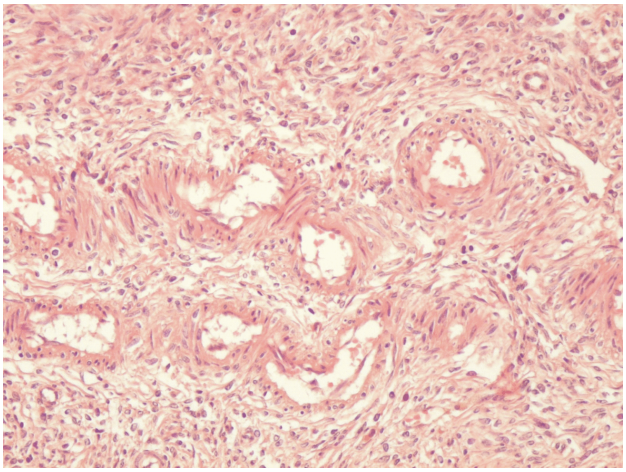

Figure 6 – ***Endometrial stroma with spiral arterioles (HE stain, ×100).***

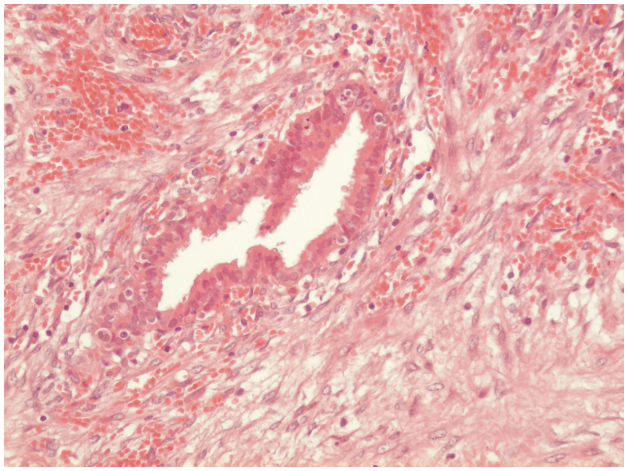

Figure 7 – ***Endometrial stroma with numerous areas of hemorrhage (HE stain, ×200).***

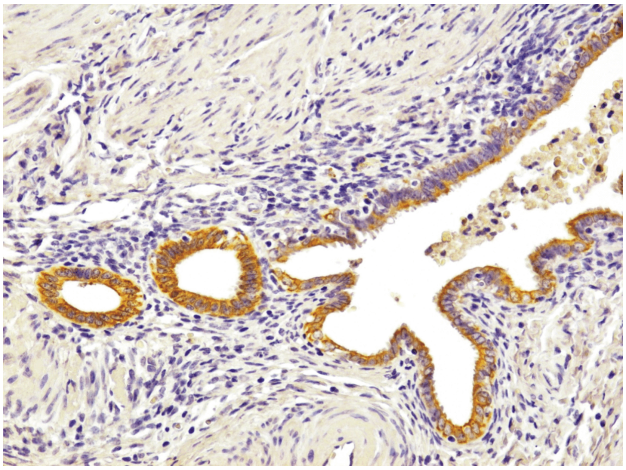

Figure 8 – ***Glandular cells intensely positive for CK7 (CK7 immunohistochemistry, ×200).***

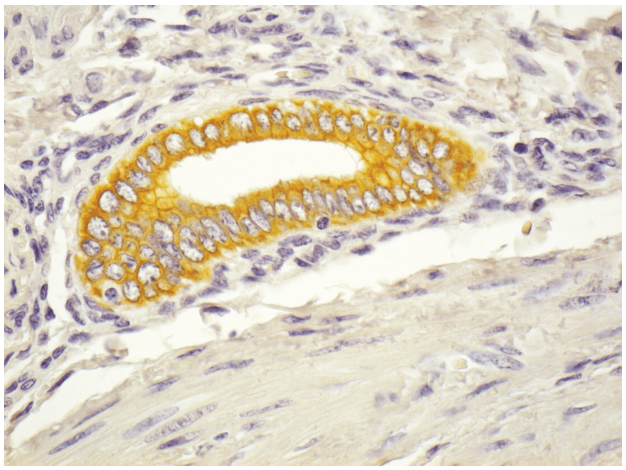

Figure 9 – ***Endometrial glands with intensely positive cytoplasmic reaction to CK18 (CK18 immunohistochemistry, ×400).***

