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Estrogen Profiles of Premenopausal Women with Breast Cancer[1]

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ABSTRACT

Population surveys have demonstrated an inverse relationship between breast cancer incidence rates and the urine "estriol ratio," the concentration of estriol relative to the sum of the concentrations of estrone and estradiol. In this study, the urine estriol ratio was evaluated in premenopausal breast cancer patients and control women from Boston and San Francisco. Although at least 2 years had passed since last use of oral contraceptives, women with a history of oral contraceptive use for 19 months or longer excreted estrogen in low concentrations compared to nonusers and so were excluded. Among the remaining 73 cases and 55 controls, the cases had lower estriol ratios and higher estrone and estradiol levels than did controls. However, these differences, which averaged about 10%, were not statistically significant. Thus the hypothesis that a low estriol ratio is a cause of breast cancer is given only minimal support. Among women in their 40's, the excretion of estrogens is subject to many influences and is difficult to study. The many determinants of estrogen excretion, including age and oral contraceptive use, should be accommodated in the design of future studies of the estriol ratio.

INTRODUCTION

It has been suggested that a woman's risk of breast cancer is inversely related to her urine estriol ratio (5, 12). This ratio is the amount of estriol excreted, relative to the sum of the amount of estrone and estradiol. This suggestion has been supported by population surveys showing that the estriol ratio and breast cancer incidence rates are correlated inversely (6, 13, 14).

Further support has come from the observation that young primiparas, who have low breast cancer rates, have a high estriol ratio (4). In contrast, case-control studies have not consistently supported the association of a high estriol ratio with low breast cancer risk. However, these studies are difficult to interpret inasmuch as they are small or relate to women who are postmenopausal or have active breast cancer. We therefore designed a case-control study to overcome these limitations.

MATERIALS AND METHODS

Cases were premenopausal women identified via tumor registries of hospitals in the Greater Boston area and in the San Francisco Bay area. These women had a histologically confirmed diagnosis of breast cancer, were at least 6 months postsurgery or post-radiation therapy, and had no known residual tumor when studied.

In Boston, controls were friends of the cases or had been subjects in a previous study (14). In San Francisco, controls were selected from among women hospitalized with certain nongynecological conditions, as described elsewhere (8). In both areas, the cases and controls were matched for age (within 5 years) and for gravidity (ever-never). In addition, all subjects were white, were under the age of 50, and had not used OC[3] in the 24 months prior to study. A woman was excluded if she was regularly taking any hormone or medication other than vitamins and dietary supplements. Also excluded were women who had had uterine or ovarian surgery or who had not had regular menses in the 6 months prior to interview.

Each potential subject was interviewed at home regarding the admissibility criteria and her reproductive history. The women studied provided a timed, 12-hr overnight urine specimen on the 10th and 21st days of a menstrual cycle. Urine aliquots, identified only by code, were frozen and shipped to the University of Melbourne for biochemical assay of the estrogens and of pregnanediol as previously described (13, 14). For determination of the replicability of the assay procedures, duplicate aliquots from 10% of the specimens were assayed. Replicability was as reported previously (14).

The distribution of the concentrations of the individual estrogens and of the estriol ratio, estriol/(estrone + estradiol), are log normal. They are described by their geometric mean and its 95% confidence limits. For other variables, the arithmetic mean and its S.E. are given. Most significance tests used are based on comparing the difference between 2 means, as a standard normal deviate, with the S.E. of that difference. Where a test for trend is used, it is the χ^2 for linear trend.

RESULTS

Preliminary analyses showed appreciable variation in estrogen concentrations accumulated through OC use (Table 1). Despite the lapse of at least 2 years after cessation of OC use, women who had used them for 19 months or more had estrogen concentrations lower than those of nonusers of OC. For estrone and estradiol, this reduction was especially marked among the cases where it averaged 30% compared to 9% for the controls. However, for estriol the reduction was nearly equal for cases and controls, 32 and 29%, respectively (women who had used OC for less than

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[3] The abbreviation used is: OC, oral contraceptives.

