Lysosomal evaluation of endometrioma capsule epithelium and endometrium of patients with or without endometriosis

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Summary

Objective: To evaluate the number of lysosomes in glandular epithelia of the endometrioma capsule and endometrium from patients with or without endometriosis using a histochemical method (acidic phosphate determination) under light microscopy.

Method: We studied 31 selected patients with similar clinical features, except for the occurrence of menorrhagia enhanced in patients with endometriosis. All patients were submitted to video laparoscopic procedures and endometrial biopsies through hysteroscopy during the secretory phase. After surgery, the patients were divided into two groups for hysteroscopic data: GI – without endometriosis (control, n = 16) and GII – with endometriosis (n = 15). In GII, the lysosomes of the endometrioma were also counted (GIIa).

Results: The GI sample was comprised only of endometrial biopsies. We found that the number of lysosomes in the topic endometrial tissue of patients with endometriosis was significantly larger than the number in endometrioma. Also, there were more lysosomes in the endometrium of patients with endometriosis than in endometrioma-bearing patients.

Conclusion: Our data suggest that variations in the number of lysosomes may underlie the pathogenesis of endometriosis.

Key word: Lysosomes; Endometrioma; Endometriosis; Endometrium.

Introduction

Endometriosis is a clinical and pathologic entity initially described by Rokitanski in 1860 that is characterized by the presence of tissue resembling functioning endometrial glands and stroma outside the uterine cavity [1]. In addition, Sampson was responsible for the classification of this affection as a non-neoplastic disease [2]. In fact, endometriosis is one of the most enigmatic and problematic maladies affecting women of reproductive age, being associated with pain and infertility. Though it is a non-malignant disorder, endometriosis features cellular proliferation, cellular invasion, and neoangiogenesis [3]. Endometriotic tissue is most commonly implanted on visceral and peritoneal surfaces within the female pelvis. Although being one of the most frequently encountered gynecological diseases, its exact pathophysiology remains largely controversial [4].

Russel first reported endometrioma as the typical lesion of endometriosis, characterized by a dense and fibrous capsule with epithelium, which is often located on the ovaries [1]. The fluid which remains inside the capsule is generally thick and dark. Although the etiology of this disease remains unclear, some authors believe that the shedded endometrial fragments follow the blood out through all open channels, including the tubes, into the peritoneal cavity [1-3]. In fact, women with endometriosis have been found to have an alteration of the immune system that might allow the endometrial cells to implant into the peritoneum [5-7]. Another hypothesis for explaining this disease is celomic metaplasia [1, 8]. Clinically, endometriosis is a cause of chronic pelvic pain and infertility; however, some patients are asymptomatic. Therefore, the real prevalence is uncertain [9-14].

It is well known that endometriosis depends on circulating hormonal levels such as estrogen for its development and progression. In fact, some authors have identified sex steroid receptors in that tissue [15]. However, a study of 196 women showed that approximately 13% of the endometrial implants were histologically synchronous with the corresponding intrauterine endometrium, thus suggesting that the hormonal responsiveness of endometrial implants is unpredictable [16]. On the other hand, some histological differences have been demonstrated between the endometrium and endometrial ectopic implants [17]. This fact may explain why some patients respond inconsistently to hormonal treatment. Therefore, there remains a piece to be found of the large endometriosis puzzle.

It was demonstrated that hormonal therapy inhibition of the proliferation and secretion of endometriosis is related to enhanced activity of the lysosomal system in the epithelial cells of some endometriotic foci [18]. Consequently, lysosomes might be involved in the pathogenesis of this disease. In addition, other studies used lysosomal counting as a marker of hormonal activity on the endometrium [19-21]. The maximum number of lysosomes is detected during a woman's peri-ovulatory period and minimal amounts are found during the beginning of the proliferative phase [19]. These values match blood estrogen levels;

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accordingly, lysosomal counting may be useful as a marker of estrogen activity on the tissue. In addition, other experiments have shown that estrogen administration increased the number of lysosomes whereas progesterone had the opposite effect [20]. Also, treatment with the antiestrogen drug tamoxifen reduced the amount of lysosomes in human mammary gland tissue [21]. These facts support the view that lysosomes are cell organelles under hormonal regulation. Thus, evaluation of the number of lysosomes in patients with endometriosis may help clarify the reason why endometrioma may be non-responsive to hormonal treatment. This study analyzes the number of lysosomes in endometrioma epithelium and compares the endometrial tissue of patients with and without endometriosis in this regard.

