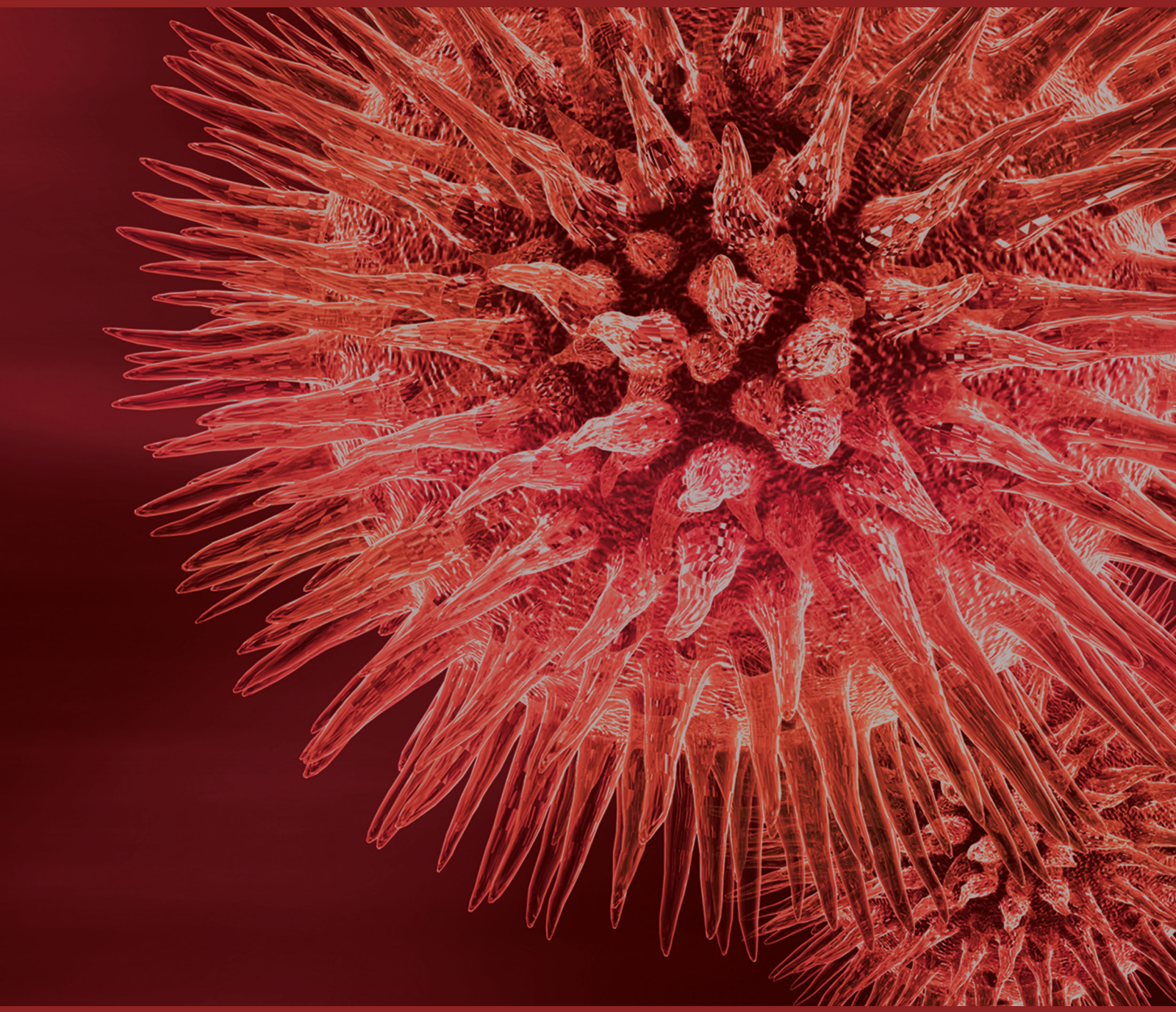
BioMed Research International

Endometriosis: Novel Models, Diagnosis, and Treatment

Guest Editors: Renato Seracchioli, Giulia Montanari, Mohamed Mabrouk, and Joseph Nassif



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Editorial

Endometriosis: Novel Models, Diagnosis, and Treatment

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Endometriosis remains a cause of significant morbidity in reproductive-aged women resulting in pelvic pain, pelvic masses, and infertility. Endometriosis is defined as the presence of endometrial glands and stroma outside of their normal intrauterine location, most commonly in the dependent portions of the pelvis. Endometriosis is treated with medical therapies, surgery or both. The medical therapies include oral contraceptive pills, progestins, gonadotropin releasing hormone analogues, and danazol. All of these medical therapies induce a hormonal steady state that results in an environment not conducive to the growth of endometriosis. Surgical therapies for endometriosis-associated pain include the removal of endometriotic implants and adhesions with restoration of normal anatomy. Laparoscopy is an effective surgical approach with the goal of excising visible endometriosis. Since endometriosis is a chronic condition, it is not uncommon for recurrences to occur. While endometriosis remains an enigmatic disease, the introduction of new pharmacologic agents and newer endoscopic methods of surgical treatment has facilitated and improved the overall management of this disease.

This special issue contains some papers that refer to molecular and cellular mechanisms of endometriosis physiopathology and some papers that refer to biomarker development for improved early diagnosis and risk of disease, while the other papers refer minimally to invasive treatment for endometriosis and new medical therapies. Finally, there are some manuscripts about prevention of endometriosis and novel models in endometriosis research.

In the paper entitled "ABO and Rhesus Blood Groups and Risk of Endometriosis in a French Caucasian Population of 633 Patients Living in the Same Geographic Area," B. Borghese et al. found that Rh-negative women have a higher risk of endometriosis; ABO blood group does not influence the risk of endometriosis.

In the paper entitled "Are Mood and Anxiety Disorders and Alexithymia Associated with Endometriosis? A Preliminary Study," G. Cavaggioni et al. found that some psychopathological aspects, such as psychoemotional distress and alexithymia, are more frequent in women with endometriosis and might amplify pain symptoms in these patients.

In the paper entitled "Antiangiogenesis Therapy of Endometriosis Using PAMAM as a Gene Vector in a Noninvasive Animal Model," N. Wang et al. found that endostatin-loaded PAMAM inhibits the development of endometriosis through an antiangiogenic mechanism and can be observed through the noninvasive endometriosis mode.

In the paper entitled "Looking for Celiac Disease in Italian Women with Endometriosis: A Case Control Study," L. Santoro et al evidenced that, in Italian population, an increased prevalence of celiac disease among patients with endometriosis is found, although this trend does not reach the statistical significance.

In the paper entitled "Endometriosis Patients in the Postmenopausal Period: Pre- and Postmenopausal Factors Influencing Postmenopausal Health," D. Haas et al. suggested that physical fitness and freedom from physical restrictions, a good social environment, and psychological care in both the premenopausal and postmenopausal periods lead to marked improvements in the postmenopausal period with regard to pain, dyspareunia, and influence on sexual life in endometriosis patients.

In the paper entitled "Evaluation of the Usefulness of the MRI Jelly Method for Diagnosing Complete Cul-de-Sac Obliteration," I. Kikuchi et al. conducted a single-center

study to evaluate the usefulness of the magnetic resonance (MR) imaging jelly method for diagnosing endometriosis-associated adhesions in the Pouch of Douglas. The sensitivity and specificity of MR with jelly administration were 95.2% and 88.9%. Opacity produced by the jelly increased the sensitivity and specificity.

In the paper entitled "Medical Treatments for Endometriosis-Associated Pelvic Pain," G. Zito et al. reviewed the pharmacological agents which have been tested for the treatment of endometriosis-associated pelvic pain. Some of them were ineffective and others proved unfit for clinical use due to significant side effects, while some others seem to be very promising but should be investigated in RCTs. Following the results of the controlled studies available, to date, the first line treatment for endometriosis associated pain is still represented by oral contraceptives used continuously. Progestins represent an acceptable alternative. GnRH analogues may be used as second line treatment, but significant side effects should be taken into account. Other agents such as GnRH-antagonist, aromatase inhibitors, immunomodulators, selective progesterone receptor modulators, and histone deacetylase inhibitors seem to be very promising.

In the paper entitled "Long-Term Outcome after Laparoscopic Bowel Resections for Deep Infiltrating Endometriosis: A Single-Center Experience after 900 Cases," G. Ruffo et al. confirm that bowel resections for endometriosis are correlated with an acceptable complication rate even at long-term follow-up and that symptoms significantly improve over time, except for rectal bleeding and dysuria.

In the paper entitled "A Deregulated CCN Matricellular Proteins Network in Endometriotic Tissues," B. Borghese et al. demonstrated that the CCN protein network is deregulated in the ectopic endometrium of women with endometriosis toward a fibrotic process.

In the paper entitled "The Effects of Sunitinib on Endometriosis: An Experimental Rat Model Study" by T. Akman et al., the volume of the endometriotic implants was reduced after sunitinib treatment. Adhesion formation decreased significantly. Therefore, sunitinib treatment seems effective for endometriotic peritoneal lesions. The effects of sunitinib in rat models give hope to improved treatment of human endometriosis to prevent pain symptoms.

In the paper entitled "Endometriosis and Nutrition: Analysis of Food Intake in Patients with Histologically Proven Endometriosis," B. Kaiseret al. showed that nutrition counseling can help to prevent endometriosis and to maintain the situation following surgery. It may influence the course of endometriosis by reducing the pain and keeping the recurrence rate low.

In the paper entitled "Frequently Misdiagnosed Extrapelvic Sites of Endometriosis: Clinical Cases and Review of the Literature," E. Fuggetta et al. underlined that extrapelvic endometriosis is a rare condition defined as the presence of endometriotic stroma and glands outside the pelvis and elsewhere in the body. Because of the lower occurrence rate of extrapelvic endometriosis, as well as its unusual sites of involvement, it is often confused with other pathologic conditions. This is the reason why the diagnosis and management are difficult and challenging.

In the paper entitled "Endometriosis: Alternative Methods of Medical Treatment," A. Tejerizo-García et al. found that hormonal treatments currently available are effective in the relief of pain associated with endometriosis. Among new hormonal drugs, aromatase inhibitors seem to be effective in the relief of pain associated with another treatment in women who did not respond to other treatments. GnRH-antagonist is expected to be as effective as GnRH agonist, but with easier administration (oral). As there is a need to find effective treatments which do not block ovarian function, antiangiogenic factors could be important components of endometriosis therapy in the future.

In the paper entitled "Correlation between Dioxin and Endometriosis: Unraveling the Pathogenesis of the Disease," O. Triolo et al. aimed to review evidence about the effect of TCDD on eutopic and ectopic endometrium, in order to unravel the machinery behind the dysregulation of immune and hormonal homeostasis caused by this environmental toxicant.

In the paper entitled "Lymphatic Spread in Deep Infiltrating Endometriosis of the Rectosigmoid Bowel," C. Letzkus and S. Rimbach described a case of deep infiltrating endometriosis of the rectosigmoid, which supports the concept of lymphatic spread from a clinical-pathological point of view by identifying and describing endometriotic implants in a perirectal lymph node and its afferent lymph vessel. The observed case is compared to results of a literature review with regard to the pathogenetic significance of lymphatic spread in endometriosis.

In the paper entitled "An Update of MR Imaging in Endometriosis," C. van Kuijk et al. aimed to review MR imaging findings of (deep infiltrating) endometriosis in order to explore and summarize experiences in the field of MR imaging in endometriosis. MR imaging in addition to transvaginal ultrasonography (TVS) shows accurate diagnosis of endometriosis and may be predominantly useful for the analysis of patients suspected of deep infiltrating endometriosis (DIE).

In the paper entitled "PJGR Hot-Dog Implant: A New Feasible Rat Model for Surgically Induced Internal and External Endometriosis," P. G. Ramos et al. studied the possibility of inducing internal or external endometriosis in Wistar rats through a new microsurgical model known as "PJGR Hot-Dog." This new implant model could enhance our understanding of the mechanisms involved in the development of endometriosis, both internal and external.

Acknowledgments

We would like to thank the authors for their excellent contributions and patience in assisting us. Finally, the fundamental work of all the reviewers of these papers is also very warmly acknowledged.

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Giulia Montanari
Mohamed Mabrouk
Joseph Nassif

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Research Article

ABO and Rhesus Blood Groups and Risk of Endometriosis in a French Caucasian Population of 633 Patients Living in the Same Geographic Area

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Objectives. The identification of epidemiological factors increasing the risk of endometriosis could shorten the time to diagnosis. Specific blood groups may be more common in patients with endometriosis. *Study Design*. We designed a cross-sectional study of 633 Caucasian women living in the same geographic area. Study group included 311 patients with histologically proven endometriosis. Control group included 322 patients without endometriosis as checked during surgery. Frequencies of ABO and Rhesus groups in the study and control groups were compared using univariate and multivariate analyses. *Results*. We observed a higher proportion of Rh-negative women in the study group, as compared to healthy controls. Multivariate analysis showed that Rh-negative women are twice as likely to develop endometriosis (aOR = 1.90; 95% CI: 1.20–2.90). There was no significant difference in ABO group distribution between patients and controls. There was no difference when taking into account either the clinical forms (superficial endometriosis, endometrioma, and deep infiltration endometriosis) or the rAFS stages. *Conclusion*. Rh-negative women are twice as likely to develop endometriosis. Chromosome 1p, which contains the genes coding for the Rhesus, could also harbor endometriosis susceptibility genes.

1. Introduction

Endometriosis is a chronic gynecological disease that severely affects quality of life [1]. High healthcare costs and repeated surgery are two hallmarks of the disease. One explanation for that is late diagnosis. The time between onset of symptoms and medical diagnosis is more than eight years in most industrialized countries [2]. Finding risk factors, especially for the most severe forms of the disease, is a crucial issue that may contribute to shortening the time from the initial symptoms to the diagnosis. Epidemiological risk factors for endometriosis have been consistently reported: a low body mass index (BMI), a family history of endometriosis, a personal history of severe and lasting dysmenorrhea at the time of adolescence, and the need to use oral contraceptives for alleviating dysmenorrhea that failed to respond to nonsteroidal anti-inflammatory drugs [3–5]. It is useful to gather this information when evaluating women experiencing infertility and/or pelvic pain [6].

With the exception of fetomaternal alloimmunization and hemolysins in relation to blood transfusion, the relationship between blood groups and human diseases, such as cancer or inflammatory diseases, has been controversial [7, 8]. To date three studies have investigated the association between endometriosis and blood groups, with varying results [9–11]. The discrepancies were so striking that an editorial has been

published on this topic, reporting population stratification bias and ethnicity as potential explanations [12]. In addition, in these studies, the distinction between endometriosis subtypes has not been taken into account for the analyses. Yet, three forms of endometriosis are well recognized and are fundamentally different from each other: superficial peritoneal endometriosis (SUP), ovarian endometrioma (OMA), and deeply infiltrating endometriosis (DIE) [13].

Consequently, we decided to set up a cross-sectional study with a design minimizing the abovementioned bias. We analyzed the blood groups distribution only in Caucasian women with and without endometriosis and originating from the same geographic area. We studied each clinical subtype (SUP, OMA, and DIE) separately. We also evaluated the frequency of ABO and Rhesus blood groups according to the revised American Fertility Society (rAFS) classification.

2. Materials and Methods

We conducted a cross-sectional study using data from a prospectively managed database. The structure of this database has already been detailed and published elsewhere [3]. Briefly we included all nonpregnant patients under 42 years old who were operated on (by laparotomy or operative laparoscopy) in our institution between January 2004 and November 2010. For the present study, we excluded from the cohort patients with cancer, non-Caucasian patients, and patients living outside Paris area (i.e., outside the region Île de France). Indications for surgery, sometimes more than one for each patient, were the following: (i) preoperative assessment of endometriosis by magnetic resonance imaging and/or ultrasound; (ii) pelvic pain, defined as the presence, for at least 6 months, of dysmenorrhea and/or intermenstrual pelvic pain and/or dyspareunia of moderate to severe intensity; (iii) infertility defined as at least 12 months of unprotected intercourse not resulting in pregnancy; (iv) pelvic mass (benign ovarian cyst, uterine myoma, etc.); and (v) others: uterine bleeding, request for tubal ligation, tubal infection, and so forth. Patients visually diagnosed with endometriosis but without histologic confirmation were considered to be ineligible to participate in the study. A total of 633 patients were diagnosed with the presence ($n = 311$, study group) or absence ($n = 322$, control group) of endometriosis. Depending on pathologic findings, endometriotic lesions were divided into SUP, OMA, and DIE, with the latter being defined as lesions that infiltrate the *muscularis propria* of bladder, vagina, intestine, or ureter [13]. Because these lesions are frequently associated [14], the final staging was given by the worst lesion found in each patient, that is, from least to most severe: SUP, OMA, and DIE. During surgery, extension of endometriosis was also scored according to the rAFS classification [15]. Control group included 322 patients without any lesion of endometriosis, as checked by an exhaustive inspection of the peritoneal surface and abdominopelvic organs at the time of surgery. Demographic data, medical and surgical history, type and duration of symptoms, and ethnicity were collected by face-to-face standardized questionnaires conducted by the surgeon during the month before surgery, as previously reported [16]. Information on ABO and Rh blood groups was obtained from medical records. If not available, blood groups were systematically determined by conventional techniques during the preoperative assessment.

We calculated the sample size by OpenEpi, Version 2, open source calculator-SSCohort (http://www.openepi.com/OE2.3/SampleSize/SSCohort.htm). Regarding the ABO groups, we assumed an odds ratio (OR) of 3.0 (data from [9]) between cases and controls, a type I error of 0.05, and a power of 0.80. We calculated the sample size to 320 (with a case-control ratio of 1 : 1). Regarding the Rhesus factor we assumed an OR of 0.6 (data from [10]) between cases and controls, a type I error of 0.05, and a power of 0.80. We calculated the sample size to 626 (ratio 1 : 1). Therefore, with 633 participants, our study appeared sufficiently dimensioned.

Statistical analysis was performed using SPSS 13.0 (SPSS, Chicago, IL). Continuous data were presented as mean ± standard deviation (SD). Student's t-tests were carried out when necessary. The chi-square and Fisher exact tests were used for categorical data. We used OR and corresponding 95% confidence intervals (CI) to compare the distribution of ABO groups and Rhesus factor in the entire study group (all endometriosis patients) and in the different forms and stages of endometriosis, as compared to the control group. We also performed an unconditional logistic regression to control for potential confounding factors, such as age, pain, BMI, blood groups, gravidity, and parity. We performed a stepwise logistic regression analysis, in which a P value of 0.5 was used as entry criteria and a P value of 0.2 was the threshold for the covariate to stay in the model [17]. A P value of <0.05 was considered to be statistically significant. Assuming a two-sided significance level of 95% and a power of 80%, this study is powered to detect a difference of 11% between the two groups with a total sample size of 633 and two equal groups. The institutional review board at our center approved the study protocol, and each individual signed an informed consent form. Standards for reporting of cross-sectional study have been followed in accordance with the STROBE statement (http://www.strobe-statement.org).

3. Results

Patients with histologically proven endometriosis (study group: $n = 311$) were distributed as follows: (i) SUP: 53 patients (17.0%); (ii) OMA: 110 patients (35.4%); (iii) DIE: 148 patients (47.6%). Classification according to the rAFS stages was as follows: (i) stage I: 56 patients (18.0%); (ii) stage II: 67 patients (21.5%); (iii) stage III: 100 patients (32.2%); (iv) stage IV: 88 patients (28.3%). Control group consisted of 322 patients without endometriosis at the time of surgery. Indications for surgery in the control group were as follows: (i) benign ovarian cysts ($n = 99$), (ii) uterine myomas ($n = 98$), (iii) chronic pelvic pain ($n = 57$), (iv) pelvic inflammatory disease ($n = 5$), (v) infertility ($n = 47$), (vi) ovarian torsion ($n = 2$), and (vii) others ($n = 14$). Significant differences between cases and controls were observed in gravidity, parity, weight, BMI, and preoperative pain scores, as expected [3, 5, 16]. Conversely, age, height, and infertility status were comparable in both groups.

Table 1: Distribution of ABO and Rh blood groups in women with and without endometriosis.

	Endometriosis *n* (%)	Controls *n* (%)	Crude OR (95% CI)	aOR (95% CI)[a]	*P* value[b]
ABO groups					
A	148 (47.5)	139 (43.2)	1.20 (0.86–1.68)	1.18 (0.82–1.70)	0.270
AB	10 (3.0)	10 (3.1)	1.10 (0.45–2.80)	1.24 (0.47–3.29)	0.780
B	32 (10.3)	36 (11.2)	1.01 (0.58–1.70)	1.04 (0.58–1.80)	0.980
O	121 (38.9)	137 (42.6)	1		
Rhesus					
Negative	72 (23.1%)	49 (15.2)	1.27 (1.07–1.52)	1.90 (1.20–2.90)	0.011
Positive	239 (76.9%)	273 (84.8)	1		
Total	**311**	**322**			

[a] Logistic binary regression (age, pain, BMI, blood type and rhesus, parity, and gestity); aOR: adjusted odds ratio.
[b] Pearson chi-square.

Table 2: Distribution of combined ABO and Rh blood groups in women with and without endometriosis.

	Endometriosis *n* (%)	Controls *n* (%)	Total *n* (%)	*P* value[a]
Blood group				
A positive	117 (37.6)	116 (36.0)	233 (36.8)	
A negative	31 (10.0)	23 (7.1)	54 (8.5)	
B positive	22 (7.1)	32 (9.9)	54 (8.5)	
B negative	10 (3.2)	4 (1.2)	14 (2.2)	0.138
AB positive	7 (2.3)	6 (1.9)	13 (2.1)	
AB negative	3 (1.0)	4 (1.2)	7 (1.1)	
O positive	93 (29.9)	119 (37.0)	212 (33.5)	
O negative	28 (9.0)	18 (5.6)	46 (7.3)	
Total	**311 (100.0)**	**322 (100.0)**	**633 (100.0)**	

[a] Pearson chi-square.

The distribution of ABO and Rh blood groups in women with and without endometriosis is shown in Table 1. There was no significant difference in ABO groups between patients and controls. On the other hand, a statistically significant difference was detected for the Rhesus factor ($P = 0.011$, Pearson chi-square). Women with the disease were more frequently Rhesus negative group as compared to the controls (23.1% versus 15.2%, resp.). Crude OR for endometriosis among the Rhesus negative patients was 1.27 (95% CI: 1.07–1.52). After multivariate analysis, taking into account age, pain, BMI, ABO group, parity, and gravidity, the difference remained significant. Adjusted OR (aOR) for endometriosis was 1.90 (95% CI: 1.20–2.90). When combining Rhesus and ABO groups, no difference in distribution was observed between cases and controls ($P = 0.138$, Pearson chi-square) (Table 2).

The distribution of ABO and Rhesus blood groups among the different clinical forms of endometriosis (SUP, OMA, and DIE) is shown in Table 3. There was no difference between the groups, either for ABO group or Rhesus factor ($P = 0.34$ and 0.26, resp., Pearson chi-square).

Finally, analyses of blood groups distribution according to the rAFS classification did not reveal any statistically significant differences between the rAFS stages (Table 4).

4. Comment

In this cross-sectional study of 663 Caucasian patients living in Paris area and referred to our institution for surgery, we observed a higher proportion of Rh-negative women in patients with histologically proven endometriosis, as compared to healthy controls. After multivariate analysis, Rh-negative women are twice as likely to have endometriosis (aOR = 1.90; 95% CI: 1.20–2.90). This result is reported for the first time in a French Caucasian population. Data available for other populations are slightly different [9–11]. In a series of 231 patients with endometriosis and 166 women without endometriosis from the Yale University School of Medicine, Matalliotakis et al. found a 2.9-fold increased risk for endometriosis in women with blood group A (OR = 2.9; 95% CI: 1.85–4.52) [9]. In a Korean population of 186 women with endometriosis, Kim et al. found a preponderance of

Table 3: Distribution of ABO and Rh blood groups in women with endometriosis according to the clinical subtypes of disease.

	SUP *n* (%)	OMA *n* (%)	DIE *n* (%)	*P* value[a]
ABO groups				0.34
A	28 (53)	47 (43)	73 (49)	
AB	2 (4)	6 (5)	2 (1)	
B	5 (9)	15 (14)	12 (8)	
O	18 (34)	42 (38)	61 (41)	
Rhesus				0.26
Positive	37 (70)	83 (75)	119 (80)	
Negative	16 (30)	27 (25)	29 (20)	
Total	**53**	**110**	**148**	

[a]Pearson chi-square.
SUP: superficial endometriosis; OMA: endometrioma; DIE: deep infiltrating endometriosis.

Table 4: Distribution of ABO and Rh blood groups in women with endometriosis according to the rAFS classification.

	rAFS I *n* (%)	rAFS II *n* (%)	rAFS III *n* (%)	rAFS IV *n* (%)	*P* value[a]
ABO groups					0.26
A	27 (48)	37 (55)	40 (40)	44 (51)	
AB	2 (5)	0 (0)	6 (6)	2 (2)	
B	4 (11)	6 (9)	16 (16)	7 (8)	
O	23 (36)	24 (36)	28 (38)	34 (39)	
Rhesus					0.79
Positive	41 (73)	51 (76)	80 (80)	66 (76)	
Negative	15 (27)	16 (24)	20 (20)	22 (24)	
Total	**66**	**67**	**100**	**88**	

[a]Pearson chi-square.
rAFS: revised American Fertility Society classification.

group A among women with endometriosis (OR = 1.6; 95% CI: 0.8–3.3). Demir et al. did not confirm this result in a Turkish population of 304 women with endometriosis and 42 controls [10, 11]. Regarding the Rhesus factor, only Demir et al. reported a higher proportion of Rh-positive women among women with endometriosis, as compared to healthy women (84 versus 76%, resp.; $P = 0.03$) [10]. As stressed by Tabei, these discrepancies are probably related to different frequencies of blood groups among subpopulations or ethnic groups [12].

