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ORIGINAL ARTICLE

OC-125 immunostaining in endometriotic lesion samples

Caio Parente Barbosa · Ângela Mara Bentes de Souza · Bianca Bianco · Denise Maria Christofolini · Fernanda Abani Mafra · Geraldo Rodrigues de Lima

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Abstract
Purpose To determine the presence of OC-125 staining in endometriotic lesions and to verify whether there is an association with endometriosis stage.
Methods Thirteen patients from the Family Planning programs (group I) and 53 patients from the Chronic Pelvic Pain outpatient clinic (group II) were studied. Endometriotic lesions were excised from areas of endometriosis incidence and studied by histopathological assay and by immunohistochemistry for OC-125 staining.
Results The histopathological study disclosed that all patients from group I had minimal/mild endometriosis. In group II, 39.6% had minimal/mild endometriosis, and 60.4% had moderate/severe endometriosis. OC-125 staining was negative in all samples from group I. In group II, OC-125 staining was positive in 52.4% patients with minimal/mild endometriosis and in 81.2% with moderate/severe endometriosis.
Conclusion The data suggest that the OC-125 antibody is probably related to endometriosis activity and, consequently, to the progression and severity of the illness.

Keywords Endometriosis · OC-125 immunoreactivity · Laparoscopy surgery · CA-125 · Pelvic pain

C. P. Barbosa (✉) · Â. M. B. de Souza · B. Bianco · D. M. Christofolini · F. A. Mafra
Disciplina de Genética e Reprodução Humana, Departamento de Ginecologia e Obstetrícia, Faculdade de Medicina do ABC, FMABC, Avenida Príncipe de Gales, 821, Prédio CEPES, 2°. Andar, Santo André, São Paulo CEP 09060-650, Brazil
e-mail: caiopb@uol.com.br

B. Bianco
e-mail: bianca.bianco@hotmail.com

G. R. de Lima
Division of Gynecology, Department of Gynecology, Universidade Federal de São Paulo, São Paulo, São Paulo, Brazil

Introduction

Endometriosis is a condition that often leads to a variety of symptoms that range from pain to infertility, but it is also found in women who are asymptomatic. Management of this disease remains challenging, as the prevalence in clinical populations varies from a 4% occurrence of largely asymptomatic endometriosis found in women undergoing tubal sterilization to 60% of patients with chronic pelvic pain [1].

Endometriosis is a polygenic/multifactorial disease, including hormonal and immunological as well as genetic factors [2]. The natural history of this disease is still to be clarified. Endometriosis may have a dynamic and moderately progressive behavior, with periods of progression and regression and active remodeling among different types of lesions. Consensus can be easily established regarding the relationship of moderate and severe endometriosis with chronic pelvic pain and/or infertility [3]. Indeed, most of the patients present with ovarian endometriosis and/or peritoneal lesions. However, because of the limited information currently available about the activity of lesions described in studies on mild and minimal endometriosis, any absolute statement on these endometriosis stages and chronic pelvic pain is probably inappropriate at this time [4, 5].

The only way to positively determine the existence of peritoneal endometriosis is by surgical inspection and

histological confirmation. There is no reliable method so far for discriminating in which cases endometriosis is a disease and in which it is an incidental finding. Cancer antigen 125 (CA-125) plasma concentrations are largely used to diagnose endometriosis, but it has proven problematic, especially in patients with minimal and mild endometriosis [6]. CA-125 is a glycoprotein of epithelial origin, which is produced in the celomic epithelium during embryonic development and is encoded by the *MUC16* gene [1, 7]. It may be elevated in several benign diseases and in patients with non-ovarian malignancies, including cancers of the endometrium, lung, breast, pancreas and colon, but it also occurs in the serum of healthy males and females at low concentrations [8].

Bast et al. [9] developed a monoclonal antibody (OC-125) against ovarian cancer cells from human epithelial lineages, by immunizing female rats with cystadenocarcinoma cells. After immunization, the spleens of these rats were fused with myeloma cells, obtaining hybridomas. The OC-125 antibody produced from these hybridomas reacts with the different epithelial cells of ovarian cancer, but does not react neither with other tumors nor with normal ovarian epithelium. CA-125 is the antigenic expression of this antibody and the use of this antibody may help to detect endometrial antigens in peritoneal lesions.