Material and Methods

This prospective study was designed to investigate the lysosomal counts in endometrioma comparing those found in an otherwise healthy sample of the endometrial tissue of the same individuals and those found in endometriosis-free patients. All patients attended screening and baseline visits and were subsequently enrolled in the Infertility Outpatient Section at the Department of Gynecology of the Federal University of São Paulo - Escola Paulista de Medicina, S.Paulo, Brazil.

We collected clinical data from all patients. Height and weight were measured; the body mass index (BMI, calculated as kg/m^2) was used as an estimate of obesity. The eligible criteria for this study were women who would submit to laparoscopic procedures due to infertility with pelvic chronic pain, and non-users of lipid-lowering drugs, antidiabetic medications, or any hormonal treatment (within less than 6 months). The exclusion criteria were Cushing's syndrome, late-onset 21-hydroxylase deficiency, thyroid dysfunction, hyperprolactinemia, androgen secreting tumors, diabetes mellitus, renal or hepatic chronic disease, Müllerian malformations, acute or chronic inflammatory processes of the endometrium, endometrial polyps, dysfunctional menstrual bleeding, myomas, endometrial hyperplasia, and synechia. The study started October 1999 and lasted 12 months. All patients signed an informed consent form for their participation in the study after reading the protocol of the study. The methods and the protocol were approved by the Ethics Committee of the Institutional Review Board.

All patients were submitted to hysteroscopy with endometrial biopsy for investigation of infertility and diagnostic laparoscopy during the early secretory phase of the menstrual cycle. Endometriosis was confirmed by means of biopsy of the endometrioma capsule or endometriotic implants. After surgery, the patients were divided into two groups for hysteroscopic data: GI-without endometriosis (control, n = 16) and GII-with endometriosis (n = 15). In GII the lysosomes of the endometrium (GII) and the endometrioma were also counted (GIIa, n=12).

Fragments of the endometrial samples and endometrioma were fixed in formaldehyde and submitted to anatomopathological study. The remaining fragments were fixed in formaldehyde-calcium-saccharose in order to study the lysosomes. The lysosomes were identified by the cytochemical method of acid phosphatase detection by Gomori [22]. The methods of Martins *et al.* [19], Freitas *et al.* [20], and Facina *et al.* [21] were used to count lysosomes. The lysosomes were counted in normal endometrium (GI), in the endometrium of patients with endometriosis (GII) and in the endometrioma (GIIa). In the endometrium the lysosomes were counted in the glandular epithelial cells.

Data analysis

Values are expressed as mean ± S.D. Results were analyzed by the paired Student's *t*-test to compare the differences in lysosome counts between the endometrial and endometrioma tissue within the same patient, and the unpaired *t*-test was used to analyze the values between patients with and without endometriosis and the clinical data. Differences were considered significant at the $p < 0.05$ level. All statistical tests were done using GraphPad Prism version 3.00 for Windows (GraphPad Software, San Diego, CA).

Results

The clinical data of patients are summarized in Table 1. No appreciable differences were detected between GI and GII in menstrual cycles, age, age of menarche, race, number of pregnancies, race, and body mass index. However, the number of patients with menorrhagia in GI was lower than GII ($p < 0.02$).

