Endogenous Sex Hormones, Prolactin, and Mammographic Features of Breast Tissue in Premenopausal Women [1,2]

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ABSTRACT—The relationship between levels of certain endogenous hormones and the mammographic features of breast tissue was evaluated in 110 premenopausal women who were referred to hospital-based radiology units for a routine xeromammogram. This analysis was based on specimens collected during the luteal phase of the menstrual cycle. Plasma 17β-estradiol, progesterone, and prolactin were measured, as well as urine estrone, estradiol, estriol, and pregnanediol. The main xeromammographic features of the breast assessed were Wolfe's parenchymal pattern and the percentages of the breast showing nodular densities and homogenous density. In these data, endogenous hormones were not strongly associated with mammographic features of breast tissue. However, women with the N1 and P1 parenchymal patterns had somewhat higher levels of estrogens and prolactin and somewhat lower levels of progesterone than women with the P2 and DY patterns.—JNCI 1986; 77:617-620.

Certain morphologic features of the breast seen on mammograms are associated with an increased risk of breast cancer: Wolfe's P2 and DY parenchymal patterns (*1*) and extensive nodular or homogenous densities (*2*). These associations are particularly strong in younger women (*2, 3*). Premenopausal women with high plasma estradiol or prolactin or low progesterone appear to have an elevated risk of breast cancer [Meyer F, Morrison AS, MacMahon B, et al: Manuscript submitted for publication; (*4, 5*)]. Since endogenous hormones, especially estrogens and prolactin, play an important role in the development and maturation of breast tissue (*6-8*), they might also influence the appearance of the breast parenchyma on mammograms. The present study evaluated the relationship between endogenous estrogens, progesterone, and prolactin and the mammographic features of breast tissue in premenopausal women.

METHODS

This study is based on the control series of a case-control study of endogenous hormones and breast cancer risk (Meyer F, Morrison AS, MacMahon B, et al: Manuscript submitted for publication). Subjects in the present analysis were white women who had a first routine xeromammogram at one of three centers in Boston: Brigham and Women's Hospital, Faulkner Hospital, or Massachusetts General Hospital. Women examined because of breast signs or symptoms and those with a history of malignant or benign breast disease were not included. For each woman, urine and blood specimens were collected. At the time of specimen collection, subjects reported having regular menses had not used oral contraceptives for at least 2 years and never received other exogenous estrogens. Women who had been pregnant were eligible if at least 8 months had passed since the delivery. Those who were regularly taking any medication other than vitamins were excluded unless they could stop for 3 days prior to the specimen collection.

Each woman collected two overnight (at least 8 hr) urine specimens: one in the follicular phase of the menstrual cycle (day 6) and another in the luteal phase of the same cycle (days 20-22). Between 8 and 10 a.m. on the morning after the collection of the luteal urine specimen, a 20-ml blood sample was obtained. Blood and urine specimens were kept cool until reaching the laboratory, where plasma and urine aliquots were prepared, frozen, and stored at −20°C. The date of the subsequent menses was ascertained.

From January 1982 through May 1983, 127 women, aged 32-52, completed the collection procedure. In this report, we present the analysis of luteal phase data collected on the 110 women whose cycle length was between 22 and 34 days and whose specimen was obtained 3-13 days before the onset of the subsequent menses. Of these women, 103 provided a blood sample.

Hormone assays.—All biological specimens were shipped frozen to the University of Melbourne. 17β-Estradiol, progesterone, and prolactin in plasma were measured by radioimmunoassay. Prolactin levels are expressed as milliunits per liter in terms of the MRC 81/541 standard. Urinary E_1, E_2, and E_3 were measured by a modification of the spectrophotofluorimetry method described by Brown (*9*). Pregnanediol assay was done by gas-liquid chromatography by means of the method of Cox (*10*). Urinary estrogen and pregnanediol levels are

ABBREVIATIONS USED: E_1 = estrone; E_2 = estradiol; E_3 = estriol.

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expressed as their weight per gram of creatinine. Creatinine was measured by the alkaline picric acid method.

Mammogram reading.—All xeromammograms were reviewed by one of us (J. B.) without reference to any other information. The mammograms (lateral and craniocaudal views) of one breast, chosen at random, were evaluated. The main mammographic characteristics of the breast tissue assessed included the parenchymal pattern as defined by Wolfe (*1*), the percentage of the volume of the breast showing nodular densities, and the percentage showing homogenous density (*2*). Breast size, the shortest distance between the base of the nipple and the chest wall, was also recorded.

