

## Assessment of the Endometrium in Postmenopausal Breast Cancer Patients on Tamoxifen: An Ultrasound, Hystroscopic, Histopathological and Flow Cytometric Study

OSAMA A. SHAWKI, M.D.<sup>1</sup>; ABEER A. BAHNASSY, M.D.<sup>2</sup>; AHMAD M. FAHMY, M.D.<sup>2</sup>; SABRY SHARAWY, Ph.D.<sup>3</sup>; RABAB GAFFAR, M.D.<sup>4</sup> and HUSSEIN M. KHALED, M.D.<sup>4</sup>

The Departments of Gynecology & Obstetrics<sup>1</sup>, Kasr El-Eini School of Medicine, Cairo University, Pathology<sup>2</sup>, Biochemistry Unit<sup>3</sup>, Cancer Biology and Medical Oncology<sup>4</sup>, NCI, Cairo University.

### ABSTRACT

**Objectives:** Breast cancer patients receiving tamoxifen have an increased risk of developing premalignant and malignant endometrial lesions; hence, screening techniques are advocated.

**Methods:** We performed a prospective study on 100 postmenopausal breast cancer patients (PMBCP), 80 tamoxifen users and 20 controls, to elucidate the endometrial changes associated with tamoxifen use and to evaluate the sensitivity of transvaginal ultrasonography (TVU) and hysteroscopy as screening tools in this group of patients. The duration of tamoxifen exposure ranged from 5-72 months (mean = 30 m).

**Results:** Endometrial thickness ranged from 1-26 mm (mean = 6.4 mm) with a significant difference between tamoxifen users and nonusers (13.5 & 4.2 mm respectively,  $p < 0.001$ ). Also, there was a significant correlation between endometrial thickness and the duration of tamoxifen exposure ( $p = 0.006$ ). Hysteroscopy and endometrial biopsy were performed in 96 patients, 20 of them revealed serious endometrial changes, of whom 18 were confirmed by histology. The remaining 76 had benign features. There was a significant difference between tamoxifen treated and non-treated patients regarding the frequency of pathologic abnormalities. The concordance between TVU and hysteroscopy was 73.3%, TVU and histopathology was 70.6% and between hysteroscopy and histopathology was 93.8%.

**Conclusion:** There is an increased incidence of endometrial premalignant and malignant conditions in tamoxifen treated PMBCP. However, TVU alone is not sufficient for screening such patients due to the high false positive rates. Therefore, patients with  $\geq 5$  mm thickness should perform hystroscopic evaluation with endometrial biopsy at least whenever bleeding is reported. Flow cytometry is useful in the follow-up of PMBCP using tamoxifen since aneuploid peaks and/or a high S-phase fraction (SPF) were detected in cases of atypical hyperplasia and prolif-

erative endometrium which precede the development of endometrial carcinoma.

**Key Words:** Tamoxifen - Breast cancer - Endometrial carcinoma - Hysteroscopy.

### INTRODUCTION

Tamoxifen is a non-steroidal triphenylethylene derivative that is widely used as an endocrine treatment of patients with advanced breast cancer due to its anti-estrogenic effect. It inhibits the binding of estradiol to the estrogen receptors (ER) and increases the concentration of progesterone receptors (PR) in some patients [1,2]. However, tamoxifen is not a pure estrogen antagonist. Several studies show that, in the low estrogen environment of menopause it exerts a weak estrogenic effect on vaginal epithelium [3], myometrium [4] and endometrium [5]. Also, many reports suggested an association between tamoxifen treatment and the induction of endometrial pathology such as hyperplasia, polyps and carcinoma [6].

A relative increase in the risk of endometrial carcinoma associated with tamoxifen use was observed for the first time in 1989 [7]. Several reports have been published since then showing a 1.3 to 7.5-fold increased risk in postmenopausal tamoxifen-using compared to nonusing breast cancer patients [8,9]. In 1996, the IARC [10] concluded that tamoxifen is carcinogenic in humans and in the same year Hemminki et al. [11] demonstrated the formation of DNA-adducts in the human endometrium of tamoxifen

users. This has stimulated the development of screening strategies for endometrial carcinoma in postmenopausal tamoxifen users.

Transvaginal ultrasonography (TVU) is a well-established, simple, non-invasive and accurate method for the evaluation of the endometrium in otherwise healthy women with postmenopausal bleeding [12]. Most authors recommend a cutoff value for the thickness of the endometrial double layer of 4-5 mm below which the risk of endometrial carcinoma is negligible [12,13]. Several studies demonstrated an increased endometrial thickness on TVU in postmenopausal breast cancer patients (PMBCP) on tamoxifen associated with an abnormal sonographic appearance of irregular echogenicity indicating polyps, hyperplasia, or carcinoma [14,15]. However, the previously described cutoff value for endometrial thickness in healthy postmenopausal women does not seem to apply to this group of patients. In addition, TVU may not be an accurate procedure because of the high false positive values in asymptomatic postmenopausal women [3]. Therefore, hysteroscopy was proposed as an additional screening method in such cases. Furthermore, hysteroscopy is the only technique that allows direct visualization of the endometrium as well as an eye-directed biopsy [3,16,17,18]. However, investigators have noted discordance between sonographic, hysteroscopic and histological endometrial findings in 45%-90% of asymptomatic postmenopausal patients [5,14,18 & 19].

