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Correlation of endogenous hormonal levels, fibroglandular tissue volume and percent density measured using 3D MRI during one menstrual cycle

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Background: We measured breast density (BD) on MRI and correlated with endogenous hormonal levels.

Patients and methods: Twenty-four premenopausal women received four weekly breast MRI. A blood sample was collected on the same day of MRI. BD was measured using a computer-based algorithm. The generalized estimation equation method was applied to model mean fibroglandular tissue volume (FV) and mean percent density (PD) from predictor variables including estradiol, progesterone, and week during a cycle.

Results: In week 3, a borderline significant correlation between estradiol and PD ($r = 0.43$, $P = 0.04$), estradiol and FV ($r = 0.40$, $P = 0.05$) and between progesterone and FV ($r = 0.42$, $P = 0.04$) was noted. The FV and PD measured in weeks 4 and 1 were higher than in weeks 2 and 3, adjusted for variation in endogenous estradiol and progesterone, indicating

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that the hormone change could not account for the changes in density. No lag effect of endogenous hormone on the change of FV or PD was noted (all *P*-values > 0.05).

Conclusions: Our results showed that BD is not strongly associated with the endogenous hormone. Their association with breast cancer risk was likely coming from different mechanisms, and they should be considered as independent risk factors.

Key words: breast density, endogenous hormone, fibroglandular tissue volume, lag effect, MRI, percent density

introduction

Endogenous sex hormone concentrations in premenopausal women fluctuate over the course of the menstrual cycle (MC). Differences in the histological characteristics of breast tissues have been reported between follicular and luteal phases [1–3]. The difference in mammographic density (MD) between the follicular and luteal phases may impact on cancer detection [4]. Only a few studies have examined estrogen and progesterone levels in premenopausal women in relation to MD, but the findings were inconsistent [5–10]. Nonetheless, all reported studies in the literature suggested that the relationship was not strong.

MD is limited by the 2D nature with the problem of overlapping tissue. Compared with MD, the 3D MRI-based density analysis [11–15] provides a more precise method for assessing the volumetric change of breast tissue. MRI has been used to evaluate the changes of breast tissue and water content within the MC [16–23]. In a previous study, we investigated the fluctuation of breast density (BD) using four weekly MRI studies done in a cycle, and found a higher density variation in premenopausal compared with postmenopausal women [24]. In this study, we further investigated the relationship between measured fibroglandular tissue volume (FV) and percent density (PD) in premenopausal women with the fluctuation of endogenous hormonal level analyzed from the blood samples taken on the same day of the MRI.

materials and methods

subjects

This study was approved by the Institutional Review Board and was HIPAA compliant. Twenty-four healthy premenopausal Asian women (age range 23–48 years, mean 29.4 year olds) were recruited for this correlative study. These normal volunteers were recruited from the colleagues and friends of one of the coauthors (CS) in her institution. All subjects provided written informed consent. The BMI for these 24 subjects ranged from 17.2 to 25.8 (mean ± STD = 20.6 ± 2.1). At the time of participation in this study, all subjects were healthy, not pregnant, and without symptoms of breast diseases. All subjects had regular MCs of ~28–30 days. Their menarche was from 10 to 14 year olds (mean 12.6 year olds). Eighteen of the 24 women were nulliparous. Two women were primiparous and four were multiparous. At the time of the study, none was taking contraceptive pills nor receiving hormonal therapy. None was smoker or alcoholic. One subject had a history of hormonal therapy for a lumpy sensation, but had ceased the treatment 6 months before this study.

MR imaging acquisition and endogenous hormone quantification

The MR images for the quantification of BD were acquired on a 1.5 T MR scanner (Siemens, Somatom, Erlangen, Germany). All women received weekly MRI for 4 consecutive weeks, with a total of 4 scans. Based on their self-reported starting of the menstruation, the first MRI study that was done after menstruation was assigned as week 1. The scans done in the following 3 weeks are then noted in sequence as weeks 2, 3, and 4. The sequential study was done every 7 days using an identical protocol. Since this study was carried out to quantify the breast tissue and PD, but not to diagnose breast lesions, the MR studies were carried out without the injection of a contrast agent. Three-dimensional gradient echo nonfat-suppressed T_1-weighted images (FOV = 350 mm, slice thickness = 2 mm, TR/TE = 11/4.7 ms, flip angle = 20 degrees, and matrix size 256 × 256) were used for measurements of FV and PD.

