OBJECTIVE: To evaluate the immunoexpression of estrogen receptor (ER) and progesterone receptor (PR) in gland and stromal cells of endometrial polyps in postmenopausal women.

STUDY DESIGN: Thirty postmenopausal patients underwent operative hysteroscopies because of benign endometrial polyps. The polyps were identified and subsequently completely removed. A section of normal-appearing endometrium adjacent to the polyp base was also obtained for the control group. The presence of ER and PR was investigated in the gland and stromal cells in the polyps and adjacent endometrium using immunohistochemistry. The slides were evaluated by semiquantitative analysis.

RESULTS: Endometrium and endometrial polyps showed a significantly higher proportion of positive cells in the glands than in the stroma for both ER (p < 0.000 and p < 0.000, respectively) and PR (p = 0.002 and p = 0.002, respectively). Polyps showed a significantly higher proportion of positive cells in glands and stroma than in the endometrium, concerning ER (p < 0.000 and p = 0.001, respectively) and also for PR (p = 0.021 and p = 0.008, respectively).

CONCLUSION: Our data suggest that steroid receptors present a crucial role in the physiopathology of the endometrial polyps in postmenopausal women. (Anal Quant Cytol Histol 2011;33:61–67)

Keywords: endometrial polyps, hormone receptors, immunohistochemistry, postmenopause.

Endometrial polyps are outgrowths of endometrial tissue attached to the inner wall of the uterus and protruding into the uterine cavity.1,2 They are composed of varying amounts of glands and dense and fibrotic stroma, containing thick-walled blood vessels covered by endometrial epithelium.2,3

The diagnosis of endometrial polyps is becoming more common than previously assumed. Currently, outpatient evaluation using imaging techniques (including ultrasonography and hysteroscopy) has revealed that polyps are a common finding. Tradi-
tionally, dilatation and curettage were performed to evaluate the uterine cavity; however, because these are blind procedures, endometrial polyps have been missed in a high percentage of cases.4,5

Endometrial polyps usually occur in women in their 40s and 50s, although they can be found earlier. In postmenopausal asymptomatic women, presence of endometrial polyps may exceed 20%.4,5 Endometrial polyps are usually benign, although some may be precancerous or cancerous. The incidence of malignancy in endometrial polyps is low, ranging from 0% to 13%. Advanced age, menopause, obesity, and diabetes increase the risk for endometrial polyp malignancy.6,7

The pathogenesis of endometrial polyps is poorly understood at present; it is known, however, that they develop as a consequence of focal stroma and glandular overgrowth. The stimulus for this occurrence is unknown, and several hypotheses have been formulated.

Review of the literature shows that there is evidence for a hormonal basis. Polyps have never been reported before menarche and are most commonly diagnosed in the fifth decade. Epidemiologic studies demonstrate that the risk factors for women with endometrial polyps are late menopause, obesity, use of tamoxifen, or hormone replacement therapy.8,9

Some studies on the pathogenesis of the endometrial polyps have pointed to a role of estrogen receptor (ER) and progesterone receptor (PR), which seem to be unbalanced in this condition. However, these studies showed somewhat conflicting findings.8,10-12

In view of this scenario, the objective of the present study was to evaluate and compare the immunoreexpression of ER and PR in the glandular epithelium and in the stroma of polyps and their adjacent endometrium in postmenopausal women.

Materials and Methods

All patients included in the study were patients at Santa Casa of São Paulo Hospital in the period ranging from January 2006 to December 2006. The specimens of the study group were obtained prospectively from a total of 30 postmenopausal women undergoing surgical hysteroscopy for resection of endometrial polyps. Patients from the study group were 50 to 74 years of age (mean, 59.2 ± 6.2). The mean age at menopause was 50.1 ± 2 years (46–54). Patients had been in menopause for 2–24 years (mean, 9.1 ± 5.7). Among the study group, 23 (76.7%) women experienced postmenopausal vaginal bleeding.

A standardized history was obtained and complete physical examination performed for each woman. Informed consent was obtained from each patient, and the hospital ethics committee approved the research. The exclusion criteria adopted were malignant neoplasia, endometrial hyperplasia, and the use of medication with hormonal effects at any time after menopause.

Operative hysteroscopies were performed using a 10-mm 26 French Storz Resectoscope (Karl Storz, Tuttlingen, Germany) and a monopolar cutting loop. In brief, under spinal block anesthesia, the cervix was dilated and the uterine cavity was then distended with a solution of 3% mannitol up to a pressure of 100 mm Hg. The polyp(s) or any focally growing lesion of the uterine cavity were identified and subsequently completely removed. A section of normal-appearing endometrium adjacent to the polyp base was also obtained as the control group.