The present study was performed to investigate the frequency of tamoxifen-induced endometrial pathology in PMBCP by comparing those who had been on tamoxifen treatment with those who had not taken tamoxifen. We also compared the degree of accuracy of TVU and hysteroscopy as two screening methods for the evaluation of endometrial changes in PMBCP using tamoxifen. The results of both techniques were compared and correlated to histopathology. The predictive value of DNA content and S-phase fraction (SPF) analysis in the determination of endometrial changes was also assessed.

## PATIENTS AND METHODS

*Patients:* During the period from January 1996 to January 2002, we evaluated 100 cases

of PMBCP who agreed to participate in the study. Cases were recruited from Radiotherapy Department, School of Medicine, Cairo University and the Medical Oncology Department, NCI, Cairo. All patients were defined as postmenopausal by amenorrhea for  $\geq 1$  year and postmenopausal estradiol levels  $< 0.10$  nmol/L. Serum samples for determination of estradiol were obtained at the day of gynecological examination and determined by radioimmunoassay. Of the studied patients, 80 (80%) were treated with 20 mg/day tamoxifen for a duration of 12-27 months with a median of 22.5 months (mean  $\pm$  SD =  $30 \pm 13.2$  months). The other 20 patients (20%) who were included in the study did not receive tamoxifen treatment. Patients were further categorized into two groups, the first group comprised 56 patients presenting with abnormal vaginal bleeding and/or discharge (47 tamoxifen-users and 9 nonusers) and the second group comprised 44 asymptomatic patients who were included in the study for comparison. None of the patients received estrogen replacement therapy. The age of the patients ranged from 49-74 (mean  $\pm$  SD =  $55 \pm 6.9$ ). The clinical work-up of patients included detailed gynecologic history-taking, pelvic examination and TVU at presentation.

*Transvaginal ultrasonography (TVU):* A first screening TVU was performed in all patients using Aloka 620, sector scanner with a 5.0-MHz transvaginal transducer (Aloka, Tokyo, Japan). Gynecological examination and TVU were performed in the lithotomy position with an empty bladder. The uterus was scanned both sagittally and coronally to measure the uterine size in three dimensions and to assess the regularity of the endometrium. The maximum width was calculated. Endometrial thickness was recorded by measuring the double layer at the widest point anteroposterior across the uterine cavity. When endometrial surfaces were opposed the total thickness was measured and divided by 2. In case of a fluid-filled cavity, the monolayer of endometrium of the anterior wall without fluid was measured and added to the endometrial thickness of the posterior wall. TVU was repeated every 6 months for all patients under study.

*Hysteroscopy:* Ninety-six patients were offered a hysteroscopy with dilatation and curet-

tage or endometrial biopsy under local or general anesthesia, whereas in 4 patients this was not possible due to the presence of cervical stenosis. Hysteroscopy and endometrial biopsy were performed due to the appearance of abnormal ultrasonographic findings, the occurrence of abnormal vaginal bleeding or routinely at the end of the study for the correlation with TVU.

Hysteroscopy was done using new continuous-flow 5 mm rod lenses operative office hysteroscopy (Karl Storz, Tuttlingen, Germany) under general anesthesia. The uterine cavity was distended using glycine, with the intrauterine pressure set at 45 mm Hg, resulting in the balance of irrigation flow around 200 ml/minute and vacuum of 0.2 bars. The specimens of endometrial curettage were taken by Novak curette from all aspects of the endometrial cavity and were sent for histopathologic evaluation and flow cytometric (FCM) DNA analysis.

*Histopathology:* Endometrial tissues were fixed in 10% neutral buffered formalin, paraffin embedded and 3 micron sections were stained with hematoxyline and eosin. A normal histological finding was defined as the presence of endometrial mucosa composed of epithelial glands and stromal fibroblasts. Glands and stroma were separately described by the examining pathologist. An abnormal histologic finding was defined as a specific histopathological finding and/or the presence of proliferative endometrial glands. Endometrial epithelium was classified as atrophic, normal or hyperblastic with or without atypia, glands were morphologically described as normal or dilated and the stroma was classified as normal, hyperplastic or condensed. In endometrial carcinoma, determination of the histologic type, grade and depth of myometrial invasion was attempted. Staging and grading of tumors were performed according to the classification of 1988 International Federation of Obstetrics and Gynecology (FIGO) and the World Health Organization (WHO), respectively [19].

*Flow cytometry:* Fresh and paraffin-embedded tissues (67 and 33 cases respectively) were processed for FCM-DNA analysis as previously described [20,21]. Cellular DNA content and S phase fraction (SPF) were analyzed on a FAC scan FCM (Becton-Dickinson, San Jose,

CA) using an excitation wavelength of 488 nm and 15 mw argon ion laser. DNA histograms were analyzed with the cell Quest program. Samples were classified as diploid if only one G0/G1 peak was seen in the expected region of the histogram (the same area obtained by a normal blood lymphocyte control in fresh tissues or a normal internal control in paraffin-embedded tissues). If more than one G0/G1 peak was seen, it was considered DNA aneuploid. DNA tetraploid cases were included in the DNA-aneuploid group. These were separated from diploid tumors by finding more than 10% of the cells at the G2/M (upper limit for G2/M fraction). The SPF was defined as the cell percentage at the area between G0/G1 and G2/M and it was automatically determined. The CV range for G0/G1 peak was  $3\pm 1.2$  (in fresh tissue samples) and  $7.4\pm 1.5$  (for paraffin embedded tissue samples). DNA histograms with a wide G0/G1 peak and CVs greater than the upper limit were considered un-interpretable.