Statistical analysis.—Analysis was done after logarithmic transformation of hormone values. The relationship between endogenous hormones and the mammographic characteristics of breast tissue was evaluated by comparing the geometric means of hormone levels for women with different mammographic features. Statistical significance of differences between mean hormone values across categories was assessed by analysis of variance (*11*). Three women were taking oral contraceptives at the time of mammography, and three others had a pregnancy after the mammography. Because their exclusion did not materially change the results, these 6 subjects were kept in the study population. In these data and in previous reports, age, parity, and weight were associated with mammographic characteristics of breast tissue and with endogenous hormone levels (*12–19*). The influence of these potential confounding factors on the relationship between endogenous hormones and mammographic features was evaluated by stratified analysis and by analysis of covariance. Adjustment for age, parity, and weight did not change the direction and only slightly reduced the magnitude of observed differences. Therefore, only the crude analysis is presented.

RESULTS

Selected personal characteristics of women according to breast parenchymal pattern are presented in table 1. Age and height were similar in the 3 groups as was the length of the menstrual cycle in which specimens were collected. There were more nulliparous women in the DY group. As expected, body weight was higher for women with the N1 and P1 pattern than for those with the P2 or DY patterns.

TABLE 1.—*Mean and SD or percentage of selected personal characteristics according to breast parenchymal pattern*

Specification	Breast parenchymal pattern					
	N1 and P1 (n=16)		P2 (n=58)		DY (n=36)	
	Mean	SD	Mean	SD	Mean	SD
Age at interview, yr	41.5	5.0	42.7	4.6	40.4	4.5
Body weight, kg	69.7	10.5	63.9	11.8	58.1	7.5
Body height, cm	162	5	165	6	164	8
Cycle length, days	27.6	2.3	27.0	3.1	26.3	2.1
No. of pregnancies	3.4	1.9	3.2	2.0	2.7	2.2
Percent nulliparous	0		5		22	

TABLE 2.—*Distribution of women according to breast parenchymal pattern and to the presence and extent of ND and HD densities*[a]

Parenchymal pattern	Mammographic densities present				
	ND only				
	Percent of breast with ND		ND + HD	HD only	Total
	<60	≥60			
N1 and P1	16	0	0	0	16
P2	16	35	7	0	58
DY	2	8	16	10	36
Total	34	43	23	10	110

[a] ND = nodular; HD = homogenous.

The distribution of subjects according to Wolfe's parenchymal pattern and to the presence and extent of nodular and homogenous densities is presented in table 2. The parenchymal pattern classification is based on characteristics of nodular densities and homogenous density. Therefore, there is a close link between the parenchymal pattern and the presence and extent of the two types of density.

The relation of endogenous hormones to the parenchymal pattern is presented in table 3. Estrogen levels, measured in plasma and urine, are higher in women with the N1 and P1 pattern than in those with the P2 or DY patterns. The differences in estrogen levels between parenchymal patterns were smaller for plasma estradiol than for urinary estrogens. Women with the N1 and P1 pattern had lower concentrations of plasma progesterone and urinary pregnanediol than those with P2 or DY patterns. Plasma prolactin levels were higher among women with N1 and P1 than among those with P2 or DY patterns.

Mean hormone levels according to the presence and extent of nodular and homogenous densities are shown in table 4. Variations in hormone concentrations according to mammographic densities were small. Women whose mammogram showed only homogenous density had higher plasma E_2 but lower urinary estrogens than those with other mammographic features. Among subjects who had nodular densities only, mean plasma E_2 increased but mean urinary E_1 and E_2 decreased as the extent of nodular densities increased. Progesterone and pregnanediol were lower among women with nodular densities in less than 60% of the breast volume, but their mean levels did not vary consistently across the other mammographic categories. Mean plasma prolactin was higher in women with nodular densities only and lower in those with homogenous density only. None of the differences between means presented in tables 3 and 4 was statistically significant ($P<.05$).

Hormone concentrations varied considerably over the time interval 3–13 days from specimen collection to the onset of the next menses. The relationships between hormone levels and mammographic features were there-

TABLE 3.—*Geometric mean of endogenous hormones in plasma and urine according to breast parenchymal pattern*

Endogenous hormones	Parenchymal pattern[a]		
	N1 and P1 Mean (95% CL)	P2 Mean (95% CL)	DY Mean (95% CL)
Plasma			
E_2, pg/ml	185 (150-227)	184 (165-204)	181 (163-202)
Progesterone, ng/ml	7.7 (4.5-13.3)	10.1 (8.8-11.6)	11.7 (9.8-14.1)
Prolactin, mU/liter	235 (174-317)	180 (166-198)	174 (146-208)
No. of women	15	55	33
Urine			
E_1, μg/g creatinine	14.3 (11.2-18.3)	10.8 (9.7-12.1)	10.2 (8.6-12.1)
E_2, μg/g creatinine	6.7 (5.3-8.6)	5.2 (4.6-5.8)	4.8 (4.1-5.7)
E_3, μg/g creatinine	21.5 (15.2-30.5)	18.7 (15.9-22.1)	15.6 (12.8-18.8)
Pregnanediol, mg/g creatinine	1.8 (1.3-2.5)	2.3 (2.0-2.6)	2.4 (1.9-3.1)
No. of women	16	58	36

[a] CL = confidence limits.

fore assessed among the 85 women whose specimens were obtained at the time of the luteal peak, 5-10 days before the next menses. Results were similar to those presented. Plasma progesterone was significantly ($P<.05$) lower among women with the N1 and P1 parenchymal patterns and among those with nodular densities in less than 60% of the breast volume.

