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Immunohistochemical Study of Estrogen and Progesterone Receptors and the Proliferation Marker Ki-67 in Abdominal Wall Endometriosis

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Authors' contributions

This work was carried out in collaboration among all authors. Authors FDCM, MOF and JEJ made substantial contributions to conception and design, acquisition of data and analysis and interpretation of data. Authors FDCM, JEJ and ADSM participated in drafting the article or revising it critically for important intellectual content. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: The scar from pelvic surgery in the abdominal wall can be a site of endometriotic implants in 0.03 to 1% of patients. This study aims to evaluate the immunohistochemical expression of the estrogen receptor (ER), progesterone receptor (PR), and cell proliferation marker Ki-67 in patients with abdominal wall endometriosis.
Materials and Methods: We investigated seven women with abdominal wall endometriosis who underwent surgery to remove a lesion at the Assis Chateaubriand Maternity, Fortaleza, Brazil. From tissue blocks, histological sections were subjected to immunohistochemistry to identify ER, PR, and Ki-67. For statistical significance, an unpaired *t*-test was applied with a 95% confidence interval.
Results: The mean patient age was 30.4 ± 1.13 years. ER expression in the epithelium and stroma had a mean score of 33.45 and 17.14, respectively, and the difference was not significant. PR had a

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mean epithelial score of 175.71 and a mean stromal score of 72.29, significantly different (p = 0.0339). Ki-67 had epithelial and stromal scores of 14.14 and 12.14, respectively, which were not significantly different.

Conclusions: Abdominal wall endometriosis presents ER and variable PR expression in the epithelium and stroma. The Ki-67 marker demonstrated a reduced proliferation index.

Keywords: Endometriosis; abdominal wall; estrogen receptor; progesterone receptor; Ki-67.

1. INTRODUCTION

Extraperitoneal endometriosis is a rare condition that may affect the central nervous system, lung, and pleura, stomach, bladder, small and large intestine, appendix, kidney, gallbladder, abdominal wall, vagina, and perineum [1-3]. A pelvic surgery scar in the abdominal wall can be the site of endometriotic implants with an incidence from 0.03 to 1% [4]. Endometrial tissue can be implanted directly into the scar during surgical procedures, or the condition could happen through metaplasia and/or lymphatic and hematogenous spread [5,6]. There are cases with no history of previous surgery, making it an even more enigmatic issue [7,8]. The possibility of association with malignancy, particularly clear cell carcinoma, has been reported [9,10].

Clinically abdominal wall endometriosis is characterized by the appearance of firm nodules, commonly subcutaneous, which may be painful [11].Diagnostic methods are based on imaging (ultrasonography, computed tomography, and magnetic resonance imaging) [12] and histopathological studies (fine-needle aspiration or excisional biopsy) [13].

Histologically the lesion is characterized by numerous glandular structures coated by columnar epithelium with stromal component and hemosiderin-laden macrophages [1,14].

Apparently, pelvic endometriosis lesions have a steroid receptor expression pattern compared to that of the topical endometrium [15]. However, neither the expression of steroid receptors nor proliferation markers concerning abdominal wall endometriosis have been studied.

This study's objective was to evaluate the immunohistochemical expression of ER, PR, and Ki-67 in cases of abdominal wall endometriosis.

2. MATERIALS AND METHODS

It was a cross-sectional, non-interventional study done for seven cases of histological blocks of abdominal wall (subcutaneous) endometriosis nodules submitted to surgery for lesion excision in the Assis Chateaubriand Maternity, Gynecology Service, Fortaleza, Brazil from January 2010 to December 2015.

The tissues were processed for microscopic examination [16]. Histological sections with 4 micrometers thickness were obtained and, put on slides previously coated with 10% Poly-L-lysine (Sigma, USA). Briefly, according to the manufacturer's protocol, antigen retrieval was performed, then blocking the endogenous peroxidase for 10 min, incubation with the primary antibody at the appropriate dilution for each antibody (ER, PR, and Ki-67), and at the maximum time of 60 minutes at room temperature. Incubation with polymer-coupled secondary antibody (Envision ™, mouse or rabbit, DAKO, USA) was followed for 45 min, followed by development on the chromogen substrate 3,3'- diaminobenzidine (DAB), mild counter-staining with Harris hematoxylin (1 min) and assembly with synthetic balm.