When considering the severity of endometriosis, as defined by the rAFS classification, no study, including our study, has shown any statistically significant association between blood groups and disease stages. In the present study, we separated for the first time the clinical forms of endometriosis but could not find any association between blood groups and SUP, OMA, or DIE.

Some limitations in our study should be considered. First, the fact that all patients were submitted to surgery may introduce selection bias, as these women were probably the most severe cases. Unfortunately this point remains unsolved in most studies published on the subject [18]. It must be borne in mind that our results are only applicable to this specific population. Secondly, it is likely that our study was underpowered to detect a slight difference in subsets of patients, especially in SUP, where the number of patients was low. Further studies, targeting specific forms of the disease and including a large number of patients for each subtype, are needed to confirm our results. Lastly, all patients in the control group had a benign gynecological condition. Some of them could be associated to a specific blood group. However, the distribution of ABO and Rhesus groups among controls was similar to that of the French population, according to the French Blood Service data (http://www.dondusang.net/rewrite/article/3160/about-blood/blood-group-basics/blood-groups.htm?idRubrique=1178). Also, in the study of Kim and colleagues, there was no significant association between blood groups and fibroids, ectopic pregnancy, or female infertility [11].

Beyond these issues, our study has specific strengths. With 633 patients recruited, our study has the largest sample size to date. The application of strict histological and surgical criteria allowed us a highly accurate and undoubted selection of cases and controls. Following the recommendations of Tabei et al., we selected the control group in the same population as the patient group, that is, in a population originating from the same geographic area and from the same ethnicity [12]. We included only Caucasian women living in Paris in this study because the frequency of blood groups may vary according to ethnicity and living area, as stressed by Tabei. Of course, this topic should be evaluated in other populations to have a global view of blood groups distribution in endometriosis. However, our results can probably be extended to the whole French population, since distribution of ABO and Rhesus groups in our control group was similar to that in France.

Obviously, it is in the nature of such an investigation, in which a link is sought but not prespecified as being biologically plausible, to find statistically significant associations quite by chance. The association that we found between Rh-negative factor and endometriosis was not strong enough (OR < 2) to suggest a causal relation. However, as the value of the aOR was not far from 2, it deserves at least consideration. In general, finding an excess of Rhesus negative subjects among patients with endometriosis suggests a genetic predisposition. Rhesus negativity has been reported as a potential risk factor for esophageal and gastric cancers [19, 20]. Rhesus negativity may also predispose to cancers in the lung [21], mouth [22], breast [23, 24], and endometrium [25]. As endometriosis shares some behavioral resemblance with tumor cells, including invasion, survival, evasion from immune clearance, or establishment of a blood supply, some common pathways may be implicated in patients with Rhesus negative phenotype [26]. Moreover, the Rh blood group locus is found on the short arm of chromosome 1 (1p34-36), at a site reported to constitute a susceptibility locus for skin malignant melanomas [27] and containing at least one tumor suppressor gene [28]. An increase in risk of malignant melanoma has been observed in Rh-negative subjects [29]. Incidentally, an

association between endometriosis and cutaneous melanoma has been repeatedly observed [30–32]. Comparative genomic hybridization (CGH) analysis revealed loss of DNA copy number on 1p in SUP and OMA [33]. Finally, the potential effect of nitric oxide has been recently reported in Rh-negative subjects [19]. Nitric oxide has been implicated both in the development of endometriosis and in the neoplastic degeneration of OMA [34, 35].

In conclusion, Rhesus negativity could represent a risk factor for endometriosis in a Caucasian population. Biologic rationale for this association is consistent and could lead to the improvement of our knowledge of endometriosis pathogenesis. This observation could also contribute to shortening the time to diagnosis as being part of a more global score to predict the risk of endometriosis, which would include other known risk factors.

Conflict of Interests

The authors report no conflict of interests.

Authors' Contribution

Bruno Borghese and Charles Chapron conceived and designed the study. Bruno Borghese, Mélanie Chartier, Carlos Souza, Dominique de Ziegler, and Charles Chapron analyzed and interpreted the data. Bruno Borghese wrote the paper. Bruno Borghese, Pietro Santulli, Marie-Christine Lafay-Pillet, and Charles Chapron contributed to data collection and/or performed surgical procedures. All authors approved the final version of the paper.

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Review Article

Medical Treatments for Endometriosis-Associated Pelvic Pain

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The main sequelae of endometriosis are represented by infertility and chronic pelvic pain. Chronic pelvic pain causes disability and distress with a very high economic impact. In the last decades, an impressive amount of pharmacological agents have been tested for the treatment of endometriosis-associated pelvic pain. However, only a few of these have been introduced into clinical practice. Following the results of the controlled studies available, to date, the first-line treatment for endometriosis associated pain is still represented by oral contraceptives used continuously. Progestins represent an acceptable alternative. In women with rectovaginal lesions or colorectal endometriosis, norethisterone acetate at low dosage should be preferred. GnRH analogues may be used as second-line treatment, but significant side effects should be taken into account. Nonsteroidal anti-inflammatory drugs are widely used, but there is inconclusive evidence for their efficacy in relieving endometriosis-associated pelvic pain. Other agents such as GnRH antagonist, aromatase inhibitors, immunomodulators, selective progesterone receptor modulators, and histone deacetylase inhibitors seem to be very promising, but there is not enough evidence to support their introduction into routine clinical practice. Some other agents, such as peroxisome proliferator activated receptors-γ ligands, antiangiogenic agents, and melatonin have been proven to be efficacious in animal studies, but they have not yet been tested in clinical studies.

1. Introduction

Endometriosis is a chronic disease of unknown etiology that affects approximately 10% of women in reproductive age [1]. The main sequelae of endometriosis are represented by infertility and chronic pelvic pain. Up to 40% of infertile women and one-third of women who undergo laparoscopy for chronic pelvic pain have endometriosis [1, 2]. Chronic pelvic pain causes disability and distress with a very high economic impact [3]. In the last decades several studies have been conducted in order to introduce new drugs into clinical practice for treating endometriosis-associated pelvic pain. In this paper the efficacy of older, emerging, and experimental pharmacological agents will be reviewed.

Pharmacological agents for treatment of endometriosis-associated pelvic pain are as follows.

First-Line Treatment. The choice should be based on patient preferences, side effects, efficacy, costs, and availability. For oral contraceptives, further benefits such as contraceptive protection, long-term safety, and control of menstrual cycle should be considered. Progestins used were medroxyprogesterone acetate, norethisterone acetate, dienogest, etonogestrel implant, and IUD-levonorgestrel (rectovaginal endometriosis).

Second-Line Treatment. Due to side effects, they should only be prescribed to women for whom other treatments have proven ineffective. For nonsteroidal anti-inflammatory drugs, significant side effects, including inhibition of ovulation and risk of peptic ulceration and cardiovascular disease, should be considered. For GnRH analogues significant side effects, such as bone loss and hypoestrogenic symptoms, should be considered. For danazol, severe side effects, such as thrombosis and hyperandrogenism, should be considered. For gestrinone, severe side effects, such as thrombosis and hyperandrogenism, should be considered.

Emerging Treatments. More clinical studies are needed, especially to assess their long-term efficacy and side effects. As

for aromatase inhibitors (AIs), due to the severe side effects, they should only be prescribed to women for whom other treatments have proven ineffective. For GnRH antagonist and selective progesterone receptor modulators (SPRMs), more clinical trials are required. For histone deacetylase inhibitors, only two small pilot studies are available to date. The benefits of Selective Estrogen Receptor Modulators (SERMs) and immunomodulators have not been demonstrated to date.

Experimental Treatments. No clinical studies are available. For peroxisome proliferator activated receptors- (PPARs-) gamma ligands, only in vitro studies are available. For antiangiogenic agents and melatonin only animal studies are available.

2. Oral Contraceptives

Despite limited evidence of effectiveness, oral contraceptives are considered as first-line medical treatment for endometriosis-associated chronic pelvic pain [4, 5]; their use is based on the evidence of a clinical improvement of the disease during pregnancy [6]. They inhibit the production of gonadal estrogen via a negative feedback mechanism. Moreover, by suppressing ovarian activity, they also lead to a reduction in estrogen-induced production of prostaglandins, decreasing the inflammation associated with endometriosis. Some authors suggest the ultralow dosage [15 mcg of ethinyl estradiol (EE)], in association with 60 mcg of gestodene, for a period of 24 days, providing only 4 days off [7]. In this way, the recovery phase of estrogen synthesis (typically 7 days) is almost absent. Thus, a more stable inhibition of the production of ovarian hormones and a steady suppression of the endometrium growth are achieved [7]. In terms of side effects, lower doses of EE can lead to an increased intermenstrual bleeding, especially in the first few months of therapy, but do not affect either their contraceptive efficacy or their protective effect on endometrial and ovarian cancer and breast pathology. In a recent randomized controlled trial, it has been shown that low-dose oral contraceptive pill is more effective than placebo in controlling dysmenorrhea and in decreasing the ovarian endometrioma size [8]. The uninterrupted use of oral contraceptive pill appears to be associated with a greater pain score reduction [9]. Furthermore, the continuous administration represents a valid, safe, and economical therapeutic coverage that might be used in patients who have undergone conservative surgery for endometriosis [10, 11]. Some limitations in the use of oral contraceptives are represented by the fast recovery of the disease after the treatment interruption and the increased risk for thromboembolic events that could affect women smokers aged >35 years [12]. They can also be used together with the GnRH analogues in the "add-back therapy," in order to balance the strong hypoestrogenism caused by the latters. In fact, they protect the bone density decrement and make women relieved of the unpleasant symptoms (above all hot flushes and vaginal discomfort) [13, 14]. However, hormone replacement treatment should be considered the first choice "add-back therapy," since it provides lower stimulation of endometriotic tissue [5].

3. Progestins

Progestins have been used in the treatment of endometriosis for over 30 years. Thanks to central and peripheral mechanisms, the mitogenic action and estrogen-induced proliferation are lacking. Furthermore, the endometrium, firstly, undergoes a secretory transformation and then to a decidualization, and, finally, it becomes atrophic, thus creating a pseudopregnancy state [15, 16].

A recent Cochrane review has shown that the use of medroxyprogesterone acetate (MPA) at a dose of 100 mg/day is more effective in controlling pain if compared with placebo, but it is burdened by several side effects (menstrual irregularities, amenorrhea, weight gain, and breast tenderness) [17]. Therefore, the authors have concluded that the use of progesteron, both oral and depot form, does not seem to be more effective than the others treatments (e.g., low-dose estroprogestin or leuprolide acetate) in controlling symptoms [17].

In recent years, the use of the levonorgestrel-releasing intrauterine device (IUD-LNG) has aroused interest. Its use in the treatment of endometriosis of the rectovaginal septum provides a significant reduction in dysmenorrhea, pelvic pain, and deep dyspareunia, as well as the size of the endometriotic implants, showing levels of efficacy comparable to GnRH analogues [18, 19]. Furthermore, it appears to be effective in preventing the recurrence of endometriosis after surgical treatment [20]. Petta et al. suggested that its use would be a favourable treatment for chronic pelvic pain, because it determines a long state of hypoestrogenism, requiring only one medical intervention for its introduction every 5 years [21]. At a second-look laparoscopy, a reduction in pelvic endometriotic lesions in 60% of patients treated with LNG-IUD and in 37.5% of those treated with GnRH agonist was observed [21]. However, this difference was not significant probably due to the small sample size evaluated [21]. Clinical trials that compared the use of LNG-IUD and depot medroxyprogesterone acetate (DMPA), administered for a period of three years, showed better compliance in patients who used the IUD [22]. Moreover, bone gain was observed with LNG-IUS, whereas bone loss was reported with DMPA [22].

Recently, DMPA has been compared with an etonogestrel subcutaneous implantation (Implanon) in a pilot study [23]. During a follow-up period of 1 year, a significant decrease in pain intensity for both treatment options was observed. However, after 6 months, the average decrease in pain was 68% in the Implanon group and 53% in the DMPA group, with a side-effect profile and an overall degree of satisfaction comparable for both treatments. Therefore, Implanon might be a valid therapeutic option for pain management in women with endometriosis [23].

Norethisterone acetate (or norethindrone acetate, NETA) is a 19-nortestosterone derivative. It causes a hypoestrogenism by suppressing gonadotropins, inhibiting ovulation, and developing amenorrhea with eventual decidualization and atrophy of the endometrium [24]. Early studies showed that NETA was effective in reducing chronic pelvic pain in women with laparoscopically confirmed endometriosis [25].

Norethisterone acetate offers various advantages for the long-term treatment of endometriosis. It allows good control of uterine bleeding; it has positive effect on calcium metabolism and no negative effects on the lipoprotein metabolism at low dosages [26]. The continuous administration of NETA for the treatment of endometriosis is approved by the US Food and Drug Administration. In a randomized study on 90 women with symptomatic rectovaginal endometriosis, oral NETA 2.5 mg/day has been compared with oral EE 0.01 mg plus cyproterone acetate 3 mg/day [27]. After twelve months of continuous treatment, seven women (16%) in the EE plus cyproterone acetate arm and five (11%) in the norethindrone acetate arm had dropped out, owing to adverse effects or treatment inefficacy. In the remaining patients, dysmenorrhea, deep dyspareunia, nonmenstrual pelvic pain, and dyschezia scores were substantially reduced without major differences between the two treatment groups [27]. A prospective study on 82 women with pain symptoms caused by rectovaginal endometriosis has shown that NETA combined with letrozole was more effective in reducing pain and deep dyspareunia than NETA alone [28]. However, a higher incidence of adverse effects, without improving patients' satisfaction or influencing recurrence of pain, have been observed with the combined regimen. In a prospective study including 40 women with colorectal endometriosis who had pain and gastrointestinal symptoms, low dose of NETA (2.5 mg/day) for 12 months provided pain relief and amelioration of gastrointestinal symptoms [29].

3.1. Dienogest. Dienogest is a derivative of 19-nortestosterone that combines the pharmacological properties of 19-nortestosterone derivatives with those of natural progesterone derivatives [30]. Therefore, it shows a high selectivity for the progesteron receptors and a powerful progestinic effect on the endometrium [30]. Furthermore, it has beneficial antiandrogenic properties, typical of the derivatives of progesteron, which cause minimal changes in serum lipid profile and carbohydrates metabolism [31]. Currently, its oral use is approved for medical treatment of endometriosis in Europe, Japan, and Australia [31]. It has been suggested that the effectiveness of dienogest in the treatment of endometriosis depends on its ability to create a hypoestrogenic and hyperprogestinic endocrine environment, which, initially, causes the decidualization of ectopic endometrial tissue. Subsequently, for prolonged treatments, dienogest causes an atrophy of the lesions. It inhibits the estradiol level increase, through the inhibition of the growth of ovarian follicles [31]. Thus, in patients with normal ovulatory cycles, ovulation is completely suppressed. However, there are no clinical trials that have proven the contraceptive efficacy of the drug in monotherapy [32]. Regarding the optimal dose, in a European randomized controlled trial (RCT), the authors concluded that 2 mg/day represents the optimal dosage, because it is well tolerated and less encumbered by side effects, in particular abnormal uterine bleeding, and it has little influence on bone mineral density (BMD) [33]. In a recent, multicenter, randomized, double-blind study, the efficacy of dienogest 2 mg/day has been compared versus placebo. Dienogest was significantly more effective than placebo for reducing endometriosis-associated pelvic pain [34]. An open-label extension of this study for up to 53 weeks showed that long-term dienogest has a favorable efficacy and safety profile, with progressive decrease in pain and bleeding irregularities [35]. Furthermore, the decrease of pelvic pain persisted for at least 24 weeks after therapy discontinuation. These effects should be due to the multiple mechanisms of action of the drug that reduces the growth and the neoangiogenesis of the lesions and provides an anti-inflammatory activity [35]. Another RCT compared dienogest with leuprolide acetate and showed that the two drugs were equivalent in reducing pelvic pain [36]. However, dienogest was associated with a lower incidence of vasomotor symptoms and a lower impact on bone metabolism than leuprolide acetate, with a minimum change in BMD [36]. Similar results were found by using triptorelin and buserelin acetate [37, 38].

3.2. Danazol. Danazol is a synthetic androgen derivative of 17α-ethinyltestosterone, commercially introduced about 30 years ago with a specific indication for the treatment of endometriosis [39]. It carries out a multifactorial biological action inducing a hypoestrogenic-hyperandrogenic state, which is very hostile to the endometriotic tissue growth. Several studies have demonstrated the efficacy of danazol in reducing the pain associated with endometriosis [40]. However, its oral use is limited by significant side effects such as weight gain, muscle cramps, acne, seborrhoea, decreased breast size, hirsutism, and deepening of the voice, all strongly related to the androgenic action [41]. The vaginal administration, through a vaginal ring or gel or intrauterine device extended-release, has been tested in patients with deep endometriosis with encouraging results [42]. However, it should be taken into account that some data suggest that danazol may increase the risk of ovarian cancer among women with endometriosis [43].

3.3. Gestrinone. Gestrinone, a synthetic trienic 19-norsteroid, acts by inhibiting the pituitary gland and, consequently, the release of gonadotrophins [44]. The resulting ovarian suppression determines atrophy of both endometrium and endometriosis lesions. It also has antiprogestinic, antiestrogenic, and androgenic action. Several studies have shown the effectiveness of gestrinone in reducing the pain associated with endometriosis [45]. However, its use is limited by the high percentage of anabolic and androgenic effects [46].

4. GnRH Analogues

GnRH analogues (GnRH-a) suppress estrogen ovarian production through a downregulation of GnRH receptors at pituitary level, causing a profound hypoestrogenism, and, consequently, amenorrhea and a hypoatrophic regression of the heterotopic endometrium. This effect is readily reversible after stopping GnRH-a administration. They are considered as a second-line treatment in case of failure of therapy with oral contraceptives or progestins or when they are

not tolerated or contraindicated. GnRH analogues provide a reduction of symptoms in about 50% of cases [47], and their administration after surgical treatment prolongs the pain-free interval [48, 49]. The treatment for 3 months with a GnRH-a may reduce the painful symptoms for about 6 months [48]. Among the limitations of their use, there are the high rate of recurrence of pelvic pain (5 years after withdrawal of therapy is at 75%) and the side effects, such us deterioration in the lipid profile, depression, flushes, urogenital atrophy, loss of libido, and bone mass decrease [46]. The latter may be avoided by an "add-back therapy" that involves the use of hormone replacement treatment (HRT) alone or in combination with biphosphonates or other antiresorptive agents [50].

5. GnRH Antagonist

The use of GnRH antagonists (GnRH-anta) in the treatment of endometriosis has been recently introduced, with optimistic results [51]. They reduce estrogen levels in order to inhibit the pain symptoms but without triggering side effects consequent to estrogen deprivation. Furthermore, in contrast to GnRH-a, they do not determine the initial stimulation of the pituitary-ovarian axis with the resulting gonadotropic peak [52]. However, it is uncertain whether this specific property can actually improve the results of analogues. A recent phase 2, randomized, double-blind, placebo-controlled study has shown that a new GnRH antagonist (Elagolix) has an acceptable efficacy and safety profile [53]. More clinical trials are required before such agents should be introduced into clinical practice.

6. Nonsteroidal Anti-Inflammatory Drugs

Nonsteroidal anti-inflammatory drugs (NSAIDs) are the most commonly used first-line treatment for endometriosis [4, 5]. However, there is inconclusive evidence to show whether or not they are effective in relieving pain associated with endometriosis [54]. Furthermore, there is no evidence on whether any individual NSAID is more effective than another [54]. The expression of COX-2 has recently been demonstrated in ectopic endometrial cells in concentrations higher than eutopic endometrium [55]. The release of prostaglandins (PGs) in ectopic endometrial cells seems to be involved in the pathogenesis of endometriosis, and high concentrations of PGs were found in the peritoneal fluid of the affected women. Nonsteroidal anti-inflammatory drugs interfere with the function of the enzyme COX-1 and COX-2, inhibiting the production of PGs, molecules involved in the genesis of endometriosis-associated pain [55]. Specific inhibitors of COX-2, as rofecoxib, have also the property to block the growth of ectopic cells and induce apoptosis, with equivalent result to one achieved with GnRH-a [56]. Rofecoxib, at doses of 25 mg/day for 6 months, was more effective than placebo, providing an adequate and safe pain relief in cases of mild or moderate endometriosis [57]. To date, there are no sufficient clinical data to prove the NSAID drugs as effective in the treatment of endometriosis-associated pain. They are also associated with several side effects including peptic ulcer and anovulation, if taken at midcycle [55].

7. Aromatase Inhibitors

An overexpression of the aromatase enzyme, the main responsible factor for estrogen synthesis in ectopic endometrium, has been demonstrated in endometrial tissue [58]. Aromatase catalyzes the conversion of the steroidal precursors into estrogens, which stimulate the expression of the enzyme COX-2. The estrogens produced in the endometrial tissue through aromatase promote the growth and invasion of endometrial lesion and favour the onset of pain and prostaglandin-mediated inflammation [59]. This local production of estrogen may explain the progression of endometriosis during therapy with GnRH-a that act only at the level of the ovarian production of estrogen [60]; aromatase inhibitors (AIs), on the contrary, lead to a reduction of extraovarian estrogen concentration [61]. The estrogen plasma levels in women taking 1–5 mg of letrozole or anastrozole daily are reduced by 97–99% [62].

There are three generations of AIs. The third generation AIs, including letrozole, anastrazole, and exemestane, are triazole derivatives and have a selective, potent, and reversible action [61]. Their side effects are represented mainly by headache, stiffness or joint pains, nausea, diarrhea, and flushing. The long-term use of these drugs favours the onset of bone fractures, osteopenia, and osteoporosis [62]. However, in premenopausal women, the bone loss can be reversed by an "add-back therapy" [63, 64]. By reducing the production of extra ovarian estrogens, AIs stimulate an increased secretion of FSH from pituitary gland, promoting an increased ovarian production of estrogens and follicular recruitment. When used in premenopausal women, it is important to associate drugs that lead to a downregulation of ovarian activity, such as progestins, GnRH analogues, or oral contraceptives, in order to counteract the potential formation of follicular cysts [62–64]. The combination of conventional therapy and AIs determines the block of the production of estrogens both in ovarian and extraovarian endometriotic foci, reducing the painful symptoms. They have been used in a pilot study evaluating 12 women with rectovaginal endometriosis, who had pelvic pain resistant to conventional treatments: after 6 months of treatment with letrozole (2.5 mg/day), norethisterone acetate (2.5 mg/day), calcium citrate, and vitamin D, there has been a significant reduction in abdominal-pelvic pain and the disappearance of endometriotic lesions at second-look surgery [65]. A subsequent study, from the same group, showed that the association of letrozole with norethisterone acetate provides pelvic pain control more effectively than norethisterone acetate alone [28]. Pelvic pain, however, tends to recur after discontinuation of treatment, just as after the discontinuation of GnRH-a [66].

8. Selective Estrogen Receptor Modulators

Selective estrogen receptor modulators (SERMs) interact with estrogen receptors as agonists or antagonists depending on the target tissue [67]. In patients with endometriosis, the rationale for their use is related to the estrogen-antagonistic activity at endometrial level and estrogen-agonistic activity on bone and plasma lipoproteins [67]. Although studies on

animals looked very promising [68, 69], currently available data in humans on SERMs do not support their clinical use. In fact, a double-blind prospective study comparing raloxifene with placebo was halted early because the raloxifene group had statistically significantly earlier pain and necessity of a second surgery [70].

9. Immunomodulators

According to Sampson's theory, the retrograde menstruation is one of the essential pathogenic events in the development of endometriosis. It is argued that the immune system exerts a critical role in the development of a local immune response against endometrial cells in the peritoneal cavity. Studies both in vivo and in vitro suggest that the ectopic endometrial tissue have some features that allow it to escape immunosurveillance mechanisms [71]. In particular, a significant increase of cytokines and growth factors in peritoneal fluid, some alterations of the activity of B lymphocytes, increased antibody response, and an increase of concentration and activity of peritoneal macrophages have been observed [72, 73]. Based on these concepts, the use of two different types of immunomodulators in the treatment of endometriosis has been advocated: agents capable of stimulating the cell-mediated immune response and agents capable of reducing the inflammatory response.

9.1. Agents Capable of Stimulating the Cell-Mediated Immune Response. Immunomodulators agents investigated in the field of endometriosis include interleukin-12 (IL-12), interferon (IFN), and two synthetic immunomodulators (the analogue of guanosine, Loxoribine, and the agonist of nicotinic cholinergic receptor, Levamisole). Interleukin-12 is a heterodimeric cytokine that acts on T and NK cells inducing the production of INF-γ, which in turn increases the cytotoxic activity of NK cells. A mouse model in which endometriosis was induced by the injection in the peritoneal space of syngenic endometrial fragment was developed [74]. To evaluate the ability of IL-12 to prevent the development of the disease, mice were treated with and without recombinant forms of cytokines by daily intraperitoneal injections. With an examination of the peritoneum done 3 weeks later, it was found that the overall weight and the total area of the lesions were significantly lower in mice treated with IL-12 compared to the untreated controls [74]. In order to assess the efficacy of a IL-2 in humans, Acién et al. conducted two RCT that have shown poor results both in terms of resolution of pain and reduction of endometriotic lesions [75, 76]. Ingelmo et al. proposed a murine study to test the hypothesis that the immunomodulation with recombinant human IFN-α-2b plays a beneficial effect on the growth of endometrial foci [77]. The result was that these cytokines, in short regimens of administration, significantly reduce endometrial implants with a long-term effect. By using a similar model of endometriosis, Keenan et al. showed that loxoribine, but not Levamisole, determines the regression of both stromal and epithelial component of the endometrial graft [78]. Unfortunately, the available data in humans are still few. Acién et al. evaluated the efficacy of IFN-α-2b left in the pouch of Douglas after surgery, in women with endometriosis who were undergoing conservative laparotomy [79]. They observed recurrence of the disease in 42.3% of patients treated with IFN-α-2b against only 15.4% of cases without interferon [79]. Thus, these results conflict with those of experimental studies. This, probably reflects the need to more fully understand the complex immunological mechanisms involved in the pathogenesis of endometriosis.