Wolfe's parenchymal patterns and the extent of nodular densities and homogenous density are strongly associated with breast size (*14*). After adjustment for breast size, the differences in hormone levels between parenchymal patterns were reduced. The largest differences in hormone levels were observed between women with N1 and P1 and those with DY pattern. There was a more than 50% reduction in the difference between these 2 groups for urinary E_1, E_2, prolactin, and progesterone. Differences in other hormones were not changed after controlling for breast size.

TABLE 4.—*Geometric mean of endogenous hormones in plasma and urine according to the presence and extent of ND and HD densities on xeromammograms*[a]

Endogenous hormones	Mammographic densities present[b]			
	ND only		ND + HD	HD only
	Percent of breast with ND			
	<60 Mean (95% CL)	≥60 Mean (95% CL)	Mean (95% CL)	Mean (95% CL)
Plasma				
E_2, pg/ml	178 (152-208)	184 (168-203)	181 (153-215)	198 (157-251)
Progesterone, ng/ml	8.2 (6.1-10.9)	11.4 (9.6-13.4)	11.7 (9.5-14.4)	9.9 (6.9-14.2)
Prolactin, mU/liter	212 (180-252)	178 (159-199)	172 (137-217)	164 (125-214)
No. of women	32	41	21	9
Urine				
E_1, μg/g creatinine	11.7 (9.7-14.1)	11.3 (10.2-12.7)	10.6 (8.5-13.1)	8.9 (6.2-12.7)
E_2, μg/g creatinine	5.7 (4.6-6.9)	5.2 (4.7-5.8)	5.1 (4.2-6.3)	4.4 (3.2-6.7)
E_3, μg/g creatinine	18.4 (14.5-23.2)	20.2 (16.9-23.9)	17.1 (13.9-21.1)	11.3 (7.0-18.1)
Pregnanediol, mg/g creatinine	2.1 (1.7-2.5)	2.5 (2.1-3.0)	2.2 (1.6-3.1)	2.2 (1.4-3.6)
No. of women	34	43	23	10

[a] ND = nodular; HD = homogenous.
[b] CL = confidence limits.

DISCUSSION

In the present study, no clear association was observed between endogenous hormones and mammographic characteristics of breast tissue that are related to breast cancer risk. Moreover, women with the low-risk parenchymal patterns N1 and P1 had higher levels of estrogens and prolactin and lower levels of progesterone than women with the high-risk P2 and DY patterns. The reverse would have been expected if endogenous hormones were causal determinants of the high-risk mammographic features.

The mammary gland is a complex endocrine target organ. Growth of the ductal system depends on the action of estrogens, prolactin, and adrenal corticosteroids. Lobulo-alveolar proliferation requires both estrogens and progesterone in the presence of prolactin. The epithelium and the connective tissue of the breast are responsive to fluctuations in endogenous hormones (*8*).

This study may have failed to uncover a true association between the hormones investigated and mammographic features. Specimen collection for hormone assay was done on the average 2 years after the mammography. Therefore, the measurements might not clearly reflect biological relationships between the hormones and breast morphology. However, characteristics of the menstrual cycle, and thus, hormone levels appear to be fairly constant throughout a woman's reproductive life apart from the few years after menarche and those immediately preceding menopause (*20, 21*). Although mammographic characteristics of breast tissue are known to evolve with age (*2, 12, 13, 22*), changes over a 2-year period should be relatively small even in premenopausal women.

Perhaps small variations in endogenous sex hormones and prolactin have relatively little influence on mammographic features. The parenchymal pattern and the extent of nodular densities appear to correspond to marked fibrosis and atypia in the terminal ductal lobular unit [(*23*); Brisson J, Morrison AS, Burstein N, et al: Manuscript submitted for publication]. Such marked histologic changes may be less responsive to variations in hormone levels observed in this study. Moreover, mammograms may not be sensitive and specific enough to detect small fluctuations in characteristics of breast tissue that accompany physiological variations in endogenous hormones.

Larger variations in endogenous hormones seem to have an influence on mammographic features. The radiologic appearance of breast tissue changed in women with benign breast disorders after 6-12 months of use of Danazol, an impeded androgen that reduces ovarian hormone production through hypothalamic pituitary suppression (*24*). Events that dramatically alter endogenous sex hormones and prolactin levels, such as pregnancy and menopause, are known to be associated with modifications of mammographic features (*13, 14, 23*). Moreover, these modifications may be long lasting. The effect of pregnancy on mammographic features can be observed several decades after the delivery.

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