Before the MR scanning, a blood sample was collected on the same day. Serum estradiol (E2) and progesterone levels were measured using the competitive binding immunoenzymatic assay kits (Beckman Coulter, Inc., Fullerton, CA).

breast and fibroglandular tissue segmentation

A semiautomatic computer-based algorithm [14, 25] was used for the segmentation of the breast region and the fibroglandular tissue by an experienced operator (CS). After completing the segmentation from all imaging slices, the FV is calculated. PD was calculated by normalizing FV to the breast volume (BV) ×100%.

statistical analysis

To compare FV and PD among weeks during a cycle, the mean difference and standard deviation of differences was calculated for the six pairs of weeks, i.e. week 1 vs. weeks 2, 3, and 4; week 2 vs. weeks 3 and 4; and week 3 vs. week 4. Paired *t*-tests were applied to test the null hypothesis that the mean difference in values was zero. To adjust for multiple comparisons made using data obtained from same women and assure an overall significance level of 0.05, the Bonferroni–Holm procedure was applied [26].

The correlations between FV, PD, estradiol, and progesterone levels obtained at each week of the study were assessed using Pearson's correlation coefficients. Also, we applied generalized estimating equations (GEE) to estimate and compare the expected (mean) FV and mean PD obtained at the 4 weeks, adjusting for variation in E2 and progesterone. In contrast to ordinary linear regression for which values measured in individual subjects are assumed to be independent, the GEE method takes in account the correlation between FV measured within individual subjects at different weeks during the cycle. The method was also used to model mean PD from E2 level (or progesterone), measurement week, and the interaction between E2 (or progesterone) and measurement week. Alternatively, to examine a potential consistent weekly lag in the effect of E2 or progesterone on FV or PD, values for E2 (or progesterone) during the previous week were used to predict values of FV and PD for the current week, and the GEE model was applied assuming that the within-subject correlation between repeated measurements of FV (or PD) was the same for each subject.

In addition, we examined within-subject effects for E2, progesterone, FV, and PD. E2 levels measured during weeks 2 and 3 were expected to be higher than those measured during weeks 1 and 4. To examine this hypothesis, the proportion of women for whom the mean E2 for weeks 2 and 3 was higher

than the mean E2 for weeks 1 and 4 was calculated. Similarly, progesterone levels measured during weeks 3 and 4 were expected to be higher than those measured during weeks 1 and 2; thus, the proportion of women for which the mean progesterone level for weeks 3 and 4 was higher than the mean for weeks 1 and 2 was computed. Finally, measurements of mean FV and mean PD during weeks 1 and 4 were expected to be higher than those measured during weeks 2 and 3; thus, the proportions of women for whom mean FV and mean PD for weeks 1 and 4 were higher than the means for weeks 2 and 3 were calculated. The 95% exact binomial confidence interval of each proportion was calculated.

results

measurement of estradiol and progesterone levels

The range and group averages of estradiol and progesterone measured on the same dates of the four MRI studies are shown in Table 1. Overall the results of the hormonal measurements were comparable to the physiology of a healthy woman with peak of estradiol at around the timing of ovulation (day 14) and peak of progesterone at the last week of the MC. There was a wide intersubject variation of estradiol and progesterone levels measured at the same week of the MC.

comparison of FV and PD among weeks during a cycle

Mean values for FV and the PD measured at week 4 were significantly higher than those measured at weeks 2 and 3 (nominal $P = 0.01$ and $P = 0.002$, respectively, for FV; and nominal $P = 0.01$ and $P = 0.005$, respectively, for PD). The mean FV (but not PD) measured at week 1 was also significantly higher than those for weeks 2 and 3, after adjustment for multiple comparisons (nominal $P = 0.04$ and $P = 0.03$, respectively) (Table 2). There was a strong correlation of measured FV and PD in the 4 weeks ($r = 0.62$, $P = 0.001$; $r = 0.60$, $P = 0.002$; $r = 0.61$, $P = 0.002$; and $r = 0.62$, $P = 0.001$, respectively).

correlation of FV and PD with estradiol and progesterone

There was a significant correlation between E2 and progesterone in week 3 ($r = 0.43$, $P = 0.03$) and week 4 ($r = 0.72$, $P < 0.0001$). In week 3, there was also a significant correlation between E2 and PD ($r = 0.43$, $P = 0.04$), E2 and FV ($r = 0.40$, $P = 0.05$) and between progesterone and FV ($r = 0.42$, $P = 0.04$).