9.2. Agents Capable of Reducing the Inflammatory Response. Pentoxifylline is a methylxanthine with antioxidants and anti-inflammatory properties, used in the treatment of rheumatoid arthritis and inflammatory bowel disease. It works by reducing platelet aggregation through inhibition of platelet phosphodiesterase. In addition, by inhibiting the same enzyme in monocytes and macrophages, the drug suppresses the action of tumor necrosis factor- (TNF-) α by operating on the extracellular part of the receptor [80]. The TNF-α is the acute phase cytokine, involved in many processes such as apoptotic cell death, proliferation, differentiation, tumorigenesis, and viral replication. It is produced largely by macrophages and also by a number of other cell types including lymphoid cells, mast cells, endothelial cells, fibroblasts, and nerve cells. Its concentration is increased in peritoneal fluid of women with endometriosis. It has been observed that TNF-α can stimulate the adhesion of endometrial cells and the proliferation of ectopic and eutopic endometrial tissues in women with endometriosis [81]. Furthermore, it induces the expression of metalloproteases that favours the invasion and the angiogenesis through regulation of IL-8 expression, and it performs cytotoxic action on gametes (with a possible role in infertility) [82]. It has been demonstrated that pentoxifylline may cause suppression of endometriotic lesions by suppressing angiogenesis through vascular endothelial growth factor- (VEGF-) C and flk-1 expression [83]. Furthermore, periovulatory treatment with pentoxifylline abrogates the adverse influence of endometrial explants on fertilization in a rodent model for endometriosis [84]. Conflicting results have been obtained in human studies evaluating the effect of pentoxifylline. Some studies have concluded that there is no evidence that immunomodulation with pentoxifylline aids fertility or decreases recurrence rate of signs and symptoms in women with different stages of endometriosis [85, 86]. Other studies have demonstrated that pentoxifylline after conservative surgery for endometriosis improves VAS scores at 2 and 3 months after the procedure when compared with patients having conservative surgery only [87] and that cumulative probability of pregnancy in 6 months after laparoscopic surgery in the patients receiving pentoxifylline was higher compared with that of the patients receiving placebo [88]. A recent Cochrane review has shown that there is still not enough evidence to support the use of pentoxifylline in the management of endometriosis in terms of subfertility and relief of pain [89].

A treatment with TNF-binding protein 1 (10 mg/kg for 7 days) has been tested in a rat model [90]. A reduction of 33% and 64% in the size of endometriotic lesions, respectively, after 2 and 9 days after the end of treatment, has

been observed [90]. Recent studies have reached similar conclusions using a mouse model with endometrial tissue grafts at different sites (subcutaneous tissue, peritoneum, and ovary) [91]. Treatment with anti-TNF therapy (etanercept) has been evaluated in baboon with spontaneous endometriosis [92]. Evaluating 12 baboons treated with placebo or etanercept, a significant decrease in the amount of spontaneously occurring active endometriosis was observed in animals treated with etanercept after 8 weeks of treatment [92]. It has been reported that neutralization of TNF activity with recombinant human TNFRSF1A (r-hTBP1) was as effective as GnRH antagonist in inhibiting the development of endometriosis without hypoestrogenic effects in baboons [93]. Similar results have been obtained treating baboons with a monoclonal antibody (mAb) to TNFα [94]. A reduction of the extention of induced peritoneal endometriosis, without interfering with the spontaneous menstrual cycle, has been observed after 25 days of treatment [94]. Only one RCT has been conducted in human [95]. This trial involving 21 participants showed no evidence of an effect of infliximab on endometriotic lesions, dysmenorrhoea, dyspareunia, or pelvic tenderness [95]. Recently, a systematic review has concluded that there is not enough evidence to support the use of anti-TNF-α drugs in the treatment of pelvic pain associated with endometriosis [96]. Moreover the increase of the risk of serious infections and malignancies in patients treated with long-term anti-TNF antibody therapy should be taken into account [97, 98].

10. Peroxisome Proliferator Activated Receptor γ Ligands

The peroxisome proliferator activated receptors (PPAR) represent a group of nuclear protein receptors that act as transcription factors [99]. They regulate the expression of certain genes involved in the processes of cell differentiation, development, and metabolism of carbohydrates, lipids, and proteins. PPARγ ligands inhibit estrogen biosynthesis by blocking the cytochrome P450 [100]. It has, recently, been shown that PGE2 receptors EP2 and EP4 mediate actions of PPARγ agonist by incorporating multiple cell signaling pathways [101]. PPARγ is expressed also in endometriotic stromal cells (ESCs) [102]. Ciglitazone, pioglitazone, and rosiglitazone are thiazolidinediones, a class of compounds that show high affinity for PPARγ. Experimental studies on human endometriotic cell lines and murine models have demonstrated that the activation of PPARγ by ciglitazone and rosiglitazone may inhibit endometriosis proliferation [103–105] and that pioglitazone reduces the TNF-alpha-induced IL-8 production and the proliferation of ESCs [102]. The efficacy of pioglitazone has also been reported in a baboon endometriosis model [106]. The animals were treated with oral pioglitazone 7.5 mg or placebo daily for 24–42 days. The surface area and volume of endometriotic lesions were significantly lower in pioglitazone treated baboons than in the placebo group [106].

11. Antiangiogenic Agents

Vascular endothelial growth factor (VEGF) is the most important angiogenic factor involved in the pathogenesis of endometriosis [107]. It is a glycoprotein able to stimulate the proliferation of endothelial cells in vitro and angiogenesis in vivo. In endometriosis VEGF causes the growth of the ectopic implants. A higher level of VEGF in eutopic endometrium and red endometriotic implants of patients with endometriosis compared with healthy patients has been found; this level was correlated directly with the severity of endometriosis [108]. An increased expression of the gene encoding for VEGF in the endometrium of affected patients has also been observed [108]. Moreover, it has been shown that the peritoneal fluid from patients with endometriosis contained significantly greater amounts of VEGF than controls and that this may be critical in the pathogenesis of endometriosis [109]. In particular, only immature blood vessels (i.e., with endothelium not surrounded by pericytes) respond to VEGF. When the pericytes cover the endothelial cells, the vascular structures become mature and therefore resistant to VEGF. It has been observed that over 80% of human blood vessels present in heterotopic endometriotic lesions are pericyte-free, a percentage significantly higher than that observed in the eutopic endometrium [110]. On the basis of these considerations it has been suggested the use of antiangiogenic drugs in the treatment of endometriosis. Encouraging results have been obtained in animal models [111, 112].

12. Melatonin

In the peritoneal fluid of women with endometriosis an upregulation of free radicals (ROS) and a depletion of antioxidants have been observed [113]. Melatonin (N-acetyl-5-methoxytryptamine), the principal secretory product of the mammal pineal gland, is a scavenger of free radicals and it is a broad spectrum antioxidant [114]. The antioxidant, immunomodulatory, and anti-inflammatory effects of melatonin have been tested in an animal model [115]. Endometriosis was surgically induced in 25 rats; then a subgroup ($n = 11$) was treated with melatonin administered intraperitoneally, while another subgroup ($n = 11$) did not receive any treatment. Four weeks later, regression and atrophy of endometriotic lesions were noted in the melatonin-treated group only [115]. Following studies in pinealectomized mice have shown an increase of endometriotic lesions in the animals not treated with melatonin compared to those treated [116]. Furthermore, a higher concentration of molecules associated with oxidative stress such as malondialdehyde (MDA) and a statistically significant reduction of antioxidant activity were observed in pinealectomized mice [116].

13. Selective Progesterone Receptor Modulators (SPRMs)

Selective progesterone receptor modulators (SPRMs) represent a class of progesterone receptor ligands that display progesterone agonist, antagonist, or mixed agonist/antagonist activity on several progesterone target tissues [117]. The

SPRMs were originally derived from norethindrone by the addition of a bulky substitute in the C11 position [118]. The daily administration of SPRMs induces amenorrhea through mechanisms that have not yet been fully elucidated. SPRMs inhibit the ovulation, but estradiol secretion is not affected and circulating levels of estradiol remain in the physiological range [119]. The thickening of the arterial wall might have a role in causing amenorrhea [120]. SPRM treatment induces particular endometrial changes, named PRM-associated endometrial changes (PAECs) that have not been previously observed in clinical practice [121, 122]. Endometrium of women receiving SPRM shows scanty mitosis, no atypical hyperplasia, asymmetry of stromal and epithelial growth, and prominent cystically dilated glands [121, 122]. These findings (PAECs) appear to be the result of a mixed estrogen (mitotic) and progestin (secretory) activity. Regression of endometriotic lesions with SPRM treatment has been observed in animal models [123, 124]. Few clinical studies have been so far conducted with SPRMs. An early report on 9 women showed that 50 mg of mifepristone for 6 months was effective in improving the symptoms and causing regression of endometriosis without significant side effects [125].

Asoprisnil has been tested in two randomized, placebo-controlled, dose-finding phase II studies. In the first study, women with laparoscopic diagnosis of endometriosis and having moderate or severe pain received asoprisnil (5, 10, and 25 mg) or placebo for 12 weeks [126]. All three doses of the drug resulted effectively in reduction of pain at same degree. By contrast, the effect on bleeding pattern was dose-dependent. The second study concluded that 5 mg represents the minimum effective dose for pain relief [117]. No relevant adverse events were observed during treatment or follow-up period.

A 21-substituted-19-norprogestin, Telapristone acetate (CDB-4124) [127], has also been investigated in the treatment of endometriosis [128]. Twenty-nine premenopausal women were treated with incremental oral daily doses of 12.5, 25.0, or 50.0 mg [128]. The study was aimed to evaluate only endometrial changes from a short term treatment. Endometrial biopsies, obtained after 3 or 6 months, showed histologic changes that are not seen during normal menstrual cycles. Short-term CDB-4124 treatment causes disorganized endometrial gland architecture of admixed cystic and tubular glands with nonphysiologic combinations of secretory and proliferative characteristics [128]. With increasing dose and time of administration, the endometrium becomes increasingly atrophic and the cysts more regularly prominent [128]. None of the CDB-4124-treated patients developed endometrial cancer or hyperplastic lesions during the study [128]. SPRMs are generally well tolerated. Only few cases of ovarian cysts, mostly small, asymptomatic and reversible, and dose-dependent increase of liver enzyme have been reported. Common adverse effects are headache, abdominal pain, nausea, dizziness, and metrorrhagia. In particular, headache was mainly present in the first few days of treatment, during the menstrual cycle [119].

14. Histone Deacetylase Inhibitors

Recently, epigenetic alterations in endometriotic cells, including the gene methylation of progesterone receptor- (PR-) B, steroidogenic factor-1 (SF-1), and estrogen receptor- (ER-) β have been described [129, for review]. Since demethylation agents and histone deacetylase inhibitors (HDACIs) can reactivate genes silenced by promoter hypermethylation, HDACIs have been suggested for treating endometriosis [129]. It has been shown that HDACIs trichostatin A and valproic acid can inhibit proliferation of endometrial stromal cells [129]. Moreover, it has been demonstrated that both these HDACIs reduce lesion growth and hyperalgesia in experimentally induced endometriosis in mice and rats [130, 131]. In human, acid valproic has been used for treating adenomyosis in two pilot studies [132, 133]. In the first study, after 3-month treatment, all three recruited patients reported complete disappearance of dysmenorrhea, with an average of one-third reduction in uterus size [132]. In the second pilot study 12 patients with confirmed adenomyosis, dysmenorrhea, and enlarged uterus were recruited [133]. After 3 months of valproic acid treatment, they were randomly assigned to 2 groups, one receiving no further treatment and the other the insertion of a LNG-IUS, and were followed up for an additional 3 months. At the end of the study, all patients showed complete resolution of dysmenorrhea and an average reduction in uterine size by 26%, regardless of whether LNG-IUS was used or not [133]. Thus, valproic acid seems to be a promising drug for treating adenomyosis.

15. Conclusion

In the last decades, an impressive amount of pharmacological agents have been tested for the treatment of endometriosis-associated pelvic pain. Some of them resulted ineffective, others proved unfit for clinical use due to significant side effects, while some others seem to be very promising but should be investigated in RCTs. Only very few have been introduced in clinical practice. Following the results of the controlled studies available, to date, the first-line treatment for endometriosis-associated pain is still represented by oral contraceptives used continuously. Progestins represent an acceptable alternative. In women with rectovaginal lesions or colorectal endometriosis, NETA at low dosage should be preferred. GnRH analogues may be used as second-line treatment, but significant side effects should be taken into account. Nonsteroidal anti-inflammatory drugs are widely used, but there is inconclusive evidence for their efficacy in relieving endometriosis-associated pelvic pain. Other agents such as GnRH antagonist, aromatase inhibitors, immunomodulators, selective progesterone receptor modulators, and histone deacetylase inhibitors seem to be very promising, but there is not enough evidence to support their introduction into routine clinical practice. Some other agents, such as peroxisome proliferator activated receptors-γ ligands, antiangiogenic agents, and melatonin have been proven to be efficacious in animal studies, but they have not yet been tested in clinical researches.

Conflict of Interests

The authors declare that no conflict of interests exists.

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Research Article

Antiangiogenesis Therapy of Endometriosis Using PAMAM as a Gene Vector in a Noninvasive Animal Model

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Objective. To evaluate the characteristics and antiangiogenic effects of endostatin-loaded PAMAM on endometriosis in a noninvasive animal model. *Materials and Methods*. A noninvasive animal model was established by injecting adenovirus-GFP transfected endometrial stromal and glandular epithelial cells subcutaneously into nude mice. Endostatin-loaded PAMAM was prepared and identified by transmission electron microscopy. For *in vitro* studies, the DNA protection and cytotoxicity of PAMAM were investigated and compared with Lipofectamine 2000. For *in vivo* study, endostatin-loaded PAMAM was injected into the noninvasive model and evaluated by continuously observing the fluorescent lesion, lesion weight, microvessel density and VEGF immunostaining. *Results*. Compared with Lipofectamine 2000, PAMAM and HC PAMAM-ES group, MC PAMAM-ES group and LC PAMAM-ES group demonstrated a better stromal cells protective such that MC PAMAM-ES group of CCK8 was 0.617 ± 0.122 at 24 hr and 0.668 ± 0.143 at 48 hr and LC PAMAM-ES group of CCK8 was 0.499 ± 0.103 at 24 hr and 0.610 ± 0.080 at 48 hr in stromal cells ($P < 0.05$) but similar cytotoxicity in glandular epithelial cells *in vitro*. After 16 hrs of digestion, DNA decreased slightly under the protection of PAMAM. Endostatin-loaded PAMAM of HD PAMAM-ES group and LD PAMAM-ES group inhibited the growth of the endometriotic lesion *in vivo* at days 15, 20, 25 and 30 detected by noninvasive observation after injecting one dose endostatin of various medicines into the endometrial lesion in each mouse on day 10 ($P < 0.05$) and confirmed by lesion weight at day 30 with HD PAMAM-ES group being 0.0104 ± 0.0077 g and LD PAMAM-ES group being 0.0140 ± 0.0097 g ($P < 0.05$). Immunohistochemistry results showed that endostatin-loaded PAMAM reduced the microvessel density 3.8 ± 2.4 especially in HD PAMAM-ES group in the lesion ($P < 0.05$). *Conclusion*. Endostatin-loaded PAMAM inhibits the development of endometriosis through an antiangiogenic mechanism and can be observed through the noninvasive endometriosis model.

1. Introduction

Endometriosis, defined as functioning endometrium outside the uterine cavity, is a common disease in women of reproductive age. Patients suffering from endometriosis may develop chronic pelvic pain, dysmenorrhea, dyspareunia, and infertility [1]. The prevalence of endometriosis has increased in recent years, while the etiology and mechanism have not been completely understood. Previous research shows that retrograde shedding [2], adherence and ectopic implantation [3, 4], and angiogenesis [5, 6] of the endometrium are three important steps in the development of endometriosis. In these processes, angiogenesis plays an important role in the formation of endometriosis because growing ectopic lesions need rich blood supply. Therefore, antiangiogenesis therapy is an important approach in the management of endometriosis.

In previous studies, endostatin has been identified as a useful and safe inhibitor for angiogenesis [1, 7–11]. However, as a protein reagent, endostatin can only function for a short time and thus is not suitable for a recurrent disease such as endometriosis. Therefore, we presumed that, a long-term drug delivery system, transferring endostatin gene into endometriotic lesions might be a better strategy for the treatment of endometriosis.

On the basis of our previous studies, the 20 μg endostatin gene transduced by 65 μg Lipofectamine 2000 inhibited the growth of endometriotic lesions with some stromal cells (ESCs) inhibited without any reproductive side effects in a nude mouse model [12]. Recently, a novel compound, polyamidoamine (PAMAM) dendrimers, has been certified as a nontoxic gene vector with high transduction efficiency [13]. However, the effect of PAMAM varied in different studies and has not been confirmed in the research on endometriosis.

To test the effect of the PAMAM-transduced endostatin gene on endometriosis, we applied the same noninvasive animal model established successfully by our research group [14]. In this model, we labeled endometrial stroma cells (ESCs) and grandular epithelial cells (EECs) separately with green fluorescence to form human endometriotic lesions by isolation-transfection-incubation procedure in nude mice and followed the fluorescent label *in vivo* noninvasively. This model helped us to repeatedly observe the lesion, and the application of the image analysis software helped us to study lesion changes quantitatively. At the same time, the accurate quantitative application of PAMAM and its effect are assessed *in vitro* and *in vivo*.

2. Materials and Methods

2.1. Endometrium Sample Collection. Samples of proliferative phase endometrium which are confirmed by histology were obtained from 16 women who received hysteroscopy for diagnostic purposes in our department between April and June 2009. All participants were aged 20–45 yrs (mean age 30), without internal complications and with regular menstrual cycles and no hormonal treatment for the last 3 months before surgery. Pieces of normal endometrium were obtained in surgery and immediately transferred into 4°C DMEM/F12 (Gibco, Carlsbad, CA, USA) supplemented with 1% penicillin and streptomycin (HyClone, Logan, UT, USA). An informed written consent was given by each patient before tissue collection. This protocol was approved by the First Affiliated Hospital of Sun Yat-sen University Ethical Review Committee.

2.2. Isolation and Primary Culture of ESCs and EECs. All experiments started within 1 hr after collection of the endometrium. The isolation of endometrial cells was in accordance with the methods stated in Ryan et al. [15] with some modification. In detail, endometrial tissue was washed 3 times with PBS, transferred to DMEM/F12 (Gibco, Carlsbad, CA, USA), supplemented with 1% penicillin and streptomycin (HyClone, Logan, UT, USA), and cut into 1–2 mm^3 pieces. Then, the tissue was digested with 2 mg/mL collagenase I (Gibco, Carlsbad, CA, USA) at 37°C in 5% CO_2 for 1 hr. After digestion, a sterile 150 μm polyethylene mesh filter (Shen Yue instrument store, Guangzhou, China) was used to remove undigested debris, followed by a 45 μm cell strainer to separate EECs from ESCs. The EECs were backwashed with DMEM/F12 from the 45-μm cell strainer (Shen Yue instrument store, Guangzhou, China) onto the dish. After being centrifuged, the ESCs and EECs were resuspended with DMEM/F12 and 10% FCS then cultured in a 6-well plate.

2.3. Preparation and Identification of the Endostatin Plasmid Mixture. The preparation of the PAMAM-Es plasmid mixture was performed according to the Dendritech Company guidelines. Six PAMAM dendrimers were dissolved in water at a concentration of 1 mg/mL and stored at 4°C. The human recombined endostatin plasmid was stored at −20°C. When used, the PAMAM solution and the endostatin plasmid were both rewarmed in room temperature on the bench for 20 min and mixed together at a ratio of 3.25 μg : 1 μg (PAMAM : endostation). After 20 min of coincubation, the mixture was identified by JEM-2010HR transmission electron microscopy (JEOL, Tokyo, Japan).

As a traditional gene vector, Lipofectamine 2000 was taken to compare with PAMAM in both *in vitro* and *in vivo* studies. The preparation of the Lipofectamine-endostatin plasmid mixture (Lipo-Es) was in accordance with the routine of our laboratory. Briefly, Lipofectamine 2000 and endostatin plasmid were rewarmed in room temperature on the bench top for 20 min and mixed together at a ratio of 3.25 μg : 1 μg and cultured for another 20 min before injection.

2.4. In Vitro Studies on the DNA-Protection Effect and Cytotoxicity of PAMAM. To determine the protection of plasmid by PAMAM, 5 U of DNase I was added into a 50 μL solution containing PAMAM-Es or Lipo-Es plasmid or naked plasmid. The primary plasmid concentration in each group was 0.200 mg/mL. OD260 values were detected by biophotometer after 1, 4, 8, and 16 hrs.

Cytotoxicity of PAMAM and PAMAM-Es was processed on primary cultural ESCs or EECs. Purified cells were cultured in 96-well plates with a density of 5000 cells/well for ESCs and 20 cells mass/well for EECs, respectively. Twenty-four hours after primary culture, the medium in the 96-well plates was discarded and 100 μL of OptiMEM (Gibco BRL, Grand Island, NY, USA) was added into each well. Then, the PAMAM-Es mixture with various dilutions, the Lipo-Es mixture, and the PAMAM solution (shown in Table 1) were added into each group. After culturing for 24 hrs, 10 μL of the CCK-8 reagent was added into each well, incubated in 37°C, 5% CO_2 for 2 hrs, and detected by a Tecan biophotometer at 495 nm.

2.5. In Vivo Observation and Quantitative Analysis of GFP-Expressing Lesions in Nude Mice. According to our preliminary work [14], the isolation-transfection-incubation mixed cells injecting subcutaneous procedure was also performed in this experiment. Forty female nude mice (BALB/c), aged 6–8 weeks and weighing 17–21 g, were provided by the National Rodent Laboratory Animal Resources, Shanghai Branch (Shanghai, China). Two days before endometrial cell injection, a sterile 60-day release pellet, containing 1.7 mg of 17-beta E2 (Innovative Research of America, USA), was added to implant s.c. for every nude mice.

Table 1: Medicines used in cytotoxicity test.

Group	Plasmid (μg/mL)	Vector (μg/mL)	Number of wells
(1) HC PAMAM-Es	10	32.5	6
(2) MC PAMAM-Es	1	3.25	6
(3) LC PAMAM-Es	0.1	0.325	6
(4) Lipofectamine-Es	10	32.5	6
(5) PAMAM	—	32.5	6
(6) Blank control	—	—	6

Table 2: Medicines used in treatment of animal model.

Group	Endostatin plasmid (μg)	Vector (μg)	Number of mice
(1) HD PAMAM-Es	20	65	8
(2) LD PAMAM-Es	10	32.5	8
(3) Lipofectamine-Es	20	65	8
(4) PAMAM	—	65	8
(5) PBS	—	65 uL	8

For *in vivo* imaging of GFP-expressing endometrial lesions, animals were put on a fluorescent stereomicroscope (SZX16, Olympus, Tokyo, Japan) equipped with a 470 nm filter. Images were recorded with an Olympus DP71 digital camera (Olympus, Tokyo, Japan) fixed with a 515 nm viewing filter. Each mouse was observed 6 times on days 5, 10, 15, 20, 25, and 30 after cells implantation.

Quantitative analysis was completed primarily according to Fortin et al. [16]. In short, at each time point, three fluorescent images were acquired for each mouse. Image software was used to identify the number and intensity of pixels corresponding to the spectral signature of GFP (present only in regions of interesting where there is a lesion) and the size of each lesion. If more than one lesion was present on a mouse, the calculated surface is the sum of each individual lesion.

To investigate whether the fluorescent area in the imaged lesion is representative of the actual lesion size, we used a traditional method to measure the length (a) and width (b) of the lesion with a vernier caliper and calculated the volume of the lesion with a well-recognized formula ($V = (1/2)a \times b^2$). Then, a correlation test was applied to study the correlation between the volume of the lesion and the fluorescent area in the photo.

2.6. Treatment of Human Recombined Endostatin Plasmid Using PAMAM as a Vector. Forty nude mice were randomly divided into 5 groups according to their weight, lesion volumes, and fluorescent pixel numbers on days 5 and 10: (1) HD PAMAM-Es group: 20 μg Es/65 μg PAMAM; (2) LD PAMAM-Es group: 10 μg Es/32.5 μg PAMAM; (3) Lipofectamine-Es group: 20 μEs/65 μg Lipofectamine; (4) PAMAM group: 65 μg PAMAM; (5) PBS group: same volume PBS. Each group received different treatments (shown in Table 2) by injecting one dose of various medicines into the endometrial lesion in each mouse on day 10, just after the second noninvasive observation.

Three approaches were used to evaluate the antiangiogenesis effects of PAMAM-Es. First, the variation in fluorescent areas of the lesions before and after treatment was calculated and compared among each group. Second, volume changes among the 5 groups were analyzed. Third, after 30 days of *in vivo* observation, mice were sacrificed and lesions in different groups were excised and weighed with an electronic balance.

2.7. Evaluation of Antiangiogenesis Efficiency of PAMAM-Endostatin. After being weighed, the lesions were then fixed in 4% PFA for 24 hours at room temperature and embedded in paraffin. Sections of 5 μm were first stained with hematoxylin and eosin to evaluate the lesions' viability and quality. Microvessel density (MVD) and VEGF expression of the lesion were determined by immunohistochemistry staining using horseradish peroxidase detection system (Zhongshan, Beijing, China) with polyclonal rabbit anti-CD31 antibody (dilution of 1 : 250, Abcam) and monoclonal rabbit anti-human VEGF antibody (dilution of 1 : 250, Abcam). The slides were counterstained with hematoxylin.