19 months generally had estrogen concentrations intermediate to those in the other 2 groups). OC users, cases and controls, were 2 to 3 years younger than nonusers. This age difference is unlikely to explain the lower estrogen excretion in the OC users since younger women would be expected to have higher estrogen excretion than would older women (16). However, the cases were an average of 2.5 years older than the controls, a difference of possible importance, as we shall discuss.

Because of the pronounced effect of long-term OC use on estrogen excretion, the remaining analyses of the hormone data are restricted to nonusers and short-term users of OC. These data are shown in Table 2. In both phases of the menstrual cycle, cases had higher estrogen concentrations but a lower estriol ratio than had the controls. However, none of the case-control differences is statistically significant. Compared to a relative incidence of breast cancer of 1.0 for women in the lowest one-third of the estriol ratio distribution, women in the highest one-third had a relative incidence of 0.7 (0.3 to 1.5, 95% confidence interval), as estimated either from follicular- or luteal-phase data.

Zumoff *et al.* (18) suggested that if a low estriol ratio is a valid breast cancer risk indicator, it is because a low ratio reflects high excretion of estrone and estradiol rather than low excretion of estriol. The data in Table 2 support this idea; the estrone and estradiol values of cases are 6 to 19% higher than those of controls, and the 2 groups have similar values for estriol. However, even the sum of estrone and estradiol is not significantly elevated for cases compared to controls in either the follicular ($p = 0.22$) or the luteal phase ($p = 0.45$).

Table 2 also shows that cases had lower levels of pregnanediol excretion than did controls ($p = 0.24$). This could reflect a case-control difference in the timing of the luteal-phase urine collections, relative to the day of ovulation, but this explanation is unlikely to be correct since the mean duration of the cycle in which urine was collected was 27.1 days for cases and 26.8 days for controls. Nor does the low pregnanediol level of the cases reflect a low frequency of ovulation among them. Based on pregnanediol values of more than 1.0 mg/liter, about 80% of the cycles of both groups were ovulatory. In addition, there was no meaningful difference between cases and controls in the urine volumes passed during the 12-hr collection period, an average of 684 and 643 ml, respectively, or in the specific gravity of the urine, 1.018 and 1.019.

Table 3, which includes long-term OC users, is a comparison of cases and controls with respect to several known or suspect breast cancer risk factors. None of the differences

Table 1

Estrogen and pregnanediol concentrations for cases and controls by cycle phase and duration of OC use

Estrogens were measured in μg/liter, and pregnanediol was measured in mg/liter.

Duration of OC use	No. of women	Cycle length (days)	Age (yr)	Variable[a]								
				Follicular				Luteal[b]				
				Estrone	Estradiol	Estriol	Estriol ratio	Estrone	Estradiol	Estriol	Estriol ratio	Pregnanediol
Nonusers												
Cases	56	27.2	44.6	13.5	7.2	14.0	0.67	12.2	5.7	15.9	0.89	1.78
Controls	42	26.8	41.8	11.6	6.1	12.9	0.72	10.5	5.1	15.2	0.96	2.16
<19 mos.												
Cases	17	26.7	41.5	14.8	8.2	15.0	0.65	8.7	4.3	12.4	0.95	1.81
Controls	13	26.9	39.8	12.6	6.5	14.8	0.78	9.1	4.5	13.9	1.01	2.14
19+ mos.												
Cases	21	28.0	41.4	9.0	4.4	9.3	0.69	9.2	4.3	11.1	0.82	1.63
Controls	15	27.5	39.7	10.8	5.1	8.9	0.56	10.0	4.7	11.0	0.73	1.96

[a] Arithmetic mean for cycle length and age; geometric mean for other variables.
[b] Estriol/(estrone + estradiol).

Table 2

Estrogen and pregnanediol concentrations for nonusers and short-term users of OC according to cycle phase

Estrogens were measured in μg/liter; pregnanediol was measured in mg/liter.