Table 1. Range and group average of estradiol and progesterone measured on the same dates of the four MRI studies

	Week 1	Week 2	Week 3	Week 4
Estradiol (pg/ml, $N = 24$)				
Mean ± STD	64.5 ± 55.5	89.5 ± 71.0	123.6 ± 88.5	99.0 ± 58.1
Range	10–251	21–309	26–385	10–200
Progesterone (ng/ml, $N = 24$)				
Mean ± STD	1.38 ± 3.29	1.89 ± 2.89	6.35 ± 6.08	7.48 ± 6.93
Range	0.01–16.08	0.16–10.11	0.22–19.61	0.13–24.98

Table 2. Range and group average of FV and PD measured from the four MRI studies

	Week 1	Week 2	Week 3	Week 4
FV (ml, $N = 24$)				
Mean ± STD	50.6 ± 24.7	48.5 ± 23.8	48.1 ± 24.7	52.6 ± 28.8
Range	18.7–122.3	17.8–177.6	19.6–120.3	19.9–143.2
PD (%, $N = 24$)				
Mean ± STD	20.7 ± 7.8	20.1 ± 7.4	20.2 ± 7.8	21.4 ± 8.4
Range	8.6–33.8	9.8–31.6	8.0–32.8	8.2–36.6

comparison of FV and PD among weeks after adjusting for estradiol and progesterone

After adjusting for variation in estradiol levels, mean FV was significantly lower during week 2 ($P = 0.005$) and week 3 ($P < 0.0001$) compared with week 4; and mean PD was also significantly lower during week 2 ($P = 0.004$) and week 3 ($P = 0.0007$) compared with week 4. After adjusting for variation in progesterone levels, mean FV and mean PD was significantly lower during week 3 compared with week 4 ($P = 0.001$ and $P = 0.005$, respectively).

lag effect of estradiol and progesterone

Using GEE models, it was found that neither changes in estradiol nor changes in progesterone affected mean PD or mean FV in the subsequent week (all P-values > 0.05). For this model, predicted mean FV in week 4, was significantly higher than those during weeks 2 and 3 (all P-values < 0.01), adjusted for variation in estradiol levels of the previous week. Figure 1 shows an example of four breast MRI studies carried out in a subject, and Figure 2 shows the measured hormonal level from the blood sample of this subject. It is seen that the estradiol peaks at week 2, progesterone peaks at week 3, and the FV is the highest at week 4. Although a clear lag effect was observed in this case example, this trend was not consistently seen in all subjects.

within-subject effects

There were 15 of 24 women (62.5%) for whom the mean E2 during weeks 2 and 3 was higher than the mean E2 for weeks 1 and 4 (95% CI 0.41–0.81). There were 20 of 24 women (83.3%; 95% CI 0.63–0.95), for whom the mean progesterone for weeks 3 and 4 was higher than the mean progesterone for weeks 1 and 2. Eighteen of 24 women (75.0%; 95% CI 0.53–0.90) had higher mean PD during weeks 1 and 4 compared with that of weeks 2 and 3. In comparison, 19 of 24 (79.2%; 95% CI 0.58–0.93) women had mean FV exhibiting this pattern.

