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Aromatase inhibitors and mammographic breast density in postmenopausal women receiving hormone therapy

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Abstract

Objective: One of the main concerns regarding long-term use of hormone therapy (HT) in symptomatic menopausal women is the perceived increased risk of breast cancer. A method to reduce breast cancer risk in this population of women is urgently needed. We hypothesized that adding aromatase inhibitors (AIs) to HT would reduce local breast estrogen exposure and breast cancer risk without altering the beneficial systemic effects of HT on menopausal symptoms or bone density. The aim of this study was to investigate the effect of AIs and HT on mammographic breast density (MBD) as a surrogate marker of breast cancer risk in postmenopausal women receiving low-dose HT.

Design: This was a retrospective cohort study conducted at private clinics affiliated with a university hospital. One group of postmenopausal women (n = 28) received low-dose HT daily plus letrozole 2.5 mg three times weekly. Postmenopausal women receiving HT alone (n = 28) served as controls. MBD, the primary outcome, was measured using quantitative image analysis software as well as by visual analysis by a radiologist. Hypoestrogenic effects, adverse reactions, and bone mineral densities were secondary outcome measures.

Results: The mammograms of 18 women in the study group and 22 women in the control group were suitable for comparison. A statistically significant reduction in MBD occurred in the women who received HT plus an AI, whereas no significant change was observed in the women receiving HT alone. There was no significant increase in hypoestrogenic symptoms during the use of AIs, and bone mineral densities were not significantly reduced.

Conclusions: Adding an AI to HT may lower MBD in postmenopausal women. AIs could be good candidates for primary chemoprevention of breast cancer in postmenopausal women using HT.

Key Words: Aromatase inhibitors – Chemoprevention – Hormone therapy – Breast cancer – Mammogram – Breast density – Computer-assisted image analysis.

Breast cancer (BC) is the most common cancer in women, resulting in 10% of all cancers diagnosed and 7% of all cancer-related deaths around the world. In North America, it has been estimated that one in nine women will be diagnosed with BC.[1,2]

Epidemiological and clinical studies demonstrated an increased risk of BC with prolonged exposure to estrogen. Early menarche, delayed menopause, nulliparity, late age at first term pregnancy, and obesity after menopause are known risk factors for BC.[3] In fact, the contribution of estrogen to BC is well established and was known more than a century ago, when bilateral oophorectomy was first suggested as a method of treatment of advanced BC.[4] Blockade of estrogenic signaling has been the core of the treatment of estrogen receptor (ER)–positive and progesterone receptor–positive BC with the use of selective ER modulators.[5]

Estrogen is likely to have multiple roles in BC pathophysiology, acting as a genotoxic agent, an epigenetic procarcinogen, and an occasional inducer of genetic mutations.[6] Estrogen is a mitogenic hormone that increases the rate of cell division and thus reduces the probability for DNA repair.[7] The hydroxy metabolites of estrogen, which have been found in BC in amounts significantly greater than those in the normal breast, are believed to exert direct damage to DNA. The free radicals produced from the catecholestrogens can cause single-strand breaks, hydroxylation of the guanine bases, and DNA adducts.[8,9]

Although BC is estrogen dependent, there is no decrease in BC risk at the time of menopause when circulating levels of estrogen decrease, and in fact BC rates continue to increase with age. Eighty percent of BC occurs in women older than

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50 years of age, and it occurs in approximately one third of women older than 70 years.[10] Currently, it is accepted that estrogen in both postmenopausal women and in men plays a role as an intracrine and paracrine factor (rather than a circulatory hormone) in many peripheral sites including the breast, endometrium, brain, adipose tissue, skin, bones, vascular endothelium, and prostate.[11] In fact, the peripheral in situ biosynthesis of estrogen contributes to appproximately 75% of the total estrogen produced in premenopausal women and almost 100% in postmenopausal women.[12] Some authors, therefore, regard the serum levels of estrogens in postmenopausal women as a reflection of the nonmetabolized excess of estrogen that has escaped from the intracellular compartment.[13]

Aromatase is the rate-limiting enzyme that catalyzes the final step of estrogen synthesis from androgens. An increase in aromatase activity plays a key role in the pathogenesis of various clinical syndromes.[13-15] Overexpression of aromatase in the mammary gland of transgenic mice leads to a significant increase of preneoplastic events such as hyperplasia, dysplasia, fibroadenomas, and nuclear abnormalities.[16] Additionally, the expression of the aromatase gene is significantly higher in the adipose tissue of healthy postmenopausal women compared with premenopausal women.[17] In BC, approximately half of the intratumoral estrogens are believed to be produced inside the tumor itself or from the surrounding tissues. In postmenopausal BC patients, the intratumoral estrogens reached up to 20 times the corresponding serum levels, consistent with local aromatase activity. The transcripts of the aromatase-producing gene (CYP19) are markedly increased in postmenopausal BC patients compared with premenopausal women.[18-20] Aromatase activity was also significantly higher in breast tumor tissues and tissues adjacent to the tumors than in healthy tissues.[21] Despite the fact that the total amount of locally produced estrogen is low, the presence of aromatase in a large mass of breast tissues helps provide a concentration that can cause biological effects.[13]