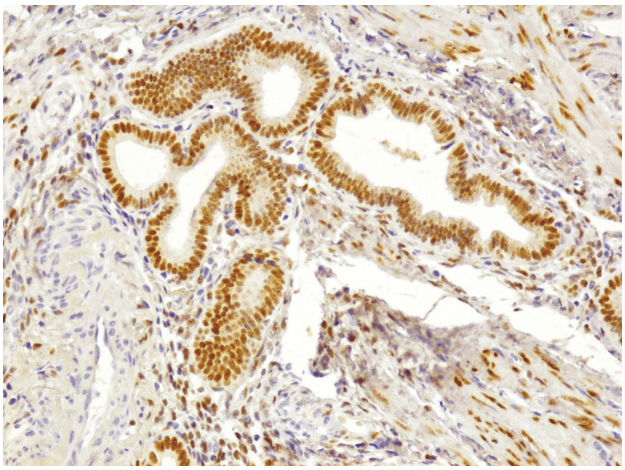

Figure 10 – ***Endometrial glands with intensely positive nuclear staining for progesterone (PR immunohistochemistry, ×100).***

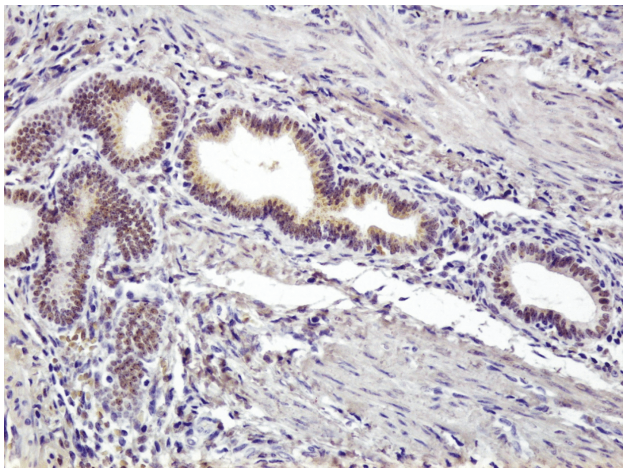

Figure 11 – ***Endometrial glands with moderately positive estrogen staining (ER immunohistochemistry, ×100).***

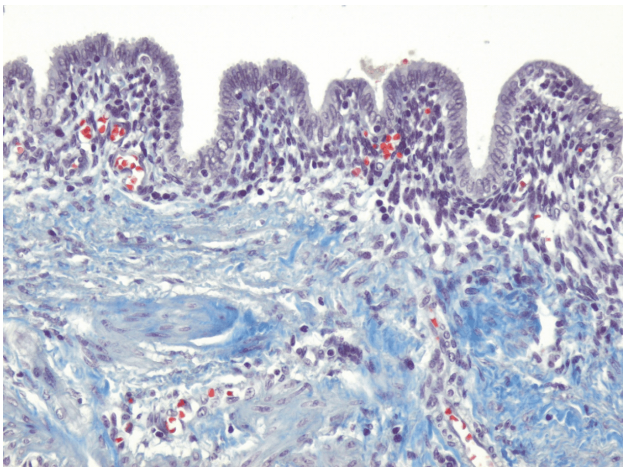

Figure 12 – ***Endometrial mucosa with scarce stroma, six months after treatment with Triptorelinum (Goldner–Szekely's trichrome stain, ×200).***

Discussion

Endometriosis is an inflammatory disease, estrogen dependent, affecting 6–10% out of the women that are at reproducing age [1]. It is characterized by the presence of endometrial tissue outside of the uterine cavity, mainly on the ovaries, and it represents one of the most common cases of chronic pelvic pain, dysmenorrhea and infertility [14, 15].

Pathophysiology of the disease is still poorly known. Numerous data have shown that estrogen is the most important known factor that stimulates the growth of endometriosis and substantial evidence indicates that both estrogen production and metabolism are altered in ways that promote the disease [3]. Estrogen in women with endometriosis derives from three major sources: secreted by the ovary into the circulation and released directly into the peritoneal cavity of ovulation, produced in adipose and skin *via* conversion of circulating androgens, and synthesized locally in endometriotic tissue, which expresses a complete set of steroidogenic enzymes, including aromatase [16].

Estrogen delivery to endometriotic implants was classically viewed as an endocrine way. We recently uncovered an autocrine positive feedback mechanism, which links the overproduction of estrogen in endometriotic stromal cells with high local production of prostaglandins. Prostaglandins are locally produced hormones involved in inflammation and pain. Both PGE2 and PGF2-alpha are overproduced in the uterine and endometriotic tissue of women with endometriosis [17, 18].