discussion

BD has been proven as an independent risk factor for development of breast cancer, as well as a surrogate marker for monitoring the effect of hormonal interventions [27–31]. Strong evidence also showed the role of endogenous estrogens in the development of breast cancer [32–35]. However, the biological
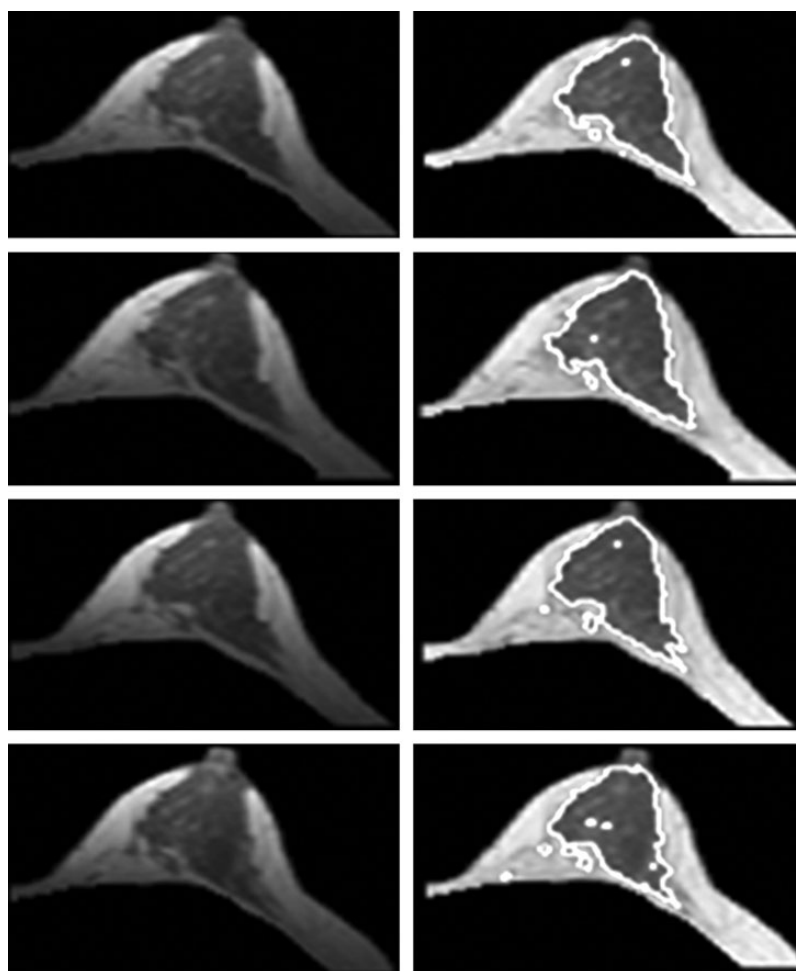
Figure 1. Illustrations of breast fibroglandular tissue from four weekly MRI studies of a healthy woman.

mechanisms by which BD and hormone are associated with breast cancer risk have not yet been clarified.

MD may change within one MC [5–10]. These studies were based on case–control design so different women in each group were compared, or used a longitudinal design based on results collected over a long period of time which might introduce additional confounding factors. In this study, we measured the density using 3D MRI and correlated the results with estradiol and progesterone measured on the same day of MRI. Such a study design is only feasible by using an imaging modality that does not involve radiation, such as MRI. Several MRI studies have reported changes in BV and FV during one MC [16, 18, 19, 24, 36]. The density analyses used in these studies did not take advantage of a well-established method for quantitative assessment. We have developed a robust 3D MRI algorithm that can precisely measure the dense tissue volume from the whole breast. MRI is known to be more sensitive than mammography to detect small changes [37].

The estrogens are powerful mitotic agents, as well as the stimulators of cell proliferation and growth [38]. Women with high total and free estradiol during the follicular phase are associated with increased risks of breast cancer [39]. The inclusion of the plasma estrogen level into the risk prediction models has been recommended [40]. As both sex hormone and

BD are associated with cancer risk, it will be interesting to investigate their fluctuations during one MC to understand the impact of measurement variation on the predicted cancer risk. Furthermore, it will be interesting to investigate how the measured changes in density and hormone level during a cycle are associated with each other.

Our unique dataset allows for investigation of the fluctuations in BD and endogenous estradiol and progesterone within one MC, as well as the relationships between them. We found that the FV measured at weeks 4 and 1 was significantly higher than that measured at weeks 2 and 3, adjusted for variation in estradiol levels. The PD measured at week 4 was also significantly higher than that measured at weeks 2 and 3, adjusted for estradiol variation. Therefore, the higher density in weeks 4 and 1 could not be explained by the estradiol level. Several MD studies have shown that there is a small, but not statistically significant, increase in the luteal phase compared with the follicular phase [8, 41–43]. Since that most proliferative activity takes place during the luteal phase of the MC [44], the higher cellular proliferation may account for the increased BD observed in weeks 4 and 1.

