Estrogen and progesterone receptor isoform distribution through the menstrual cycle in uteri with and without adenomyosis

Mohamed Khairy Mehasseb, M.B.B.Ch., M.Sc., M.D., Ph.D.,[a] *Rina Panchal, M.B.B.S.,*[a]
Anthony Henry Taylor, B.Sc., M.Sc., Ph.D.,[a] *Laurence Brown, B.Sc., F.R.C.Path.,*[b]
Stephen Charles Bell, B.Sc., F.R.C.Path., Ph.D.,[a] *and Marwan Habiba, M.B.B.Ch., M.Sc., Ph.D.*[a]

[a] Reproductive Sciences Section, Department of Cancer Studies and Molecular Medicine, University of Leicester, and [b] Histopathology Department, Leicester Royal Infirmary, University Hospital of Leicester NHS Trust, Leicester, United Kingdom

Objective: To test the hypothesis that the expression of the different isoforms of the estrogen receptor alpha (ER-α) and beta (ER-β) and the progesterone receptor A (PR-A) and B (PR-B) would be differentially modulated in uteri with adenomyosis compared with controls and that modulation would be related to the menstrual cycle.
Design: Case control, blinded comparison.
Setting: University department.
Patient(s): 54 premenopausal women with and 35 without uterine adenomyosis as the sole pathology.
Intervention(s): Multiple samples studied using immunohistochemistry for estrogen and progesterone receptors.
Main Outcome Measure(s): Histomorphometric analysis of receptor expression.
Result(s): The ER-α expression in the adenomyotic endometrium was different from that of the normal endometrium and the foci in the midsecretory phase of the cycle, but expression of ER-α in the inner and outer myometrium was not statistically significantly different. The ER-β expression was statistically significantly elevated in the adenomyotic functionalis gland during the proliferative phase and throughout the myometrium across the entire menstrual cycle. Expression of PR-A was similar to that of PR-B, with reduced expression in the basalis stroma, and inner and outer myometrium in the adenomyotic samples. The pattern of ER-β, PR-A, and PR-B expression was similar in the endometrial basalis and adenomyotic foci.
Conclusion(s): These data suggest ER-β expression and the lack of PR expression are related to the development and/or progression of adenomyosis and might explain the poor response of adenomyosis-associated menstrual symptoms to progestational agents. (Fertil Steril® 2011;95:2228–35. ©2011 by American Society for Reproductive Medicine.)

Key Words: Adenomyosis, estrogen receptors, progesterone receptors, uterus, menstrual cycle

There is considerable literature on the distribution of estrogen (ER) and progesterone (PR) receptors in the endometrium and their fluctuation during the menstrual cycle (1–8). Some of these studies have reported receptor distribution in the inner but not the outer myometrium (1–3). The cyclical changes in the uterine junctional zone (inner myometrium) as seen by magnetic resonance imaging, together with the peristaltic waves seen by videosonography, indicate that this layer is influenced by steroid hormones (9–11).

Steroid hormones have also been implicated in the pathogenesis of uterine adenomyosis (12), and local rather than systemic hyperestrogenism may be implicated (13). Whether steroid receptor fluctuations have a role remains unknown. We have recently demonstrated reduced estrogen receptor alpha (ER-α) isoform expression in a CD-1 neonatal mouse model for adenomyosis. However, similar reduction was noted after tamoxifen administration to C57/BL6J mice that did not develop the disease (14, 15).

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Reprint requests: Mohamed Khairy Mehasseb, M.B.B.Ch., M.Sc., M.D., Ph.D., Reproductive Sciences Section, Department of Cancer Studies and Molecular Medicine, Robert Kilpatrick Clinical Sciences Building, University of Leicester, Leicester, LE2 7LX, United Kingdom (E-mail: mohamed.mehasseb@doctors.net.uk).

We report here the expression patterns of the ESR1 gene product, ER-α, the ESR2 gene product, ER-β, and the expression of the PR-A and PR-B receptor isoforms that arise from the PGR (NR3C3) gene in uterine adenomyosis.