Aromatase inhibitors (AIs) are effective in reducing circulating estrogen concentrations by 97% to 99% in menopausal women[22] with a good safety profile.[23] AIs are now the gold standard for adjuvant endocrine therapy of ER–positive BC patients.[24] They have been tested in several phase III trials and impart a significant clinical advantage over previous drugs such as tamoxifen and megestrol acetate. The AI anastrozole was shown to be superior to megestrol acetate in the treatment of advanced BC resistant to tamoxifen.[25,26] In a recent consensus, the Central European Cooperative Oncology Group recommended the use of AIs as first-line treatment for postmenopausal patients with hormone receptor–positive metastatic BC.[27] Moreover, letrozole was more effective than tamoxifen for Erbb-1– and/or Erbb-2–positive, ER–positive primary BC.[24] AIs have also resulted in significant improvement of overall survival and disease-free survival in patients with ER-/progesterone receptor–positive tumors.[28]

Nonetheless, AIs are known to create a systemic hypoestrogenic state that typically produces symptoms of menopause such as hot flashes, skeletal and joint pains, mood swings, and vaginal dryness. In the long term, osteoporosis and an increased risk of fractures are also expected. Lonning et al[29] reported that using exemestane for 2 years significantly reduced bone mineral density in the femoral neck. In addition, bone resorption markers increased significantly with 3 months of letrozole when given to healthy postmenopausal women with high risk of BC.[30]

The objective of this study was to investigate the effect of AIs on mammographic breast density (MBD) in postmenopausal women receiving hormone therapy (HT). We hypothesized that AIs could have a protective effect against BC in women using HT by reducing MBD, a surrogate marker of BC risk.

METHODS

Study design

This was a preliminary retrospective cohort study that was conducted from December 2006 until August 2007. Ethics approval for this study was obtained from the Committee for Research on Human Subjects at Mount Sinai Hospital (06-0264-C). Women provided written consent after the study was explained to them.

Selection of study participants and controls

Study participants were chosen from healthy postmenopausal women being treated with HT in the Division of Reproductive Sciences, Department of Obstetrics and Gynecology, University of Toronto, Toronto, ON, Canada. The study group comprised 28 postmenopausal women who were prescribed the AI letrozole (2.5 mg/day on Monday, Wednesday, and Friday) in addition to their current HT. These women had at least two annual mammograms, one within a year before the administration of the AI and the most recent mammogram during the study. The women's initial use of AIs was not related to participation in the study, although all women were counseled regarding the theoretical and off-label nature of administering AIs to reduce their risk of BC before receiving letrozole. Women who had a history of BC were excluded. We compared the mammograms of the study group with those of two control groups for MBD. One control group included comparison of two annual mammograms in the study participants while receiving HT alone before starting the AI. This control was to determine the trend of change in MBD with advancing of age in the same study patients while taking hormone therapy only. The second control group included two annual mammograms of postmenopausal women who used HT alone for 1 or more years and who had at least two regular mammograms during this therapy. This second control group was to verify the effects of age on MBD in a separate group of women.

Assessment and outcome measures

Chart review

We examined the data of both the study and the control groups. Clinical characteristics of the study participants

and the controls were recorded. Additionally, we evaluated risk factors for BC in both groups. We used a validated Breast Cancer Risk Assessment Tool available online from the National Cancer Institute to calculate the 5-year BC risk based on the Gail model.[31] However, the Gail model does not take into account factors such as second-degree relatives with BC, family history of ovarian cancer, and BC susceptibility genetic mutations, which are considered in other risk models and considerably elevate the individual absolute risk of BC.[32] We included these risk factors in the evaluation of BC risk in the study participants. Menopausal symptoms before and after the treatment with AIs were included as well as side effects and causes of drug discontinuation. Bone mineral density records were also evaluated whenever possible.

Breast density

Two formats were used to collect in this study:

Digital mammograms. Obtained by direct digital acquisition and archived through the Picture Archiving and Communication Systems. The digital mammograms were burned on CDs with a DICOM viewer (eFilm Lite software; Merge eMed, Milwaukee, WI) that allowed manipulation of the images and exporting them to other computer programs.