Statistical analysis was performed using the  $\chi^2$  test with Fisher's exact test and unpaired Student's *t* test. A *p* value of  $< 0.05$  was regarded statistically significant.

## RESULTS

A hundred PMBCP were evaluated for the development of significant pathologic endometrial changes in tamoxifen users. Patient characteristics at referral are summarized in Table (1).

Out of the 100 patients studied, 25 (25%) had a normal, regular thin endometrium of  $< 5$  mm and 75 (75%) had an endometrial thickness of  $\geq 5$  mm. Among tamoxifen users (Group 1), 9/80 patients (11.25%) had a regular thin endometrium  $< 5$  mm and 71/80 (88.75%) had an endometrial thickness  $\geq 5$  mm, whereas in tamoxifen non-users (Group 2), 16/20 (80%) patients had  $< 5$  mm endometrial thickness and 4/20 (20%) had a  $\geq 5$  mm endometrial thickness. The mean endometrial thickness was 6.4 mm (range, 1-26; SD = 2.88) in all patients; 3 mm (range 1-5; SD = 1.03) in patients whose endometrial thickness was  $< 5$  mm and 15 mm (range 6-26; SD = 5.25) in those whose endometrial thickness was  $\geq 5$  mm. In group 1, the mean endometrial thickness was  $13.5\pm 6.10$  mm,

4.5 mm (range 1-5) in patients whose endometrial thickness was < 5 mm and 15.5 mm (range 7-26) in those whose endometrial thickness was  $\geq$  5 mm. In group 2, the mean endometrial thickness was  $4.21 \pm 1.81$  mm; 2 mm (range 1-5) in patients whose endometrial thickness was < 5 mm and 7.0 mm (range 5.5-11) in those whose endometrial thickness was  $\geq$  5 mm (Table 2).

There was a statistically significant difference in the mean endometrial thickness between tamoxifen treated and non-treated patients ( $13.50 \pm 6.10$  vs  $4.21 \pm 1.81$  respectively,  $p = 0.003$ , Table 1). However, there was no statistically significant difference in the endometrial thickness between symptomatic and asymptomatic patients ( $p = 0.635$ ). The mean endometrial thickness in tamoxifen treated asymptomatic patients was 8.6 mm (range 1-10) whereas in symptomatic patients it was 13.6 mm (range 5-26). In tamoxifen non-treated patients, the mean endometrial thickness in asymptomatic patients was 3.42 mm (range 1-4.2), whereas in symptomatic patients it was 4.65 mm (average 1.9-4.9).

In 29 out of the 75 patients whose endometrial thickness was  $\geq$  5 mm, the sonographic aspect of the endometrium was irregular with multiple cystic areas resembling Swiss cheese (Fig. 1).

Table (2) shows the distribution of patients by endometrial thickness according to age and duration of tamoxifen therapy. Two categories of endometrial thickness were used according to the cutoff value (< 5 mm) used for healthy postmenopausal subjects. A significant association between endometrial thickness and age ( $p = 0.036$ ) or tamoxifen exposure ( $p = 0.021$ ) was evident.

Hysteroscopy revealed 18 cases with endometrial polyps (EP), 20 with simple hyperplasia (SH), 14 atypical hyperplasia (AH), 6 endometrial carcinoma (EC), 2 proliferative endometrium (PE), 8 atrophic endometrium (AE), 10 cystic atrophy (CA) and 21 with normal endometrium (NE). Out of the 17 cases that were diagnosed as SH by hysteroscopy, 14 were confirmed by histology, 2 cases were diagnosed as AH and 1 as NE. One of the 14 hysteroscopically diagnosed AH cases were diagnosed as

SH on histopathologic examination and 1 out of the 6 cases diagnosed as EC by hysteroscopy was histologically diagnosed as AH. All cases diagnosed as AP, AE, CA, PE or NE by hysteroscopy were confirmed by histopathologic examination (Table 3).

The concordance between TVU and hysteroscopy in the study group was 73.3% (55/75 cases), between TVU and histopathology was 70.6% (53/75 cases), whereas between hysteroscopy and histopathology, the concordance was 93.8% (91/97 cases).

Histological examinations of all polyps were characterized by cystically-dilated glands lined with a unilayer of atrophic or metaplastic epithelium of the tubal and periglandular, collagen-rich stromal condensation (Fig. 2). No mitotic activity in the stroma was observed. In 16 patients the epithelium covering the uterine cavity was atrophic and in 7 patients, it was proliferative without atypia.

Abnormal endometrial findings; PE, EP, hyperplasia or EC were detected in 78 cases (78%). The frequency of abnormal histopathologic findings was remarkably higher in tamoxifen-treated than in non-treated patients [52/80 (65%) vs 4/20 (20%) respectively,  $p = 0.015$ ] (Table 4). All cases of EC and AH were found in tamoxifen users only and all were symptomatic. All patients who developed endometrial pathology were exposed to tamoxifen treatment for 18 months at least, whereas the 5 cases of EC were exposed to tamoxifen treatment for more than 30 months and all were symptomatic. Three patients only with an endometrial thickness < 5 mm had pathologic changes in the endometrium compared to 65 patients with  $\geq$  5 mm thickness. In contrast to tamoxifen treated patients, none of the non-treated patients showed AH or EC.