We found a borderline significant correlation between E2 and PD ($r = 0.43$, $P = 0.04$), E2 and FV ($r = 0.40$, $P = 0.05$), and progesterone and FV ($r = 0.42$, $P = 0.04$) in week 3. No
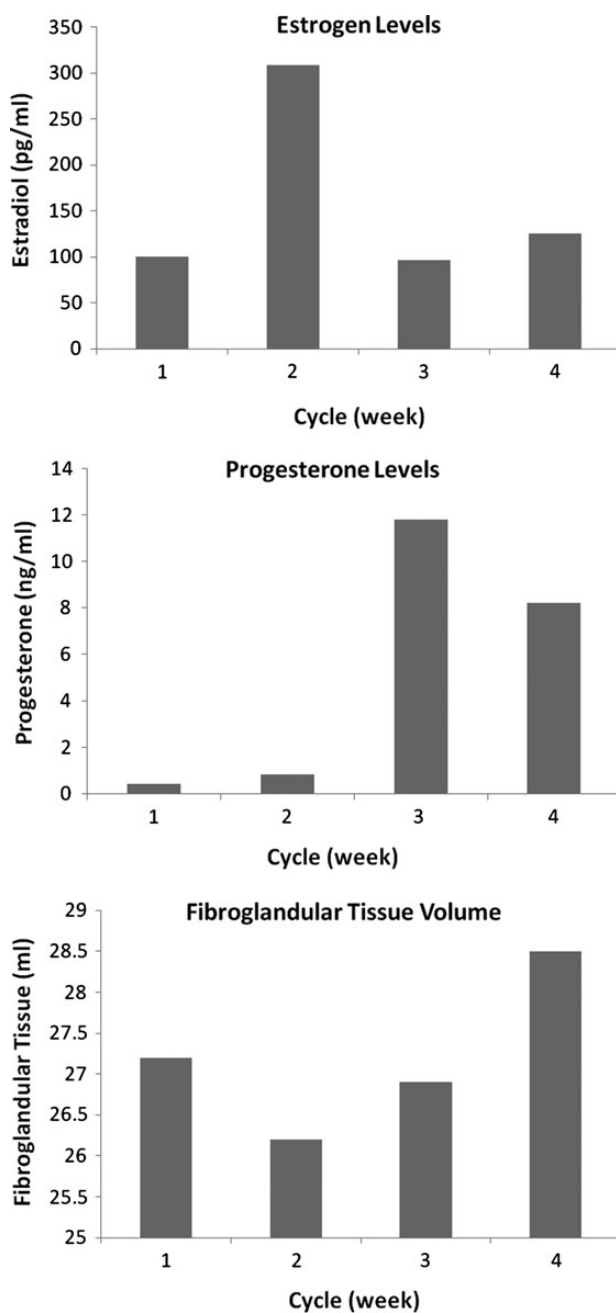
Figure 2. Estrogen, progesterone and FV measured in the woman shown in Figure 1. The E2 were 100, 309, 96, and 125 pg/ml, and progesterone were 0.44, 0.85, 11.80, and 8.23 ng/ml, from weeks 1 to 4, respectively. The corresponding FV were 27.2, 26.2, 26.9, and 28.5 ml, respectively. The corresponding PD were 12.4, 11.2, 12.2, and 12.5%, respectively. Obviously, a clear lag effect was noted in this case example. This trend was, however, not consistently seen in all subjects in our study.

significant correlation was found in other weeks. According to the hormonal results shown in Table 2, week 3 is at the transition of follicular phase to luteal phase when both the levels of estradiol and progesterone are high. It is thus a plausible explanation that the breast responds to both endogenous estrogen and progesterone, thus the combined high level at this phase has a stronger effect on BD at week 3. However, as the P-

values are close to 0.05, the association is not strong, only at borderline significance level. The results from previous studies in the literature also did not show a strong association of the effect of circulating hormones on BD in premenopausal women [5–10]. A study showed that PD and the dense tissue area during the luteal phase had borderline significant correlation with progesterone ($P = 0.06$ and $P = 0.07$, respectively) [5]. Our results show that the correlation is dependent on the measurement time during one MC (only at week 3), and this might be a possible reason to explain the diverse findings in these studies.