Analog mammograms. Obtained by the conventional film-screen x-ray systems using an analogue acquisition technique. Digitization was done to convert the analogue mammograms into digital formats suitable for further computer analysis. We used Kodak LS85 film digitizer (Eastman Kodak Co., Rochester, NY). This is a high-resolution film digitizer that produces digital signals as accurate as 0.001 optical densities. For some patients, we used flatbed scanners: Agfa DuoScan (Agfa-Gevaert Group, Mortsel, Belgium) and ScanMaker i900 (Microtek International, Inc., Carson, CA). To have identical scanning settings, each woman's mammograms were compared using the same scanner and the same scanning parameters set in the corresponding scanning software.

Only mammograms produced using the same format were able to be usefully compared. That is, digital versus digital or analogue versus analogue formats were compared before and after treatment.

Quantitative image analysis of breast density

We obtained or scanned available projections of both breasts. For the MBD analysis by computer software

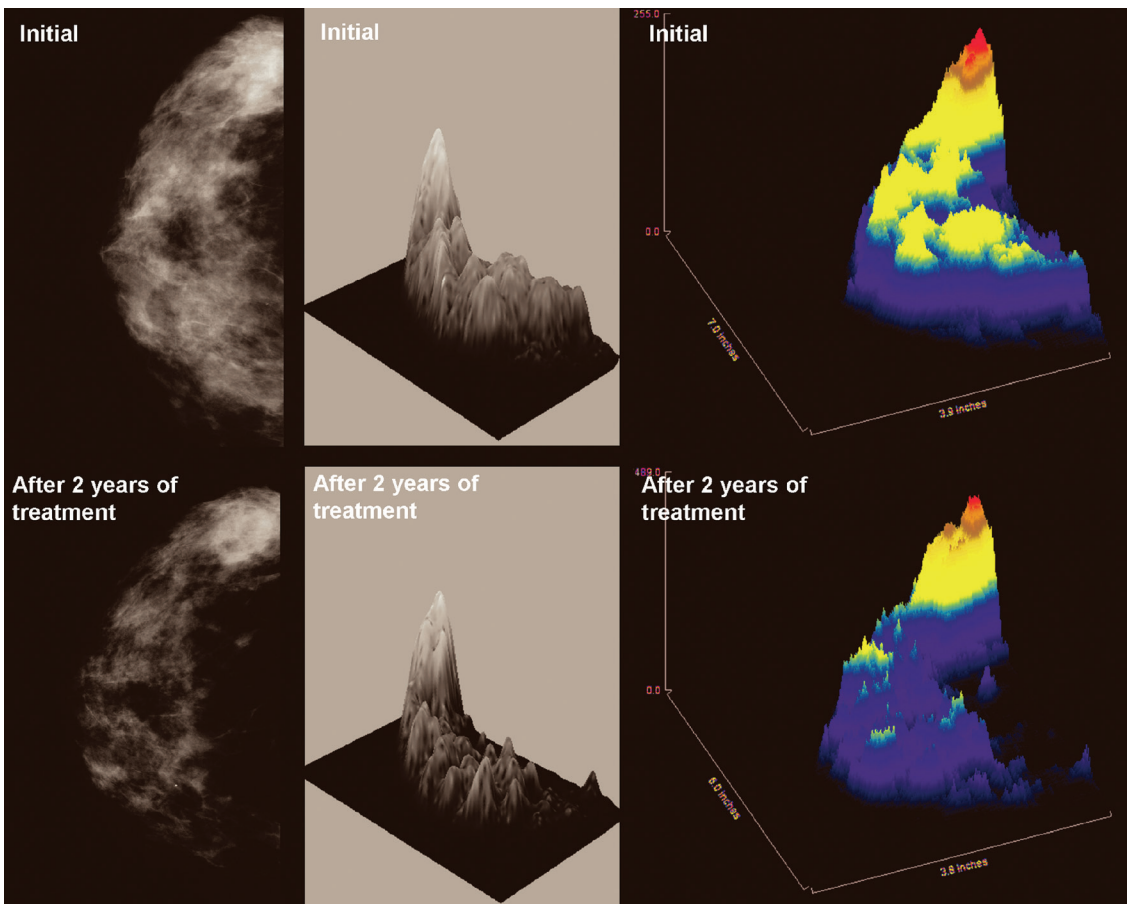

FIG. 1. Mammographic breast density in the aromatase inhibitors plus hormone therapy group represented by the ImageJ software. Interactive three-dimensional surface plotting (second column) shows mammographic breast density in gray scale. Broad selection of colors that can be assigned for each of 256 possible displayed pixel values (third column) can be obtained by the look-up table tool.