Aneuploid peaks were detected in 23 cases; 5 EC, 16 AH and 2 EP. All cases of EC revealed aneuploid peaks, 2 polyploid and 3 hypertriploid. Whereas, the 2 cases with EP showed tetraploid peaks. Out of the 16 AH, 11 were hypertriploid and 5 were near diploid. A high SPF (> 10) was reported in all cases of EC, PE and AH, in 2 cases of SH and in 3 cases with EP, two of them revealed aneuploid peaks (Fig. 3).

Table (1): Patients characteristics in relation to tamoxifen use.

	Total group	Tamoxifen users (Group 1)	Tamoxifen non-users (Group 2)	p value
Total number	100	80	20	
Age in years (range)	55 (49-74)	63 (49-74)	57 (51-69)	0.665
Duration of amenorrhea (months)	40 (12-72)	45 (12-102)	49 (14-112)	0.892
Estradiol in nmol/L	0.06 (0-0.1)	0.08 (0-1.1)	0.054 (0-0.06)	0.581
Endometrial thickness in mm (range)	6.4 (1-26)	13.5±6.10 (7-11)	4.21±1.8 (1.1-5)*	0.003
Number of hysteroscopy	96	78	18	
Number of histological examinations	96	78	18	
Vaginal bleeding	56 (56%)	47 (58%)	9 (45%)	0.175
<i>Ploidy status:</i>				
Diploid	77	58 (72.5%)	19 (95%)	0.015
Aneuploid	23	22 (27.5%)	1 (5%)	
<i>SPF:</i>				
High ≥ 10%	28	24 (30%)	4 (20%)	0.414
Low < 10	72	56 (70%)	16 (80%)	

Table (2): The relation between endometrial thickness, age of patients and exposure to tamoxifen.

	< 5 mm No. = 11	≥ 5 mm No. = 69	Total No. = 80	p value
<i>Age (years):</i>				
< 60	8 (72.7%)	7 (10.1%)	15	.036
≥ 60	3 (27.3%)	62 (89.9%)	65	
<i>Tamoxifen exposure:</i>				
12-45 m	5 (50%)	8 (50%)	13	0.021
> 45 m	6 (3.8%)	61 (96.1%)	67	

Table (3): Comparison between hysteroscopic and histopathologic findings.

Histopathology	Hysteroscopy							Total No. = 96
	NE No. = 21	AE & CA No. = 18	PE No. = 2	EP No. = 18	SH No. = 17	AH No. = 14	AC No. = 6	
NE	21	–	–	–	1	–	–	22
AE & CA	–	18	–	–	–	–	–	18
PE	–	–	2	–	–	–	–	2
EP	–	–	–	18	–	–	–	18
SH	–	–	–	–	14	1	–	15
AH	–	–	–	–	2	13	1	16
AC	–	–	–	–	–	–	5	5

NE: Normal endometrium      AE: Atrophic endometrium      CA: Cystic atrophy      PE: Proliferative endometrium  
 EP: Endometrial polyp      SH: Simple hyperplasia      AH: Atypical hyperplasia      AC: Adenocarcinoma

Table (4): Histopathologic findings of endometrial curettage in tamoxifen treated and tamoxifen non-treated postmenopausal breast cancer patients.

Histopathology	Tamoxifen-users No. = 80	Tamoxifen-nonusers No. = 20	Total No. = 100
NE	11	11	22
AE & CA	13	5	18
PE	1	1	2
EP	18	0	18
SH	12	3	15
AH	16	0	16
AC	5	0	5

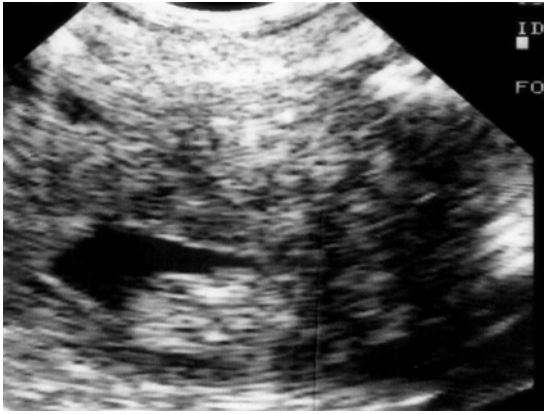


Fig. (1): Swiss-cheese appearance at transvaginal ultrasonography in a postmenopausal breast cancer patient receiving tamoxifen.

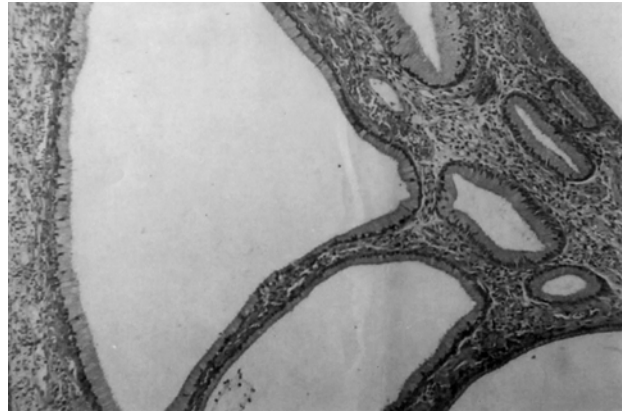


Fig. (2): Hematoxylin and eosin stained section showing the histology of the endometrium in an endometrial polyp discovered by hysteroscopy in a postmenopausal breast cancer patient receiving tamoxifen.