Two blinded observers examined the tissue with a microscope (IX71, Olympus, Tokyo, Japan). For the MVD calculations, the regions with the highest microvessel density (hot spots) were scanned at low magnification (×40) as described by Weidner [17] and counted at a ×400 magnification in a blinded fashion. For each slide, microvessels were counted twice in 5 different high magnifications and the average was used as the final value.

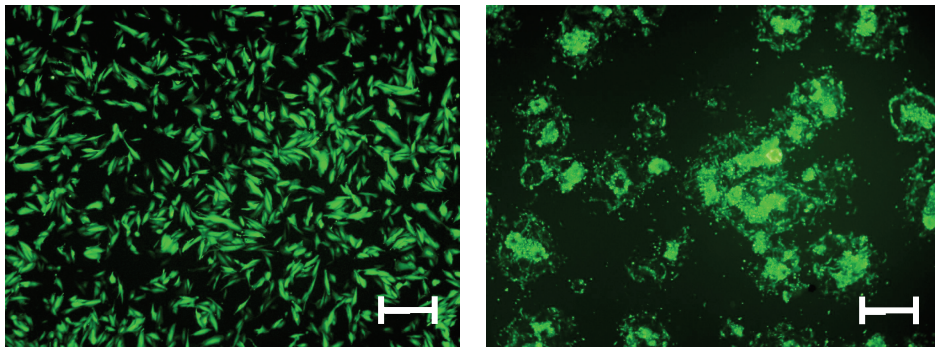
For VEGF analysis, slides were first scanned at low magnification (×40), and five fields of the immunostained sections were randomly chosen for histomorphometry at ×400 magnification. A semiquantitative evaluation of immunohistochemical staining for VEGF was performed according to the method described by Donnez et al. [18], involving the analysis of the distribution and the intensity of staining within the endothelium and glandular epithelium or stroma. The histologic scores (H) for VEGF were calculated using the formula $H = \Sigma P_i$, where i is the intensity ranging from 0 (negative cells) to 3 (deeply staining cells) and P is the percentage of staining cells for each given i, with P values of 1, 2, 3, 4, and 5 indicating <15%, 15–50%, 50–85%, >85%, and 100% positive-staining cells, respectively. The staining result was expressed as the mean ± standard deviation.

2.8. Statistics. Data were analyzed by SPSS 13.0 software. For the cytotoxicity study *in vitro*, OD values of various treatments were described as the mean ± SD and analyzed by one-way ANOVA. For noninvasive *in vivo* studies, the corelationship between fluorescent pixel numbers and the volumes of each lesion was tested. A two-way ANOVA analysis (main effect: group and time; interaction: group and time) was applied to compare lesion fluorescent pixel numbers among groups. For the invasive study, lesion weight, MVD count, and VEGF histology scores were compared among groups using one-way ANOVA. In this study, all the ANOVA tests were followed by a post hoc Bonferroni test

Table 3: Cytotoxicity test of ESCs and EECs by CCK-8 Kit (absorbance, mean ± S.D).

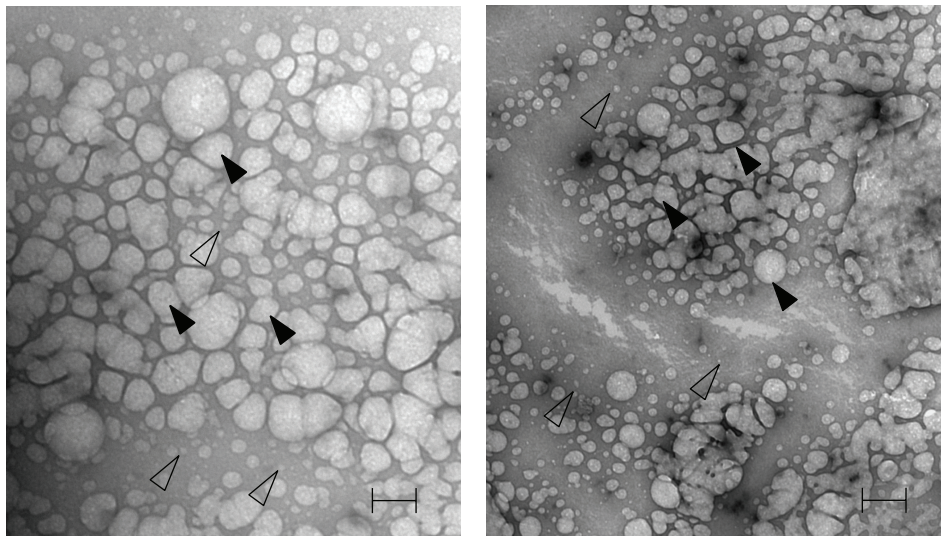
	Stromal cell		Glandular epithelial cell	
	24 h	48 h	24 h	48 h
Control	0.625 ± 0.155	0.648 ± 0.117	0.409 ± 0.252	0.233 ± 0.114
HC PAMAM-Es	0.118 ± 0.019[a]	0.088 ± 0.007[a]	0.385 ± 0.218	0.325 ± 0.060
MC PAMAM-Es	0.617 ± 0.122	0.668 ± 0.143	0.395 ± 0.156	0.308 ± 0.192
LC PAMAM-Es	0.499 ± 0.103	0.610 ± 0.080	0.337 ± 0.167	0.290 ± 0.071
Lipo-Es	0.202 ± 0.081[a]	0.136 ± 0.049[a]	0.347 ± 0.171	0.255 ± 0.063
PAMAM	0.167 ± 0.083[a]	0.096 ± 0.018[a]	0.328 ± 0.138	0.265 ± 0.054
F	31.444	71.95	0.193	0.651
P	0.000	0.000	0.963	0.663

[a]Statistic significant difference compared with control group.



(a) (b)

Figure 1: Primary cultural stromal cells (a) and glandular epithelial cell masses (b) expressed green fluorescent after adenovirus-eGFP transfection for 18 h (100x, Bar = 200 μm).



(a) (b)

Figure 2: Structure of PAMAM and PAMAM-Es was similar under transmission electron microscopy (37000x, BAR = 200 nm). (a) Morphology of PAMAM under TEM. (b) Morphology of PAMAM-Es under transmission electron microscopy. Open arrows: PAMAM monomer (diameter: 5–10 nm); close arrows: PAMAM polymerize compound (diameter: 50–200 nm).

Table 4: Area of fluorescent lesion in treatment and control groups at different time point (pixel).

Group	Day 5	Day 10	Day 15	Day 20	Day 25	Day 30
(1) HD PAMAM-Es[a]	223104 ± 60101	146451 ± 54606	83812 ± 52424	59549 ± 49634	33301 ± 36204	29676 ± 36394
(2) LD PAMAM-Es[b]	179970 ± 64910	111880 ± 59332	91058 ± 73949	55692 ± 46240	46290 ± 39019	43015 ± 41680
(3) Lipofectamine-ES[a,b]	194664 ± 62033	163435 ± 56293	113506 ± 50036	117513 ± 62957	120533 ± 71327	131120 ± 71257
(4) PAMAM[a,b]	200465 ± 58021	154130 ± 60652	128977 ± 62570	138480 ± 85150	160714 ± 105618	161172 ± 92100
(5) PBS[a,b]	184576 ± 57799	159941 ± 78005	130626 ± 50700	126370 ± 58317	111729 ± 44760	123874 ± 62956

[a]Statistic significant difference between Group 1 and Group 3, Group 1 and Group 4, and Group 1 and Group 5 by Bonferroni test. [b]Statistic significant difference between Group 2 and Group 3, Group 2 and Group 4, and Group 2 and Group 5 by Bonferroni test. (Injecting one dose of various medicines into the endometrial lesion in each group mouse on day 10.)

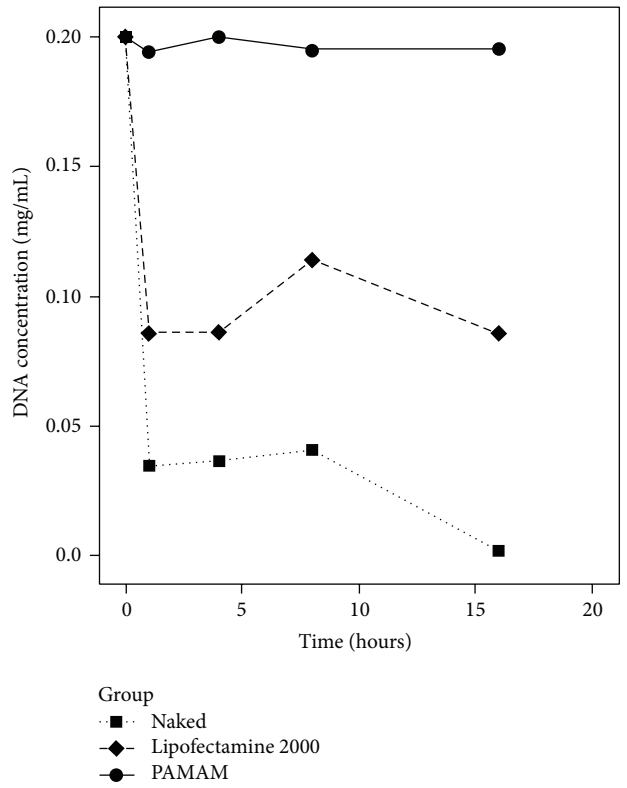


Figure 3: DNA concentration after digestion by DNase I (mg/mL).

Table 5: Lesion weight of different groups (gram, mean ± SD).

Group	Lesion weight
(1) HD PAMAM-Es[a]	0.0104 ± 0.0077
(2) LD PAMAM-Es[b]	0.0140 ± 0.0097
(3) Lipofectamine-Es	0.0253 ± 0.0158
(4) PAMAM[a]	0.0350 ± 0.0245
(5) PBS[a,b]	0.0378 ± 0.0170

[a]Statistic significant difference between Group 1 and Group 4 and Group 1 and Group 5 by Bonferroni test. [b]Statistic significant difference between Group 2 and Group 5.

to detect differences between groups. Two-tailed values of $P < 0.05$ were considered statistically significant.

Table 6: Numbers of MVD (CD31-stained vessels per ×400 magnification) and VEGF *H* score (per ×400 magnification) in endometriosis lesions in nude mice (mean ± SD).

Group	MVD	VEGF *H* score
(1) HD PAMAM-Es	3.8 ± 2.4[a]	3.8 ± 3.1
(2) LD PAMAM-Es	10.5 ± 3.9	4.7 ± 2.6
(3) Lipofectamine-Es	11.4 ± 4.7	5.2 ± 3.2
(4) PAMAM	11.9 ± 6.7[a]	7.0 ± 3.1
(5) PBS	12.1 ± 4.3[a]	8.1 ± 2.3

[a]Statistic significant difference between Group 1 and Group 4 and Group 5.

3. Results

3.1. The Characteristics of ESCs and EECs in Primary Culture and after Viral Transfection. ESCs attached to the 6-well plate 12 hrs after placement began to express GFP. The intensity of fluorescence increased until 18 hrs and was maintained at a high level (Figure 1). According to our previous result, after 18 hrs of incubation, both ESCs and EECs had high GFP positive rates and low apoptosis rates [14]. Thus, ESCs and EECs were harvested 18 hrs after transfection and collected to build a noninvasive animal model.

3.2. Morphology, DNA Protection Effect, and Cytotoxicity of PAMAM-Es. The morphology of PAMAM and PAMAM-Es was detected by transmission electron microscopy (Figure 2). The diameter of the PAMAM monomer ranged from 5 to 10 nm, consistent with the description of the product (5.4 nm). The PAMAM monomer polymerized and formed compounds with diameters ranging from 50 to 200 nm. No differences were found between PAMAM and PAMAM-Es in morphology under transmission electron microscopy.

In the study on the DNA protective effects, as shown in Figure 3, the DNA concentration of the PAMAM-Es mixture was much higher than the Lipofectamine-Es mixture and the naked endostatin plasmid after digestion by DNase I. After 16 hrs of digestion, the naked plasmid had almost broken down, and the plasmid protected by Lipofectamine reduced to approximately half of the primary concentration, while DNA decreased slightly under the protection of PAMAM.

To analyze cytotoxicity in various reagents to ESCs and EECs, OD495 absorbance, representing live cell activity, was compared among each group via one-way ANOVA. As shown in Table 3, toxicity levels among 6 types of reagents to ESCs,

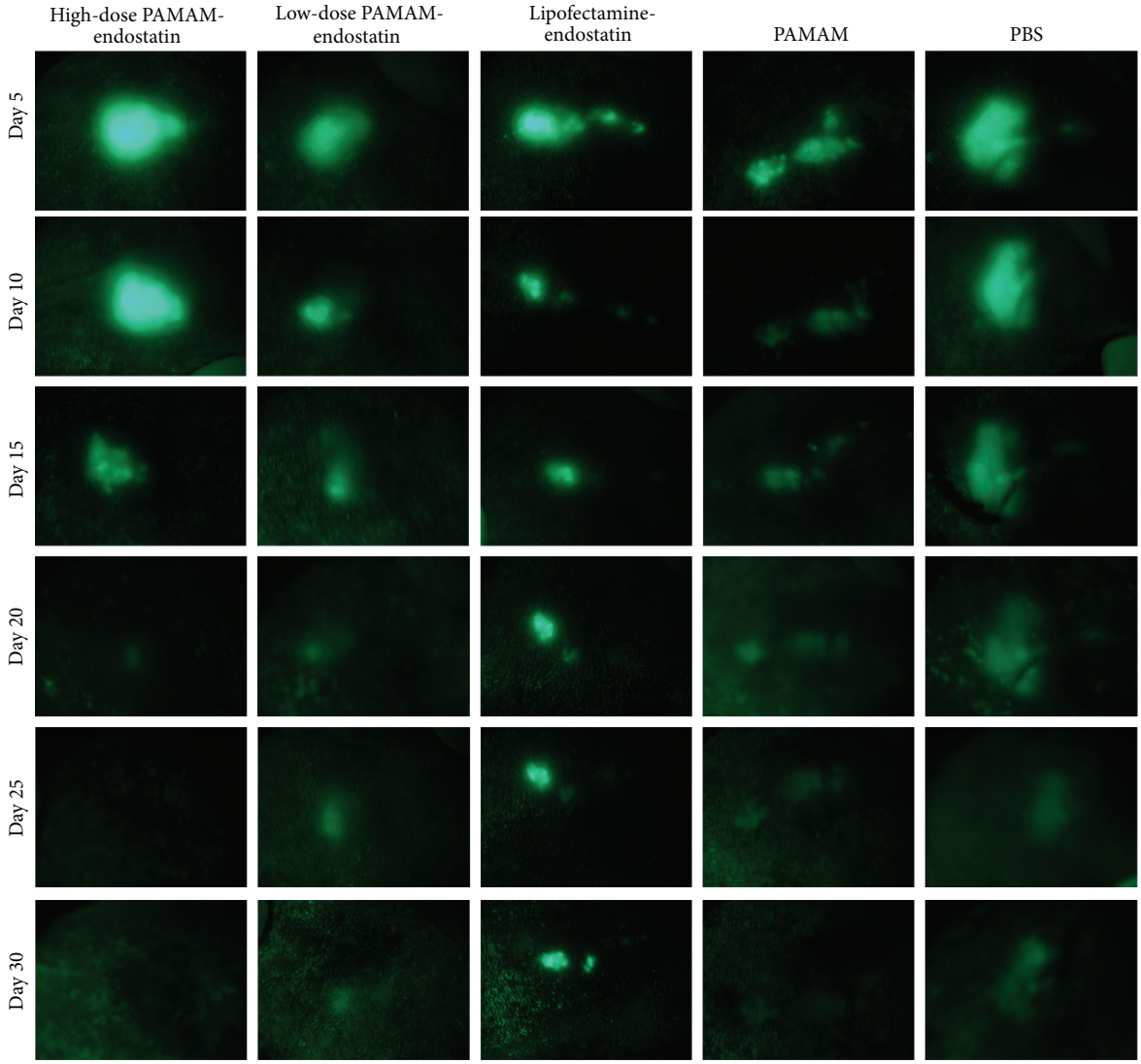


Figure 4: *In vivo* observation by fluorescent microscopy of nude mice in each group.

rather than to EECs, had statistically significant differences at both 24 and 48 hrs. The post hoc Bonferroni test showed that, when compared with the control group, high concentrations of PAMAM-Es, Lipo-Es, and PAMAM groups significantly inhibited the activity and growth of ESCs, while the inhibition of ESCs with moderate concentrations of PAMAM-Es and low concentrations of PAMAM-Es groups was not significant and demonstrated a better stromal cells protective.

3.3. In Vivo Observation and Therapeutic Effects of PAMAM-Endostatin. Using fluorescent microscopy, positive lesions on the models are easily detected (Figure 4). All fluorescent images were quantitatively analyzed. To identify the fluorescent area in the photos, we studied the corelationship between the fluorescent area in the image and the volume of the same lesion, as measured by the vernier caliper and calculated with a formula. The correlation test illustrated that, when combining data from five groups, there is a statistically significant positive correlation between the fluorescent pixel numbers and the lesion volumes (Figure 5, with a $R = 0.590$ and $P < 0.001$). This result demonstrated that the fluorescent area was representative of the lesion size in the noninvasive *in vivo* study. Therefore, we used the quantitatively analyzed fluorescent area to study the therapeutic effects of PAMAM-Es on endometriosis.

First, to evaluate the homogeneity of pretreatment states among groups, lesion volumes and fluorescent areas on days 5 and 10 were compared by one-way ANOVA. The results showed that no significant differences existed between the lesion volumes and the fluorescent areas among groups.

Then, we compared the inhibition of PAMAM-Es to endometriotic lesions with Lipofectamine-Es, PAMAM, and PBS controls. Two-way ANOVA tests showed that there was a statistically significant difference among the groups (Table 4). The post hoc Bonferroni test demonstrated that high-dose and low-dose PAMAM-Es reduced the size of the lesions compared with other reagent groups.

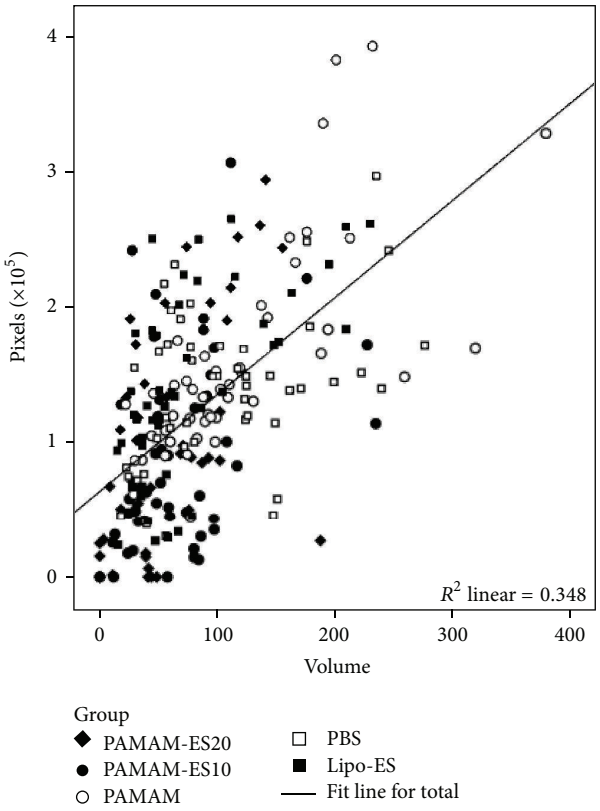


Figure 5: Correlation between fluorescent pixel number and lesion volume.

After 30 days of noninvasive observation, mice were sacrificed and the lesions were collected (Figure 6) and weighed. The one-way ANOVA test showed that the tumor burden among each group had a statistically significant difference. In the post hoc Bonferroni test, the HD PAMAM-Es group had a statistically significant lower lesion weight than PAMAM and PBS controls and the LD PAMAM-Es group had a statistically significant lower lesion weight than PBS controls (Table 5).

3.4. Evaluation of the Antiangiogenesis Efficiency of PAMAM-Es. To investigate whether PAMAM-Es inhibited endometriotic lesions through an antiangiogenesis mechanism, two well-recognized angiogenesis biomarkers, CD31 and VEGF, were detected by immunohistochemistry (Figure 7) on every lesion and compared among the groups. There was a statistically significant difference in MVD, calculated as CD31-stained vessels per ×400 magnification, among the 5 groups in the ANOVA test ($P = 0.023$). HD PAMAM-Es group showed a greater antiangiogenesis effect than both the PAMAM and PBS groups in the post hoc Bonferroni test (Table 6). The ANOVA test found no difference in the VEGF H score among the 5 groups ($P = 0.092$) (Table 6).

4. Discussion

Although several approaches can be applied, there is no ideal therapy method for endometriosis. Hormonal treatment has substantial side effects, whereas it does not remove the ectopic lesion radically. Conservative surgery can excise the ectopic lesion, but the disease recurs in part of patients. Radical surgery can directly affect reproducibility. Thus, new methods should be investigated to treat endometriosis [19]. Considering the characteristic of endometriosis, an ideal reagent should have followed these features: (1) reducing or removing the ectopic lesion; (2) reducing side effects, especially to the reproductive system; (3) preventing recurrence.

Endostatin is considered the most effective inhibitor of microvessel growth [1] and has been used to treat endometriosis [1, 7–11, 20, 21]. Our previous results [12] showed that 20 μg Lipofectamine mediated endostatin for lesion injection could significantly reduce the volume of the ectopic lesion by destroying established vessels after 21 days treatment or inhibiting angiogenesis factor-VEGF mRNA at day 3 treatment in the lesion. However, the effect of Lipofectamine mediated endostatin can last a period but still existed ESCs inhibited potentially. Common lesions in nude mice should wait near 28 days enough to form a 4 mm diameter lesion to observe the treatment result. To resolve this problem, we attempted to change noninvasive model and more effective vector in the experiment treatment.

The green fluorescent human endometriosis lesion model which was supported on isolation-transfection-incubation procedure by injecting GFP-Adenovirus transfected human ESCs and EECs subcutaneously into nude mice was continued to be used for noninvasively evaluating the effect of various treatments [14]. Except for high transfection rate and low apoptosis rate, there were several other advantages. First, GFP labeling enabled us to repeatedly observe the lesion in a noninvasive manner; thus, changes in lesions after treatment could be detected directly rather than from necropsy, and continuous data rather than endpoint information could be more accurately analyzed. Second, injection of separated cells rather than a whole tissue increased the reliability of the results. Researchers usually implanted whole pieces of transfected and nontransfected endometrium to build an endometriosis model. In these cases, although the same pieces of tissue were used in each host animal, the exact number of cells injected was unknown. However, in our study, the number of injected cells was controlled and the baseline of each host mouse was the same.

To the best of our knowledge, it was the first study applying polyamidoamine (PAMAM) dendrimers as a gene vector in the antiangiogenesis therapy of endometriosis. We primarily tested the cytotoxicity of the PAMAM-Es compound on ESCs and EECs *in vitro*. CCK-8 studies demonstrated that stromal cells were sensitive to high concentrations (HC) of PAMAM-Es, PAMAM, and Lipofectamine 2000-Es but not by moderate (MC) or low concentrations (LC) of PAMAM-Es demonstrating such that MC PAMAM-Es and LC PAMAM-Es reagent could be more safely used for treatment than Lipofectamine 2000 that we have used before. On the DNA protective effects, DNA decreased slightly under the protection of PAMAM. On the basis of *in vitro* experiment, the dose of endostatin used in the MC PAMAM-Es group was 0.00001 μg per stromal cell *in vitro*, which is calculated to be applied with HD PAMAM-Es group (20 μg

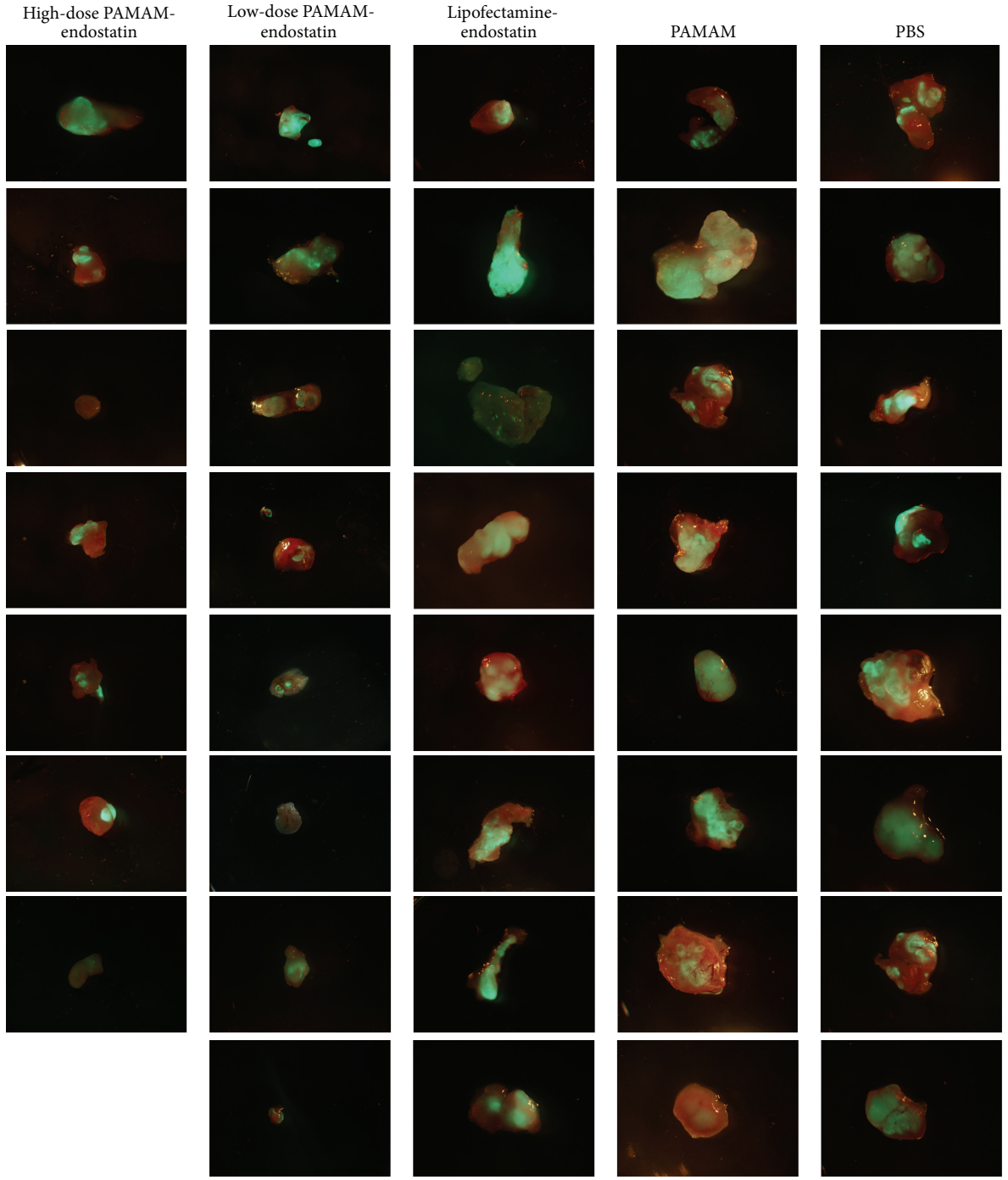


Figure 6: Fluorescent lesions observed after necropsy (combined by fluorescent imaging and white light picture).