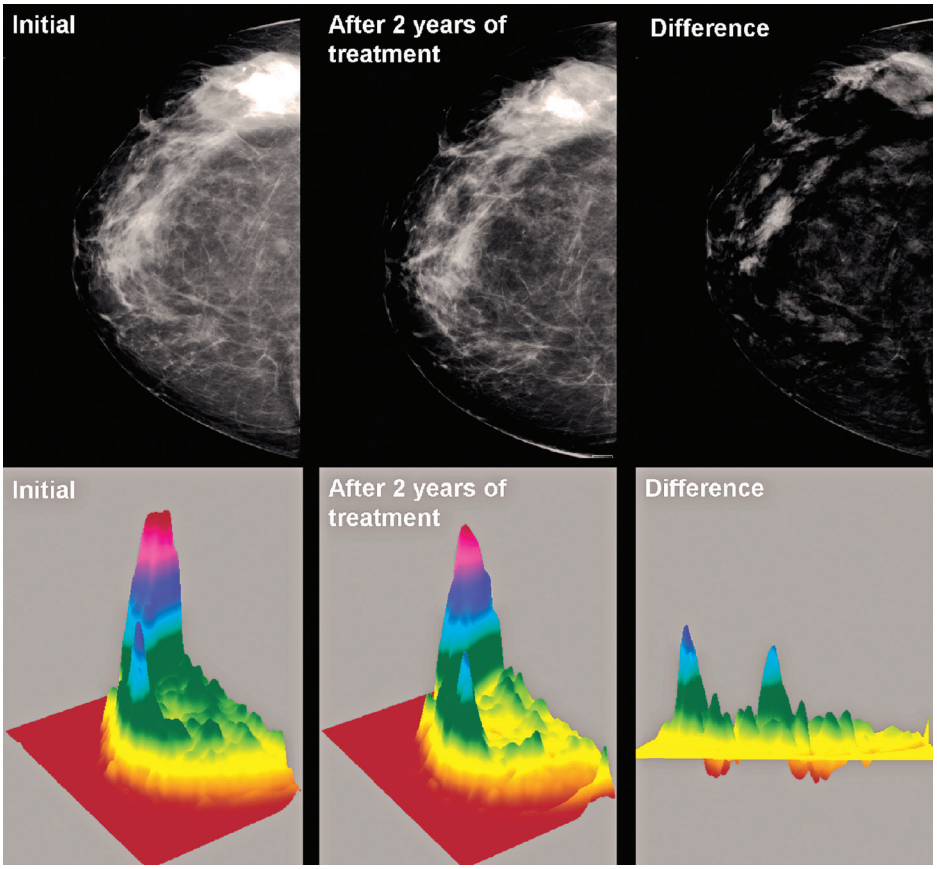

FIG. 2. Mammographic breast density in the aromatase inhibitors plus hormone therapy group represented by the ImageJ software. Image subtraction shows the sites of increased or decreased breast densities between the two images before and after the aromatase inhibitor.

program, we used the right craniocaudal projections or the left craniocaudal projections when the former were not available based on our own data (unpublished) and previous data of the high symmetry existing between different projections.[33-35] One film for each woman receiving HT before the start of the AI treatment and the most recent mammogram during the combination of HT and AI were compared. In our study, we used two image analysis software programs to assess MBD:

ImageQuant (IQ) Version 5 (Molecular Dynamics, Sunnyvale, CA). This is a multipurpose program that offers different modules for various types of samples. Each set of mammograms assigned for comparison was viewed simultaneously, and the gray scale was adjusted to clearly visualize the breast edge. The same outline of the total area of the breast was used in both images. Chest wall structures were excluded from the outline. IQ calculates the area using the number of pixels for the outlined region. Then it assigns each pixel a numerical value called pixel intensity according to the absorbance of the image at that point, which in turn is an indication of breast tissue density. Finally, pixel intensity is integrated over all the pixels in the outlined area and presented as the integrated pixel intensity (IPI).

IQ includes various types of background correction methods that subtract the background noise from the IPI to result in the approximate volume estimate of the MBD. We set the local average option in all measurements, so that the program calculated a background value equal to the average of all the points beneath the outline.

ImageJ. This is a free public domain Java image-processing program offered by the National Institutes of Health. It can be used either as an online applet or can be downloaded (http://rsb.info.nih.gov/ij/). Using ImageJ, we were able to measure the IPIs inside an outlined area similar to IQ. In addition, ImageJ provided a visual representation of the changes in breast density by its surface plot function. Interactive three-dimensional surface plotting is also available as a plug-in. MBD in gray-scale images could be additionally represented in a broad selection of colors that can be assigned for each of 256 possible displayed pixel values (Figs. 1 and 2).

