Effect of tamoxifen on postmenopausal endometrium

T. Kalampokas[1], C. Sofoudis[1], C. Anastasopoulos[2], I. Boutas[1], S. Melloy[1], A. Kondi-Pafiti[1], E. Kalampokas[1], D. Botsis[1], N. Salakos[2]

[1]2nd Department of Obstetrics and Gynecology University of Athens, Aretaieion Hospital, Athens
[2]Department of Obstetrics and Gynecology, University of Crete, Herakleion, Crete (Greece)

Summary

Purpose: The objective of this study was to evaluate the effect of tamoxifen on the endometrium of 45 postmenopausal women with breast cancer, as evidenced by hysteroscopic, ultrasound, histological methods, and by immunohistochemical investigation of the expression of Bcl-2 and Ki67. *Materials and Methods:* Forty-five postmenopausal women with breast cancer (ER and/or PgR positive) undergoing tamoxifen therapy for six to 48 months, were selected from the files of the 2nd Department of Obstetrics and Gynecology, University of Athens Aretaieion Hospital, among a total of 120 patients treated from 2004-2009. *Results:* The ultrasound findings during the follow-up period revealed 18 cases of thickened endometrium, 14 cases of suspected polyps, one case with accumulation of endometrial fluid, and 12 cases of heterogeneous endometrial echo texture. The patients had undergone hysteroscopy because of thickened endometrium (18/45 patients), postmenopausal bleeding (14/45 patients), and polyps (13/45 patients). The endometrial tissue samples were examined in the Pathology Department of Aretaieion Hospital and showed in 23 cases with adenomatous endometrial polyps, 15 cases with endometrial cystic atrophy, two cases with adenomatous hyperplasia, and five cases with mucosal endometrial adhesions. Immunohistochemical investigation of Bcl-2 and KJ67 expression was undertaken on paraffin blocks and showed elevated expression in the cases with endometrial polyps and hyperplasia, in contrast to atrophic endometria. *Conclusion:* Long-term tamoxifen therapy of postmenopausal women with breast cancer is associated with uterine pathology. Ultrasonography alone is useful in asymptomatic patients selecting cases with increased endometrial thickness for further investigation. Hysteroscopy is an accurate method for diagnosing endometrial disease because it provides a direct view of the uterine cavity, reveals focal lesions, and enables targeted biopsies to be performed at the same time. Pathological findings show elevated expression of Ki67 and Bcl-2 in hyperplastic endometria and adenomatous polyps, consistent with an elevated glandular cell proliferation due to tamoxifen effect.

Key words: Tamoxifen; Ultrasonography; Hysteroscopy; Endometrium; Polyps; Hyperplasia; Bcl-2; Ki67.

Introduction

The non-steroidal anti-estrogen tamoxifen is probably the most widely-used agent for breast cancer that exhibits estrogen receptors [1]. The decline of breast cancer mortality in developed countries may be attributed to the use of tamoxifen but long term use has been associated with endometrial thickening and endometrial pathology. Patients who take tamoxifen as hormonal therapy for breast cancer have a two to three times higher relative risk to develop endometrial cancer, the risk depending on the duration of the treatment and the dose itself [2]. It is recommended that all patients who receive tamoxifen for a long term must be enrolled in a ultrasonography screening, and in cases with specific ultrasonographic characteristics, must undergo further endometrial evaluation by an office biopsy, sonohysteroscopy, dilatation, hysteroscopy and curettage [3].

In the 2nd Department of Obstetrics and Gynecology, University of Athens, Areteion Hospital, a systematic evaluation of women with breast cancer receiving tamoxifen was undertaken in 2004 and all findings were evaluated ultrasonographic, hysteroscopic, histologic, and specific immunohistochemical findings about the endometrial hormonal receptor status and apoptosis-related agents. Bcl-2 is a proto-oncogene that prolongs the survival of cells by inhibiting apoptosis and it is strongly expressed in endometrial simple and complex hyperplasia and adenomatous polyps [4].

Ki-67 is a nuclear and nucleolar protein associated with cell proliferation normally observed during the proliferative phase of the cycle [5].

The aim of this study was to correlate the ultrasonographic with the hysteroscopic and histological findings of the endometria of postmenopausal patients who received tamoxifen for breast cancer and investigate the expression of Bcl-2 and Ki67, as well in the endometrial specimen.