Receptor and cell proliferation marker scores were calculated according to the staining intensity (from zero to three) and percent stained cells. The intensity level was multiplied with the percentage of cells, reaching a score ranging from 0 to 300 [16]. The expression of receptors between the epithelial region and the stromal region of each abdominal wall endometriosis node was compared.

For statistical significance, an unpaired t-test was applied for a 95% confidence interval.

3. RESULTS

Among the studied cases of extra-pelvic endometriosis, all were in a post-cesarean abdominal scar. The patients' mean age was 30.4 ± 1.13 years, and nodule excision was performed in the second phase of the menstrual cycle.

The results of immunohistochemistry were presented as follows (Table 1):

Thus, the expression of ER in epithelium and stroma had a mean score of 33.45 and 17.14, respectively, and the difference was not considered significant ($p>0.05$). PR had a mean epithelial score of 175.71 and in the stroma of 72.29, which was significantly different ($p = 0.0339$). Ki-67, on the other hand, had a score on the epithelium and the stroma of 14.14 and 12.14; the difference was not considered significant ($p>0.05$). (Table 1) (Fig. 3).

4. DISCUSSION

According to Ecker et al. [17] there is a shortage of abdominal wall endometriosis publications. Meanly studying. There is no study comparing estrogen/progesterone receptors and proliferation index in the epithelium with that in the stroma. In the present study about the expression of ER, PR and Ki-67 among seven abdominal wall endometriosis nodules, there was a significant variation in the lesion's epithelial part or in its stromal site. Even PR in epithelial tissue, which showed the highest expression scores, had a zero score case. Despite this, 6 of the seven patients had a score higher than 100 for PR in the epithelium (mean 175.71). This receptor in the stroma was expressed in all cases with a score of at least 20 (mean 72.29). ER in the epithelial cells had a case of zero score, and the others had no score higher than 100 (mean 33.43), while in the stroma, this expression was lower (mean 17,14). Therefore, there is a much greater PR expression, especially in epithelial cells and a little lower in stromal cells, but both higher than ER expression, i.e., apparent synchrony with the menstrual cycle phase in 6/7 cases (85, 7%). ER and PR had more expression in epithelium than in stroma but with no statistical significance.

In general, foci of endometriosis express steroid estrogen (ER), progesterone (PR), and androgen (AR) receptors. The steroid receptor expression pattern has been compared between endometriosis tissue and the topical endometrium [15]. The electron microscopy analysis showed that 1/3 of the endometriosis implants are out of phase with the menstrual cycle [17], optical microscopy showed that only 13% of the endometriosis implants were synchronous with the corresponding endometrium [18]. Other studies [19-21] have shown a consistent reduction in ER and RP expression in endometriosis implants, while Jones et al. [22] refer to an increased ER expression in endometriosis implants. A recent study of immunohistochemistry of pelvic and extra pelvic endometriosis showed heterogeneity, revealing lesion type-specific differences and case-by-case variability in the expression of ovarian hormone receptors. The authors considered the necessity of personalized medicine to approach case by case [23].

Non-separation of the identification of ER types in alpha and beta is a limiting factor in this study. However, in a systematic review conducted by May et al. (2011) [24], the authors observed no difference observed in ER, alpha or beta, investigated separately in cases of endometriosis.

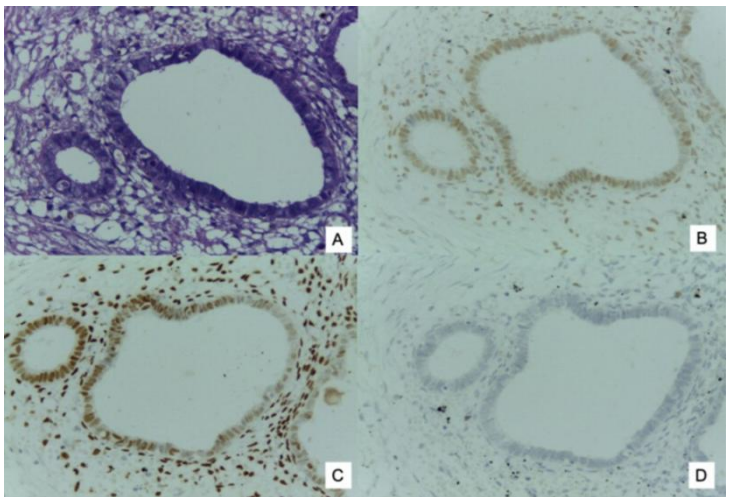


Fig.1. Illustration of hematoxylin-eosin stained biopsy (100x) (A) and immunohistochemical expression (100x) of RE (B), RP(C) and KI-67(D) in epithelial and stromal cells in case of endometriosis of abdominal wall corresponding to case 4