MATERIALS AND METHODS

Patients and Samples

This study was approved by the Leicestershire and Rutland Local Research Ethics Committee. After informed consent had been obtained from the patients, uteri from premenopausal women who were not using exogenous hormones were selected for the study. Uteri were removed by abdominal or laparoscopic hysterectomy. All hysterectomies were performed for subjective menorrhagia with or without dysmenorrhea. No patient in either group was asymptomatic. None of the participants had fibroids or endometrial abnormalities on ultrasound or on preoperative and postoperative biopsy, and none had endometriosis. All participants were parous, except for one woman in the adenomyosis group. There was no statistically significant difference in the mean age of the women in the adenomyosis group (41.7 years) and the control group (40.4 years).

Full-thickness histologic sections including the serosa were obtained from the anterior uterine wall near the fundus. Adenomyosis was defined by the presence of endometrial glands and stroma in >25% of myometrial thickness (16). The specimens were classified into early, middle, or late proliferative (EP, MP, LP) and early, middle, or late secretory (ES, MS, LS) phase by use of established histologic criteria (17). The study included 35 control uteri

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(EP = 5, MP = 6, LP = 5, ES = 8, MS = 6, and LS = 5) and 54 uteri with adenomyosis (EP = 10, MP = 11, LP = 6, ES = 8, MS = 9, and LS = 10).

Immunohistochemistry

Serial 5-μm sections were stained for ER-α, ER-β, PR-A, and PR-B using standard immunohistochemistry techniques. Briefly, sections were dewaxed and rehydrated. Epitope antigen retrieval was performed by use of microwave energy in 10 mM citrate buffer (pH 6.0) for 20 minutes. Endogenous peroxidase, avidin-biotin, and nonspecific binding were blocked respectively with hydrogen peroxide, Avidin-biotin blocking kit (Vector Laboratories, Peterborough, UK), and 3% bovine serum albumin (Sigma-Aldrich, Poole, Dorset, UK). Sections were then incubated overnight at 4°C with the primary monoclonal antibodies against ER-β (MS-ERB13-PX1; Genetex, Abcam, Cambridge, UK), 1:500 v/v; or ER-α (NCL-L-ER-6F11), 1:50 v/v; PR-A (NCL-PGR-312), 1:200 v/v; or PR-B (NCL-PGR-B), 1:200 v/v (all from Novocastra, Newcastle upon Tyne, UK) diluted in phosphate-buffered saline (PBS) containing bovine serum albumin (BSA) and normal rabbit serum (1:10 v/v). After washing in PBS containing 0.05% TWEEN 20 (Sigma-Aldrich), sections were incubated with biotinylated rabbit anti-mouse antibodies (1:400 v/v) (Dako, Cambridge, UK) for 90 minutes. Immunoreactivity was visualized using avidin-biotin complexes (ABC Elite; Vector Laboratories) and 3,3′-diaminobenzidine/H_2O_2 (Vector Laboratories). Finally, sections were lightly counterstained with Mayer's hematoxylin (Sigma-Aldrich), dehydrated, cleared, and mounted with XAM mounting medium (BDH, Poole, UK) (18, 19). Negative control slides were run in parallel using isotype IgG (Vector Laboratories) at the same concentration as the respective primary antibody. No staining for Fc-region antibody binding was found with any of the primary antibodies tested.

Image Capture and Analysis

Image capture and analysis was performed using an Axioplan light microscope (Carl Zeiss, Jena, Germany) and an image capture system, based on a single chip color video camera (Sony DXC-151P, Sony Inc., Tokyo, Japan), a camera adapter (Sony CMA-151P, Sony Inc.), and a Meteor 2 MMC graphics display. Digital image analysis was performed using Axiovision image analysis software (version 4.0; Carl Zeiss). Standardization was performed by maintaining the same illumination and settings throughout the experiment.