The samples of the removed polyps and adjacent endometrium were fixed in 10% formalin for the medium period of 24 hours. The entire tissue fragments were dehydrated in ethyl alcohol and xylene and then paraffin embedded for analysis (both specimens for each patient were put in the same paraffin block). Specimens were cut in serial sections of 4-μm thickness. All sections were stained with hematoxylin-eosin (H-E). Sections were evaluated under light microscope and were reviewed by the same pathologist to confirm the histopathologic diagnosis.

Identification of expression of ERs and PRs was performed by immunohistochemical study by the streptavidin-biotin-peroxidase complex method. All immunohistochemical reactions were performed at the same time. For these reactions, two sections were cut for each case (ER and PR) in 3-μm thickness. After deparaffinization in toluene (two times during 10 minutes) and in alcohol solutions (5 minutes in 100%, 5 minutes in 95%, and 5 minutes in 75% alcohol), sections were washed in tap water. The deparaffinized sections were treated with hydrogen peroxide to block endogenous peroxidase activity and then washed in phosphate-buffered saline (PBS).

For each case, the two sections were incubated with specific antibodies as follows: (1) ER monoclonal antibodies, prepared in mice (dilution 1:150; Dako, Carpinteria, California, U.S.A.; clone 1 D 5); (2) PR monoclonal antibodies, prepared in mice (di-
olution 1:100; Dako, clone 16) for 18 hours (overnight) at 4°C. Sections were washed three times in PBS and incubated for 1 hour with biotinylated goat anti-mice immunoglobulin (Dako, K0492) at 37°C. Sections were washed again three times in PBS and incubated with streptavidin-biotin-peroxidase complex for 30 minutes, to label the antibodies.

The antigen-antibody complex was visualized by incubating for 5 minutes with diaminobenzidine (0.6 mg/mL buffer) and hydrogen peroxide. Sections were washed in sterile distilled water, counterstained with Harris hematoxylin and mounted for light microscopy. The reactions were considered positive when the cells became stained in brown. Appropriate positive and negative controls were included in each case.

Each section prepared by the immunohistochemical method was evaluated under light microscope (Axioskop 40, Zeiss, Thornwood, New York, U.S.A.) adapted to a microcamera and screen. The number of positive cells (with expression of ERs and PRs) was counted at ×400 magnification. The calculation of the area, in square millimeters, for morphometric analysis was performed with help of a Neubauer chamber; it was established that each area of the ×400 magnification represented 0.094 mm². A 10-field count was performed in the polyp and adjacent endometrium independently; in each case the stained glandular cells and the stroma were counted independently. The cells count was expressed as numerical densities, that is, the number of positive stained cells per square millimeter of epithelium.13

For the statistical analysis of results the Wilcoxon signed ranks test, t-test and Pearson correlation coefficient were applied as appropriate. Levels of p < 0.05 were considered significant.

Results

From the 30 cases evaluated in the present study, ERs and PRs were found both in the stroma and in the glandular epithelium of the polyps, as well as in the stroma and glandular epithelium of the adjacent endometrium. The cases with proportion of positive cells for each marker and histopathologic groups are summarized in Tables I and II.

Adjacent endometrium and endometrial polyps showed a significantly higher proportion of positive cells in the glands than in the stroma for both ERs (p < 0.000 and p < 0.000, respectively; t-test confirmed by Wilcoxon signed ranks test) and PRs (p = 0.002 and p = 0.002, respectively; t-test confirmed by Wilcoxon signed ranks test).

However, polyps showed significantly higher proportion of positive cells in glands and stroma than in the endometrium, concerning ER (p < 0.000 and p = 0.001, respectively; t-test confirmed by Wilcoxon signed ranks test) and also for PR (p = 0.021 and p = 0.008, respectively; t-test confirmed by Wilcoxon signed ranks test).