PGE2 is the most potent known physiological stimulator of estrogen biosynthesis in endometriosis. This prostanoid was capable of inducing aromatase activity up to 44-fold the baseline level. PGE2 can cause a diverse range of actions that are mainly determined by the subtype of receptor used in that tissue. These actions were later explained by the discovery of different PGE (EP) receptor subtypes in both normal and ectopic endometrium (EP1, EP2, EP3, and EP4) that, in turn, are linked to different signal transduction pathways [19].

The treatment for endometriosis is mainly surgical, at the end of the menstrual cycle when ectopic endometrial tissue changes are minimal.

However, in cases with pelvic endometriosis, most studies recognize that it is possible that islands of endometrial tissue remain.

Therefore, in our study, surgical treatment or endoscopic cystectomy was completed with medication, in this case Medroxyprogesterone or Triptorelinum.

Macroscopic and microscopic results revealed that treatment with Medroxyprogesterone prevented local recurrence of endometriosis, while Triptorelinum treatment gave lower results, all patients presenting recurrence of endometriosis in the ovaries.

Progesterone and progesterone derivatives were and are used to treat endometriosis to relieve pain by suppressing ovarian estrogen biosynthesis, thereby blocking tumor growth and inflammation [20].

Also, gonadotropin releasing hormone agonists (GnRH) are commonly used to treat endometriosis for pelvic pain and to reduce the progression of endometrial implants [21].

Some authors have shown that approximately 9% of women with endometriosis do not respond to progesterone therapy for unknown reasons [1, 22, 23].

The molecular basis of progesterone resistance in endometriosis may be related to an overall reduction in the levels of progesterone receptor (PR). In normal endometrium, progesterone acts *via* PR by stimulating retinoic acid production on stromal cells to induce 17β-hydroxysteroid dehydrogenase type 2 (HSD17B2) activity in the epithelium. HSD17B2 is an extremely efficient enzyme and rapidly metabolizes the biologically potent estrogen E2 to weakly estrogenic estrone. In endometriotic tissue, progesterone is incapable of inducing epithelial HSD17B2 expression due to a defect in stromal cells to produce retinoic acid. The end result is a deficient metabolism of E2 in endometriosis giving rise to high local concentrations of this mitogen [24].

In our study, microscopic aspects of ectopic endometrial tissue were characteristic of typical endometriosis, characterized by the presence of endometrial tissue without atypical cells or tissue.

However, there were histological differences between one case or another, issues that we have considered to be influenced by the endocrine status of each person, especially by the balance between estrogen and progesterone or better summed up as utero-ovarian cycle phase (proliferative or secretory), at the date of the excision of biological samples.

Immunomarkers for the estrogen and progesterone receptors allowed us to note that the immunohistochemical reaction to progesterone was very intense, being present in the endometrial epithelium and in cells from the stroma, while the estrogen response was moderate.

For the differential diagnosis of endometriosis from digestive metastatic adenocarcinomas we performed immunomarking with CK7 and CK18, which were homogeneous and highly reactive in the endometrial epithelium.

Similar data were obtained by other authors in the study of endometriosis [25].

Conclusions

Both clinical and histological findings show that progestatives are better than Triptorelinum in endometriosis recurrence. Treatment with Medroxyprogesterone for six months prevented recurrence of pelvic endometriosis in all patients.

Acknowledgments

This paper is partially supported by the Sectoral Operational Programme – Human Resources Development, financed from the European Social Fund and by the Romanian Government under the contract number POSDRU/89/1.5/S/64109.

Contribution Note

All authors have equally contributed to this work.