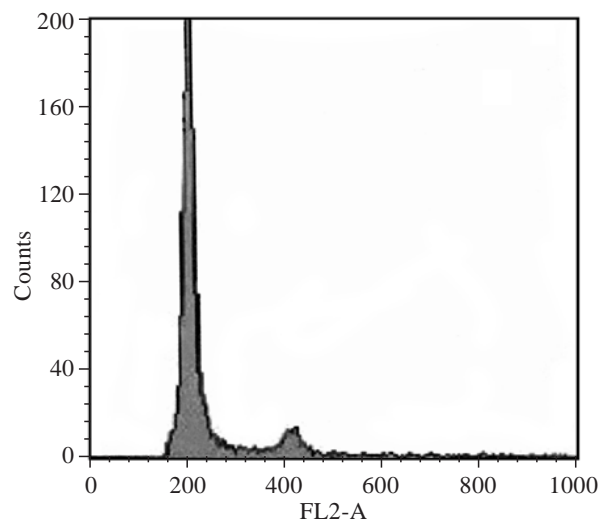
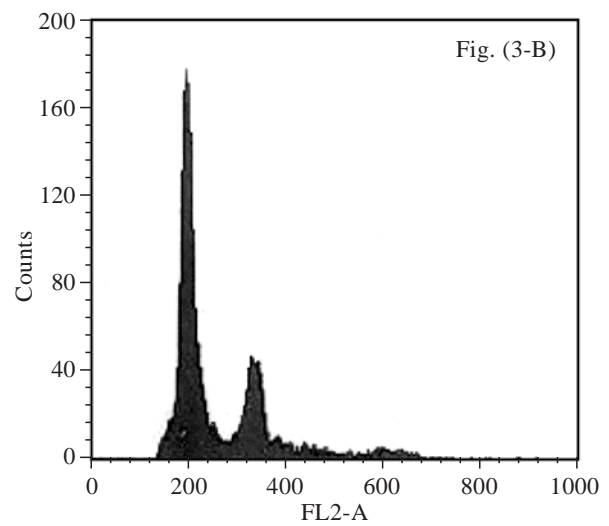
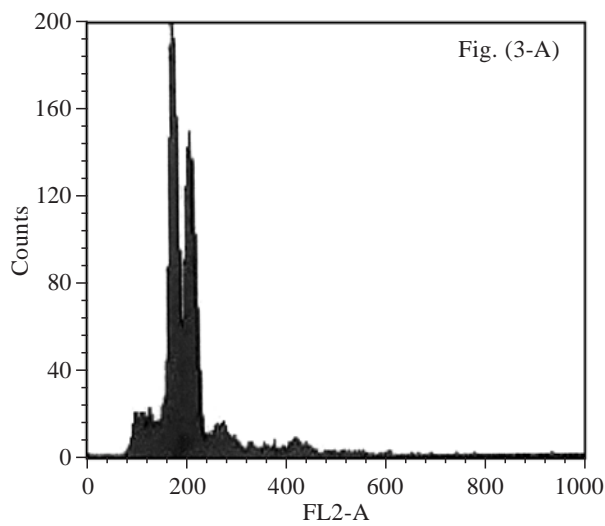


Fig. (3): Flowcytometric DNA analysis for 3 cases of a postmenopausal breast cancer patient receiving tamoxifen. A- A diploid case of a polyp showing high S phase fraction, B- An aneuploid (near diploid) case of atypical hyperplasia with high S phase fraction and C- An aneuploid (tri-tetraploid) case of invasive endometrial carcinoma with high S phase fraction.

## DISCUSSION

Concern about the oncogenic potential of tamoxifen on the endometria of patients with breast cancer is justified by the existing data from controlled studies [22,23]. However, cost-benefit considerations suggest that the current practice of adjuvant tamoxifen therapy should not be discontinued [1].

In the present study, abnormal endometrial changes were demonstrated in 78% of the studied cases with a significant difference between tamoxifen treated (62%) and non-treated patients (4%). Especially noted are premalignant or malignant endometrial lesions which were detected in tamoxifen-treated patients only. This remarkably high incidence in tamoxifen treated patients including 21 patients with premalignant or malignant endometrial lesions is consistent with previous reports [6,24,25,26].

Cohen et al. [27] mentioned that the frequency of endometrial histopathologic findings is remarkable in tamoxifen-treated postmenopausal patients with a risk ratio of 4.6 and Ozsener et al. [25] reported the occurrence of endometrial carcinoma and premalignant changes in 46/104 cases (44.2%) of tamoxifen-treated PMBCP. This was further confirmed by Zabro et al. [24] who reported significant endometrial abnormalities in 26.9% of their 219 breast cancer cases after 2 years of tamoxifen exposure, whereas none of the non-treated cases had any endometrial side effects.