While considering only the polyps score, PRs showed significantly higher values than ERs for both glands (p = 0.040; t-test confirmed by Wilcoxon signed ranks test) and stroma (p = 0.001; t-test confirmed by Wilcoxon signed ranks test). The same situation occurred for the endometrium score where the PRs showed significantly higher values than ERs for both glands (p = 0.006; t-test confirmed

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Smallest value</th>
<th>Maximum value</th>
<th>Mean value</th>
<th>Median value</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polyp gland</td>
<td>8.5</td>
<td>3,042.5</td>
<td>1,499.2</td>
<td>1,517.0</td>
<td>812.6</td>
</tr>
<tr>
<td>Polyp stroma</td>
<td>0</td>
<td>2,180.8</td>
<td>575.3</td>
<td>380.3</td>
<td>571.4</td>
</tr>
<tr>
<td>Endometrium gland</td>
<td>0</td>
<td>2,744.7</td>
<td>780.7</td>
<td>640.9</td>
<td>721.1</td>
</tr>
<tr>
<td>Endometrium stroma</td>
<td>0</td>
<td>1,372.3</td>
<td>187.3</td>
<td>65.4</td>
<td>280.3</td>
</tr>
</tbody>
</table>

Table II Immunohistochemical Data on PR Expression for Endometrial Polyps and Adjacent Endometrium

<table>
<thead>
<tr>
<th>Tissue</th>
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<th>Maximum value</th>
<th>Mean value</th>
<th>Median value</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polyp gland</td>
<td>56.2</td>
<td>3,787.2</td>
<td>1,794.6</td>
<td>1,994.7</td>
<td>981.9</td>
</tr>
<tr>
<td>Polyp stroma</td>
<td>12.9</td>
<td>2,826.3</td>
<td>1,026.9</td>
<td>778.7</td>
<td>826.7</td>
</tr>
<tr>
<td>Endometrium gland</td>
<td>0</td>
<td>4,436.1</td>
<td>1,297.8</td>
<td>1,274.2</td>
<td>1,039.5</td>
</tr>
<tr>
<td>Endometrium stroma</td>
<td>0</td>
<td>2,277.6</td>
<td>705.6</td>
<td>545.8</td>
<td>657.1</td>
</tr>
</tbody>
</table>
by Wilcoxon signed ranks test) and stroma (p < 0.000; t-test confirmed by Wilcoxon signed ranks test).

To determine whether there is an increase in the number of ERs and PRs in glands and stroma of both endometrium and endometrial polyps according to aging, Pearson correlation coefficient was calculated. Significant results were obtained, except for PR in glands of the polyp and ER in endometrial stroma. Table III shows the values for these correlations and their respective p values.

Also, to check whether there is an increase in the number of ERs and PRs in glands and stroma of both endometrium and endometrial polyps in relation to postmenopausal time, the Pearson correlation coefficient was also calculated. Significant values in general, except for PRs in glands of the polyp, ERs in endometrial glands and stroma, were observed. Table IV shows the values for these correlations and their respective p values.

Discussion

Today there is not agreement on the management of postmenopausal patients with endometrial polyps.8 The etiology and pathogenesis of endometrial polyps are not fully understood, but polyps are believed to be a risk factor for endometrial cancer and thus detection should be taken seriously. As mentioned previously, the stimulus for its occurrence is unknown and several hypotheses have been formulated.6,14,15

A clonal rearrangement of chromosome 6p21 is common in the mesenchymal (stromal) cells in the polyp. Endometrial cells do not have the chromosome 6 rearrangement. One possible explanation for these findings is that an endometrial polyp begins when a stromal cell undergoes a rearrangement in chromosome 6p21, resulting in an abnormal signal the polyps to grow. The stromal elements proliferate and bring the endometrial glands.16

A study performed by Nogueira et al3 evaluated the immunoeexpression of p63 in 36 specimens of endometrial polyps and adjacent endometrium in postmenopausal women in order to determine whether p63 is expressed differently in postmenopausal endometrial polyps than in the adjacent endometrium. The 63-kD membrane protein p63 is one protein that plays an important role in regulating epithelial proliferation and differentiation, which is also a marker of basal and reserve cells in the female genital tract. In this study the authors provide evidence that a basal cell immunophenotype is maintained in the endometrial polyps seen in postmenopausal women, suggesting that p63 plays a role in the pathogenesis of such polyps. The majority of endometrial polyp samples (94.4%) presented nuclear immunostaining for p63, whereas only 5.6% of adjacent endometrium samples were positive for p63.

In postmenopausal women, endometrial polyps appear as circumscribed areas of focal hyperplasia and are not associated with high estrogen levels because the adjacent endometrium is usually atrophic.8 Based on these previous findings we believe that it is very important to study the role of steroidal receptors in the origin and growth of endometrial polyps as focal lesions of endometrium could be related to local modifications of these receptors.