Table 1. Results of immunohistochemistry expression of RE, RP, and KI-67 in epithelial and stromal cells in cases of abdominal wall endometriosis

	Estrogen Receptor						Progesterone Receptor						Ki-67					
	Epithelial cells			Stroma cells			Epithelial cells			Stroma cells			Epithelial cells			Stroma cells		
Case	**%**	**I**	**S**	**%**	**I**	**S**	**%**	**I**	**S**	**%**	**I**	**S**	**%**	**I**	**S**	**%**	**I**	**S**
1	25	2	50	12	2	24	40	3	120	20	3	60	1	2	2	5	3	15
2	15	1	15	1	1	1	80	3	240	12	3	36	1	2	2	1	2	2
3	21	1	21	1	1	1	70	3	210	30	3	90	0	0	0	0	0	0
4*	8	1	8	4	1	4	60	2	120	10	3	30	0	0	0	0	0	0
5	50	1	50	30	1	30	90	3	270	90	2	180	15	3	45	4	2	8
6	0	0	0	0	0	0	0	0	0	10	1	20	0	0	0	15	3	45
7**	45	2	90	30	2	60	90	3	270	30	3	90	30	3	90	5	3	15

% = percent stained cells; I = staining intensity; S = score (% x I)

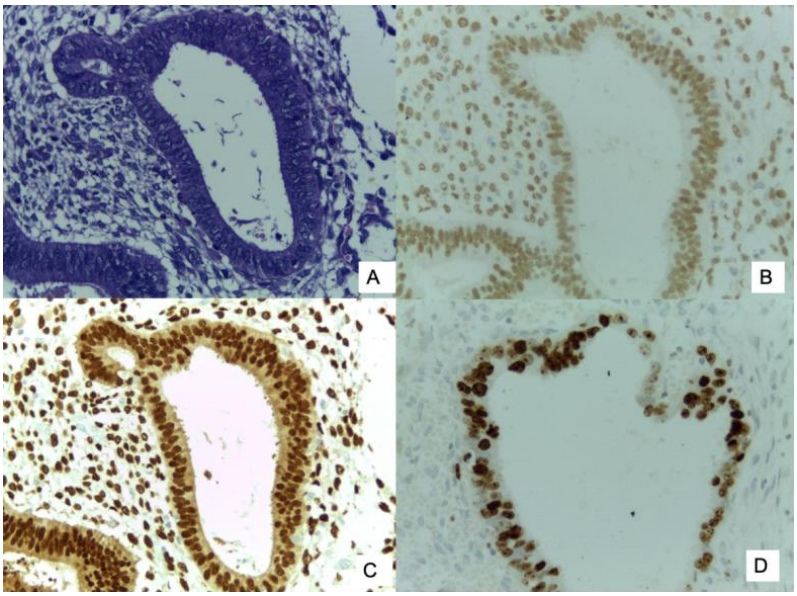
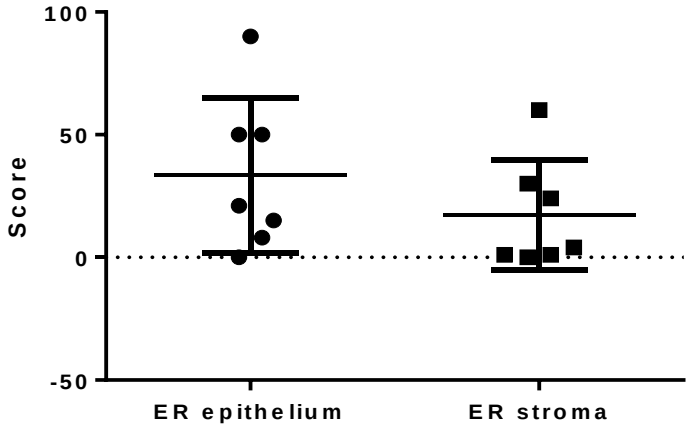
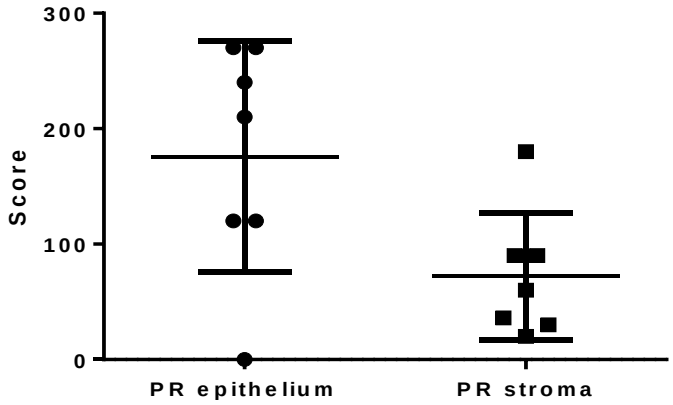