Es/2 $*$ 10^6 ESCs) *in vivo*. *In vivo* noninvasive observations in the nude mice model illustrated that PAMAM-Es with a dose-dependent effect had been more effective on inhibiting the growth of endometriotic lesions and avoiding ESCs affected than the Lipofectamine Es.

To uncover whether PAMAM-Es inhibited the growth of endometriotic lesions because of its antiangiogenesis effect, we employed the fluorescent pixel number which was positive correlation to lesion volume and the immunohistochemistry to detect the microvessel density of the lesion. The results demonstrated further that 20 μg PAMAM-Es significantly directly reduced the microvessel density leading to a decrease in fluorescent pixel number. These noninvasive observing results support our previous research not only on the endostatin gene therapy but also on PAMAM mediated for the management of endometriosis. There are primarily two strategies for antiangiogenesis therapy. One is to prevent angiogenesis in the newly developing lesion; the other is to

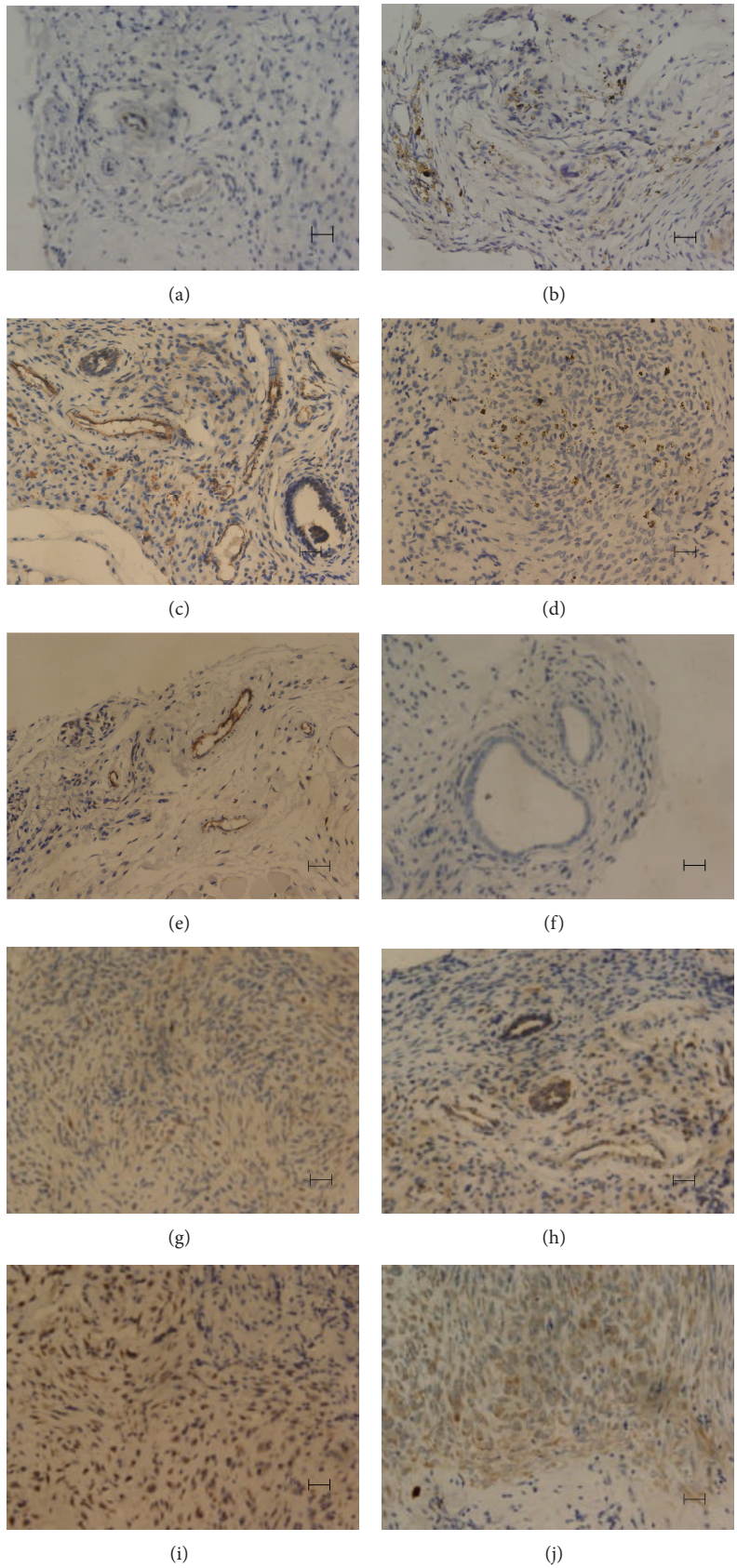


Figure 7: CD31 and VEGF expression in endometrial lesions in nude mice (400x, Bar = 100 μm) (a–e): CD31 expression in microvessels in the implanting lesions ((a) HD Pamam-Es; (b) LD PAMAM-Es; (c) Lipofectamine-Es; (d) PAMAM; (e) PBS); (f–j): VEGF expression in cells of the implanting lesions ((f) HD Pamam-Es; (g) LD PAMAM-Es; (h) Lipofectamine-Es; (i) PAMAM; (j) PBS).

inhibit vascular growth in the established lesion. Therefore, in experimental endometriosis, antiangiogenesis reagents were used before or at the time of transplantation as preventive measures or after lesion formation as treatment. Eggermont et al. demonstrated that newly developing vessels were usually established between 5 and 8 days after graft implantation [22]. Thus, our data suggested that, after transduction and expression, endostatin could also inhibit established vessels in the endometriotic lesion after 10 days implantation.

Further, VEGF is considered a pivotal angiogenic factor by inducing endothelial cell-specific mitogenic and vascular permeability activities [20, 23]. Immunostaining showed that PAMAM-Es did not induce a reduction directly in VEGF expression after 20 days treatment as same as the lipofectamine Es but short effect should be confirmed in the future experiment.

In summary, compared with the traditional gene carrier Lipofectamine *in vitro* and *in vivo,* study in the present research demonstrates that PAMAM is an ideal vector in gene therapy for the treatment of endometriosis. Endostatin-loaded PAMAM inhibits the development of endometriosis through an antiangiogenic mechanism and can be observed through the green fluorescent endometriosis model.

5. Conclusions

Endostatin-loaded PAMAM inhibits the development of endometriosis through an antiangiogenic mechanism and can be observed through the noninvasive endometriosis model.

Disclosure

Ningning Wang and Bin Liu are cofirst authors.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Clinical Study

Are Mood and Anxiety Disorders and Alexithymia Associated with Endometriosis? A Preliminary Study

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Objective. The aim of this preliminary study was to determine whether psychiatric disorders, psychopathological symptoms, and alexithymia are associated with endometriosis in an Italian population. *Study Design.* A preliminary study comprising 37 Italian patients with surgically confirmed endometriosis and 43 controls, without clinical and ultrasound signs of endometriosis, was carried out. Both patients and controls were evaluated for the presence/absence of psychiatric disorders, psychopathological symptoms, alexithymia, and pain symptoms (nonmenstrual pelvic pain, dysmenorrhea, and dyspareunia). *Results.* Statistically significant differences were found between cases and controls for prevalence of mood and anxiety disorders, malfunctioning on obsessive-compulsive subscale ($P < 0.01$) and depression subscale ($P < 0.05$) of the Symptom Checklist-90-Revisited (SCL-90-R), and higher alexithymia levels ($P < 0.01$). Patients with endometriosis-associated pain showed greater prevalence of psychiatric disorders compared to pain-free patients but that difference was not significant. Significant correlation was found between malfunctioning in some SCL-90-R dimensions and pelvic pain, dysmenorrhea, and dyspareunia scores at the visual analog score (VAS). *Conclusion.* Some psychopathological aspects, such as psychoemotional distress and alexithymia, are more frequent in women with endometriosis and might amplify pain symptoms in these patients.

1. Introduction

Endometriosis is a gynecological condition characterized by the presence of ectopic endometrial tissue (endometrial glands and stroma) outside of the uterus associated with pelvic pain and infertility [1]. The disease affects 6–10% of women in reproductive age, 50–60% of women and adolescent girls with pelvic pain, and more than 50% of infertile women [2, 3] and has a severe impact on the quality of life and work ability of employed women, representing a significant socioeconomic burden [4–7]. Endometriosis varies from a mild disease with only peritoneal lesions to a severe form involving both ovaries associated with infiltrating lesions and extensive adhesions. Women with endometriosis may have a range of pelvic and abdominal pain symptoms, including dysmenorrhea, dyspareunia, nonmenstrual (chronic) pelvic pain, pain at ovulation, dyschezia, and dysuria [8–10]. Endometriosis and its symptoms tend to recur after treatment [11]. Pain symptoms significantly vary among patients and do not always correlate with the severity of endometriosis [8–13] suggesting that other factors, such as psychological factors, altered stress response, and emotional factors, may influence

the perception of pain [14]. Different studies have shown an increased prevalence of depressive symptoms and anxiety in women with endometriosis highlighting the importance of mood disorders in the perception of pain in these women [15–17].

The aim of the present study was to preliminarily evaluate the role of psychopathological symptoms, comorbid psychiatric disorders, and alexithymia in endometriosis patients compared with healthy women.

2. Materials and Methods

2.1. Patients and Controls. Thirty-seven Italian Caucasian women with endometriosis (mean age 35 ± 7.6 years), ranging from unskilled workers to university graduates, were included in the study. Patients were recruited from the Department of Gynecology and Obstetrics, "Sapienza" University of Rome, Italy. Diagnosis of endometriosis was achieved by laparoscopy and histologic analysis. Twenty-one of them (56.8%) had ovarian endometrioma, while sixteen (43.2%) had ovarian endometrioma and peritoneal endometriosis. No other comorbid physical conditions were present. As healthy controls, 43 women from the same ethnic area, referred to the gynecological clinic for a gynecological control, were enrolled, mean age of 34.9 ± 10.1 years and medical history, vaginal pelvic examination, and ultrasound (US) imaging with color Doppler flow evaluation negative for endometriosis. The ethical committee of the "Sapienza" University of Rome approved the study protocol and all subjects provided their informed consent.

2.2. Symptoms and Pain Assessment. Patients were evaluated for the presence/absence of symptoms (nonmenstrual pelvic pain, dysmenorrhea, and dyspareunia). Pain intensity was assessed by a 10-point visual analogue scale (VAS) with 0 representing no pain and 10 representing the worst pain [18].

2.3. Psychometric Testing. Assessment of psychiatric disorders was performed using the Structured Clinical Interview for DSM IV Axis I Disorders Clinical Version (SCID-I cv) [19] for each subject, after a clinical interview performed by a psychiatrist experienced in the use of this instrument.

The presence of psychopathological symptoms was investigated using the Symptom Checklist-90-Revised (SCL-90-R), a 90-item self-report instrument that has been designed to evaluate a broad range of psychological problems and symptoms of psychopathology [20]. We considered 9 subscales: somatization (SOM), obsessive-compulsive (OC), interpersonal sensitivity (SENS), depression (DEP), anxiety (ANX), hostility (HOS), phobic anxiety (PHOB), paranoid ideation (PAR), and psychoticism (PSY) (24). A score > 60 T points was indicative of malfunctioning.

The Toronto Alexithymia Scale (TAS-20) was used to assess alexithymia. It consists of 20 self-report items and has 3 subscales: Difficulty Describing Feelings (F1), Difficulty Identifying Feelings (F2), and Externally Oriented Thinking (F3). A TAS-20 total score ≥ 61 is diagnostic for alexithymia [21].

2.4. Statistical Analysis. Basic statistical analyses were performed with the Statistical Product and Service Solutions software (SPSS) version 17.0 WIN program (SPSS, Chicago, IL). The χ^2 or Fisher's exact tests were used to compare differences between categorical variables, whereas those between continuous variables were determined by Student's t-test or Mann-Whitney U test. Spearman's rho correlation coefficient was applied to analyze nonparametric correlations. A P value < 0.05 was considered statistically significant. Test results from the nine SCL-90-R subscales were processed by ARC90 v1.2.1 software [22].

3. Results

Thirty-seven patients and 43 controls underwent psychiatric evaluation. The two groups were homogeneous for age (patients mean age 35.0±7.6, controls mean age 34.9±10.1; $t = -0.034$, $P = 0.973$), educational status ($\chi^2 = 5.335$; $P = 0.149$), marital status ($\chi^2 = 6.713$; $P = 0.082$), and occupational status ($\chi^2 = 5.915$; $P = 0.657$). Pain evaluation showed higher dyspareunia and pelvic pain VAS mean scores in patients with endometriosis than in controls, while no differences were observed for dysmenorrhea VAS mean scores (Table 1).

The frequency of psychiatric disorders was 54.0% in patients and 18.6% in controls ($\chi^2 = 10.985$; $P = 0.001$); the relative percentages of each disorder are shown in Table 2. No significant difference was observed between the two groups regarding the presence of a specific psychiatric disorder; while grouping the disorders in categories the frequencies of mood and anxiety disorders were significantly higher in patients than in controls (Table 3). No significant difference in psychiatric comorbidity between pelvic pain, dysmenorrhea, and dyspareunia VAS subgroups (VAS = 0–5 and VAS = 6–10) in the overall sample was found (data not shown). However, considering only the patients group a statistically significant difference was observed in the frequency of mood and anxiety disorders between women with pelvic pain (10 subjects) and women without pelvic pain (8 subjects) (70.0% and 37.5%, resp.; Fisher's exact test, $P = 0.0342$).

Regarding the SCL-90-R and the TAS-20 instruments, four tests in the patient group were discarded because of being invalid. Table 4 shows the frequencies distribution in subjects with scores > cut-off. No statistically significant difference in the frequency distribution between cases and controls was observed, except for obsessive-compulsive ($\chi^2 = 15.005$; $P < 0.01$) and depression subscales ($\chi^2 = 4.035$; $P = 0.045$). Mean ranks comparison performed with Mann-Whitney U test showed statistically significant differences between patients and controls group in relation to TAS-20 total score ($P < 0.01$), Difficulty Identifying Feeling ($P < 0.05$), and Externally Oriented Thinking ($P < 0.01$). Comparing SCL-90-R and TAS-20 results in the presence/absence of comorbid psychiatric disorder groups, no differences were found in frequency distribution and mean ranks comparison. In the patients group, the analysis of VAS mean scores (chronic pelvic pain, dysmenorrhea, and dyspareunia) in absence/presence of comorbid psychiatric disorders (also

Table 1: Pain intensity evaluation.

	Dyspareunia VAS scores		Dysmenorrhea VAS scores		Pelvic pain VAS scores	
	Mean ± SD	P	Mean ± SD	P	Mean ± SD	P
Patients	2.5 ± 3.2	0.003	5.3 ± 3.8	0.365	2.1 ± 3.6	<0.001
Controls	0.7 ± 2.1		4.6 ± 3.5		0.02 ± 0.2	

Table 2: DSM IV-TR Axis I disorders.

Diagnosis	Patients		Controls	
	n	%	n	%
Psychotic disorder NOS	1	(2.7%)	0	(/)
Dysthymic disorder	3	(8.1%)	1	(2.3%)
Major depressive disorder	2	(5.4%)	3	(7.0%)
Depressive disorder NOS	1	(2.7%)	0	(/)
Bipolar II disorder	1	(2.7%)	0	(/)
Generalized anxiety disorder	1	(2.7%)	1	(2.3%)
Panic disorder without agoraphobia	1	(2.7%)	0	(/)
Specific phobia	3	(8.1%)	2	(4.7%)
Social phobia	1	(2.7%)	0	(/)
Obsessive-compulsive disorder	1	(2.7%)	0	(/)
Anxiety disorder NOS	4	(10.8%)	0	(/)
Eating disorder NOS	0	(/)	1	(2.3%)
Undifferentiated somatoform disorder	1	(2.7%)	0	(/)
No diagnosis or condition on Axis I	17	(46.0%)	35	(81.4%)

($\chi^2 = 19.289$; $P = 0.114$).

Table 3: DSM IV-TR Axis I disorders.

Diagnosis	Patients		Controls	
	n	%	n	%
Psychotic disorders	1	(2.7%)	0	(/)
Mood disorders	7	(18.9%)	4	(9.3%)
Anxiety disorders	11	(29.7%)	3	(7.0%)
Eating disorders	0	(/)	1	(2.3%)
Somatoform disorders	1	(2.7%)	0	(/)
No diagnosis or condition on Axis I	17	(46.0%)	35	(81.4%)

($\chi^2 = 14.251$; $P = 0.014$).

specifically for depressive spectrum disorders), malfunctioning in SCL-90-R subscales, and alexithymia in TAS-20 showed that significant differences were present for chronic pelvic pain and dysmenorrhea (Student's t-test; Table 5). Statistically significant correlation was observed between chronic pelvic pain and TAS-20 total score and between dyspareunia, Difficulty Describing Feelings, and Difficulty Identifying Feelings (Table 6). The analysis of VAS mean scores did not show statistically significant results for any of the investigated variables in the control group.

Finally, the correlation between the type of endometriosis (ovarian endometrioma or ovarian endometrioma and peritoneal endometriosis) and psychopathological symptoms or VAS mean scores did not show significant results.

Table 4: Frequencies of scores > cut-offs in SCL-90-R subscales and TAS-20 total score.

	Scores > cut-offs		χ^2	P
	Patients	Controls		
Somatization	11 (33.3%)	11 (25.6%)	0.546	0.460
Obsessive-compulsive	10 (30.3%)	0 (0%)	15.005	0.000**
Interpersonal sensitivity	13 (39.4%)	10 (23.3%)	2.304	0.129
Depression	13 (39.4%)	8 (18.6%)	4.035	0.045*
Anxiety	8 (24.2%)	6 (14.0%)	1.315	0.251
Hostility	5 (15.2%)	10 (23.3%)	0.774	0.379
Phobic anxiety	1 (3.0%)	4 (9.3%)	1.195	0.274
Paranoid ideation	9 (27.3%)	13 (30.2%)	0.080	0.778
Psychoticism	5 (15.2%)	5 (11.6%)	0.203	0.652
TAS-20 total score	6 (18.2%)	5 (11.6%)	0.353	0.552

*Frequencies difference is significant at the 0.05 level.
**Frequencies difference is significant at the 0.01 level.

4. Discussion

This preliminary study aimed to explore the possible role of psychiatric comorbidity, psychopathological symptoms, and alexithymia in endometriosis and their correlation with pain symptoms.

Even if in our study no statistically significant differences were observed between cases and controls regarding frequency of a specific Axis I DSM IV-TR diagnosis, the prevalence of psychiatric disorders was statistically higher in patients than in controls and a statistically significant difference was also found for mood and anxiety disorders and malfunctioning on obsessive-compulsive and depression SCL-90 subscales.

In the present study psychiatric comorbidity of patients with endometriosis was lower than that reported by previous studies [15–17]. Although not statistically significant, the higher prevalence of mood and anxiety disorders observed in patients with chronic pelvic pain compared with those with no pain confirmed previous findings [15, 23, 24]; however our observations were in contrast with some studies showing no difference between these subgroups in terms of frequencies of depression and anxiety disorders [25] or mental health status [26]. Since the small sample sizes (10 versus 8 subjects) of our subgroups might be a strong limitation, any outcome should be cautiously considered.

Data analysis revealed some interesting results: (1) patients with endometriosis showed higher scores on TAS-20 total score, Difficulty Identifying Feeling, and Externally Oriented Thinking (that indicates an inadequate introspective ability) than controls; (2) among patients with endometriosis, greater chronic pelvic pain VAS score was observed in

Table 5: Pelvic pain and dysmenorrhea VAS mean scores comparison in relation to presence of psychiatric disorders, malfunctioning on SCL-90 subscales, and alexithymia (TAS-20 total score) in endometriosis patients.

		Pelvic pain		Dysmenorrhea	
		Mean ± SD	*P*	Mean ± SD	*P*
Comorbid psychiatric disorder	absent	1.3 ± 2.8	0.286	3.9 ± 4.1	0.049*
	present	2.6 ± 4.1		6.4 ± 3.3	
SOM	<60 T points	0.68 ± 2.2	0.003**	4.1 ± 3.7	0.011*
	>60 T points	4.4 ± 4.4		7.5 ± 4.0	
OC	<60 T points	0.65 ± 2.2	0.001**	4.4 ± 4.7	0.048*
	>60 T points	4.8 ± 4.4		7.2 ± 3.4	
SENS	<60 T points	0.90 ± 2.8	0.038*	5.2 ± 3.6	0.865
	>60 T points	3.4 ± 4.0		5.4 ± 4.2	
ANX	<60 T points	0.96 ± 2.7	0.004**	4.6 ± 3.8	0.107
	>60 T points	4.9 ± 2.3		7.1 ± 3.4	
TAS-20	≤60	1.1 ± 2.7	0.004**	4.7 ± 3.7	0.376
	≥61	5.5 ± 4.5		6.5 ± 4.1	

*Means difference is significant at the 0.05 level.
**Means difference is significant at the 0.01 level.

Table 6: Correlation between pelvic pain and dyspareunia VAS scores and TAS-20, Difficulty Describing Feelings (F1), and Difficulty Identifying Feeling (F2) scores in endometriosis patients. Spearman's rho correlations.

		TAS-20 total score	F1	F2
Pelvic pain	Correlation coefficient	0.359*	0.247	0.251
	Sig. (2-tailed)	0.040	0.165	0.158
	N	33	33	33
Dyspareunia	Correlation coefficient	0.104	0.345*	−0.357*
	Sig. (2-tailed)	0.565	0.049	0.041
	N	33	33	33

*Correlation is significant at the 0.05 level.

patients with alexithymia (TAS-20 total score ≥ 61); (3) a statistically significant correlation was found between TAS-20 total score, Difficulty Describing Feelings, and Difficulty Identifying Feelings and chronic pelvic pain and dyspareunia VAS scores in patients group. These findings could suggest that the difficulty to recognize feelings and emotions in endometriosis patients may facilitate somatization, that is, the expression of psychological problems on a somatic level. Similar assumptions could be done also in relation to malfunctioning in somatization and sensitivity SCL-90-R subscales, correlated with higher chronic pelvic pain and dysmenorrhea (only somatization) VAS scores. Finally, the observed greater chronic pelvic pain VAS scores in patients with malfunctioning in anxiety and obsessive-compulsive SCL-90-R subscales were in line with what was shown by previous studies regarding the possible association with the presence of psychiatric comorbidity (above all of anxiety spectrum disorders) and pelvic pain in patients with endometriosis. In addition, our study highlighted the prevalence of a greater dysmenorrhea VAS score in patients with comorbid psychiatric disorders and those with malfunctioning in obsessive-compulsive SCL-90-R subscales, while no significant correlations were found between the type of endometriosis and the presence of psychopathological symptoms or pain symptoms.

5. Conclusions

Even if in our study we could not find a specific association with certain psychopathological features and the presence of endometriosis, however the subgroups of patients with chronic pelvic pain, dysmenorrhea, and dyspareunia were characterized by the presence of a specific grade of psychopathology that could play a role in pain perception and reaction.

The small sample size of this preliminary study certainly represented a limitation and did not allow us to determine a specific causality relation between some psychological features and endometriosis onset or pain perception or to exactly identify specific risk factors. Therefore, these preliminary findings need to be confirmed by further investigations and increasing the sample size.