	No. of women	Variable[a]					
		Estrone	Estradiol	Estiol	Estrone + estradiol	Estriol ratio	Pregnanediol
Follicular phase							
Cases	73	13.8 (11.6–16.5)	7.4 (6.2–8.9)	14.2 (11.5–17.6)	21.3 (17.9–25.5)	0.66 (0.58–0.76)	
Controls	55	11.8 (9.7–14.4)	6.2 (5.1–7.6)	13.3 (10.3–17.2)	18.1 (15.0–22.0)	0.73 (0.63–0.85)	
Luteal phase							
Cases	73	11.3 (9.6–13.3)	5.3 (4.5–6.3)	15.0 (12.7–17.8)	16.7 (14.2–19.7)	0.90 (0.79–1.02)	1.78 (1.43–2.23)
Controls	55	10.2 (8.4–12.4)	5.0 (4.1–6.0)	14.9 (12.1–18.3)	15.2 (12.6–18.5)	0.97 (0.83–1.14)	2.15 (1.75–2.66)

[a] Geometric mean for all variables; 95% confidence interval of the mean in parentheses.

shown is statistically significant. However, when age at first birth was treated as a trichotomy, the trend of increasing breast cancer incidence with increasing age at first birth was statistically significant ($p = 0.04$); compared to a relative incidence of unity for women who had a first birth prior to age 24, the relative incidence was 1.4 for women who had a first birth from 24 to 28 years of age and 2.7 for women who had a first birth at age 29 or older.

DISCUSSION

The results of this study lend only moderate support to the idea that the estriol ratio or patterns of estrogen metabolism are related to breast cancer risk. Cases did have lower estriol ratios and higher concentrations of estrone and estradiol than did the controls. However, these differences were small, about 10%, and were not statistically significant. However, several factors may have operated to cause the strength of the association to be underestimated. A discussion of these difficulties is warranted in the hope that they can be circumvented in future investigations.

The first difficulty is the apparently long-term residual effect of OC use on estrogen excretion. Prior studies of small numbers of younger women have shown that estrogen metabolism is normal even shortly after cessation of OC use. However, most of the women studied had used OC for less than 2 years (2, 11). This study suggests that older women who have used OC for at least 19 months have an appreciable reduction in estrogen excretion for at least 2 years following cessation of use. This finding has numerous

Table 3

Arithmetic mean of several breast cancer risk factors for all cases and controls

	No. of women	Wt (kg)	Height (cm)	Age (yr) At interview	Age (yr) At menarche	Age (yr) At first birth[a]	Quetelet's index[b]
Cases	94	61.1 ± 1.1[c]	164.7 ± 0.6	43.6 ± 0.5	13.2 ± 0.2	25.8 ± 0.5	22.5 ± 0.4
Controls	70	61.0 ± 1.2	163.5 ± 0.8	41.4 ± 0.6	131.1 ± 0.1	24.7 ± 0.5	22.9 ± 0.4

[a] Excludes 5 nulliparous cases and 4 nulliparous controls.
[b] Weight (kg) × 10,000/height² (cm).
[c] Mean ± S.E.

Table 4 A comparison of case-control studies of specific estrogens and breast cancer.