Therefore, surveillance of patients under tamoxifen was advocated, however there is no consensus on the methodology to be used [1]. Abdominal or TVU had been widely used in the past. However, most of the previously published reports using TVU were criticized because the majority of studied subjects had no histological assessment of the endometrium and the absence of endometrial carcinoma was excluded on the basis of endometrial sonography only and/or cancer registry follow-up [28]. Although the authors claimed that, the sensitivity of endometrial sonography and the strict monitoring through cancer registry were an acceptable standard to exclude the presence of carcinoma, endometrial cancer cases might have been missed in this way [29]. Moreover, tamoxifen therapy may cause an apparent increase in the endometrial thickness when measured by sonog-

raphy which is not confirmed by routine histopathologic examination of endometrial biopsies [30]. A possible explanation for this phenomenon was provided by Goldstein [14] and Hulka [31] who mentioned that tamoxifen exerts its effect on the non-epithelial or sub-endometrial substrata only. These changes might be impossible to differentiate from endometrial hyperplasia at sonography since both of them are equally depicted as a band of increased echogenicity clearly separated from the surrounding hypoechoic myometrium [28]. Therefore, recent studies advocate the use of additional techniques to confirm TVU findings especially in asymptomatic patients or those with increased endometrial thickness [3,24,25,26].

In the present study, hysteroscopy with endometrial biopsy and FCM were performed in 96 patients after explaining the aim of the study, the steps and the possible complications of the techniques. The results of hysteroscopy, TVU and flow cytometry were correlated to each other and evaluated in relation to histopathology to determine the sensitivity of each of them. We found a significant difference in the mean endometrial thickness between tamoxifen-treated and non-treated patients. Similarly, there was a significant correlation between the endometrial thickness and the duration of tamoxifen exposure. Our results in these regards are in agreement with previously published data [6,28].

The mean endometrial thickness reported in the present study for patients with premalignant and malignant lesions (16.5 mm) is comparable to that reported by Nasari and Coast, (18.2 mm) [32]. In addition, all patients with endometrial abnormalities had taken tamoxifen for more than 18 months and 10 patients with premalignant and malignant lesions had tamoxifen for more than 2 years. The later finding is consistent with previous data showing that women using tamoxifen for more than 2 years have higher risk of endometrial cancer than non users [33,34].

The results of the present work showed an obvious discordance between TVU, hysteroscopy and histopathology. Whereas (75%) PMBCP showed irregular thickening of the endometrium on TVU, only 57 (57%) could be explained by hysteroscopy and 55 (55%) by histopathology. Histologic examination of the uterus in patients

who underwent hysterectomy as a result of their endometrial pathology (11 cases) gave an explanation for this discrepancy. The thickness and appearance on ultrasound and hysteroscopy corresponded with the histological endometrial layer comprising epithelial atrophy with cystic glands and stromal condensation [3,35,36]. The dense stroma in which large cysts are lined with one layer of flattened epithelium corresponds with the Swiss-cheese aspect on TVU.

Flow cytometry is a rapid and accurate method that permits quantitative DNA analysis and SPF determination in few hours [20]. To our knowledge, the present study is the first report regarding the application of FCM in the assessment of the endometrium in tamoxifen-treated patients. The results of flow cytometric DNA analysis in the present work are novel and have an important clinical impact. We found a significant association between flowcytometric DNA analysis, hysteroscopy and histopathology ( $p = 0.024$  and  $p = 0.001$ , respectively). The fact that all EC and AH cases were aneuploid with high SPF points to the possibility of using FCM for rapid identification of cases with significant histopathologic changes. Moreover, the two cases of EP that revealed aneuploid peaks also had high SPF indicating the necessity of following these patients at regular intervals since they represent high risk cases that could proceed to carcinoma. An additional important observation is that, all EC and AH cases fall in the polyploid, near diploid and/or hypertriploid range, whereas the 2 cases with EP showed tetraploid peaks which usually carry better prognosis.

In conclusion, the present study sheds light on the association between long-term tamoxifen therapy and the development of significant endometrial changes in PMBCP. It shows a high frequency of endometrial abnormalities in PMBCP using tamoxifen as an adjuvant therapy and an apparent increase of endometrial thickness, when assessed by sonography compared to tamoxifen nonusers. Of special concern are the symptomatic group and those with thick endometrium. This indicates that, tamoxifen might be a potentially malignant in the postmenopausal endometrium. However, the exact mechanism(s) are still not fully understood. It has been postulated that endometrial proliferation or hyperplasia which is usually associated

with continuous unopposed estrogen stimulation may predispose to endometrial malignancy [37]. Since none of our patients received any hormonal treatment, it could be assumed that the high prevalence of pathological endometrial changes is due to continuous and unopposed tamoxifen rather than estrogen exposure especially in the absence of progesterone effect in postmenopausal women. Some previous studies illustrated an estrogen mimetic effect of tamoxifen in endometrial tissues which can probably contribute to the increased incidence of premalignant and malignant lesions in tamoxifen treated patients. However, recent reports suggest that tamoxifen may have multiple carcinogenic effects as well [38,39]. One of such mechanisms could be achieved through the DNA-damaging effect of the 4-OH-tamoxifen, a tamoxifen metabolite which is present in the serum of patients taking tamoxifen. Stimulation of cell proliferation in the presence of a DNA damaging agent significantly increases mutation rates and accelerates tumor progression [38]. Zhong et al. [39] postulated that, the combination of the cell proliferation-inducing estrogen mimetic effects of tamoxifen and DNA damage brought about by 4-OH tamoxifen makes tamoxifen a potent carcinogen. However, cigarette smoke which also contains both tumor promoting and DNA damaging agents takes more than 20 years before significant increases in the lung cancer are observed. Therefore, it is likely that there are other effects of tamoxifen that make it even more potent carcinogen capable of inducing endometrial cancers in shorter periods. They demonstrated a TPA mimetic effect of tamoxifen that could induce additional carcinogenic properties responsible for the rapid tumor-producing ability of tamoxifen. The TPA was defined as a tumor promoting phorbol ester which depletes the  $\delta$  isoform of the protein kinase C.