Materials and Methods

Forty-five postmenopausal patients were selected among 120 patients with breast cancer (ER-positive) who received tamoxifen for at least six months and were examined at the 2nd Department of Obstetrics and Gynecology, University of Athens Areteion Hospital, from 2004-2009. Patients that had already received chemotherapy, as well as cases with negative breast cancer hormonal receptors were excluded from the study [6]. Twenty-six patients were asymptomatic and 19 presented abnormal vaginal bleeding. All patients were enrolled in a surveillance program which included ultrasound examination, hysteroscopy, and endometrial biopsy [7-9].

Ultrasonography was performed with a vaginal probe at 65 MHz. The widest endometrial thickness was measured on the midline sagittal scan, including the double layer of the endometrium. If endometrial fluid was present, the anterior and posterior layers were measured separately and added together.

The patients underwent diagnostic hysteroscopy with a 3.5

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mm sheath. The authors classified hysteroscopical findings as normal and abnormal.

Normal findings included functional endometrium and atrophic edometrium with or without cystic changes. Abnormal findings included unevenly thickened mucosa with or without irregular spaced gland openings, vascular atypias, single or multiple polyps, and malignant endometrial growth. Diagnostic hysteroscopy was performed using saline infusion as a distension medium.

Endometrial tissue (sampling) was obtained from all patients and a routine pathological examination with Hematoxylin-Eosin (H&E) stained histological sections was performed at the Pathology Laboratory of Aretaieion Hospital. Additional sections of neutral formalin-fixed and paraffin-embedded tissue blocks were processed by an immunohistochemical method using an appropriate system. For the investigation of the expression of Ki67, an ab-neomarker clone SP6 was used and for the Bcl-2, an ab-Cellmarque clone 124. As positive and negative controls, sections from tonsils were used [10]. The intensity of Bcl-2 staining was semi-quantitatively evaluated as negative (-) and as positive (+) when strong and distinct [11]. Ki-67 immunostain-positive nuclei were counted per 100 cells and the expression was graded as negative (-) or positive (+, < 25% cells), and ++ (when > 25% cells). Only the glandular endometrial cells were evaluated for Bcl-2 and Ki67 expression [12].

Statistical analysis was performed with MedCalc software version 11.4.4.0 sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of the probability of entering competence with respect to the various parameters was calculated.

Results

The demographic and clinical characteristics from the patient's files are as follows: the patients age was 63 ± 8.9 years and the menarche age was 12.3 ± 1.8 years, the age of the menopausal 26.8 ± 4.9, and parity reported was 2.1 ± 2.9. Sixteen of the 45 patients (34, 8%) reported one or two pregnancies, nine patients (21%) reported three or more pregnancies, and 11/45 (25%) reported one or more abortions [13]. Breast feeding was reported in 32 cases (61.1%). The body mass index (BMI) was between 26.8 ± 4.9% of the cases. Thirteen of the 45 (28%) patients were asymptomatic, 19/45 (42%) were symptomatic reporting vaginal bleeding, five of the 45 patients (11.1%) had type II diabetes and five of the 45 patients (11.1%) had hypertension. All women had been previously treated for infiltrating breast cancer (Er+) by surgery (radical or partial mastectomy or tumorectomy with axillary node dissection) and tamoxifen (dose 20 - 40 mg/day). The duration of the tamoxifen use was six to 48 months.

All the women had undergone hysteroscopy and all the endometrial samples were histologically examined. Hysteroscopy was indicated because of the thickened endometrium in 40% (18 patients), postmenopausal bleeding in 31.1% (14 patients), and suspected polyps in 28% (13 patients) [14]. The ultrasonographic and hysteroscopic findings are presented in Tables 1 and 2.

Histological examination revealed in 23 cases (48.8%) fragments of endometrial adenomatous polyps, in 15 cases (33.3%) with atrophic changes, of simple and cystic type, and in two cases (4.4%) simple and focally complex hyperplasia without atypia. In five cases (11.1%) the specimens were inadequate for histological examination. The immunhistochemical investigation showed a focal positive Bcl-2 cytoplasmic immunostain reaction in 19/22 cases of adenomatous polyps [15]. In all cases (3/3) there was adenomatous hyperplasia and in five cases there was cystic atrophy. A positive (+) Ki-67 nuclear immunoreaction was observed in five to 15 percent of the glandular cells of endometrial polyps and hyperplastic endometrium, but not in atrophic endometrial tissue (Tables 3 and 4).