The percentage of positively stained cells per high power field was obtained by counting the number of positive (brown) and negative (blue) stained cells (Supplemental Fig. 1, available online) in 10 randomly selected fields per layer at ×400 magnification and multiplying the proportion of positive cells by 100% (20). We examined the endometrial functionalis and basalis (glands and stroma), innermost or subendometrial myometrium and outer myometrium, and adenomyotic foci separately. Endothelial and vascular cells were omitted from the count.

Statistical Analysis

The analysis was performed using Graphpad Instat 3 and Graphpad Prism 5 software (GraphPad Software, San Diego, CA). The Kolmogorov-Smirnov test was used to test for normality and Student's unpaired *t*-test and analysis of variance (ANOVA) (with Tukey's correction) were used for comparisons. $P<.05$ was considered statistically significant (20).

RESULTS

ER-α Expression

The ER-α immunostaining was solely nuclear. In control uteri, 80% to 99% of the cells in both the endometrium and myometrium stained positive, except for the secretory phase of the functionalis where the percentage statistically significantly decreased to approximately 50% to 60% ($P<.05$) (Fig. 1). In adenomyotic foci, ER-α staining did not vary through the menstrual cycle in either the glands or stroma. There were no cyclical changes in ER-α expression in the innermost or outer myometrium in adenomyosis.

In the adenomyotic functionalis glands and stroma, there was a statistically significant ($P<.001$) decrease in ER-α expression during the MS phase. In both adenomyosis and control samples, there was no consistent cyclical variation in ER-α expression in either the basalis or innermost or outer myometrium, although the number of positive cells in the basalis stroma was statistically significantly lower ($P<.05$) in four out of the six phases of the cycle.

ER-β expression

Immunoreactive ER-β was also mainly nuclear with minor cytoplasmic staining. Expression was higher in the glandular epithelium (Fig. 2). In the proliferative phase, ER-β expression was statistically significantly higher in the functionalis glands of adenomyosis compared with controls ($P<.01$) (see Fig. 2). Expression was similarly higher in adenomyosis basalis stroma or innermost or outer myometrium compared with control tissue where expression was weak and showed no statistically significant variation with the phase of the cycle.

PR-A Expression

The PR-A staining was confined to the nucleus. In the control functionalis and basalis, PR-A expression was high (>90%) during the proliferative and ES phase, but decreased markedly ($P<.001$) during the MS and LS phases (Fig. 3). The PR-A expression in the functionalis and basalis stroma, and innermost or outer myometrium of the normal uterus was highly consistent from patient to patient, and showed no statistically significant variation with the phase of the cycle (see Fig. 3); expression was higher in these layers compared with adenomyosis. Expression of PR-A in adenomyotic foci was statistically significantly higher in the glands compared with the stroma (see Fig. 3).

PR-B expression

Staining for PR-B was also confined to the nucleus. The expression of PR-B was similar to that for PR-A (Fig. 4). In the glandular epithelium, expression was similar in adenomyosis and controls. But expression was lower in adenomyosis in the functionalis stroma during the secretory phase and in the basalis stroma and both the inner and outer myometrium.

DISCUSSION

The immunoreactive staining distribution described here for the control group is in general agreement with published literature (1–3, 6, 8, 18, 21, 22), although there are discrepancies between published reports on ER-β expression (22–26). The reasons for these differences have been previously discussed elsewhere (27), and may be related to sample collection, methodologies, or the source of antibody. We used antibodies approved for the diagnosis of tumor receptor status (ER-α, PR-A, PR-B). For ER-β, we used an antibody recommended for paraffin-embedded tissues (28). The expression of nuclear ER-α, PR-A, and PR-B, with nuclear and cytoplasmic ER-β staining was also in good agreement with the literature. We used the classic histologic criteria of Noyes et al. (17) to assemble our samples into six groups spanning the menstrual cycle. We did not study the hormonal changes in these women, and there remains a possibility that ovarian dysfunction may be responsible for the observed differences in receptor expression. However, that was not the focus of our research, and previous research has not identified abnormal ovarian function in adenomyosis but rather suggested a role for local steroid synthesis (29, 30).