Table 1. – *Clinical characteristics of patients before the surgical procedures.*

	GI (n = 16)	GII (n = 15)
Menstrual cycles (n. per year)	9.1 ± 0.3	10.7 ± 0.8
Age	28.5 ± 2.1	29.7 ± 1.8
Age at menarche	11.2 ± 1.1	12.2 ± 0.5
Number of pregnancies	0.4 ± 0.5	0.8 ± 0.7
Race		
Caucasian	16	15
Menorrhagia	2/16	13/15*
Body mass index (kg/m^2)	22.1 ± 0.5	23.4 ± 0.4

n. = number; *p < 0.02 compared to GII.

The patients with endometriosis presented in Figure 1 show lysosomes in the glandular epithelial cells of the endometrioma and endometrium as revealed by the Gomori method [22]. The largest amount of lysosomes is located on the apical region of the endometrial cells. The amount of organelle in the endometrial cells is significantly larger than that in endometriomas.

The lysosomal count of endometrial cells in GI was significantly higher than in GIIa ($p < 0.001$). The number of lysosomes in the endometrioma (GIIa) was found to be lower than in GII ($p < 0.001$).

Discussion

Endometriosis is a complex disease with many pathophysiologic mechanisms, which also involves the immunological system. In fact, few studies have shown a link between this disease and the deficit in natural killer cells which presented antigens for macrophages [23-25]. In addition, some studies have demonstrated that lymphocytes obtained from endometriosis-free patients were significantly more efficient in cytolysis of isolated endometrial stromal cells than were lymphocytes obtained from endometriosis-bearing patients [26, 27]. However, these

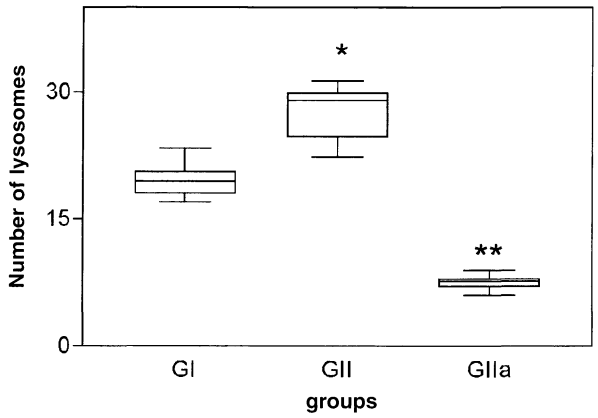

Figure 1. – Lysosomal countings in glandular epithelial cells of the endometrioma and endometrium. GI – endometria of patients without endometriosis (n = 16); GII – endometria of patients with endometriosis (n = 15); GIIa – endometrioma (n = 12). *p < 0.001 compared to GI; **p < 0.001 compared both to GI and GII.

immunological phenomena appear to be different from the cellular events in vivo. The epithelium of endometrioma had fewer lysosomes than the epithelium of the endometrium of patients with and without endometriosis (Figure 1). This finding might explain, at least in part, the resistance of endometrioma to hormonal therapy. This hypothesis may be supported by the fact that the number of lysosomes is related to cytolysis, focal death, and degradation of the matrix [17, 19, 28].

Some studies have demonstrated that the number of lysosomes reflected cellular metabolic response of some tissues to estrogenic effects [29, 30]. Women with endometriosis may respond differently to endogenous sexual steroids and present more degradation of the endometrial matrix than normal women. Herein, our data showed that women with endometriosis had a higher number of lysosomes in the endometrium than those without endometriosis. This result may support the idea that women with endometriosis might present more degradation of the endometrial matrix than those without this disease. Therefore, this fact may increase the blood flow to the abdominal cavity (retrograde menstruation) [1, 3, 5] and then these patients may be prone to develop endometriotic foci. Additionally, the endometrioma capsule presented less lysosomes than the eutopic endometrium, thus indicating that the metabolism and response to estrogens is changed in the abdominal cavity after implantation.

Our data showed that patients with endometriosis presented a difference in lysosomal number in the endometrium compared to patients without endometriosis. Also, the endometrioma epithelial cells presented fewer numbers of lysosomes than endometrial epithelial cells. These facts suggest that lysosomes may be involved in endometriosis pathogenesis.

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