References

[1] Giudice LC, Kao LC, *Endometriosis*, Lancet, 2004, 364(9447):1789–1799.
[2] Bulun SE, *Endometriosis*, N Engl J Med, 2009, 360(3):268–279.
[3] Olive DL, Schwartz LB, *Endometriosis*, N Engl J Med, 1993, 328(24):1759–1769.
[4] Gurates B, Bulun SE, *Endometriosis: the ultimate hormonal disease*, Semin Reprod Med, 2003, 21(2):125–134.
[5] Sampson JA, *Peritoneal endometriosis due to menstrual dissemination of endometrial tissue into the peritoneal cavity*, Am J Obstet Gynecol, 1927, 14:422–469.
[6] Ebert AD, Fuhr N, David M, Schneppel L, Papadopoulos T, *Histological confirmation of endometriosis in a 9-year-old girl suffering from unexplained cyclic pelvic pain since her eighth year of life*, Gynecol Obstet Invest, 2009, 67(3):158–161.
[7] Díez García R, Prieto Díez M, Aguilar Tremoya F, *Neonatal ovarian endometriosis. Its conservative treatment*, An Esp Pediatr, 1996, 44(4):397–398.
[8] Marsh EE, Laufer MR, *Endometriosis in premenarcheal girls who do not have an associated obstructive anomaly*, Fertil Steril, 2005, 83(3):758–760.
[9] Balci O, Karatayli R, Capar M, *An incidental coexistence of Mayer–Rokitansky–Kuster–Hauser syndrome with pelvic ectopic kidney and perirenal endometrioma*, Saudi Med J, 2008, 29(9):1340–1341.
[10] Nisolle M, Donnez J, *Peritoneal endometriosis, ovarian endometriosis, and adenomyotic nodules of the rectovaginal septum are three different entities*, Fertil Steril, 1997, 68(4): 585–596.
[11] Nap AW, Groothuis PG, Demir AY, Evers JL, Dunselman GA, *Pathogenesis of endometriosis*, Best Pract Res Clin Obstet Gynaecol, 2004, 18(2):233–244.
[12] Vercellini P, Aimi G, Panazza S, Vicentini S, Pisacreta A, Crosignani PG, *Deep endometriosis conundrum: evidence in favour of a peritoneal origin*, Fertil Steril, 2000, 73(5):1043–1046.
[13] Cristescu C, Veliscu A, Marinescu B, Damian C, *Endometriosis: medroxiprogesterone versus triptorelinum*, Gineco.ro, 2012, 28(2):66–68.
[14] Eskenazi B, Warner ML, *Epidemiology of endometriosis*, Obstet Gynecol Clin North Am, 1997, 24(2):235–258.
[15] Traşcă ET, Traşcă E, Tiţu A, Riza ML, Busuioc I, *Ureteral stenosis due to endometriosis*, Rom J Morphol Embryol, 2012, 53(2):433–437.
[16] ***, *Revised American Fertility Society classification of endometriosis: 1985*, Fertil Steril, 1985, 43(3):351–352.
[17] Bulun SE, Zeitoun KM, Takayama K, Sasano H, *Estrogen biosynthesis in endometriosis: molecular basis and clinical relevance*, J Mol Endocrinol, 2000, 25(1):35–42.
[18] Milne SA, Jabbour HN, *Prostaglandin (PG) F(2alpha) receptor expression and signaling in human endometrium: role of PGF(2alpha) in epithelial cell proliferation*, J Clin Endocrinol Metab, 2003, 88(4):1825–1832.
[19] Hofmann GE, Rao CV, Barrows GH, Sanfilippo JS, *Topography of human uterine prostaglandin E and F2 alpha receptors and their profiles during pathological states*, J Clin Endocrinol Metab, 1983, 57(2):360–366.
[20] Olive DL, Pritts EA, *Treatment of endometriosis*, N Engl J Med, 2001, 345(4):266-275.

[21] Brown J, Pan A, Hart RJ, *Gonadotrophin-releasing hormone analogues for pain associated with endometriosis*, Cochrane Database Syst Rev, 2010, 12:CD008475.
[22] Vercellini P, Cortesi I, Crosignani PG, *Progestins for symptomatic endometriosis: a critical analysis of the evidence*, Fertil Steril, 1997, 68(3):393–401.
[23] Bulun SE, Cheng YH, Yin P, Imir G, Utsunomiya H, Attar E, Innes J, Julie Kim J, *Progesterone resistance in endometriosis: link to failure to metabolize estradiol*, Mol Cell Endocrinol, 2006, 248(1–2):94–103.
[24] Pavone ME, Reierstad S, Sun H, Milad M, Bulun SE, Cheng YH, *Altered retinoid uptake and action contributes to cell survival in endometriosis*, J Clin Endocrinol Metab, 2010, 95(11):E300–E309.
[25] Kyamidis K, Lora V, Kanitakis J, *Spontaneous cutaneous umbilical endometriosis: report of a new case with immuno-histochemical study and literature review*, Dermatol Online J, 2011, 17(7):5.

Corresponding author
Anca Pătraşcu, Associate Professor, MD, PhD, Department of Obstetrics and Gynecology, University of Medicine and Pharmacy of Craiova, 2 Petru Rareş Street, 200349 Craiova, Romania; Phone +40744–704 641, e-mail: patrascuanca@yahoo.com

Received: October 10th, 2012

Accepted: February 5th, 2013