In the present study the endometrium and endometrial polyps showed a significantly higher proportion of positive cells in the glands than in the stroma for both ERs and PRs. While

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Pearson correlation</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER: Polyp gland</td>
<td>0.453</td>
<td>0.012a</td>
</tr>
<tr>
<td>ER: Polyp stroma</td>
<td>0.531</td>
<td>0.003a</td>
</tr>
<tr>
<td>PR: Polyp gland</td>
<td>0.308</td>
<td>0.098</td>
</tr>
<tr>
<td>PR: Polyp stroma</td>
<td>0.682</td>
<td>&lt; 0.000a</td>
</tr>
<tr>
<td>ER: Endometrium gland</td>
<td>0.374</td>
<td>0.042a</td>
</tr>
<tr>
<td>ER: Endometrium stroma</td>
<td>0.121</td>
<td>0.524</td>
</tr>
<tr>
<td>PR: Endometrium gland</td>
<td>0.414</td>
<td>0.023a</td>
</tr>
<tr>
<td>PR: Endometrium stroma</td>
<td>0.577</td>
<td>0.001a</td>
</tr>
</tbody>
</table>

*aSignificant p value.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Pearson correlation</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER: Polyp gland</td>
<td>0.422</td>
<td>0.020a</td>
</tr>
<tr>
<td>ER: Polyp stroma</td>
<td>0.525</td>
<td>0.003a</td>
</tr>
<tr>
<td>PR: Polyp gland</td>
<td>0.331</td>
<td>0.074</td>
</tr>
<tr>
<td>PR: Polyp stroma</td>
<td>0.616</td>
<td>&lt; 0.000a</td>
</tr>
<tr>
<td>ER: Endometrium gland</td>
<td>0.242</td>
<td>0.197</td>
</tr>
<tr>
<td>ER: Endometrium stroma</td>
<td>0.107</td>
<td>0.572</td>
</tr>
<tr>
<td>PR: Endometrium gland</td>
<td>0.404</td>
<td>0.027a</td>
</tr>
<tr>
<td>PR: Endometrium stroma</td>
<td>0.534</td>
<td>0.002a</td>
</tr>
</tbody>
</table>

*aSignificant p value.
considering only polyp score, the PRs showed significantly higher values than the ERs for both glands and stroma. The same situation happened for endometrium score.

The cellular mechanism involved in development of endometrial polyps is poorly understood, and the presence of a higher expression of steroidal receptors in the glands and stroma of endometrial polyps than in the adjacent endometrium suggests a higher sensitivity of these structures to steroid hormones. So, their development could be possible without high estrogen levels.\textsuperscript{8,11} The significantly higher levels of ERs and PRs in gland cells than in the stroma of endometrial polyps and adjacent endometrium support the notion that the glands are the most important structures in the response to hormones.

In the literature, some few studies were found evaluating the immunexpression of the steroidal receptors in endometrial polyps compared to the adjacent endometrium in postmenopausal women. These studies are mentioned in the following section, and the results show conflicting findings in relation to those of the present study.

Mittal et al\textsuperscript{17} investigated 14 cases of endometrial polyps, in which normal cycling endometrium was also present on the same slide, to evaluate whether endometrial polyps result from localized overexpression of ERs or reduced expression of PRs. They showed that fewer stromal cells in polyps expressed ERs and PRs compared with cycling endometrium. Stroma in polyps also had significantly reduced intensity of staining for PRs, but not for ERs. There were no significant differences in expression of ERs and PRs in the endometrial glands in endometrial polyps compared with normal endometrium. The authors concluded that endometrial polyps may result from a decrease in ER and PR expression in stromal cells, which may be relatively insensitive to cyclic hormonal changes seen in the rest of the endometrium.

In 2004, Sant’Ana de Almeida et al\textsuperscript{8} in a study similar to the present one, evaluated 44 postmenopausal patients who underwent operative hysteroscopy because of benign endometrial polyps. The authors observed that in the glandular epithelium, the median of the ER and PR scores were higher in the endometrial polyps than in the endometrium. In the stroma, the median of the ER score was higher in the endometrial polyps than in the endometrium; the median of the PR score in the polyps did not differ from that in the endometrium. They suggest that steroid receptors present a crucial role in the physiopathology of the endometrial polyps in postmenopausal women, especially the ERs.