However, this study highlighted some significant characteristics. Indeed, these results suggested that some mood and psychiatric characteristics such as mood and anxiety disorders, higher alexithymia levels, and malfunctioning on obsessive-compulsive and depression dimension are more frequent in women with endometriosis than in general population. Moreover, these psychopathological conditions could be correlated with moderate-severe pain symptoms and could influence pain perception in endometriosis patients.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Research Article

Endometriosis Patients in the Postmenopausal Period: Pre- and Postmenopausal Factors Influencing Postmenopausal Health

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Objective. To evaluate patients' health status and the course of endometriosis from the premenopausal to the postmenopausal period and evaluate influencing factors that may be relevant. *Methods*. Questionnaire completed by 35 postmenopausal women in whom endometriosis had been histologically confirmed premenopausally. Correlation and regression analyses were carried out to identify factors relevant to their postmenopausal health status. *Results*. Overall, there was clear improvement in typical endometriosis symptoms and sexual life. Clear associations ($P < 0.005$) were observed between premenopausal factors like physical limitations caused by the disease, impaired social contacts and psychological problems, and postmenopausal pain and impairment of sexual life. Three statistical models for assessing pain and impairment of sexual life in the postmenopausal period were calculated on the basis of clinical symptoms in the premenopausal period, with a very high degree of accuracy ($P < 0.001$; $R^2 = 0.833/0.857/0.931$). *Conclusions*. The results of the survey strongly suggest that physical fitness and freedom from physical restrictions, a good social environment, and psychological care in both the premenopausal and postmenopausal periods lead to marked improvements in the postmenopausal period with regard to pain, dyspareunia, and influence on sexual life in endometriosis patients.

1. Introduction

Endometriosis is one of the most common gynecological diseases, with an estimated current incidence of 40,000 new patients per year in Germany [1]. Worldwide, nearly 80 million women are affected by the disease. Data in the medical literature suggest that the prevalence of endometriosis is 10–15% in all women of reproductive age [1–3].

Several theories on the etiology and pathogenesis of endometriosis have been proposed, but a definitive explanation of the pathophysiological mechanism involved has not yet been found. Three basic theories are under discussion: the theory of cell transplantation [4], the theory of metaplasia [5], and the theory of the endometrial-subendometrial unit or "archimetra" [6]. Immunological, endocrinological, genetic, and inflammatory factors also appear to be essential elements in the pathogenesis of endometriosis [7–14]. However, estrogen dependence is considered to be central to the pathophysiological process and persistence of the lesions [11, 15]. The latter concept has led to the widely held belief that endometriosis is a disease of premenopausal women that is "cured" by the menopause [16, 17].

Cases have occasionally been reported in the literature describing endometriosis in the postmenopausal period in patients with or without hormone replacement therapy [18–24]. However, the current state of the data is inadequate to allow any assessment of this and the mechanisms underlying the entity have not been explained [25].

The aim of the present study was to evaluate health status and the course of endometriosis from the premenopausal to the postmenopausal periods. In addition, relevant factors influencing this were to be identified. Using a statistical model, an attempt was made to calculate the expected health status in the postmenopausal period on the basis of premenopausal clinical symptoms.

2. Methods

Institutional review board (IRB) approval was obtained (ref. number K-20-12). Data for 35 endometriosis patients who were postmenopausal at the time of responding to a questionnaire were collected and statistically analyzed in this epidemiological study. Before the menopause, all of the participants had undergone surgery for endometriosis, with the findings confirmed histologically.

The inclusion criteria were histologically confirmed: premenopausal endometriosis and age ≥55 years, with the last menstruation being at least 12 months previously. Patients with bilateral adnexectomy who were not receiving hormone replacement therapy were also included. Exclusion criteria were questionnaires that were not fully completed and patients under the age of 55 who had undergone hysterectomy premenopausally. The hysterectomy would have made it impossible to obtain a menstrual history, giving rise to bias in relation to menopausal status.

A total of 150 questionnaires were presented to two self-help groups (the Austrian Endometriosis Association and the German Endometriosis Association) and were also made available in our own outpatient gynecological department. Forty-one women decided to participate in the study anonymously. The anonymous questionnaire letter boxes were opened at the end of 6 months and the forms were checked for completeness. Six of the 41 questionnaires had not been fully completed or did not match the inclusion criteria. All of the questions were explicitly related to endometriosis. The patients were thus instructed to respond to the questions—for example, in relation to "psychological problems"—exclusively in relation to endometriosis. As an alternative response, the patients were also given the option "due to a different cause."

The questionnaire included a total of 147 questions, divided into three parts. Part 1 (29 questions) was concerned with the patient's general medical history (18 questions), including social and family history and also surgical history (11 questions). Part 2 (54 questions) inquired into symptoms (21 questions) and complaints (33 questions) in the period before the menopause. Part 3 (64 questions) was concerned exclusively with questions about symptoms (24 questions) and complaints (40 questions) in the postmenopausal period. A visual analogue scale (best grade: 0, poorest grade: 10) was used for responses to questions about pain and impairment of sexual life (best grade: 0, poorest grade: 10).

2.1. Statistical Analysis. The exact Wilcoxon test was used to compare the patients' general condition before and after the menopause. Spearman's rank correlation coefficients and point biserial correlation coefficients were calculated to assess correlations. Multiple stepwise regression analyses were used to investigate the predictability of the intensity of general pain in the postmenopausal period (0–10), pain intensity during sexual intercourse in the postmenopausal period (0–10), and the influence of endometriosis on sexual life in the postmenopausal period (0–10) relative to variables from the premenopausal period (all of these independent variables are listed in Supplemental Digital Content 1 available online at http://dx.doi.org/10.1155/2014/746705). Type 1 error was not adjusted for multiple testing, and all P values presented are therefore only descriptive. The open-source R statistical software package, version 2.14.1 (Institute for Statistics and Mathematics, University of Vienna, Austria), was used for statistical analysis.

Table 1: Patients ($n = 35$).

	Mean ± SD
Postmenopausal period (in years)	10.8 ± 11.14
Pain intensity since menarche (0–10)	6.3 ± 2.85
Premenopausal abdominal pain (0–10)	7.2 ± 2.81
Postmenopausal abdominal pain (0–10)	3.3 ± 3.40
Premenopausal dyspareunia (0–10)	4.1 ± 2.94
Postmenopausal dyspareunia (0–10)	2.3 ± 2.87
Operations (numbers)	2.7 ± 1.69
Premenopausal effect on sexual life (0–10)	5.3 ± 3.65
Postmenopausal effect on sexual life (0–10)	2.8 ± 3.73

3. Results

The group of patients consisted of 35 women aged 37–79 years. The patients' average age was 53.9 ± 9.78 years. Their average age at the onset of menopause was 43.03 years. The median time from menopause to completing the questionnaire was 11 years (Table 1).

Each participant had undergone a mean of 2.74 ± 1.69 gynecological operations due to endometriosis at the time of the questionnaire.

All of the patients (100%) stated that they were enjoying or had enjoyed their occupations, including nine (25.7%) who were retired at the time of the questionnaire.

To the question of how often the participants had been pregnant, nine (26%) responded that they had never been pregnant. Ten (28%) had been pregnant once, nine (26%) twice, and seven (20%) more than twice. Nine participants (26%) had not given birth to any children, 13 (37%) had given birth once, 11 (31%) had had two children, and two (6%) had given birth to more than two children.

There were no relevant differences from the normal population with regard to concomitant diseases. The large number of 21 patients with allergies (60%) was notable. Six participants (17.1%) stated that they were regular smokers at the time of the questionnaire, while 16 (45.7%) had been smokers in the past.

With regard to family history, nine patients (25.7%) stated that their mothers had had dysmenorrhea; three of

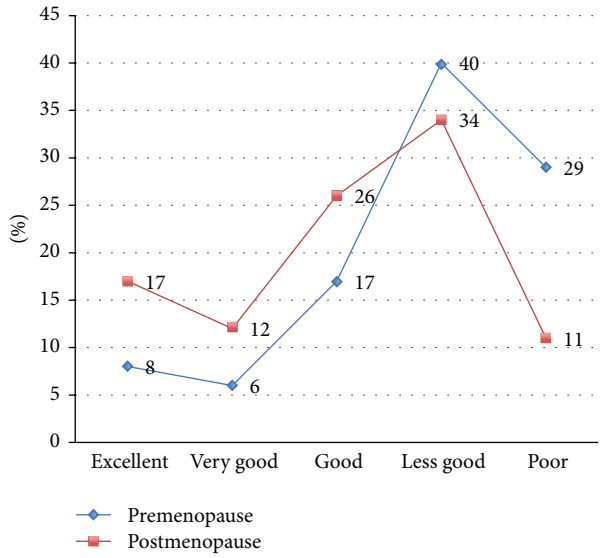

Figure 1: Comparison of the general condition of endometriosis patients in the premenopausal and postmenopausal periods.

the mothers had histologically confirmed endometriosis. Six patients (17.1%) reported that a sister had dysmenorrhea; five of the six sisters had histologically confirmed endometriosis. Eight patients (22.9%) stated that their daughters had dysmenorrhea; three of the daughters had histologically confirmed endometriosis.

3.1. General Health Status. Eleven patients (31.4%) described their general state of health in the premenopausal period as "excellent" to "good," while 24 patients (68.6%) described it as "not so good" to "poor." In the postmenopausal period, 18 patients (51.4%) described their general state of health as "excellent" to "good," while 17 patients (48.6%) described it as "not so good" to "poor" (Figure 1).

When comparing their premenopausal and postmenopausal status, in both, 26 of the 35 patients (74.3%, resp.) stated that they had limitations in strenuous physical activities, while 25 versus 23 (71.4% versus 68.6%) stated that they had limitations in moderate physical activities. Impairment was experienced by 21 versus 20 patients (60% versus 57.1%) when carrying a shopping bag, by 19 versus 17 (54.3% versus 48.6%) when climbing several steps, and by seven versus nine (20% versus 25.7%) when climbing a single step. Impairment when "bending/kneeling/stooping" was experienced by 19 versus 18 of the patients (54.3% versus 51.4%) and difficulty in walking approximately 1 km by 18 versus 12 of the patients (51.4% versus 34%). Whereas 62.9% of the patients attributed these restrictions to endometriosis in the premenopausal period, 22.9% of them attributed their physical limitations to endometriosis in the postmenopausal period.

In the premenopausal period, psychological problems were reported by 51.4% of the patients and restriction of social contacts by 62.9%, and as many as 80% described occupational restrictions due to endometriosis. The corresponding postmenopausal figures were 20%, 17.1%, and 20%.

Table 2: Symptoms.

Symptoms	Premenopausal (n, %)	Postmenopausal (n, %)
Intestinal cramp	21 (60%)	11 (31.4%)
Dyschezia	18 (51.4%)	9 (25.7%)
Diarrhea	11 (31.4%)	9 (25.7%)
Constipation	14 (40%)	10 (28.6%)
Mucus in feces	16 (45.7%)	6 (17.1%)
Blood in feces	8 (22.9%)	3 (8.6%)
Dysuria	11 (31.4%)	10 (28.6%)
Hematuria	6 (17.1%)	0 (0%)
Increased urinary urgency	15 (42.9%)	12 (34.3%)
Incontinence	13 (37.1%)	18 (51.4%)
Retrosymphyseal pain	10 (28.6%)	8 (22.9%)

3.2. Pain History. Thirty-two patients (91.4%) had had dysmenorrhea since the menarche, with a median intensity of 7.0. Thirty-three patients (94.3%) reported abdominal pain in the premenopausal period, with a median pain intensity of 8.0. As many as 22 patients (62.9%) reported pain in the postmenopausal period, with a median of 3.0. These patients all attributed the pain to the endometriosis. Seven of the 22 patients also reported pain due to other causes.

Twenty-five of the patients (71.4%) had already had dyspareunia premenopausally, and it persisted postmenopausally in 19 patients (54.3%). The median values for its intensity were 5.0 and 2.0, respectively.

Twenty-eight patients (80%) reported that their sexual life had been affected by the endometriosis in the premenopausal period, and the figure was still as high as 19 patients (54.3%) in the postmenopausal period. The median values for this effect were 5.0 and 1.0 (Table 1). Other symptoms the patients experienced are summed up in Table 2.

Premenopausally, 21 patients (60%) had regularly taken analgetics; 10 (28.6%) had taken gonadotropin-releasing hormone (GnRH) analogues; and 15 patients (42.9%) had taken the contraceptive pill. The mean for the total period of drug intake was 40.9 months. Twenty-three of the patients (65.7%) stated that taking medication had not led to any improvement in symptoms, and side effects developed in 37.1%.

A total of 26 patients (74.3%) had undergone hysterectomy, 10 of them with bilateral adnexectomies. Three patients had a bilateral adnexectomy without hysterectomy. At least one laparoscopy was carried out on 32 patients and 16 patients had at least one laparotomy. Thus, 13 patients had at least one laparoscopy and a laparotomy.

3.3. Correlation Analyses. Table 3 shows the most notable associations between the general intensity of pain, pain intensity during sexual intercourse, and influence on sexual life in the postmenopausal period, on the one hand, and various premenopausal and postmenopausal variables, on the other hand. All of the parameters are listed in Supplemental Digital Content 1. Factors that correlated poorly with the target variables were concomitant diseases and bowel symptoms,

Table 3: Correlation coefficients for associations between parameters for the patient's medical history and health status, on the one hand, and postmenopausal impairment of sexual life and pain intensity, on the other hand.

	General intensity of pain	Pain intensity during sexual intercourse	Impairment of sexual life
General medical history			
Allergies	0.386*	0.171	0.210
Hysterectomy without adnexa	−0.335*	−0.284	−0.271
Hysterectomy with bilateral adnexectomy	0.412*	0.447**	0.445**
Bilateral adnexectomy	0.258	0.425*	0.424*
Premenopausal period			
General pain	0.276	0.135	0.244
General pain intensity	−0.022	0.228	0.226
Pain during sexual intercourse	0.284	0.354*	0.287
Pain intensity during sexual intercourse	0.370*	0.385*	0.287
Effect of endometriosis on sexual life	0.385*	0.625**	0.607**
Poor general condition	0.483**	0.248	0.244
Physical restriction due to endometriosis	0.500**	0.427*	0.336*
Physical restrictions during			
Strenuous activities	0.460**	0.359*	0.300
Moderately strenuous activities	0.516**	0.410*	0.287
Carrying shopping bag	0.461**	0.366*	0.341*
Climbing steps	0.453**	0.564**	0.362*
Climbing one step	0.353*	0.359*	0.306
Bending/kneeling/stooping	0.643**	0.588**	0.401*
Walking 1 km	0.548**	0.496**	0.385*
Bathing/dressing	0.256	0.260	0.089
Psychological problems	0.530**	0.514**	0.453**
Impairment of social contacts	0.500**	0.507**	0.506**
Impairment of everyday living/work	0.182	0.433**	0.496**
Postmenopausal period			
Pain during urination	0.409*	0.443**	0.482**
Increased urinary urgency	0.396*	0.438**	0.367*
Physical restriction due to endometriosis	0.676**	0.691**	0.605**
Physical restrictions during			
Strenuous activities	0.553**	0.499**	0.402*
Moderately strenuous activities	0.596**	0.512**	0.501**
Carrying shopping bag	0.553**	0.396*	0.467**
Climbing steps	0.606**	0.389*	0.170
Climbing one step	0.516**	0.376*	0.293
Bending/kneeling/stooping	0.658**	0.643**	0.456**
Walking 1 km	0.644**	0.441**	0.349*
Bathing/dressing	0.406*	0.092	0.031
Psychological problems	0.471**	0.435**	0.407*
Impairment of everyday living/work	0.706**	0.459**	0.548**

* $P < 0.05$.
** $P < 0.01$.

family history, all forms of drug intake including the period of medication and alternative therapies, pregnancies, and parity. In addition, only a slight association was noted between the number, type, and method (surgical technique) of operations and postmenopausal target variables, with the exception of hysterectomy and adnexectomy or combinations of them (Table 3). There were no correlations worth mentioning between bladder symptoms in the premenopausal period and those in the postmenopausal period.

3.4. Regression Analyses. Multiple stepwise regression analyses resulted in statistical models with remarkably high levels of predictive power and accuracy for predicting the general intensity of pain in the postmenopausal period ($P < 0.001$; $R^2 = 0.833$), pain intensity during sexual intercourse in the postmenopausal period ($P < 0.001$; $R^2 = 0.857$), and influence on sexual life in the postmenopausal period ($P < 0.001$; $R^2 = 0.931$) on the basis of information available premenopausally (for details, see Supplemental Digital Content 2).

4. Discussion

It is interesting that concomitant diseases and bowel symptoms (Table 2), family history, all forms of medication including their duration and alternative therapies, pregnancy, and parity proved to be quite unimportant influencing factors relative to the postmenopausal target variables mentioned. The number, type, and method (surgical techniques) of operations carried out also hardly correlated at all with the target variables "general pain experienced," "pain during sexual intercourse," and "disturbance of sexual life," with the exception of hysterectomy and adnexectomy and the combinations of them listed in Table 3. It is notable here that hysterectomy with the adnexa or bilateral adnexectomy led to marked deterioration of symptoms during the postmenopausal period.

As Figure 1 shows, there was a clear improvement in the patients' general condition when the premenopausal and postmenopausal periods are compared. However, it should be pointed out that this parameter is probably composed of several factors. It appears that a poor general state of health during the premenopausal period markedly correlates with more severe general pain in the postmenopausal period (Table 3).

Clear improvement with regard to pain, dyspareunia, and influence on sexual life is seen in the postmenopausal period (Table 1). However, it is also notable here that general pain and its intensity in the premenopausal period are not significantly associated with any postmenopausal findings. By contrast, dyspareunia and influence on sexual life in the premenopausal period certainly correlate well with symptoms in the postmenopausal period (Table 3).

We would interpret these results as follows. As endometriosis is a long-term disease that usually has a course lasting several years, the symptoms and complaints in the premenopausal period can become chronic and can thus have an effect on postmenopausal life [26]. Dyspareunia and a negative effect on sexual life in the premenopausal period may thus perhaps be able to leave "inner scars" that have negative effects on the postmenopausal period. On this view, general pain that is not necessarily associated with sexual life appears to have a less marked effect on the period after the menopause.

The results with regard to psychological problems, impairment of social contacts, and impairment of everyday life and working life due to endometriosis in the premenopausal period are surprising. Effects of these are seen very clearly during the postmenopausal period in the deterioration in general pain experienced, pain during sexual intercourse, and disturbances of sexual life. There is also evidence in the literature in this connection showing that chronic pelvic pain (CPP) can have a negative influence on family life, sexual life, and social life [26]. The target variables in the postmenopausal period understandably also deteriorate from the psychological point of view (Table 3). The patients were asked to relate psychological problems, impairment of social contacts, and impairments of everyday life and working life only to the endometriosis. It might be questionable whether it is really possible for patients to assign such psychological factors to endometriosis in isolation and objectively. However, the data suggest that the patients were in fact able to do this, since a marked decline in these factors was observed in the postmenopausal period.

Similarly surprising were the results with regard to physical restrictions (Table 3). Almost all physical restrictions in the premenopausal and postmenopausal periods correlate very strongly with the postmenopausal target variables (general pain experienced, pain during sexual intercourse, and disturbances of sexual life). This clearly shows how important maintenance of physical fitness is even in the premenopausal period.

Stress plays a very important role in the clinical picture of endometriosis [27, 28]. It has been shown in an animal model that stress leads to a deterioration in endometriosis [29]. It has been well demonstrated that stress is often linked to nicotine consumption [30]. This is also reflected in the present study, in which the proportion of smokers was notably high (46%) in comparison with that in the general population in Austria (19% of women over the age of 15) [31].

There was also a high proportion of patients with allergies (60%). Comparable prevalence figures among women in the general population are 25% in Austria [32] and 29% in Switzerland [33]. Studies have shown that endometriosis patients suffer significantly more often from immunodeficiencies, asthma, and allergies [34]. This might also be attributable to increased stress caused by the endometriosis, leading to a deterioration in the immune system [35, 36]. However, it has already been clearly shown that physical exercise and psychological care lead to a marked reduction in the level of stress in endometriosis patients [37].

On the basis of the present results, it can be concluded that physical fitness and an absence of physical symptoms, a good social environment, and psychological care not only during the premenopausal period but also in the postmenopausal period as well lead to marked improvement with regard to pain, dyspareunia, and effects on sexual life.

In Germany, there are already two rehabilitation centers for endometriosis patients that have been certified by the Endometriosis Research Foundation (*Stiftung Endometriose-Forschung* (SEF)) [38]. The centers focus on physical exercise, physiotherapy, and psychological care.

If the results of the present study are confirmed by further research, there will be an urgent need for accessible institutions of this type to be established in every country in the world. This is particularly the case in view of the fact that endometriosis has been identified as a high cost factor in studies investigating economic targets, which have indicated that a potential cause of this might be inadequate infrastructure [39–43].

The good predictability of the target parameters, "general intensity of pain in the postmenopausal period," "pain intensity during sexual intercourse in the postmenopausal period," and "influence on sexual life in the postmenopausal period" relative to premenopausal factors, might be due to endometriosis patients' very good ability to recall the symptoms and complaints that they had during the premenopausal period (Supplemental Digital Content 2).

Despite the high level of statistical accuracy of the models, the current state of knowledge and the questionable representativeness of the data discussed above do not make it currently possible to draw any detailed conclusions regarding the actual course of the disease. Therapeutic decisions should on no account be made on the basis of these models. Endometriosis in itself represents an extremely complex and polymorphous disease, and it is influenced by the patients' individual characters. The absolute focus should always be on individual consideration and treatment of each and every patient.

A major limitation of the present study is the relatively small number of cases included. This is due to the difficulty of accessing a relevant group of patients with treatments that may already lie decades in the past. In addition, the fact that evidently only a small proportion of candidate patients decided to contribute their information and complete the questionnaire substantially increases the chances that individuals with exceptionally positive or exceptionally negative experiences may be overrepresented (recall bias). Furthermore, the choice of a self-help group for the investigation increases the risk of a selection bias. However, irrespective of this limitation on the validity of the study, it would be valuable to take the very marked and partly surprising results of this survey as an interesting approach that needs to be investigated in further research.

5. Conclusions

Physical fitness and freedom from physical symptoms, a good social environment, and psychological care not only in the premenopausal period but also in the postmenopausal period lead to a marked improvement with regard to pain, dyspareunia, and effects on sexual life during the postmenopausal period in patients with endometriosis. Rehabilitation clinics for endometriosis patients, aimed at maintaining their physical fitness and providing psychological care, should be available and accessible. In view of the patients' good memory of their complaints and symptoms during the premenopausal period, it is possible to anticipate complaints and their severity in the postmenopausal period relatively precisely using these premenopausal factors alone—and with this information, it may be possible to influence them in a positive way.

Conflict of Interests

The authors hereby state that there was no conflict of interests.

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Clinical Study

Long-Term Outcome after Laparoscopic Bowel Resections for Deep Infiltrating Endometriosis: A Single-Center Experience after 900 Cases

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Background. Laparoscopic bowel resections for endometriosis are safe and effective but only short-term follow-up has been evaluated. In the present study long-term outcome in terms of intestinal and urinary function, fertility, chronic pain, and recurrence was assessed. *Materials and Methods*. From January 2002 to December 2010 nine hundred patients underwent laparoscopic bowel resection for endometriosis, and on 774 (86%) a questionnaire was administered. Patients were divided into 3 groups on the strength of the operation date. Postoperative diarrhea, constipation, rectal bleeding, tenesmus, dyschezia, dysuria, dyspareunia, fertility, and recurrence of disease were assessed. *Results*. The median follow-up was 54 months (range 1–120). All the evaluated symptoms significantly improved over time, with $P = 0.0001$ for dyspareunia, constipation, and pelvic pain and $P = 0.004$ for diarrhea. Nonsignificant improvement was reported for dysuria and rectal bleeding (with $P = 0.452$ and $P = 0.097$, resp.). *Conclusions*. The present results confirm that bowel resections for endometriosis are correlated with an acceptable complication rate even at long-term follow-up and that symptoms significantly improve over time, except for rectal bleeding and dysuria, the latter associated with a neurological damage.

1. Introduction

Endometriosis is a benign disease but it can seriously worsen quality of life. Its incidence is quite high, affecting 6–10% of women in childbearing age [1, 2]. Deep infiltrating endometriosis (DIE) is a form of endometriosis in which the pathologic tissue can penetrate up to 5 mm under the surface of the affected structure [1, 2]. The incidence of DIE is reported in 20% of all cases of endometriosis and the gastrointestinal tract results involved in 5.3–12% [3, 4]. The most frequent localization is the rectosigmoid junction and it has been estimated in 65% of cases; other common localizations are the ileocaecal junction in 20% and the rectum in 15%. The endometriotic tissue can involve the submucosal layer but the infiltration of the mucosa is very rare.

Bowel endometriosis may cause severe symptoms such as diarrhea, constipation, abdominal pain, bleeding, dyschezia, and rarely bowel obstruction. The best therapeutic approach is still controversial but several studies have demonstrated that surgery offers improvement of symptoms, better quality of life, and acceptable postoperative fertility rates [5–7]. For this purpose it is essential to establish a long-term outcome to evaluate intestinal and urinary dysfunctions, quality of life, and fertility rate [8, 9].

Recently, a nerve-sparing approach laparoscopic bowel resection for DIE was proposed to preserve bladder, rectal, and sexual functions [10, 11].

Since Nezhat described in 2001 the first laparoscopic bowel resection for endometriosis [12], many studies have been published on this topic and recently, Daraï et al. have

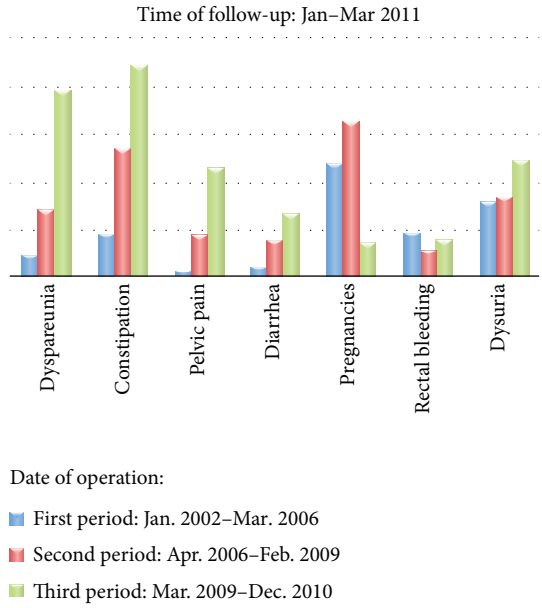

Figure 1

demonstrated, in a prospective trial, that laparoscopy is a safe option in the treatment of bowel endometriosis and offers a high pregnancy rate and a good quality of life [13].