However, our data demonstrates a discrepancy between TVU, hysteroscopy and histopathology and a significant association between FCM, hysteroscopy and histopathology. Consequently, TVU alone is not an effective screening test for endometrial pathology in PMBCP and should be confirmed by other more accurate techniques. Hysteroscopy, FCM-DNA analysis and/or histologic assessment of the endometrium should be performed to detect endometrial lesions especially in patients with thickened endometrium or when gynecological symptoms

occur. In addition, our results provide evidence for the usefulness of using FCM in monitoring PMBCP using tamoxifen since abnormal; aneuploid peaks and/or a high SPF were detected in almost all cases of EC, AH and PE. It can also help in the early detection of abnormal endometrial changes through regular follow-up of cases with abnormal DNA profile even if these cases did not show endometrial pathology since they usually carry an increased malignant potentiality.

### REFERENCES

- 1- Nola M., Jukie S., Iltie-Forko J., Babie D., Uzarevie B., Petroveeki M., et al.: Effect of tamoxifen on steroid hormone receptors and hormone concentration and the results of DNA analysis by flow cytometry in endometrial carcinoma. *Gynecol. Oncol.* 1999, 72: 331-336.
- 2- Cohen I., Beyth Y., Altaras M., Shapira J., Tepper R., Cardoba M., et al.: Estrogen and progesterone receptor expression in postmenopausal tamoxifen exposed endometrial pathologies. *Gynecol. Oncol.* 1997, 67: 8-15.
- 3- Mourits M., Van der Zee A., Willemse P., Ten Hoor K., Hollema H. and De Vries E.: Discrepancy between ultrasonography and hysteroscopy and histology of endometrium in postmenopausal breast cancer patients using tamoxifen. *Gyn. Oncol.* 1999, 73: 21-26.
- 4- Powles T., Jones A. and Ashley S.: The Royal Marsden Hospital pilot tamoxifen chemoprevention trial. *Breast Cancer res Treat.* 1994, 31: 73-82.
- 5- Lahti E., Blanco G., Kauppila A., Apaja-Sarkkinen M., Taskinen P. and Laatikainen T.: Endometrial changes in postmenopausal breast cancer patients receiving tamoxifen. *Obstet. Gynecol.* 1993, 81: 660-664.
- 6- Cheng F., Lin H., Torng L. and Huang C.: Comparison of endometrial changes among asymptomatic tamoxifen-treated premenopausal and postmenopausal breast cancer patients. *Gynecol. Oncol.* 1997, 66: 233-237.
- 7- Fornander T., Rutqvist L., Cedermark B., Glas U., Mattsson A., Silfversward C., et al.: Adjuvant tamoxifen in early breast cancer: occurrence of new primary cancers. *Lancet* 1989, 19: 117-120.
- 8- Sasco A., Chaplain G., Amoros E. and Saez S.: Endometrial cancer following breast cancer: effect of tamoxifen and castration by radiotherapy. *Epidemiology* 1996, 7: 9-13.
- 9- Van Leewen E., Benraadt J. and Coeberg W.: Risk of endometrial cancer following breast cancer treatment with tamoxifen. *Lancet* 1994, 343: 448-452.
- 10- IARC on tamoxifen (news). *Environ Health Perspect* 1996, 104: 688.
- 11- Hemminki K., Rajaniemi H., Lidahl B. and Moberger B.: Tamoxifen-induced DNA adducts in endometrial samples from breast cancer patients. *Cancer Res.* 1996, 56: 4374-4377.
- 12- Karlsson B., Granberg S., Wikland M., Ylostalo P., Torvid K., Marsal K. and Valentin L.: Transvaginal ultrasonograph of the endometrium in women with postmenopausal bleeding-a Nordic multicentered study. *Am. J. Obstet. Gynecol.* 1995, 172: 1488-1494.
- 13- Granberg S., Wikland M. and Karlsson B.: Endometrial thickness as measured by endovaginal ultrasonography for identifying endometrial abnormality. *Am. J. Obstet. Gynecol.* 1991, 164: 47-52.
- 14- Goldstein S.: Unusual ultrasonographic appearance of the uterus in patients receiving tamoxifen. *Am. J. Obstet. Gynecol.* 1994, 170: 447-451.
- 15- Exacoustos C., Zupi E., Cangi B., Chiaretti M., Arduini D. and Romanini C.: Endometrial evaluation in postmenopausal breast cancer patients receiving tamoxifen: an ultrasound, color flow Doppler, hysteroscopic and histological study. *Ultrasound Obstet Gynecol.* 1995, 6: 435-442.
- 16- Timmerman D., Deprest J. and Bourne T.: A randomized trial on the use of ultrasonography or office hysteroscopy for endometrial assessment in postmenopausal patients with breast cancer who were treated with tamoxifen. *Am. J. Obstet. Gynecol.* 1998, 179: 62-70.
- 17- Ceci O., Bettocchi S., Marelllo F., Di Venere R., Pellegrino A., Laricchia L. and Vimercati A.: Hysteroscopic evaluation of the endometrium in postmenopausal women taking tamoxifen. *Am. J. Assoc. Gynecol. Laparosc.* 2000, 7 (2): 185-189.
- 18- Cohen I., Beyth Y. and Tepper R.: The role of ultrasound in the detection of endometrial pathologies in asymptomatic postmenopausal breast cancer patients with tamoxifen treatment. *Obstet. Gynecol. Surv.* 1998, 53: 429-438.
- 19- Longacre T., Kempson L. and Hendrickson M.: Endometrial hyperplasia metaplasia and carcinoma, in Fox H., Wells M. (eds): Haines and Taylor Obstetrical and Gynecological Pathology. New York. Churchill Livingstone 1995, pp: 421-510.
- 20- Vindelov I., Christenson I. and Nissen N.: Standardization of high resolution FCM-DNA analysis by simultaneous use of chicken and trout RBCs as internal reference standards. *Cytometry* 1983, 3 (5): 328-31.
- 21- Fallenius A., Franzen S. and Auer G.: Predictive value of nuclear DNA content in breast cancer in relation to clinical and morphological factors. A retrospective study of 227 consecutive cases *Cancer* 1988, 62 (3): 521-30.
- 22- Fornander T., Rutqvist I., Ledermark B., Glas U., Mattsson A. and Silfversward C.: Adjuvant tamoxifen in early breast cancer: Occurrence of new primary cancers. *Lancet* 1989, 1: 117-120.
- 23- Fisher B., Constantino J., Redmond C., Fisher R., Wickerham L. and Cronin H.: Endometrial cancer in tamoxifen-treated BC patients: Findings from the NSBP B. 14. *J. Natl. Cancer Inst.* 1994, 86: 527-537.