Two years later, Belisario et al\textsuperscript{11} compared the relationship of body mass index (BMI) and the immunexpression of ER and PR in endometrial polyps and endometrium in gland and stromal cells of 35 postmenopausal women. They observed that BMI was significantly higher among patients with lower expression of ERs in the glands of endometrium. Endometrial polyps and adjacent endometrium showed a significantly higher proportion of positive cells in the glands than in the stroma, for both ERs and PRs. Glands and stromal cells showed a significantly higher proportion of positive cells in the polyps than in the endometrium for ER. The authors concluded that the higher proportion of positive gland cells for ER in endometrial polyps compared to endometrium supports implication of these receptors in the pathogenesis of polyps. Association of higher BMI with lower expression of ER in endometrial glands, but not in endometrial polyps, may indicate that factors influencing ER expression do not affect ER, supporting an autonomous function of polyps.

Inceboz et al\textsuperscript{18} while evaluating 36 postmenopausal women found that in both the glandular epithelium and stroma of endometrial polyps, ERs and PRs, Ki-67 and bcl-2 showed significantly more positive staining than the inactive endometrium from the control group. The authors postulate that estrogen may have a role in the development of postmenopausal endometrial polyps, either by direct stimulation of localized proliferation or by stimulation of proliferation via other pathways, such as activation of Ki-67 or through inhibition of apoptosis via bcl-2.

Lopes et al\textsuperscript{19} investigated the presence of ERs and PRs in the glandular epithelium and stroma of endometrial polyps in 48 women who underwent hysteroscopic polypectomy. It was verified that in immunohistochemistry, the concentrations of both ERs and PRs in glandular epithelium were significantly higher in the endometrial polyp than in the normal endometrium. The concentrations of ERs and PRs in the stroma were similar in the polyp and endometrium. The concentrations of these receptors in the glandular epithelium and stroma were similar in postmenopausal and premenopausal patients.

In the past year Gul et al\textsuperscript{20} investigated the differences in steroid receptor expression patterns
between glandular and stromal portions in endometrial polyps among 25 postmenopausal and 25 premenopausal patients and the relationship between the receptor expression in endometrial polyps and clinical parameters. Comparison in postmenopausal patients showed that glandular ER and PR expression were both significantly greater than stromal ER and PR expression. Proliferative phase endometrial polyps also demonstrated significantly greater expression of PRs in glandular epithelium compared with that in stroma. However, stromal and glandular ER expression did not differ among premenopausal patients. Stromal PR expression was lower in older patients, and there was a relationship between low estrogen hormone levels and lower stromal PR expression.

The present study examined whether there was an increase in ER and PR numbers in glands and stroma of both endometrium and endometrial polyps according to patient age. Significant results were obtained, except for PRs in glands of the polyp and ERs in endometrial stroma. Nevertheless, a weak to moderate coefficient of Pearson correlation was found. As mentioned earlier, the significantly higher levels of ERs and PRs in gland cells than in the stroma of endometrial polyps and adjacent endometrium support the notion that the glands are the most important structures in response to hormones. An ER increase in glands of the polyp and not in PR was observed; this could explain the genesis of these structures because a larger amount of ERs could lead to higher proliferation.

Only one study was found evaluating ERs and PRs according to patient age. The immunohistochemical reactivity of the postmenopausal endometrium was studied by Koshiyama et al. using monoclonal antibodies against ERs and PRs in 33 postmenopausal patients. The authors found that the endometrium was thicker in patients who were postmenopausal for 1–10 years than in patients who were postmenopausal for > 10 years. The average age of the patients with ER-positive reactivity in the glands was significantly lower than that of the patients with ER-negative reactivity. Furthermore, the endometrial thickness of the patients with ER- or PR-positive reactivity in the glands was significantly greater than that of the patients with ER- or PR-negative reactivity. They conclude that ERs in postmenopausal endometrium decrease gradually with increased aging and the presence of ERs and PRs in the gland cell seemed likely to determine the thickness of the endometrium. However, these authors did not study endometrial polyps.

The present study also examined whether there was an increase in ERs and PRs in the glands and the stroma of both endometrium and endometrial polyps in relation to postmenopausal time. Significant results were obtained, except for PRs in glands of the polyp and ERs in endometrial glands and stroma. A weak to moderate coefficient of Pearson correlation was found. As a result of the results shown, it could be postulated that the increase in ERs in both the gland and the stroma of endometrial polyps in relation to postmenopausal time suggests a possible explanation of its high incidence in this period. No study was found investigating such fact.

In conclusion, the present study shows that the immunohistochemical detection of ERs and PRs in glands and stroma of endometrial polyps showed a significantly higher proportion of positive cells than in the adjacent endometrium. These data, added to the other studies discussed, could suggest that steroid hormones play a crucial role in the physiopathology of endometrial polyps in postmenopausal women.

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