In our division, since January 2002, we performed 1023 colorectal resections for bowel endometriosis; after 10 years, on the basis of this experience, we decided to analyse retrospectively our results. The aim of this study was to investigate bowel and urinary dysfunction and fertility rate in a large series of bowel laparoscopic resection for DIE.

2. Materials and Methods

Between January 2002 and December 2010, 1023 women underwent laparoscopic bowel resections for DIE at the Departments of General Surgery and Gynecology, Ospedale "Sacro Cuore-Don Calabria," Negrar, Verona. Discoid and transvaginal resections were excluded from the present study and 900 patients were considered eligible for the investigation.

All patients were postoperatively interviewed by telephone between January 2011 and March 2011 by three male surgeons, and a questionnaire was filled in forms for each patient, dividing the whole population into three subgroups of 300 patients; the first group included patients treated between 1 January 2002 and 31 March 2006, the second from 1 April 2006 to 1 February 2009, and the third up to 31 December 2010.

The questionnaire is made up of questions regarding intestinal and gynaecological symptoms: presence of diarrhea or constipation, rectal bleeding, postoperative pregnancy, dyspareunia, dysuria, and pelvic or back pain, evaluated using a 10-point visual analogue scale (0 = absent, 10 = unbearable) (Figure 1).

All women underwent a clinical multidisciplinary evaluation; they were all studied with a barium enema and a transvaginal ultrasound. The indication for surgery was made on the basis of the presence of intestinal stenosis associated with intestinal-related symptoms. All women underwent laparoscopic bowel resection after a complete multidisciplinary evaluation. The procedures were carried out when a stenosis of the intestinal lumen was radiologically showed, when gastrointestinal symptoms were described, and when the presence of DIE was intraoperatively demonstrated. Ureteroneocystostomy was performed in patients with radiological stenosis of the ureter and hydroureteronephrosis. All patients had a stage IV endometriosis according to the American Society of Reproductive Medicine AFs, 1989, and all of them underwent a bowel resection (rectal, rectosigmoid, sigmoid, ileocaecal, or ileal resection). For rectal, rectosigmoid, or sigmoid resections, an end-to-end anastomosis was performed, according to the Knight-Griffen procedure, with manual or mechanical sutures [10]. Resections were classified according to distance from the anus as high/medium (>8 cm), low (5–8 cm), or ultralow (<5 cm) and measured by intraoperative proctoscopy. Ileocaecal and ileal resection were carried out extracorporeally with a manual anastomosis through, either a sovrapubic or a left or right pararectal incision. All patients were operated on by the same surgical, gynaecological, and urological teams with surgical standardized techniques. We retrospectively analyzed diarrhea, constipation, rectal bleeding, tenesmus, dyschezia, dysuria, fertility after surgery, and relapse. Constipation was defined as fewer when three difficult, incomplete, or infrequent evacuations of dry hardened faeces per week, even with laxatives or enemas, appeared after surgery. Preoperative and postoperative scoring of symptoms was considered using a 10-point visual analogue scale (VAS; 10-point rating scale: 0 = absent, 10 = unbearable) [14]. All the data obtained by the questionnaire were investigated and inserted in a computerized database.

One-way ANOVA was performed to compare all variables in the two study groups. Categorical variables were compared by using χ^2 test. Wilcoxon Signed Rank test was adopted to compare paired scale variables. Statistical analyses were performed using SPSS for Windows 16.0 package (SPSS Inc., © Copyright IBM Corporation 2010 IBM Corporation, Route 100 Somers, NY).

3. Results

After a median follow-up of 54 months (range 1–120), 900 women were called by telephone; long-term follow-up data were available for 774 patients (86%), 210 for the first period (70%), 267 for the second (85%), and 297 for the third (99%). No significant differences in demographic data between the three groups were reported (Table 1). Median age was 27.5 (range 22–51) and median BMI (body mass index) was 23.7 (range 18.5–31.5). All women were at fertile age and 123 (15.8%) of them reported a previous pregnancy before surgery. Five hundred and eighty-three (64.8%) patients had previous abdominal surgery, laparotomy in 252 cases and laparoscopy in 331. The data related to the operation are reported in Table 2. Among the whole population (900

women), sixty-three percent of patients (567) underwent a rectosigmoid resection, 25% (225 patients) underwent a rectal resection, 5% (45 patients) underwent an ileocaecal resection, 61 patients (6.8%) underwent a double resection (rectosigmoid and ileocaecal), and 2 patients (0.2%) underwent a triple resection (rectosigmoid, ileal, and ileocaecal). One hundred and fifty-six (21.3%) patients required an ileostomy. In 5 cases the ileostomy was still present at the time of follow-up. In 60 cases (7.7%) a ureteral reimplantation was performed. The results were divided per period and reported in Table 3. After the operation, we registered 128 (16.5%) pregnancies, among which 35 (5.3%) were in the nulliparous group that consisted of 651 women; twenty-four patients (3.5%) reported at least one miscarriage. Forty-eight pregnancies were reported in the first period, 65 in the second, and 15 in the third. One hundred and sixteen patients (14.9%) reported dyspareunia (median VAS 6, range 1–10), 9 in the first period (4.2%), 29 in the second (10.8%), and 78 in the third (26.2%). Ninety-five percent of patients reported tenesmus in the first month following surgery but all of them described this symptom as completely settled at the time of follow-up. One hundred and sixty-two patients (21%) reported constipation, 18 in the first period (8.5%), 54 in the second (20.2%), and 90 in the third (30.3%); all of them underwent a rectosigmoid resection (157 anterior rectal resections with low anastomosis in 143 cases and ultralow in 14) except in 5 cases, 2 ileocolic resections and 3 combined ileocolic and rectal resections. Fifty-seven (7.3%) patients reported diarrhea; 55 of them had a rectosigmoid resection and two an ileocaecal resection. This symptom was present in 4 cases (1.9%) in the first period, in 16 cases (5.9%) in the second, and in 27 cases (9.1%) in the third. Forty-seven (6.0%) women reported mild rectal bleeding: one of them had an ileocaecal resection and 46 had a rectal resection. This symptom was present in 19 cases (9.0%) in the first period, in 12 cases (4.5%) in the second, and in 16 cases (5.3%) in the third. Sixty-four (8%) women reported pelvic pain, 3 in the first period (1.4%), 18 in the second (6.7%), and 46 in the third (15.4%); median VAS was 6 (range 1–10). One hundred and fourteen (14.7%) women described dysuria, 31 in the first period (14.7%), 34 in the second (12.7%), and 49 in the third (16.4%); median VAS was 5.5 (range 1–10). Among the 60 patients who underwent a ureteral reimplantation, only 2 of them complained of this symptom. Twenty-three women (2.9%) reported a relapse; in 16 cases (2%) it involved the gynaecological apparatus, in 5 cases (0.6%) the urinary system, and in 2 cases (0.2%) the intestinal tract. Five of them (0.5%) were reoperated on but no bowel resection was necessary. At univariate analysis for the different periods, symptoms as dyspareunia, constipation, and pelvic pain statistically improved over time with $P = 0.0001$, as well as diarrhea with $P = 0.004$. As regards dysuria and rectal bleeding, no improvement was reported over time, with $P = 0.452$ and $P = 0.097$, respectively.

Table 1: Demographic data.

Age (years)	27.5 (22–51)
BMI (Kg)	23.7 (18.5–31.5)
Previous pregnancies	123 (15.8%)
Previous abdominal surgery	583 (64.8%)

Table 2: Types of operations.

Rectosigmoid	567 (63%)
Rectal	225 (25%)
Ileocecal	45 (5%)
Rectosigmoid + ileocecal	61 (6.8%)
Rectosigmoid + ileocecal + ileal	2 (0.2%)
Ileostomy	156 (21.3%)
Ureteral reimplantation	60 (7.7%)

4. Discussion and Conclusion

Several studies demonstrate that colorectal resection for DIE is a safe and effective procedure with an acceptable postoperative complication rate and that it improves significantly quality of life; however, only short-term postoperative outcome has been evaluated [2, 4].

Many records analysed functional results after sphincter preserving colorectal resection, low anterior rectal resection, and abdominoperineal resection in treatment of rectal cancer [15]. In case of surgery for malignant disease the bowel function is related to the length of bowel resected, to preoperative chemoradiotherapy, and to the surgical technique applied as demonstrated by several studies [16]. Bowel function disorders, such as constipation and diarrhea, after colorectal resection for cancer are very common [17]. In case of colorectal resection for DIE the bowel dysfunction rate is significantly lower, probably because of the young age of the patients, because radiochemotherapy is not necessary, and because, in the majority of cases, the length of the resected colorectal segment is shorter and limited to the affected tract; in fact oncological radicality is not necessary.

Bowel movements disorders are difficult to evaluate since the latter depend on several factors among which the surgical technique can be considered the most important. Postoperative constipation can be correlated to the hypertension of the puborectalis sling, which can be frequently investigated by a radiological defecography. At our institution, patients who suffer from this type of dysfunction undergo a physiatric evaluation and a biofeedback pelvic floor therapy, which proved to be very useful for many women. Very few patients complained of rectorrhagia in our series; among them no cases of recurrence were recorded. Probably this symptom is correlated to a concomitant proctological disease; in fact its incidence in the three periods is homogeneous and it is not influenced by the length of the follow-up.

The urinary disorders after colorectal resection for endometriosis worsen significantly quality of life and the incidence is reported between 0.5 and 19.5% [8, 9]. In our series patients who suffered from dysuria were 114 (14.7%) and its rate is homogeneous in the three periods. Many studies describe urinary dysfunctions after intestinal resection for endometriosis as for laparoscopic surgery for malignant diseases; they are most probably correlated to

Table 3: Results.

	Total 774 patients	First period January 2002–March 2006 ($n = 210$)	Second period April 2006–February 2009 ($n = 267$)	Third period March 2009–December 2010 ($n = 297$)	P value	
Pregnancies	128 (16.5%)	48 (22.8%)	65 (24.3%)	15 (5.0%)	$P < 0.0001$	S
Dyspareunia	116 (14.9%)	9 (4.2%)	29 (10.8%)	78 (26.2%)	$P < 0.0001$	S
Constipation	162 (21%)	18 (8.5%)	54 (20.2%)	90 (30.3%)	$P < 0.0001$	S
Diarrhoea	57 (7.3%)	4 (1.9%)	16 (5.9%)	27 (9.1%)	$P = 0.004$	S
Rectal bleeding	47 (6.0%)	19 (9.0%)	12 (4.5%)	16 (5.3%)	$P = 0.097$	NS
Pelvic pain	64 (8%)	3 (1.4%)	18 (6.7%)	46 (15.4%)	$P < 0.0001$	S
Dysuria	114 (14.7%)	31 (14.7%)	34 (12.7%)	49 (16.4%)	$P = 0.452$	NS

inferior hypogastric plexus damage; in fact this symptom in our series did not improve over time after the operation, according to the hypothesis that the complication is correlated to a neurological impairment. Different techniques have been described in gynecological surgery to preserve these neural fibers such as the Tokyo method [18]; however very few long-term results are available. Recently, Ceccaroni et al. reported, in a single-center prospective study performed on 126 patients, good results of the nerve-sparing radical excision of DIE with segmental bowel resection in terms of reduced bladder dysfunction and higher satisfaction than the classical technique [10].

Postoperative recurrence rate in our series is very low even at long-term follow-up. If second surgery was needed for persistence or recurrence of the disease, no bowel resection was necessary to achieve a complete ablation of endometriosis.

Postoperative pregnancy rate is reported in literature between 45 and 48% [5]. In our series the rate is lower but none of our patients underwent a preoperative fertility study, and the resort to assisted conception was not investigated. Some studies demonstrated that fertility is influenced by colorectal surgery even for nongynecologic diseases; for example, Gorgun et al. reported a statistically significant difference in fertility before and after surgery in patients who underwent coloproctectomy for hemorrhagic enterocolitis [19]. Fedele et al. described how the pregnancy rate diminishes in case of recurrence of endometriosis, and actually the five patients in our study who had a reoperation for recurrence did not become pregnant [20]. Some authors described how postoperative complications could reduce the possibility of pregnancy and they advise an ovariopexy to keep the ovaries away from the affected areas [21, 22]. In the present study the correlation between postoperative complications and pregnancy rate was not investigated; therefore this issue merits further studies.

As regards complications as dyspareunia, constipation, diarrhea, and pelvic pain, these symptoms significantly improve over time, rising at a very low rate at long-term follow-up.

In conclusion the present study confirms that bowel resections for endometriosis have an acceptable postoperative complication rate even at long-term follow-up and symptoms improve over time, although our data concern a very wide range of follow-up time, which can be considered as a bias. For this reason we believe that further studies are needed to confirm our results.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Research Article

Evaluation of the Usefulness of the MRI Jelly Method for Diagnosing Complete Cul-de-Sac Obliteration

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Objective. We conducted a single-center study to evaluate the usefulness of the magnetic resonance (MR) imaging jelly method for diagnosing endometriosis-associated adhesions in the Pouch of Douglas. *Methods.* Thirty women with menstrual pain, dyspareunia, and chronic pelvic pain were enrolled in the study. All had been scheduled for laparoscopic surgery on the basis of pelvic and/or ultrasonographic (US) evaluation. All underwent MR imaging both with and without application of US jelly to the vagina and rectum. The images were compared and analyzed postsurgically in a random and blinded fashion by a radiology specialist and a radiology fellow. The radiologists' interpretations of the images were compared to the surgical findings recorded on DVDs. *Results.* Adhesions in the Pouch of Douglas were found in 21 patients. The sensitivity and specificity of MR imaging without jelly administration were 85.7% and 55.6%, respectively, for the specialist and 81.0% and 55.6%, respectively, for the fellow; with jelly administration, values were 95.2% and 88.9% for the specialist and 90.5% and 66.7% for the fellow. Opacity produced by the jelly increased the sensitivity and specificity for both radiologists. *Conclusion.* The MRI jelly method is a potentially useful, beneficial, and simple approach for diagnosing Pouch of Douglas adhesions.

1. Introduction

Endometriosis is roughly classified into three types: peritoneal endometriosis, ovarian chocolate cyst, and rectovaginal endometriosis [1]. Rectovaginal endometriosis in particular can cause the most severe symptoms including dysmenorrhea, dyspareunia, defecation pain, and chronic pelvic pain, all of which can compromise the patient's quality of life (QOL) [2]. Deep infiltrating endometriosis (DIE) is a particularly severe type of rectovaginal endometriosis. In the most severe cases, DIE results in complete cul-de-sac obliteration (CCDSO), which is evident upon laparoscopic examination [3, 4].

Adhesiolysis for CCDSO is technically demanding, and the incidence of complications is higher than when routine gynecologic laparoscopy procedures are performed because the Pouch of Douglas is adjacent to critical organs including the rectum and ureters [4]. Thus, presurgical diagnosis is very important for preoperative planning and for obtaining meaningful informed consent. However, a standard diagnostic imaging method has not yet been established for DIE.

The magnetic resonance (MR) imaging jelly method can be used to delineate the locational relationships between the uterus, vagina, and rectum and to assess adhesions in the Pouch of Douglas. The method involves application of ultrasonography (US) jelly in the vagina and rectum during MR imaging to enhance contrast [5, 6]. US jelly is characteristically hypointense on T1-weighted images and hyperintense on T2-weighted images. In the present study, we aimed to evaluate the usefulness of the MR imaging jelly method in the diagnosis of adhesions in the Pouch of Douglas.

2. Materials and Methods

2.1. Study Patients. Thirty women were enrolled in the study. Assuming a true diagnostic rate of 90% for identification of DIE on MR images obtained with jelly and a true diagnostic rate of 50% for identification of DIE on MR images obtained without jelly, we determined that a sample size of 19 patients would be needed to yield a statistical power of 80%. Taking a certain safety margin into account, a sample of 30 women was specified in the study protocol. Outpatients were recruited through our Juntendo University Hospital Department of Obstetrics and Gynecology between April 2010 and March 2012. Women presenting with menstrual pain, dyspareunia, and chronic pelvic pain were eligible for the study (1) if they received a preoperative diagnosis of benign gynecologic disease, that is, myoma, adenomyosis, and/or endometriosis by pelvic examination or by US, (2) if the diagnosed condition was indicated for laparoscopic surgery, (3) if they were scheduled to undergo such surgery at our hospital, and (4) if they provided written informed consent to participate in the study as outlined in the study protocol, which involved undergoing MR imaging twice (before and after administration of the jelly). The study protocol, including the MR imaging jelly method, was explained in writing to patients, who also provided informed consent for their imaging data and operative findings to be used for the study.

2.2. MR Imaging, with and without Jelly. We worked together with our hospital's radiology department to schedule specific times during which our study patients could be admitted for imaging. MR imaging was performed on a 1.5T scanner (VISART EX, Toshiba; Nasu, Japan) equipped with a phased array body coil to obtain T1- and T2-weighted images. Axial and sagittal T2-weighted fast spin echo images and sagittal T1-weighted spin echo images with fat saturation were obtained as 5-6 mm thick contiguous slices. All images were obtained with a 25–27 cm × 25–27 cm field of view and a 256 × 256 matrix. All images were stored in our hospital's picture archiving and communication system (PACS).

A gynecologist, one of the four gynecologists in the team of investigators, was present at each of the imaging sessions and applied the US jelly to each of the patients. Each patient was given a senna extract (0.5 g of Alosenn) to be taken at bedtime for 3 consecutive days before their scheduled exam. This was done to empty the rectum. Pelvic MR imaging was first performed routinely as described above. Then, with the patient lying on her side on the examination table in the MR imaging room, US jelly (Echo Jelly, Hitachi Aloka Medical, Ltd., Tokyo, Japan) was introduced by the gynecologist into the patient's vagina and rectum by means of our own customized 16 French Nelaton catheter attached to a single syringe. The vagina was filled with 50 mL of the jelly, and the rectum was then filled with 150 mL of the jelly diluted twice with tap water. After completion of the imaging, the patient returned home without undergoing any other procedure. The water soluble jelly was easily washed out at home during bathing.

2.3. Surgical Procedure. All 30 patients underwent laparoscopic surgery under general anesthesia with endotracheal intubation. Patients were placed in the lithotomy position, a Veress needle was inserted through the umbilical region into the peritoneal cavity, and carbon dioxide gas was used to create pneumoperitoneum. Four trocars were inserted: a 10 mm trocar for the scope at the umbilical region; two 5 mm trocars, one on either side of the iliac spine; and one 10 mm trocar in the anterior axillary line, slightly above the umbilical region.

The uterus was anteverted with the use of a Uterine Manipulator (Atom Medical Corp., Tokyo, Japan), and the posterior vaginal fornix was pushed cephalad with the tip of the shaft of the manipulator. The presence or type of CCDSO was determined according to the revised American Society for Reproductive Medicine (rASRM) classification [7]. That is, the cul-de-sac was judged to be normal when the bulge of the posterior vaginal vault was seen between the two uterosacral ligaments, partial cul-de-sac obliteration (PCDSO) was diagnosed when only part of the bulge of the posterior fornix bulge was seen, and CCDSO was diagnosed when the posterior vaginal vault could not be seen at all.

If CCDSO was diagnosed, the uterus was anteverted with a uterine manipulator, and the anterior wall of the rectum adhering to the posterior wall of the uterus was drawn cephalad with grasping forceps. The interface was opened with a monopolar needle and dissected bluntly, and the incision was repeated until the cul-de-sac was opened so that the uterosacral ligament and bulge of the posterior fornix were clearly seen.

2.4. Image Analysis. Patients' MR images were analyzed before the laparoscopic surgery was performed, and images obtained by the jelly method were used for final preoperative diagnosis. The images were interpreted with reference to six findings previously reported to be useful [6]: (1) uterine position (anteflexion or retroflexion: a retroflexed uterus was considered positive for CCDSO), (2) thickness of the posterior uterine wall (adenomyosis uteri: thickness of the muscular layer from the junctional zone ≥12 mm was considered positive), (3) ascites in the Pouch of Douglas (no ascites or ascites not reaching the level of the posterior vaginal vault was considered positive), (4) apparent tethering of the posterior vaginal vault (a beak-shaped posterior vaginal vault without a round bulge on MR images obtained with jelly was considered positive), (5) apparent tethering of the anterior wall of the rectum (a serrated anterior rectal wall without a smooth surface on MR images obtained with jelly was considered positive), and (6) a Pouch of Douglas lesion visualized as a high-intensity area on a T1-weighted image (T1WI) (a hyperintense lesion in the Pouch of Douglas between the hypointense vagina and rectum on a T1WI was considered positive). These findings were assessed within the overall context, and each radiologist made a final determination of whether CCDSO was present. Moreover, the radiologists reviewed anatomical abnormalities and/or deformities to the extent possible in making their final assessment of the presence or absence of CCDSO.

2.5. Comparisons. For the purpose of the study, MR images obtained with and without jelly (60 images in total) were extracted from the PACS and reanalyzed. The images were randomly extracted with the patients' names concealed. Furthermore, the images obtained with and without jelly were analyzed in random order.

The MR images were interpreted by the two aforementioned radiologists. One of these radiologists is a specialist who, at the time of the study, had 31 years of experience in imaging diagnosis, and the other is a fellow who, at the time of the study, had 2 years of experience in imaging diagnosis and had completed a 2-year residency. They independently analyzed the randomly extracted images and recorded their findings on a report sheet. The 6 findings described above and the final imaging diagnosis, that is, the presence or absence of CCDSO, were recorded on the sheet. A gynecologist (the first author) reviewed DVD recordings of the patients' laparoscopic surgeries and confirmed the presence or absence of CCDSO by checking the video findings against the surgical records. The presence of CCDSO was determined by this investigator according to the rASRM classification [7]. Specifically, CCDSO depended on the visible extent of the posterior vaginal vault during the adhesiolysis surgery recorded on DVD.

2.6. Statistical Analysis. Four imaging-based diagnoses were obtained for each patient because MR images before and after opacification with jelly in the same patient were interpreted by two readers. Diagnostic sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated for each of the imaging methods as interpreted by each of the two radiologists. We compared the diagnostic accuracies of the two imaging methods by examining the difference in areas under the receiver-operating characteristic (ROC) curves drawn for each method. The ROC curves were drawn to show the trade-off between sensitivity and specificity and thus reflected the accuracy of the respective imaging methods. All statistical analyses were performed with SPSS statistical software, version 18 (IBM, Tokyo, Japan).

3. Results

The 30 enrolled patients ranged in age from 23 to 45 years (mean, 36.4 ± 8.0 years). MR imaging examinations were performed for all 30 patients according to the above-described protocol. There were no adverse effects related to the MR imaging procedures, jelly administration, or surgery. Eleven of the 30 patients were diagnosed as having myoma/adenomyosis, four of whom underwent myomectomy and seven of whom underwent hysterectomy. Seventeen patients were diagnosed as having ovarian cyst(s) and underwent cystectomy. Two patients were found to have bowel endometriosis and underwent bowel resection. Of the total 30 patients, 21 had CCDSO. All 21 patients underwent complete adhesiolysis. The mean rASRM score was 68.6 ± 43.3 (range, 0–128).

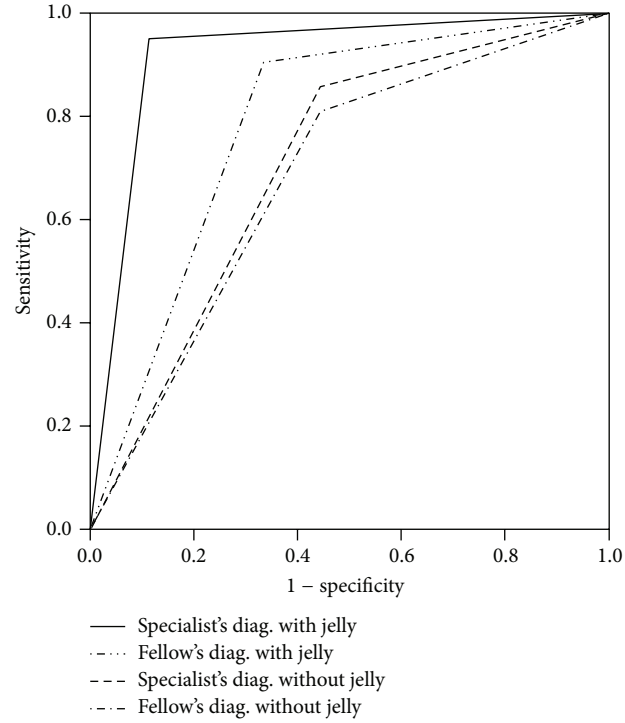


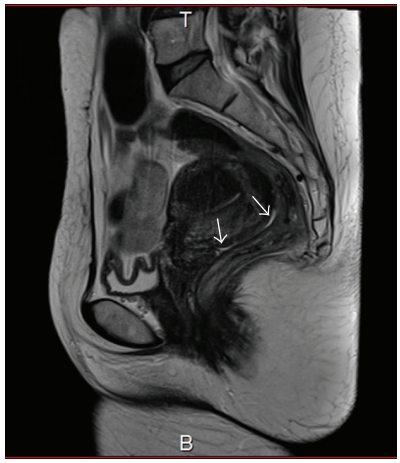
Figure 1: ROC curves. Greater sensitivity for a diagnosis of adhesions in the Pouch of Douglas was achieved for the specialist by MR imaging with jelly than by MR imaging without jelly. Greater sensitivity and specificity were achieved for the fellow by MR imaging with jelly.