Visual analysis of breast density

An experienced breast radiologist (C.P.) was responsible for analysis of the MBD by visual inspection. The radiologist was blinded to time points, treatment status, and any clinical data related to the mammograms. To assess the intraobserver reliability, all projections (right craniocaudal, left craniocaudal) and right and left mediolateral oblique views of the same woman were evaluated by the radiologist. In addition,

TABLE 1. *Clinical data for women in the treatment and control groups*

	AI + HT (n = 28)		HT controls (n = 28)		
	Mean ± SD or no. (%)	SEM	Mean ± SD or no. (%)	SEM	*P*
Age, y	61.79 ± 7.65	1.45	65.46 ± 7.52	1.47	0.075
Age at menopause, y	47.70 ± 5.99	1.15	47.60 ± 6.13	1.22	0.951
HT total duration, y	14.48 ± 6.69	1.31	13.91 ± 7.35	1.41	0.767
Menopause (natural/surgical), E + P/E	21 (75)/7 (25)		22 (78.6)/6 (21.4)		0.752
HT indication					
Vasomotor symptoms (hot flashes, night sweats)	6 (21.43)		4 (14.3)		
Sexual symptoms (vaginal dryness, dyspareunia, decreased libido)	2 (7.14)		0 (0)		
Psychological symptoms (depression, mood swings)	2 (7.14)		0 (0)		0.207
Mixed menopausal symptoms (hot flashes, vaginal dryness, skeletal pains)	10 (35.71)		18 (64.3)		
Osteoporosis	3 (10.71)		3 (10.7)		
Premature menopause	5 (17.86)		3 (10.7)		

AI, aromatase inhibitor; HT, hormone therapy; E + P/E, therapy with estrogen + progesterone or estrogen alone.

random samples (20%) of the films were reblinded and shown again to the same radiologist. The correlation coefficient for the intraobserver reliability was 0.92 for the visual scores of breast density.

Measurements of breast density were scored based on the Breast Imaging Reporting and Data System, which is a qualitative method developed by the American College of Radiology.[36] In addition, the two projections compared by the software analysis were also compared blindly by the radiologist by assigning increase, decrease, or unable to detect significant changes of the MBD for each pair of mammograms.

Data recording and statistical analysis

IQ IPI data output was numerical and exported to a spreadsheet (Microsoft Office Excel 2003). The statistical tests were performed using SPSS 14.00 for Windows (release 14.01, SPSS Inc., Chicago, IL). We used the nonparametric Wilcoxon signed-rank test to analyze related continuous variables and the nonparametric Mann-Whitney *U* test to analyze independent continuous variables. The χ^2 test was used to analyze categorical variables, and Spearman's test was used to measure bivariate correlations. $P < 0.05$ was considered statistically significant.

TABLE 2. *Aromatase inhibitors (AIs) in the study group (n = 28)*

AIs: original indications		
To reduce BC risk	25 (89.29%)	
Severe endometriosis	2 (7.14%)	
Breast tenderness	1 (3.57%)	
BC risk		
Gail score of 1.67% or higher	10	
2nd-degree relatives with BC	6	
Family history of ovarian cancer	4	
BRCA mutation carriers	2	
Others (high breast density, combination HT, age)	3	
Menopausal symptoms after using AIs		
Vasomotor symptoms (occasional night sweats)	1 (3.57%)	
Joint and skeletal pains (joint and muscle aches)	2 (7.14%)	
Sexual symptoms (decreased libido)	1 (3.57%)	
Psychological symptoms (insomnia)	2 (7.14%)	
Bone mineral density (n = 7)	Mean ± SD	*P*
Lumbar before AI	0.97 ± 0.12	0.735
Lumbar after AI	0.98 ± 0.14	
Femoral before AI	0.73 ± 0.07	0.866
Femoral after AI	0.73 ± 0.09	
Hip before AI	0.86 ± 0.07	0.028
Hip after AI	0.89 ± 0.09	

BC, breast cancer; HT, hormone therapy.