Table 5 shows sensitivity, specificity, and PPV and NPV of each test when polyps were diagnosed. The diagnostic test was histological examination. The estimate of sensitivity and specificity for hysteroscopy was 65.22 and 100.0, respectively, and the estimate of PPV and NPV was 100.0 and 76.72, respectively. The estimate of sensitivity and specificity for ultrasound was 60.87 and 100.0, respec-

Table 1. — *Ultrasound findings of 45 cases.*

Findings	Cases
Endometrial thickness	18/45 cases (40%)
Suspected polyps	14/45 cases (31.1%)
Endometrial fluid	1/45 cases (2.2%)
Heterogenous echotexture	12/45 cases (26.6%)

Table 2. — *Hysteroscopic findings of 45 cases.*

Findings	Cases
Thickened endometrium	18/45 cases (40%)
Polyps	15/45 cases (33.3%)
Suspicious lesions	2/45 cases (4.4%)
Mucosal adhesions	5/45 cases (11.1%)

Table 3. — *Histological examination findings.*

Fragments of endometrial adenomatous polyps	23/45 cases (46.6%)
Atrophic changes of simple and cystic type	15/45 cases (33.3%)
Simple and focally complex hyperplasia without atypia	2/45 cases (6.6%)

Table 4. — *Immunhistochemical findings.*

	Positive Bcl-2	Ki-67 expression
Adenomatous polyps	19/22 cases	5% - 15%
Atrophic changes	5/15 cases	< 5%
Hyperplastic changes	3/3 cases	10% - 15%

Table 5. — *Polyps histological diagnosed vs test (hysteroscopy or ultrasound).*

Test	Sensitivity (95% CI)	Specificity (95% CI)	PPV	NPV	Sign. level
Polyps hysteroscopic	65.22 (42.7 - 83.6)	100.0 (84.6 - 100.0)	100.0	76.72	< 0.0001
Polyps ultrasound	60.87 (38.5 - 80.3)	100.0 (84.6 - 100.0)	100.0	74.55	< 0.0001
Polyps immuno-histochemical	100.0 (85.2 - 100.0)	31.82 (13.9 - 54.9)	56.14	100.0	0.0017

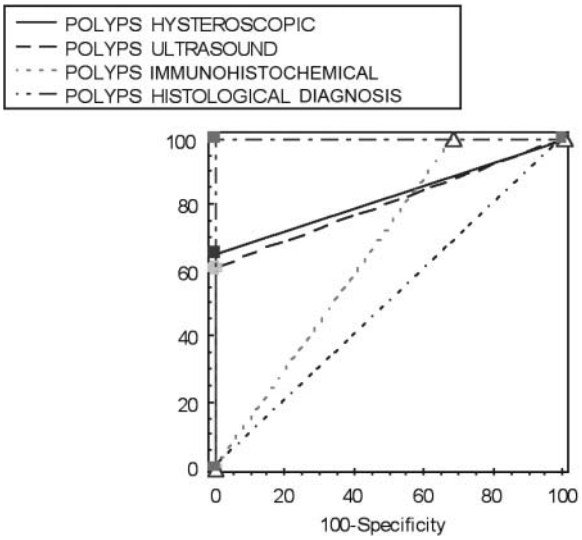

Figure 1. — Sensitivity and specificity of each test when polyps are diagnosed.

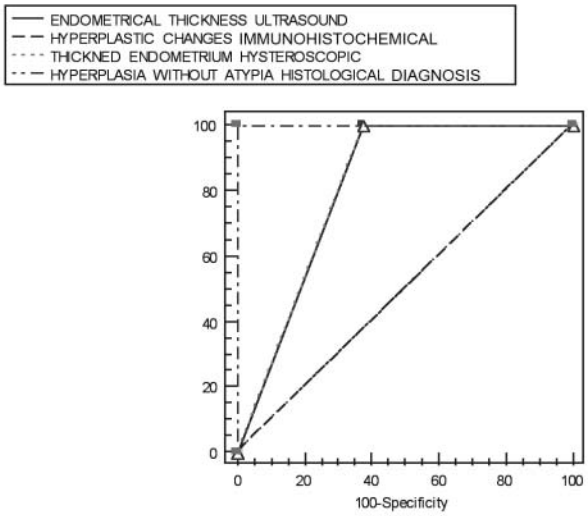

Figure 2. — Sensitivity and specificity of each test when hyperplasia is diagnosed.