We also investigated whether there is a lag effect of the hormone level on the subsequent BD. The breast densitometry community has been curious about this potential lag effect but so far no clues were found [45–47]. It was noted that neither changes in E2 nor changes in progesterone affected mean PD or mean FV in the subsequent week (all P-values > 0.05). Also, we did not find differential impact between estradiol and progesterone on the BD.

In this study, we also examined within-subject effects for E2, progesterone, FV, and PD. We did not compare the parameters week by week. Instead, we combined 2 weeks and compared each other. This is because that weekly MRI and blood sample may have wide variation. Levels of hormones and amount of fibroglandular tissue change within each week so measurements taken in day 1 of the week may not be comparable with those taken in day 7 of the same week.

A study like our present one, it is very difficult to schedule the MRI based on a particular day during one MC, especially when four weekly scans are needed. In order to design a feasible study that can be precisely executed, we invited normal volunteers who have regular MC to received weekly MRI in 4 consecutive 'weekends' when the scanner is available and when the subject can easily make time to come to this imaging study. Therefore, week 1 MRI may be done from day 1 to 7 of the MC. Although there is a variation with respect to the timing of MRI in a cycle, the study protocol was precisely executed and that data were collected in a very consistent manner, e.g. the blood draw and the MRI were done on the same day, and it was always 7 days apart between sequential measurements. Overall, the estradiol and progesterone measured from the blood sample of our subjects were consistent with the expected results: showing the rising of estradiol in week 2, and the rising of progesterone in week 3. We acknowledged the study could have been much improved and more accurate if the information on the length of the MC for each woman has been collected and the analyses have been carried out based on the specific day of the cycle when the blood sample was collected and the MRI scan was carried out, taking into account differences in the length of the cycle.

There are limitations in this study. First, the subject number is small, which may not be sufficient to observe strong associations particularly given the high variations in the measured hormone level. Second, we did not measure the sex hormone binding globulin so could not calculate the concentration of free hormone. Third, the subjects in this study were all slim Asian women with relatively small breast size and dense breast tissue and a narrow BMI range; thus, the results may not be generalizable to other populations. Fourth, breast

tissue composition is likely to reflect cumulative lifetime exposure to endogenous and other hormones rather than the values measured at a particular week in the cycle as our study showed. Despite these limitations, we believe that the reported data were novel (that has never been done before) and that the results provide valuable knowledge and can contribute in this research field.

In summary, we measured BD including FV and PD using MRI and correlated the measured density with endogenous estradiol and progesterone at different phases within one MC. There was a borderline significant correlation of FV and PD with endogenous estradiol, and FV with progesterone in week 3. The FV and PD measured in weeks 4 and 1 were higher than in weeks 2 and 3, adjusted for variation in endogenous estradiol and progesterone. Therefore, the change of BD cannot be accounted for by the fluctuation of hormone levels. The lag effect of the hormone level change on the change of BD in the following week was not significant. Overall, our results were consistent with reports in the literature showing that BD is not strongly associated with the endogenous hormone. Therefore, the association of BD and hormone level with a high cancer risk was likely coming from different mechanisms, and they should be considered as independent risk factors.

disclosure

The authors have declared no conflicts of interest.

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A randomized phase III trial on maintenance treatment with bevacizumab alone or in combination with erlotinib after chemotherapy and bevacizumab in metastatic colorectal cancer: the Nordic ACT Trial

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Background: The main objective was to study the effect on progression-free survival (PFS) of adding erlotinib to bevacizumab as maintenance treatment following chemotherapy and bevacizumab as first-line treatment of metastatic colorectal cancer (mCRC).

Patients and methods: Patients with untreated mCRC received doublet chemotherapy + bevacizumab during 18 weeks and those without tumor progression were eligible for randomization to bevacizumab + erlotinib (arm A) or bevacizumab alone (arm B), until progression or unacceptable toxic effect.

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