RESULTS

Clinical characteristics and menopausal symptoms

The characteristics of 28 postmenopausal women in the study group and 28 postmenopausal women in the control group are shown in Table 1. Study participants all received the AI letrozole (Femara, Novartis Pharmaceuticals, East Hanover, NJ) 2.5 mg orally three times weekly (Monday, Wednesday, and Friday) with the exception of two women who were given anastrozole (Arimidex, AstraZeneca Pharmaceuticals, LP) 1 mg/day because of headaches with letrozole. The women received HT together with AI for a median duration of 24 (range, 2-63) months. Women received estrogen and progestin HT if they had an intact uterus (~75%) or estrogen alone if they had had a hysterectomy (~25%). The estrogen was predominantly micronized estradiol (Estrace 1 mg), in the majority of participants. The others received transdermal estradiol. The progestin used was norethinedrone 0.35 mg/day or micronized progesterone 100 mg/day by the majority of women. The remaining women received medroxyprogesterone acetate 2.5 mg/day. In all cases, the HT was not changed during the course of the study. There was no statistically significant difference in regard to the number of women in the study and the control groups who used these regimens (Table 3). Twenty-five (89%) of the 28 women in the study were at increased risk of BC. During the study, six women had experienced one or more symptoms indicative of hypoestrogenism. Only one woman had a mild increase of hot flashes, two described an increase in joint and muscle aches, two reported insomnia without night sweats or hot flashes, and one reported decreased libido. Three women discontinued AIs after 2 months of use because of adverse effects including headaches, nausea, vomiting, and diarrhea. We could retrieve bone mineral density measurements done before and after the addition of an AI in seven women shown in Table 2. In these seven women there was no

TABLE 3. *Characteristics of women in the treatment and control groups in the mammographic breast density analysis*

	AI + HT (n = 18)		HT controls (n = 22)		
	Mean ± SD or no. (%)	SEM	Mean ± SD or no. (%)	SEM	*P*
Current age, y	63.06 ± 6.99	1.75	65.45 ± 8.28	1.77	0.354
Age at the 1st mammogram, y	59.72 ± 7.45	1.86	60.86 ± 8.75	1.86	0.796
BMI, kg/m^2	25.57 ± 5.44	1.57	24.40 ± 4.07	0.96	0.553
Time between the 1st and 2nd mammograms, y	2.44 ± 1.24	0.29	3.00 ± 1.41	0.30	0.222
HT total duration, y	14.13 ± 7.97	1.99	15.4 ± 7.4	1.61	0.770
Type of HT product					
Micronized estradiol 1 mg	88.9%		68.2 %		0.119
Norethinedrone 0.35 mg or micronized progesterone 100 mg	100.0%		81.3%		0.088
Breast cancer risk factors					
Gail risk model	1.82 ± 0.96	0.22	1.7 ± 0.66	0.14	1.00
Gail score higher than the estimated risk for the same age/race in the general US population	6 (33.3)		6 (27.3)		0.677
2nd-degree relatives with BC	5 (27.8)		3 (13.6)		0.266
Family history of ovarian cancer	3 (16.7)		1 (4.5)		0.204
BRCA mutation carriers	1 (5.6)		0 (0)		0.263

AI, aromatase inhibitor; BC, breast cancer; BMI, body mass index; HT, hormone therapy.

decrease in bone mineral density in the lumbar spine, the femoral neck, or the total hip measurement.

MBD

A total of 203 films were digitized and another 235 mammograms were obtained as digital images. The mammograms of 18 (of 28 postmenopausal women) women receiving AI and HT were eventually entered into the statistical analysis. The remaining 10 women were excluded from the MBD assessment for three different reasons. There were six women in whom one of the pretreatment or posttreatment mammograms was not available or one of them was analogue and the other was digital, precluding comparison because previous data showed that MBD is significantly lower in the case of digital compared with analogue acquisition.[37] One high-risk woman had a prophylactic bilateral mastectomy. Three other women used AIs for 2 months only and were excluded from the mammogram analysis. As a result, the minimal duration of AI use was 5 months in the study group.

Table 3 shows the characteristics of women entered in the mammogram analysis comparing the study group with the control group. There were no statistically significant differences between the study group and the control group regarding their age, body mass index, risk of BC, duration of HT, and the interval between the compared mammograms.

Image analysis of MBD

The software analysis of MBD showed a statistically significant reduction in the total IPI as well as in the percentage of dense IPI in the women who were taking an AI plus HT, whereas there were no significant changes in the IPI observed between either of the two films in the control group or between the two control films in the study group before starting an AI (Table 4).

Radiologist assessment of MBD

The radiologist was able to detect a significant change in 21 of the 56 pairs of mammograms evaluated (37.5%). Most of the time, the radiologist was able to confirm significant changes in the MBD between the two films in each woman when the mean change from the baseline IPI on image analysis was 35.6% with a median value of 27.9%. When the mean change in the total IPI on image analysis was less than 24.2% and the median was 11.3%, the radiologist was unable to visually confirm a significant change in MBD. Whenever the radiologist confirmed a significant change in MBD, there was a significant correlation ($P = 0.027$, $R = 0.482$) between

TABLE 4. *Integrated pixel intensity (IPI) of mammographic breast density in the treatment and the control groups*