The aim of this study was to determine the expression of CA-125 by OC-125 staining and to find out whether there is an association with endometriosis stage, using endometriotic lesion samples from healthy patients and from patients with chronic pelvic pain.

Materials and methods

Subjects

Patients with endometriosis were recruited consecutively from the Chronic Pelvic Pain outpatient clinic and from Family Planning Programs. This prospective observational study was approved by the Research Ethical Committee of Faculdade de Medicina do ABC. Patients were included in the study after confirmed diagnosis of endometriosis by surgical inspection and/or histological examination. The exclusion criteria were the use of hormonal medications for 3 months prior to the surgical procedure, intraoperative finding of ovarian neoplasia or pelvic inflammatory disease, pregnancy, and patients with endometriosis classified as stromal disease by histological examination. None of the patients had previous history of abdominal or pelvic surgical intervention. They were grouped as follows:

- Group I ($n = 13$): healthy patients who underwent tubal ligation by laparoscopy. In our Department, the prevalence of peritoneal lesions suggestive of endometriosis in patients submitted to tubal ligation by laparoscopy is 16% (unpublished data).
- Group II ($n = 53$): symptomatic patients with chronic pelvic pain. Indication of surgical investigation for these patients was based on the guidelines proposed by International Pelvic Pain Society [10]. Briefly, characterization of pelvic pain, laboratorial screening to exclude other causes of chronic pelvic pain such as pelvic infection, gastrointestinal and genitourinary diseases, clinical treatment (anti-inflammatory) for at least 6 months without improvement of symptoms.

Surgical procedure and peritoneal sampling

The surgical procedures of both groups were scheduled on random days of the menstrual cycle and were performed by the same surgeon (C.P.B). After pneumoperitoneum installation, a detailed inspection of pelvic peritoneal lining was performed to identify and classify (location and type) potential endometriotic lesions [11]. The lesions viewed during laparoscopy were divided into typical or pigmented (black, suggestive of chronic disease) and atypical or non-pigmented (red, suggesting active disease). Endometriosis was scored according to the revised American Fertility Society (r-AFS) classification [12].

Peritoneal samples sized 3–5 mm were excised from four areas of endometriosis incidence, such as left and right ovarian fossae, and left and right sacral-uterus ligaments. These four areas were selected as they are the most frequent sites of pelvic endometriosis. Additional peritoneal samples were collected from other areas if further lesions suggestive of endometriosis were identified during surgical inspection.

Histological criteria for endometriosis

Biopsy specimens were fixed in a 10% formalin solution and embedded in paraffin. Histological sections (3–5 mm thick) were obtained and stained with hematoxylin–eosin. The criteria for histological classification of endometriosis were identification of stromal endometrioid or epithelial elements of the Mullerian type, with or without stroma, associated with signs of hemorrhage and fibrosis.

After histological confirmation of endometriosis, the lesions were classified morphologically, based on the variations in the ectopic endometrial structures [13]. Morphological criteria for analysis were: stromal disease, when only endometrial stroma was found; well-determined disease, when glands similar to topic endometrium were found; undifferentiated disease, when the aspect of the glands was different from topic endometrium; and mixed disease, when the aspect of the glands was typical or undifferentiated [14].