Investigators	No. of Cases	No. of Controls	Menopausal Status	Disease Status of Cases	Specimen/ Phase	Estrogen Values[a] E1	E2	E3	E1+E2+E3	Estriol Ratio	Comments
Arguelles et al., 1973	47	8	Pre	Stages 1/2	Urine	10.0; 16.2	3.6; 4.5	17.2; 20.8	30.4; 41.8	1.3 ; 1.0	Ovulatory specimens for all premenopausal subjects.
	24	8	Pre	Stages 3/4	Urine	11.5; 16.2	3.8; 4.5	20.1; 20.8	35.3; 41.8	1.3 ; 1.0	
	25	10	Post	Stages 1/2	Urine	4.3; 2.6	1.1; 1.2	7.0; 7.1	12.5; 11.0	1.3 ; 1.9	
	19	10	Post	Stages 3/4	Urine	5.5; 2.6	1.9; 1.2	8.5; 7.1	16.0; 11.0	1.1 ; 1.9	
Brown, 1958	27	22	Post	Recurrent Dis.	Urine	1.5; 1.4	0.6; 0.3	5.8; 4.1	8.1; 6.0	2.8 ; 2.4	[b]
England et al., 1974	10	32	Pre	Not stated	Serum/ Pollicular		Mean difference between cases & controls 6.1				
					Serum/ Luteal		Mean difference between cases & controls 9.0				
	27	25	Post	Not stated	Serum	7.4; 5.8					
Grönroos and Aho, 1968	24	10	Post	Primary Dis.	Urine		4.3; 5.3	5.3; 5.0	9.6; 13.6	1.22; 0.59	E1 and E2 not given separately [b]
				Recurrent Dis.	Urine		6.0; 8.6	7.1; 5.0	13.1; 13.6	1.17; 0.59	
Henderson et al., 1975	49	46	Pre	Daughters of cases	Serum/ Pollicular	4.2; 3.9	4.3; 3.6				Daughters of cases and of controls
					Serum/ Luteal	8.9; 7.6	14.9; 11.0				
Lemon et al., 1966	6	15	Pre	Not stated	Urine	2.8; 5.6	4.1; 5.3	4.3; 10.2		0.6 ; 1.5	Random specimens, days 4 to 26 for all subjects
	30	8	Post	Not stated	Urine	3.7; 5.6	3.1; 3.4	5.0; 6.9		2.1 ; 3.2	
Schweppe et al., 1967	10	6	Post	Recurrent Dis.	Urine				20.3; 12.7	0.6 ; 1.3	
Tominaga et al., 1975	25	20	Pre	Recurrent Dis.	Urine	4.5; 21.2	7.7; 18.9	16.3; 40.5	26.5; 80.6	0.55; 0.60 (Estriol Proportion)	Ovulatory specimens for controls, random for cases

[a] The first value is that of the cases, the second that of controls. Serum values are in nanograms per 100 milliliters and urine values in micrograms per 24 hours.
[b] Estriol ratio estimated from means by present authors.

and important implications and requires further evaluation. However, the practical effect for the present investigation was that more than 20% of the subjects had to be excluded from the definitive analyses.

The second difficulty reflects the fact that among women in their 40's there are changes in estrogen metabolism. For example Sherman *et al.* (16) found that perimenopausal women who were having regular menstrual periods had lower serum estradiol, higher gonadotropins, and a shorter follicular phase than did normal younger women. This may explain why we found higher estrogen excretion in the follicular- than in the luteal-phase urine specimens; the reverse is found in women under age 40 (6, 13, 14). In light of this observation, the fact that the cases were on average 2.5 years older than the controls takes on significance and may account for our weekly positive results. Closer age matching should be used in future case-control studies of hormonal patterns in perimenopausal women unless the studies are large enough that age discrepancies can be overcome in the analysis of the data. It would probably also be useful to gather 3 rather than 2 urine specimens in order to estimate the length of the follicular phase and to sample the postovulatory estrogen peak during the menstrual cycle.

In view of the many factors that affect estrogen excretion and the estriol ratio, it is difficult to compare our results with those of earlier studies. However, this is attempted in Table 4. Most earlier studies used specimens collected at random through the menstrual cycle. This introduces variation leading to an underestimation of any true case-control differences in hormone levels. In at least 1 study (17), urine specimens were collected at random times for the cases but only at ovulation for the controls, and no valid comparison can be made of the 2 groups. Other studies have involved only postmenopausal women or women with metastatic breast cancer and are not readily interpreted. Henderson *et al.* (10) studied the young daughters of women with breast cancer and comparable young women. In comparison to the controls, the case daughters had moderately higher serum levels of estrone and estradiol in both phases of the menstrual cycle. England *et al.* (7) also found higher serum levels of estradiol in cases than in controls. However, Arguelles *et al.* (1) found lower urine values for all estrogens in premenopausal cases than in controls, and the controls had lower estriol ratios. Thus, useful data are sparse and inconsistent, and no conclusion can be drawn regarding the relationship between the estriol ratio and breast cancer risk. However, 3 of the 4 studies that can be interpreted show that controls excrete less estrone than do cases and/or have higher estriol ratios. It therefore seems important to conduct a study designed to offset the limitations in the present and in previous investigations.

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