The sensitivity, specificity, PPV, and NPV of MR imaging before jelly administration in the diagnosis of CCDSO, as interpreted by the radiology specialist, were 85.7% (18/21), 55.6% (5/9), 81.8% (18/22), and 62.5% (5/8), respectively. The sensitivity, specificity, PPV, and NPV of MR imaging before jelly administration in the diagnosis of CCDSO as interpreted by the radiology fellow were 81.0% (17/21), 55.6% (5/9), 81.0% (17/21), and 55.6% (5/9), respectively. Values for MR imaging with jelly administration in the diagnosis of CCDSO as interpreted by the radiology specialist were 95.2% (20/21), 88.9% (8/9), 95.2% (20/21), and 88.9% (8/9), respectively, and by the radiology fellow, they were 90.5% (19/21), 66.7% (6/9), 86.4% (19/22), and 75.0% (6/8), respectively.

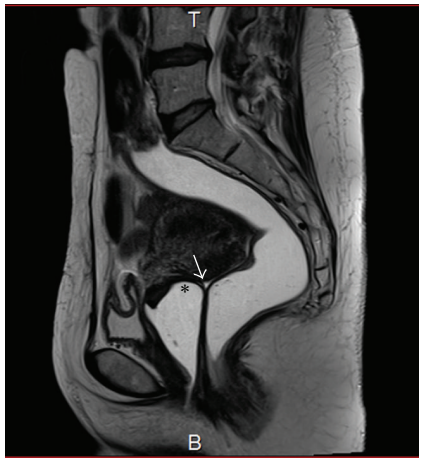
The ROC curves are presented in Figure 1. For the specialist, statistically significant results ($P = 0.001$) were achieved without jelly. However, with jelly, greater sensitivity was achieved. For the fellow, greater sensitivity and specificity were achieved with jelly than without jelly.

3.1. Example MR Images

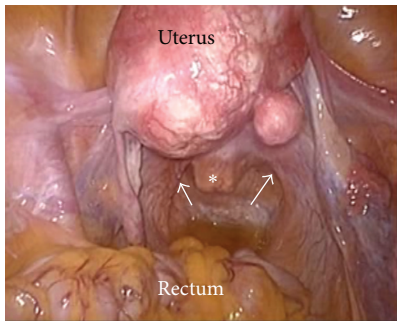
3.1.1. Absence of CCDSO. Images from a case of endometriosis without CCDSO are shown in Figures 2(a)–2(c). Figure 2(a) is an MR image obtained before jelly administration. Ascites is present in the Pouch of Douglas, but no anatomical abnormality is present. Figure 2(b) is an MR image obtained after jelly administration in the same patient.



(a)



(b)



(c)

Figure 2: (a) MR image obtained before jelly administration in a patient with endometriosis but no CCDSO. Ascites is seen in the Pouch of Douglas (white arrow). (b) MR image obtained after jelly administration in the same patient. The surface of the rectal wall has a smooth appearance, and the posterior vaginal vault (asterisk) is visualized along with the ascites in the Pouch of Douglas (white arrow). (c) Laparoscopic findings in the same patient. The posterior vaginal vault is easily visualized in the area where the handle of the manipulator (asterisk) is seen. The uterosacral ligaments are also apparent (white arrow).

The surface of the rectal wall has a smooth appearance, and the posterior vaginal vault is visualized along with the ascites in the Pouch of Douglas. The image is interpreted as showing the absence of CCDSO. Figure 2(c) is an image obtained upon laparoscopy in the same patient. The posterior vaginal vault is easily visualized in the area where the handle of the manipulator is seen. The uterosacral ligaments are also apparent, and the posterior cul-de-sac is not obliterated.

3.1.2. Presence of CCDSO. MR images from a case of ovarian cyst and typical CCDSO are shown in Figures 3(a)–3(d). Figure 3(a) is an MR image obtained before jelly administration, whereas Figure 3(b) is an MR image obtained after jelly administration. The posterior vaginal vault is tethered, and the posterior cul-de-sac is obliterated. The rectal surface is tethered, and a high-intensity lesion is seen on T1WI (Figure 3(c)). Figure 3(d) is an image obtained upon pelvic laparoscopy in the same patient. The ovarian cyst and Pouch of Douglas obliteration are clearly seen.

4. Discussion

Endometriosis is one of the most common diseases encountered in gynecological practice. Patients with endometriosis have various symptoms and conditions, and a wide variety of treatment options are available [8, 9]. CCDSO scores can be as high as 40 or more according to the rASRM scoring system, reaching a severe Stage IV condition [7, 10]. CCDSO can cause various types of pain including menstrual, chronic pelvic, and defecation pain, as well as dyspareunia, and can thereby significantly compromise QOL [9]. To date, the only definitive means of diagnosing endometriosis and assessing its severity, according to criteria such as those of the rASRM scoring system, is direct visualization during abdominal operative procedures, including laparoscopic surgery [7, 10]. Critical organs including the rectum and ureters are adjacent to the Pouch of Douglas, making injury to these structures a concern during adhesiolysis [3]. Therefore, adequate presurgical assessment and understanding of the distribution and extent of endometriosis are essential. CCDSO is not always anticipated presurgically. It can be incidentally encountered during surgery. Thus, a reliable preoperative diagnostic method is needed.

4.1. Imaging Modalities Used for Diagnosing CCDSO. Transvaginal US, rectal endoscopic US, MR imaging, and laparoscopy are reportedly used for diagnosing CCDSO, and each approach has its specific advantages [11–23]. MR imaging provides good soft tissue delineation and visualization of the entire pelvis including the support structures [18, 19]. However, nonneoplastic lesions such as adhesions involving the Pouch of Douglas are somewhat difficult to detect on MR images without contrast medium. We have reported the utility of the MR imaging jelly method for diagnosing CCDSO [5, 6]. This imaging technique facilitates the diagnosis of bowel endometriosis. A reported study showed MR imaging at 3.0T to be valuable in the diagnosis of CCDSO because it allows for detailed interpretation of images owing to

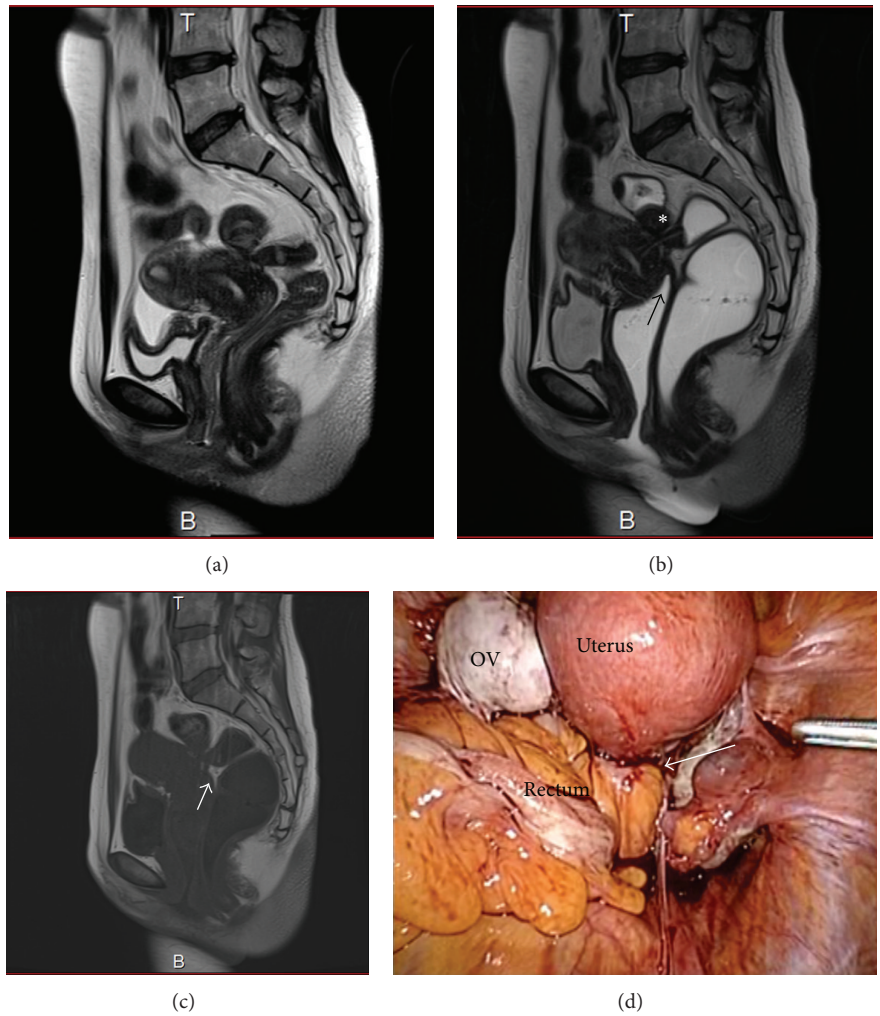


Figure 3: (a) MR image obtained before jelly administration in a patient with ovarian cyst and CCDSO. (b) MR image obtained after jelly administration in the same patient. The posterior vaginal vault is tethered (black arrow). The rectal surface is tethered (asterisk). (c) T1WI from the same patient showing a high-intensity lesion (white arrow). (d) Pelvic laparoscopy findings in the same patient. The ovarian cyst and obliterated Pouch of Douglas are seen (white arrow).

the high spatial resolution [21]. In our study, MR imaging was performed at 1.5T. A method that is similar to ours has also been reported [22, 23].

4.2. Usefulness of the MR Imaging Jelly Method. Our MR imaging jelly method was shown by the present study to be a useful diagnostic approach for deep endometriosis. Comparison of the images obtained with and without jelly administration confirmed its usefulness. The MR imaging jelly method is a convenient approach that requires no special devices, places minimal burden on patients, and is highly cost effective [5, 6]. The US jelly administered in this study is routinely used for US, making the purchase of new materials or devices unnecessary. Moreover, no adverse events occurred either in our previous studies [5, 6] or in the present study. A gynecologist administered the jelly to all 30 patients of our study; however, administration of the jelly is quite easy because only catheter insertion is necessary. No device specific to gynecologic practice is needed. Thus, not only gynecologists but also nurses and medical radiology technicians can easily be trained to administer the jelly.

4.3. Limitations. This study has several limitations. First, our study group comprised only 30 subjects. Although statistically significant results were obtained, it is possible that group homogeneity influenced our study results. We do not believe this to be the case, especially because all of the enrolled patients had symptoms such as menstrual pain but not all were found to have CCDSO. In addition, some of the patients enrolled were scheduled to undergo surgery based on a comparison of their intra-abdominal findings with the MR images used to evaluate the accuracy of the jelly method. Second, all of the images were reinterpreted after surgery. As described above, the assessments were performed in a blinded manner and in random order. However, because of the small number of subjects ($n = 30$), we cannot

be certain that the radiologists did not recall the specific patients to which the MR images belonged. Third, there may be a concern regarding reproducibility of our diagnostic method at other institutions. Of the two radiologists that interpreted MR images, one is a specialist in the field of gynecologic imaging and was closely involved in development of the radiographic method at our hospital. Thus, the other less experienced radiologist (a fellow) also interpreted the MR images, and the findings of the two radiologists were compared. However, even the less experienced radiologist achieved clinically meaningful results from the MR imaging jelly method, indicating that this method is likely to be useful for diagnosing CCDSO at other institutions.

5. Conclusion

The MR imaging jelly method was shown by our single-center study to be a useful, beneficial, and minimally invasive approach for diagnosing CCDSO. We believe that the MR imaging jelly method will be recognized as a superior diagnostic approach by patients suffering from endometriosis, by gynecologists who plan therapeutic strategies and treat these patients, by radiologists performing diagnostic imaging to provide important information for devising treatment strategies, and by all other personnel involved in the care of patients with endometriosis. The jelly method increased the sensitivity and specificity of MR imaging for diagnosis of adhesions in the Pouch of Douglas.

Ethical Approval

The study was approved by the Ethics Committee of the Juntendo University Faculty of Medicine.

Consent

All patients who participated in this study provided written informed consent for participation in the study, including the imaging protocol and use of the pertinent study data.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Research Article

Looking for Celiac Disease in Italian Women with Endometriosis: A Case Control Study

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In the last years, a potential link between endometriosis and celiac disease has been hypothesized since these disorders share some similarities, specifically concerning a potential role of oxidative stress, inflammation, and immunological dysfunctions. We investigated the prevalence of celiac disease among Italian women with endometriosis with respect to general population. Consecutive women with a laparoscopic and histological confirmed diagnosis of endometriosis were enrolled; female nurses of our institution, without a known history of endometriosis, were enrolled as controls. IgA endomysial and tissue transglutaminase antibodies measurement and serum total IgA dosage were performed in both groups. An upper digestive endoscopy with an intestinal biopsy was performed in case of antibodies positivity. Presence of infertility, miscarriage, coexistence of other autoimmune diseases, and family history of autoimmune diseases was also investigated in all subjects. Celiac disease was diagnosed in 5 of 223 women with endometriosis and in 2 of 246 controls (2.2% versus 0.8%; $P = 0.265$). Patients with endometriosis showed a largely higher rate of infertility compared to control group (27.4% versus 2.4%; $P < 0.001$). Our results confirm that also in Italian population an increased prevalence of celiac disease among patients with endometriosis is found, although this trend does not reach the statistical significance.

1. Introduction

Endometriosis is a chronic gynaecologic disorder characterized by the presence of endometrial tissue outside the uterus, mainly in the pelvic cavity. It is estimated to affect at least 5–10% of women in the reproductive age, up to 40–80% of women complaining of pelvic pain, and up to 30–50% of infertile patients [1, 2]. The pathogenesis of endometriosis is still under active investigation, and in the last few years a growing amount of data has underlined the potential role of oxidative stress, inflammation, and immunological dysfunctions in its development [3, 4]. It is noteworthy that these features seem to be not restricted only to peritoneum, being found also in the peripheral blood, so that endometriosis can be considered, in effect, as a systemic disease with a widespread inflammatory status that could also explain extrapelvic locations of endometriosis and its association with other diseases [5–9].

Celiac disease (CD) is an autoimmune enteropathy, occurring in genetically susceptible individuals, induced by the ingestion of gluten-containing foods and characterized by intestinal malabsorption and total or subtotal atrophy of intestinal villi [10]. Recent reports indicated that CD prevalence is growing, up to 2% in some Western countries [11]; in Italy a prevalence of 0.5–1% is referred to in general population [12, 13]. It is well known that CD can be associated

with other intestinal and extraintestinal diseases, in particular with autoimmune disorders, such as type 1 diabetes mellitus, autoimmune thyroiditis, rheumatoid arthritis, and Sjögren's syndrome [14–16].

Very recently, some first studies have hypothesized a potential link between endometriosis and CD, since these conditions share some similarities [17, 18]. In our study, we investigated for the first time the prevalence of CD among Italian women with endometriosis with respect to general population.

2. Materials and Methods

Consecutive women with a laparoscopic and histological confirmed diagnosis of endometriosis referring to the Department of Obstetrics and Gynaecology of Catholic University of Rome between January 1, 2012, and December 31, 2012, were considered for the study. Endometriosis severity was classified according to the American Society for Reproductive Medicine revised classification of endometriosis [19]. Female nurses of our institution, without a known history of endometriosis, were enrolled in the study as control group.

At enrollment, a venous blood sample was collected by either patients or controls for IgA endomysial (EMA) and tissue transglutaminase (t-TGA) antibodies measurement and serum total IgA dosage. Qualitative and semiquantitative detection of EMA was performed using commercially available indirect immunofluorescence antibody test (Immu-Glo, IMMCO Diagnostics, Buffalo, NY); the presence of a characteristic pattern to fluorescence microscope was scored as positive for CD. For the detection and quantification of t-TGA antibodies, commercially available ELISA kit was used (IMMCO Diagnostics, Buffalo, NY); a t-TGA titre of >25 U/mL was considered as positive for CD. Using the manufacturer recommended cut-off values, sensitivity and specificity were 90–100% and 97–100% for EMA tests, whereas t-TG kits had 98% and 97%, respectively. Serum IgA levels were determined by nephelometric method, using commercially available kit (Siemens Healthcare Diagnostic Products Gmbh, Germany). IgA levels lower than 5 mg/dL were considered abnormal.

An upper digestive endoscopy with an intestinal biopsy (at least six biopsy samples obtained from the second duodenal portion) was proposed in case of antibodies positivity. CD diagnosis was made in presence of histological findings characterized by severe or partial villous atrophy along with crypt hyperplasia as indicated by Marsh [20]; histopathology was expressed according to Marsh criteria modified by Oberhuber et al. [21].

Moreover, each subject was asked to complete a questionnaire reporting the possible presence of one or more of the following conditions: infertility (defined as the failure to conceive after one year of regular intercourse without contraception), miscarriage, coexistence of other autoimmune diseases, and family history of autoimmune diseases.

2.1. Ethics Approval. Procedures were in accordance with the ethical standards of the Helsinki Declaration of 1964, as modified by the 48th World Medical Association General Assembly in 1996. Each subject gave written informed consent to the study. The study was approved by the Ethical Committee of the Catholic University of Rome (clinical trial registration number: Prot.cm. P588 (A.1138)/C.E./2008).

2.2. Statistical Analysis. Statistical comparison of patients and controls groups was performed by means of chi-square test or Fisher's exact test as appropriate. Statistical comparison of parametric variable (age) among the groups was performed by t-test for unpaired data. All values were expressed as total count and percentage. Age was expressed as mean ± standard deviation. A P value of 0.05 or less was regarded as significant.

3. Results

A total of 315 women with laparoscopic and histological confirmed diagnosis of endometriosis were considered during the whole period study. Ninety-two (29.2%) of them refused to participate in the study, and thus 223 (70.8%) patients were finally enrolled. According to the revised American Society for Reproductive Medicine classification of endometriosis, 30 (13.4%) patients were classified as having minimal endometriosis, 71 (31.8%) patients were classified as having mild endometriosis, 48 (21.5%) patients were classified as having moderate endometriosis, and 74 (33.2%) patients were classified as having severe endometriosis [19].

Two hundred and forty-six female nurses, without a known history of endometriosis, participated in the study as controls.

Mean age was 36 ± 6.6 years in the endometriosis group and 35.1 ± 7.7 years in the control group ($P = 0.241$).

Among all subjects with antibodies positivity, none refused the endoscopic examination.

No serum IgA deficiency was found in any subject (patients or controls).

According to serological and histological findings, CD occurrence was higher in patients with endometriosis than in controls, this difference being not statistically significant: 5 (2.2%) versus 2 (0.8%), respectively ($P = 0.265$) (Table 1). By dividing 5 subjects with both CD and endometriosis according to endometriosis classification [19], CD was found in 1 patient with minimal endometriosis, 2 patients with mild endometriosis, and 2 patients with moderate endometriosis.

Table 2 shows the distribution of medical records, as reported by patients and controls in the questionnaire: patients with endometriosis showed a largely higher rate of infertility compared to control group (27.4% versus 2.4%; $P < 0.001$); all the other considered conditions were similar between the two groups.

By dividing all study population according to presence/absence of CD, familiarity for autoimmune diseases and familiarity for CD were statistically more frequent among subjects with CD (71.4% versus 8.7%; $P < 0.001$, and 42.9 versus 1.9; $P < 0.001$, resp.).

Table 1: Serological and histological findings in patients and controls.

	Endometriosis N° 223	Controls N° 246	*P*
Only EMA Ab positivity	0 (0%)	0 (0%)	1
Only t-TGA Ab positivity	0 (0%)	0 (0%)	1
Both EMA and t-TGA Ab positivity	5 (2.2%)	2 (0.8%)	0.265
Marsh-Oberhuber classification			
3a	2 (0.9%)	2 (0.8%)	1
3b	3 (1.3%)	0 (0%)	0.11

Table 2: Distribution of medical records as reported in the questionnaire in patients and controls.

Condition	Endometriosis N° 223	Controls N° 246	*P*
Infertility	61 (27.4%)	6 (2.4%)	<0.001
Abortivity	30 (13.5%)	28 (11.4%)	0.906
Autoimmune disease	20 (9.0%)	17 (6.9%)	0.838
Familial history of autoimmune disease	34 (15.2%)	35 (14.2%)	0.567
Familial history of celiac disease	12 (5.4%)	11 (4.5%)	0.978

4. Discussion

Our study reports a potential association between endometriosis and CD in Italian women, showing a trend for increased prevalence of CD in women affected by endometriosis, even if not statistically significant.

In the last few years, some recent studies about this topic have been performed, showing similar results [17, 18]. The interest arises from shared features of both CD and endometriosis, specifically concerning etiology and ongoing inflammation.

It is well known that CD is an autoimmune disorder in which an abnormal T cell response to gluten occurs. Dieterich et al. recently showed that the tissue enzyme transglutaminase is a target of immunological reaction, generating a complex of the prolamine of gluten with HLA molecule that is recognized by T helper cells [22]. It is noteworthy that CD is strongly associated with some HLA class II genes, in particular with homozygosis for HLA DQ2.5 haplotype; also a condition of heterozygosis for this haplotype associated with the presence of DQ2.2, DQ7, or DQ8 produces a higher risk of CD [23–25].

The presence of gut inflammation, resulting from the above-mentioned immunological response, together with abnormal intestinal permeability and consequent increased antigenic exposure and autoantibody production, could be also responsible for the association of CD with other autoimmune diseases: dermatitis herpetiformis, diabetes mellitus type 1, Sjögren's syndrome, rheumatoid arthritis, primary biliary cirrhosis, and sclerosing cholangitis. This theory is supported by the evidence that a number of autoantigens, normally cryptics, are processed and presented by APC to T cells, because of prolonged intestinal inflammation [26].

Another factor that could explain the association of CD with other autoimmune diseases is a common genetic background, represented by HLA haplotype; in fact, some genes in the region of major histocompatibility complex are involved in multiple autoimmune disorders, such as HLA DQA1 and DQB1 genes that can confer risk to both CD and type 1 diabetes [27].

Despite decades of extensive research, the pathogenesis of endometriosis remains not completely clarified. Actually, endometriosis is considered a multifactorial disorder, in which the *primum movens* seems to be represented by retrograde endometrial debris reflux into the peritoneal cavity that promotes increase of oxidative stress and consequent low-grade inflammation [3, 28, 29]. Peritoneal macrophages have been identified as key processes, by producing growth and angiogenic factors, as well as various proinflammatory cytokines that could be responsible for maintenance of disorder and impairment of reproductive function, as well as the systemic involvement that characterized endometriosis [30, 31]. In the last years, chronic inflammation with increased oxidative stress has been reported to be involved also in the association of endometriosis with other chronic inflammatory diseases, as atherosclerosis [32]. According to these evidences, endometriosis is now considered a chronic inflammatory disease, with inflammation not limited to peritoneal cavity but spread to systemic level, as signaled by elevated serum levels of markers as Ca-125 and C-reactive protein (CRP) [33, 34].

Also a genetic predisposition has been suggested for development and progression of endometriosis, with HLA DQ7 haplotype being reported as the first allele significantly associated with endometriosis [35, 36].

A potential role of autoimmunity for endometriosis has been suggested, as it fulfills many of the classification criteria of autoimmune diseases: polyclonal B cell activation, immunological abnormalities in T and B cell functions, defective apoptosis, tissue damage, and multiorgan involvement [37, 38]. This topic is supported also by familial occurrence, genetic predisposition, female preponderance, and increased likelihood of other autoimmune diseases, like systemic lupus erythematosus, rheumatoid arthritis, Sjogren's syndrome, multiple sclerosis, and endocrine disorders [8, 39].

Among these associations, recently some studies have evaluated the relationship with CD. The first study has determined the presence of CD in a Brazilian subpopulation of women with endometriosis suffering from infertility [17]. The authors have found that prevalence rates of positive CD serology for anti-tTG and antiendomysium IgA in a group of 120 patients with endometriosis versus 1500 controls were statistically significant (4.1% in patients versus 0.8% in controls), even if the prevalence rates of the biopsy-confirmed CD did not reach the statistical significance (2.5% cases versus 0.66% controls), showing only a positive trend, maybe also because one patient refused the endoscopic examination. They have concluded that, even if not statistically significant due to small number of cases, CD is more common in women with endometriosis with respect to controls, suggesting a potential clinical relevance. In the interpretation of their results, however, we cannot ignore that control group was constituted by blood donors; these subjects, as underlined by authors themselves, cannot be considered to be representative of the general population. In fact, some conditions, first of all the presence of anemia (one of the possible manifestations of CD), have to be excluded in subjects candidate as donors. Moreover, in this report total serum IgA assessment was not performed, making it not possible to exclude serum IgA deficiency, a condition that compromises the diagnostic power of serological assays for CD. Finally, since patients with endometriosis were enrolled among subjects referring for infertility disorders, women at higher risk of CD could have been screened, infertility being a complaint also of CD. As regards, in our study the enrollment was conducted among women with endometriosis, not necessarily involving the presence of infertility; however, an increased prevalence of infertility among patients with endometriosis with respect to controls was finally found.

Recently, a Swedish nationwide population-based study has evaluated the risk of developing endometriosis in about 11.000 women with known CD [18]. During the follow-up period of study, the authors have found an increased risk of developing endometriosis, hypothesizing that chronic inflammation characterizing CD could act as trigger in endometriosis development. It is not by chance that they reported that this risk was higher in the first year after the diagnosis of CD, when mucosal healing could not be yet achieved, despite gluten-free diet start. The greater awareness to CD presence, together with improved diagnosis and some socioeconomic factors, has led to increased prevalence of CD, especially identifying mild degrees of CD; pursuant to authors' view, pointing severity of inflammation due to CD to be crucial for endometriosis development, the presence of mild CD could modify the association with endometriosis. As regards, in our study all subjects diagnosed as affected by CD (both patients and controls), although clinically not suspected for CD, presented villous atrophy with high grades of inflammation at intestinal biopsy.

In conclusion, our results confirm the potential association between CD and endometriosis, although this trend does not reach the statistical significance. Further studies with higher number of subjects are desirable to definitively support this relationship. Actually, we can suggest screening a woman with endometriosis for CD if a valid clinical suspect is present.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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