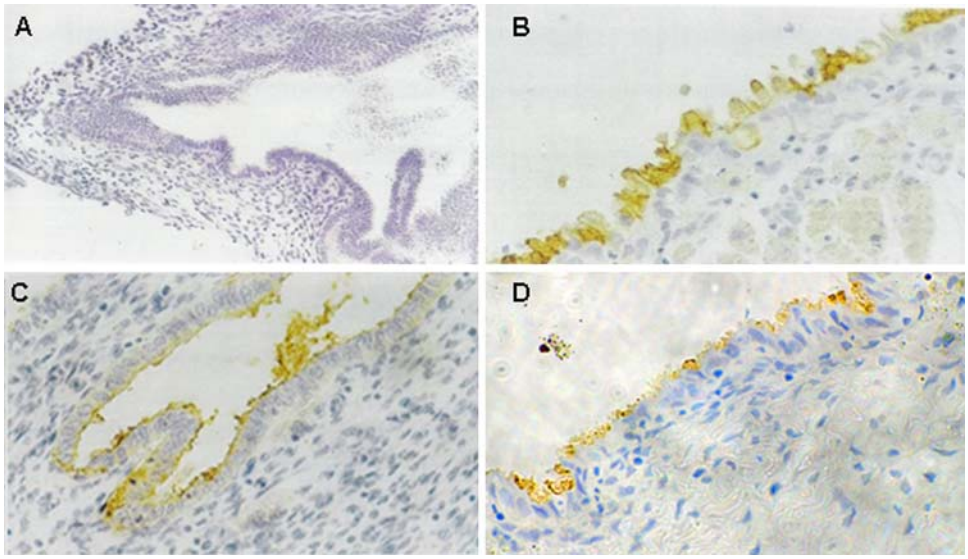

Fig. 1 Light micrographs (×400) of a pelvic lesion from an asymptomatic patient, showing negative OC-125 immunoreactivity test (**a**); ovarian endometrioma (**b**)—a positive control, endometrium (**c**) and a peritoneal endometriotic lesion (**d**) showing positive OC-125 immunoreactivity test

Immunohistochemistry assay

All biopsy specimens were fixed with 10% formalin solution and embedded in paraffin. Four-micrometer serial sections of tissue samples were subjected to immunoperoxidase biotin–avidin reaction, to determine the local expression of CA-125, using the monoclonal antibody mouse anti-human CA-125 OC-125, at a dilution of 1:20 in the Labeled Streptavidin Biotin method (LSAB) (Dako clone M11, Carpinteria, CA, USA), according to Gimeno et al. [15] with modifications. In our laboratory, tests using OC-125 staining showed negative results for normal peritoneum, normal ovary and endometrial stromal cells; and positive results for ovarian and endometrial adenocarcinoma.

All samples were analyzed field by field, using 20× and 40× objectives of an Axioskop light microscope (Zeiss, Germany). The presence of the antigen was detected qualitatively only by the brown appearance in the cytoplasm and classified as positive or negative. For all negative results, a second slide was analyzed for confirmation. Ovarian and endometrial adenocarcinoma samples were used as positive controls (Fig. 1).

Statistics

Data analysis was performed using the chi-square test. For correlation testing, Spearman's coefficient was used. They were conducted using SPSS, version 11. Statistical significance was set at $P < 0.05$ (Table 1).

Results

The mean age was 31.6 ± 5.8 years for group I and 33.6 ± 4.6 years for group II ($P > 0.05$). There were no clinical complaints in group I. The major complaints reported by the 53 women from group II were dysmenorrhea (89.0%), acyclical pelvic pain (76.4%) and dyspareunia (52.1%).

Surgical inspection and histological study

In group I, endometriotic lesions were located in the uterosacral ligaments (53.8%) and ovarian fossae (46.1%). In two cases, lesions were found in more than one site. Regarding the type of lesions, there were six (46%) cases

Table 1 OC-125 immunoreactivity test in peritoneal endometriotic lesions

Patients	Endometriosis	*n*	OC-125 immunoreactions		Ratio (%)
			Positive	Negative	
Symptomatic	I or II	21	11	10	52.3
	III or IV	32	26	6	81.25
Asymptomatic	I or II	13	0	13	0

Chi-square test ($X^2 = 5.014$); correlation was tested using Spearman's coefficient ($P < 0.01$)

classified as typical, four (31%) as atypical, and in three (23%) cases lesions were found only by histopathological study (unapparent endometriosis). All 13 patients had only peritoneal lesions scored as minimal/mild endometriosis. No ovarian endometriomas or adhesions were found in this group.

In the group II, endometriotic lesions were located in the uterosacral ligaments (56%), ovarian fossae (32.5%), vesicouterine pouch (21.5%), ovaries (19.5%), cul-de-sac (18.5%), broad ligament (7.5%), sigmoid surface (5%), uterine surface (5%), and fallopian tubes (5%). Out of the 53 patients with pelvic pain, 21 (39.62%) had minimal/mild (I/II) endometriosis and 32 (60.37%) had moderate/severe endometriosis (III/IV). Patients with minimal/mild endometriosis had only peritoneal lesions located in the uterosacral ligaments (58%) and ovarian fossae (42%), classified as typical (9, 42.8%) and atypical (8, 38%). In six cases, lesions were found in more than one site. Patients with moderate/severe endometriosis had lesions in multiple sites with all types of lesions.