Table 6. — *Hyperplasia histological diagnosed vs test.*

Test	Sensitivity (95% CI)	Specificity (95% CI)	Positive predictive value	Negative predictive value	Sign. level
Endometrial thickness ultrasound	100.0 (15.8-100.0)	62.79 (46.7-77.0)	15.96	100.0	< 0.0001
Endometrial thickness hysteroscopic	100.0 (15.8-100.0)	62.79 (46.7-77.0)	15.96	100.0	< 0.0001

tively and the estimate of PPV and NPV was 100.0 and 74.55, respectively. Both hysteroscopy and ultrasound had a high significance level < 0.0001. The estimated sensitivity and specificity for immunhistochemistry was 100.0 and 31.82, respectively, and the estimated PPV and NPV was 56.14 and 100.0, respectively, with a significance level of 0.0017 (Figure 1).

Table 6 shows sensitivity, specificity, PPV and NPV of each test when hyperplasia was diagnosed. The diagnostic test was histological examination. The estimate of sensitivity and specificity for ultrasound and hysteroscopy was both 100.0 and 62.79, respectively, and the estimate of PPV and NPV was 15.96 and 100.0, respectively with a high significance level < 0.0001. There was no statistical significance for the immunohistochemical results (Figure 2).

Discussion

Tamoxifen is widely used to treat menopausal women suffering from estrogen-receptor positive breast cancer [16, 17]. An improvement in breast cancer behavior follows tamoxifen administration, both as adjuvant treatment and in relapsed or advanced disease. In postmenopausal women tamoxifen is associated with increased proliferative activity of glandular endometrial cells and increased incidence of endometrial cancer. This is probably due to an imbalance between cellular proliferation and cellular death, as expressed by the apoptotic index as reported in recent studies [18].

The endometrial lesions that may arise during hormonal adjuvant treatment can be diagnosed through hysteroscopy and transvaginal ultrasound. Ultrasonography is useful in asymptomatic patients because it permits selection of patients with increased endometrial thickness who should undergo hysteroscopy and dilation and curettage [19]. A limitation of ultrasound is that an abnormal finding may not be diagnostic: ultrasound may not be able to distinguish between hyperplasia and malignancy and the next step in clinical treatment requires tissue sampling; therefore, hysteroscopic examination seems to be the "gold standard" in diagnostic procedures.

Hysteroscopy is a valuable method for diagnosing endometrial disease because it provides a direct view of the uterine cavity, reveals focal lesions, and enables targeted biopsies to be performed at the same time [20-22]. This is an accurate method in detecting polyps, and hyperplastic and neoplastic changes of the endometrium. Therefore, an evaluation plan using transvaginal sonography as the initial screening evaluation, followed by endometrial biopsy or, more likely, hysteroscopy, tends to become the standard of care globally [21, 23]. It remains unproven whether certain patients at higher risk for carcinoma should proceed directly to invasive evaluation.

The most common pathologic change detected in this present study is the presence of endometrial adenomatous polyps after tamoxifen use. This is in accordance with other researchers [20, 24-27], while others suggest other pathologic conditions as more common in women undergoing tamoxifen use (hyperplastic lesions, endometrial hypertrophy, etc.) [28-31].

The observed elevated levels of Bcl-2 expression in polyps and hyperplastic endometrium found in the present study, are in accordance with recent reports. Bcl-2 exerts its biological anti-apoptotic action, which, in turn, favors the accumulation of hyperplastic glandular cells being responsible for the formation of endometrial polyps [21].

Women on tamoxifen with persistent recurrent bleeding, women with significant risk factors for carcinoma, and women with life-threating hemorrhage comprise this group.

Further studies are still necessary to evaluate high-risk patients and determine whether ultrasound or biopsy is really the most cost-effective initial test.

In conclusion, the authors demonstrated that tamoxifen acts on the postmenopausal uterus as an estrogenic substance, inducing a significant increase in the endometrial thickness. They believe that these preliminary data confirm the potential value of the transvaginal sonography for the detection of the uterine pathology.

For further evaluation, especially for symptomatic patients, hysteroscopy and histological examination of endometrial samples is mandatory. Transvaginal sonography, hysteroscopy, and histological examination can identify women at risk who need further invasive diagnostic treatment.

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Address reprint requests to:
T. KALAMPOKAS, M.D.
Department of Obstetrics and Gynecology
18 Estias Street, Athens (Greece)
e-mail: kalamp@yahoo.com