	AI + HT (n = 18)			HT alone (n = 22)		
	Mean ± SD	SEM	*P*	Mean ± SD	SEM	*P*
Total IPI						
1st mammogram	$2.1 \times 10^{10} \pm 2.6 \times 10^{10}$	0.62×10^{10}	0.010	$1.5 \times 10^{10} \pm 2.4 \times 10^{10}$	0.52×10^{10}	0.910
2nd mammogram	$1.2 \times 10^{10} \pm 1.2 \times 10^{10}$	0.27×10^{10}		$1.4 \times 10^{10} \pm 2.2 \times 10^{10}$	0.46×10^{10}	
Δ Total IPI	$-0.85 \times 10^{10} \pm 1.5 \times 10^{10}$	0.36×10^{10}		$-0.14 \pm 0.80 \times 10^{10}$	0.17×10^{10}	0.013
% of total IPI change from baseline IPI	−17.48 ± 24.32	5.73		2.24 ± 16.06	3.42	0.007
% dense IPI						
1st mammogram	50.44 ± 23.33	5.49	0.008	37.66 ± 22.05	4.70	0.506
2nd mammogram	43.60 ± 22.65	5.34		36.25 ± 22.99	4.90	
Δ% dense IPI	−6.84 ± 9.00	2.12		−1.41 ± 8.17	1.74	0.044

AI, aromatase inhibitor; HT, hormone therapy.

the radiologist's trend of change and the trend shown by the image analysis software (ie, increase or decrease in MBD). The Breast Imaging Reporting and Data System scores of dense areas to the total area of the breast seemed to be less sensitive than the image analysis assessment because there was a nonsignificant change in Breast Imaging Reporting and Data System scores in both the study and control groups.

DISCUSSION

It is well established that breast density is one of the strongest risk factors for the occurrence of BC, only surpassed by age and BC susceptibility genetic mutations.[38,39] Women with the highest MBD have a four- to sixfold increase in BC risk compared with women who have the lowest MBD.[40] BC risk also increases in *BRCA* mutation carriers with an increase in MBD.[41] Mammographically dense breasts have been associated with an increase in proliferative lesions such as hyperplasia, atypia, and carcinoma in situ.[42] Reduction of MBD was associated with a reduction in BC risk.[43,44] Additionally, high breast density tends to decrease the sensitivity of mammograms for cancer detection,[45] and a reduction in breast density should make mammograms more sensitive for earlier detection.[46] Therefore, recognizing or determining factors that can modify breast density is an important approach in BC prevention.[47]

A number of studies have attempted various strategies to reduce MBD. Tamoxifen caused significant reduction in MBD in women with a high BC risk.[48,49] One study has shown a significant reduction in MBD with the use of gonadotropin-releasing hormone agonists in six *BRCA1* mutation carriers. This study included a low dose of estrogen, intermittent progesterone, and testosterone for 12 months to alleviate the marked hypoestrogenic effects of gonadotropin-releasing hormone agonists.[50] Likewise, tibolone, given for 1 year, demonstrated a significant decrease in MBD in postmenopausal women with dense breasts.[51] Another study demonstrated a nonsignificant reduction in the MBD of 30 premenopausal women who used isoflavones for 1 year.[52] Apart from pharmacological interventions, there has been one study that demonstrated that dietary changes such as a low-fat, high-carbohydrate could significantly modify breast density.[53]

Conversely, a large number of studies have investigated the effect of HT in different regimens, doses, and durations on MBD in menopausal women. Combined HT, especially with continuous progestin regimens, significantly increased MBD,[54-57] and the MBD changes decreased on HT discontinuation.[58] An HT-induced increase in MBD is known to reduce the sensitivity and specificity of mammograms.[45,59]

In one study, MBD measured by IQ software and a previously validated computer-assisted thresholding technique (Cumulus 108 software, University of Toronto) and by subjective visual analysis all had a positive correlation with the Gail risk for BC.[60] However, the planimetry and thresholding methods commonly used to measure changes in MBD calculate the percentage of the dense area to the total area of the breast, considering the breast a two-dimensional surface. This methodology applies an all-or-none rule (dense vs nondense) to the calculation so that changes in density above or below the reader-defined threshold, a subjective decision, are missed. Additionally, these methods do not account for the total size of the breast nor the total amount of fibroglandular tissue. Therefore, a small breast could have a larger percentage of dense areas, although it does not have much fibroglandular tissue in absolute terms.[61] In this regard, the advantage of our approach is the calculation of the total densities of all the breast tissue in the mammogram. IQ and ImageJ software both take into consideration the breast as a three-dimensional structure so that each pixel in the image represents a volume rather than an area unit of the breast. In other words, the same region of the breast could be recognized as being slightly more or less dense in a serial section of films of the same woman. In addition, having several background correction and subtraction tools that can adjust for dissimilarities in the dose of radiation and the processing of the films enabled more objective and consistent analysis of the MBD. Differences in positioning and compression of the breast can be also managed by adjusting the outline tools in the software. Nevertheless, our study is limited by being an observational study with a small number of participants. Prospective studies are needed to ensure that all the technical factors that might interfere with accurate measurements of MBD are controlled for and to replicate our findings before clinical recommendations can be made.