Immunohistochemistry assay

The positivity of OC-125 staining varied between the groups. In group I, OC-125 was negative in all samples. In the group II, the prevalence of positive OC-125 in patients with minimal/mild and moderate/severe endometriosis was 52.38% (11/21) and 81.25% (26/32), respectively. A correlation was found between the degree of endometriosis and the positivity of OC-125 staining in the endometriotic lesion samples ($P < 0.01$). No correlation was found between the type and location of endometriotic lesions and positivity of OC-125. Statistics were applied only to group II, as in group I all samples were negative for the OC-125 immunoreaction.

Discussion

An endometriotic lesion is defined as the presence of endometrial tissue outside the uterus, causing a variety of symptoms, including infertility, pelvic pain and dysmenorrhea [7]. Laparoscopy or laparotomy should be performed to confirm and classify the stage of endometriosis according to the revised American Fertility Society [1]. In practice, severe endometriosis can be diagnosed with high accuracy by pelvic examination and tools such as transvaginal ultrasonography [16] and magnetic resonance imaging [17]. However, these imaging tools are still not sensitive enough to detect mild endometriosis [18].

In the present study, no OC-125 immunoreactivity was observed in asymptomatic patients. Among the symptomatic patients, the antibody was detected in 52.38% of the patients with endometriosis level I or II, and in 81.25% of the patients with endometriosis level III or IV. Thus, the frequency of OC-125 was significantly higher in patients with endometriosis level III or IV than in patients with level I or II, confirming a positive relationship of this antibody with disease activity, once it was more frequently found in the more severely affected patients.

Antibody production depends, of course, on the presence of antigens. However, the critical antigen level necessary to start the production of OC-125 antibodies is still unknown [19]. In our study, we did not observe OC-125 antibody immunoreaction on the peritoneum of asymptomatic patients with and without endometriosis, but only in endometriotic lesions of symptomatic patients, arising according to the disease severity. Could OC-125 be related to the severity and prognosis of endometriosis?

Endometriosis is a major cause of infertility and pelvic pain, with a high frequency worldwide; however, its etiology, physiopathology and behavior are still obscure. Moreover, there are no biomarkers associated to neither pain and/or infertility, nor indicating the progression and remission of the disease [20].

Superficial endometriosis has been described as a cyclical and normal phenomenon in the life of a woman, but in some women the development and progression of this disease occurs as a result of immunological [7] and genetic alterations. Some authors have considered superficial endometriosis as a physiological and intermittent condition in women during their reproductive years, whereas its progression, characterized as deep infiltrative endometriosis and endometrial ovarian cysts, is considered to be the true disease [21–23]. Divergences persist regarding the natural history of endometriosis, its symptoms, extent, location and staging [24]. However, the incidence of pelvic pain, especially dysmenorrhea, in women with endometriosis plus infertility and dyspareunia, are considered the triad that characterizes the disease [22].

The peritoneal fluid of women with endometriosis shows a marked increase in the number of macrophages [25]. This increase causes higher local secretion of various products. Among these are growth factors and cytokines, which may be involved in the mechanism for the implantation and subsequent development and proliferation of endometriotic implants. Moreover, some authors have suggested that there is a correlation between serum levels of CA-125 and the proliferative activity of the epithelial cells in endometriotic lesions [26], because endometriosis is an inflammatory process associated with altered function of immune-related cells in the peritoneum and may be viewed as a local disease with systemic and subclinical inflammation [27, 28]. These alterations contribute to the mechanism of infertility, due to an intraperitoneal exudate of unknown cause, even in the presence of normal ovulatory function [20].

In conclusion, the data suggest that OC-125 immunoreactivity in endometriotic lesions is higher in women with severe endometriosis, probably reflecting the proliferative activity of epithelial cells and, consequently, the progression of the disease.

Conflict of interest statement None.

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