- 24- Zabro G., Caruso G., Zammitti M., Caruso S. and Zabro R.: The effects of tamoxifen therapy on the endometrium. *Eur. J. Gynaecol. Oncol.* 2000, 21 (1): 86-88.
- 25- Ozsener S., Ozaran A., Itil I. and Dikmen Y.: Endometrial pathology of 104 postmenopausal breast cancer patients treated with tamoxifen. *Eur. J. Gynaecol. Onco.* 1998, 19 (6): 580-583.
- 26- Tesoro R., Borgida F., MacLaurin A. and Asuncion M.: Transvaginal endometrial sonography in postmenopausal women taking tamoxifen. *Obstet. Gynecol.* 1999, 94 (2): 321-322.
- 27- Cohen I., Rosen D., Shapira J., Cordoba M., Gilboa S., Altaras M. and Yigael D.: Endometrial change with tamoxifen: comparison between tamoxifen-treated and nontreated asymptomatic, postmenopausal breast cancer patients. *Gynecol. Oncol.* 1994, 52: 185-190.
- 28- Cecchini S., Ciatto S., Bonardi R., Mazzotta A., Grazzini G., Pacini P. and Muraca M.: Screening by ultrasonography for endometrial carcinoma in postmenopausal breast cancer patients under adjuvant tamoxifen. *Gynecol. Oncol.* 1996, 60: 409-411.
- 29- Osmers R., Volksen M. and Schauer A.: Vaginosonography for early detection of endometrial carcinoma. *Lancet* 1990, 335: 1569-1571.
- 30- Ciatto S., Cecchini S., Bonardi R. and Grazzini G.: Ultrasonography surveillance of endometrium in breast cancer patients on adjuvant tamoxifen. *Lancet* 1994, 344: 60.
- 31- Hulka A. and Hall A.: Endometrial abnormalities associated with tamoxifen therapy for breast cancer: sonographic and pathologic correlation. *Am. J. Radiol.* 1993, 160: 809-812.
- 32- Nasari M. and Coast J.: Correlation of ultrasound findings and endometrial histopathology in postmenopausal women. *Br. J. Obstet. Gynecol.* 1989, 96: 133-138.
- 33- Leeuwen F., Benraad J., Coebergh J., Kiemeny L., Gimberere H., Otter R., et al.: Risk of endometrial cancer after tamoxifen treatment of breast cancer. *Lancet* 1994, 343: 448-452.
- 34- Yokusuka K., Teshima H., Katase K., Fujimoto I., Yamauchi K., Hasumi K. and Tatsuki Y.: Effects of long-term administration of tamoxifen on vaginal epithelium and complications of endometrial lesions in breast cancer patients. *Acta Obstet. Gynaecol. Jpn.* 1995, 47 (2): 125-132.
- 35- Decensi A., Fontana V., Bruno S., Gustavino C., Gatteschi B. and Costa A.: Effect of tamoxifen on endometrial proliferation. *J. Clin. Oncol.* 1996, 14: 434-440.
- 36- Ismail S.: Pathology of endometrium treated with tamoxifen. *J. Clin. Pathol.* 1994, 47: 827-833.
- 37- Kistner W.: Treatment of hyperplasia and carcinoma in situ of endometrium. *Clin. Obst. Gynecol.* 1982, 5: 63-72.
- 38- Zhong M., Lu Z., Abbas T., Hornia A., Chatakond K., Barile N., et al.: Noval tumor-promoting property of tamoxifen. *Cell growth & differentiation* 2001, 12: 187-192.
- 39- Deligdisch L.: Hormonal pathology of the endometrium. *Mod. Pathol.* 2000, 13: 285-294.