Recently, there has been an increasing interest in the concept of BC chemoprevention. The initial phase III clinical trials investigated selective ER modulators for prevention. The overall results of four major trials showed a 48% reduction in BC risk, which led to US Food and Drug Administration approval of tamoxifen for the primary prevention of BC in high-risk women in 1998.[62] However, the significant increase in venous thromboembolic events (relative risk = 1.9) and the incidence of endometrial cancer (relative risk = 2.4) were major disadvantages that have limited the use of this drug as a preventive measure and many women refuse to take it because of its toxicity.[63]

The proven benefits of AIs in the therapeutic setting put them on the top of the list of the current candidates for primary prevention of BC.[64] In fact, the potential role of AIs in prevention has been emphasized in the BC adjuvant clinical trials in which AIs caused significant reduction in the incidence of cancer in the opposite breast (43%, 46%, and 56% reduction in the ATAC, the MA-17, and the exemestane trials, respectively).[5] Furthermore, the evidence that the carcinogenic effect of estrogen is not solely receptor mediated and may also be related to estrogen's genotoxic metabolites suggests a preventive advantage of AIs over selective ER modulators. The presence of a low concentration of ERs in normal breast tissue substantiates this hypothesis.[65] Consequently, several clinical trials have started to investigate the effect of AIs on BC chemoprevention in healthy women.[66,67]

According to a recent analysis of the estrogen-alone (conjugated equine estrogens) arm of the Women's Health Initiative (WHI) study, the effect of estrogen therapy for 7.1 years included a significant reduction in the total fracture risk as well as fractures of the hip, vertebrae, and wrist bones. There was a modest albeit consistent increase in bone mineral density as well.[68] A positive correlation between serum estradiol and bone density as well as its ability to increase collagen deposition and bone mass in postmenopausal women with osteoporosis led to the recommendation of estrogen for the treatment of established osteoporosis.[69,70] Further, progesterone may augment osteoblastic activity to synergize with estrogen or to inhibit the negative effects of corticosteroids on the bone.[71]

Although various alternative drugs have been used for the relief of menopausal symptoms, no treatment so far has been shown to provide the same level of symptom relief as estrogen.[72] More recent analyses of the WHI subgroup data have demonstrated cardioprotection of HT in women between 50 and 59 years of age.[73] Many of these women are symptomatic related to hypoestrogenism. As a result, a considerable number of menopausal women are still using HT. Essentially, the estrogen therapy in postmenopausal women plays a protective role in many tissues. Partial suppression of local estrogen in the breast by using a potent AI and adding back systemic estrogen to allow a physiological circulatory level has been suggested as one of the novel approaches that could enhance the acceptability of AIs in a long-term prevention protocol.[62,74,75] This strategy could offset the increased BC risk reported in the WHI study in similar women taking HT regimens that contain progesterone.[76] For completeness, it should be noted that there was no statistically significant increased risk of BC in the combined estrogen and progesterone arm of the WHI for women with no previous use of HT.[73] In addition, although there was a decrease in BC risk in the estrogen-alone arm of the WHI study,[77] estrogen-alone treatment was also included in the current study because many of these women had an elevated BC risk due to other factors such as family history. In our study, the numbers of women who were receiving the combined or the estrogen-alone HT were comparable in both the study and control groups.

A previous small pilot study of letrozole given for 6 months to women taking HT also provided encouraging results regarding the breast proliferation marker Ki67, although there was not a significant reduction in MBD,[78] possibly because of the short duration of AI use. Our study is the first, to our knowledge, to report the use of an AI plus HT for extended durations of as long as 5 years and to show a significant reduction in MBD with AI plus HT in healthy women with no history of BC. This protocol was well tolerated with few hypoestrogenic side effects. Therefore, the use of AIs in women who are receiving HT could reduce their breast density without causing hypoestrogenic symptoms. Similarly, we speculate that a low-dose add-back HT could be added to AIs in a chemopreventive protocol to reduce any negative effect on bone mineral density in women at high risk of BC. At the moment, we are working on a randomized, placebo-controlled trial to explore the effects of combined AI and HT on breast estrogen responsive gene expression and on other markers of BC risk.

CONCLUSIONS

We propose that AIs are associated with a significant reduction in MBD in postmenopausal women using HT without producing significant hypoestrogenic symptoms.

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