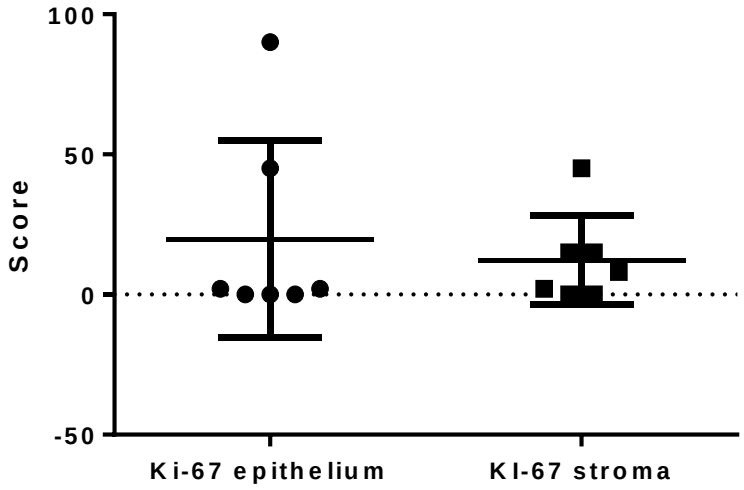
Fig. 2. Illustration of hematoxylin-eosin stained biopsy (100x) (A) and immunohistochemical expression (100x) of RE(B), RP(C) and KI-67(D) in epithelial and stromal cells in case of endometriosis of abdominal wall corresponding to case 7



Mann-Whitney test, p = 0.3974



Mann-Whitney test, p = 0.0339



Mann-Whitney test, p = 0.7465

Fig. 3. Graphic representation of the scores of Estrogen Receptor (ER), Progesterone Receptor (PR), and cell proliferation marker (Ki-67) in endometrial epithelial cells and stromal cells abdominal in abdominal endometriosis

The expression of Ki-67 in all cases was regularly low (mean score of 12.14 in the epithelium and 14.14 in the stroma), demonstrating a low proliferation of the lesion. The same was observed in pelvic endometriosis by Jones et al. [22] that proliferative activity in the ectopic endometrium remained low and constant throughout the menstrual cycle in 30 cases followed up. However, in a more recent study, in pelvic endometriosis cases, an increase in the proliferation marker expression was observed according to the lesion's severity [25]. The proliferative index characteristics in abdominal wall lesions have not been discussed, which seem to have less proliferation; since the mechanism associated with the process is predominantly fibrotic, new studies are necessary to understand its enigmatic mechanisms.

Based on the findings of a study by Hegazy and Hegazy [26] in fallopian tubes, the detection of hormonal receptors could be of significance in the potential medical management of endometriosis.

5. CONCLUSION

Thus, postoperative abdominal wall endometriosis presents RE and variable PR expression in epithelium and stroma but with PR's more significant expression. Ki-67 proliferation index was reduced, demonstrating a low proliferation of the lesion. Further studies to understand the mechanisms associated with endometriosis of the abdominal wall should be made to unveil the similarities and differences with pelvic endometriosis and what possibilities of non-surgical treatment can be adopted.

CONSENT

It is not applicable.

ETHICAL APPROVAL

This research project was submitted to the analysis of the Maternal-School Assis Chateaubriand Ethics Committee of the Federal University of Ceará, and received approval. CEP / MEAC Office No. 131/11 on August 12, 2011.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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