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SEX HORMONE LEVELS IN SERUM IN RELATION TO THE DEVELOPMENT OF BREAST CANCER

DIANE K. WYSOWSKI,[1,2] GEORGE W. COMSTOCK,[1] KNUD J. HELSING,[1] AND H. LORRIN LAU[1,3]

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In 1974, approximately 13,000 female residents of Washington County, Maryland, donated 15 ml of blood as part of a project to determine if certain serologic factors were related to the development of site-specific cancer. Sera were stored at −73 C. The present study reports the associations of serum levels of estrone, estradiol, estriol, androstenedione, progesterone, and testosterone with breast cancer among 17 premenopausal cases diagnosed 8–132 months after blood was drawn, and 39 postmenopausal cases diagnosed 6–72 months after blood was drawn. Each case was matched to four controls selected from the other serum bank donors. Matching factors were age, race, and time since last menstrual period. Cases and controls who were taking estrogen-containing preparations were excluded from this analysis. Sera were analyzed without knowledge of case-control status. Differences in levels of serum hormones between cases and controls were slight and not statistically significant.

androstenedione; breast neoplasms; estradiol; estriol; estrogens; estrone; progesterone; testosterone

Evidence exists that suggests a role for endogenous sex hormones in female breast cancer etiology: Female sex is the leading risk factor for breast cancer (1–3); the ovarian hormones, estrogen and progesterone, and the pituitary hormones, prolactin and somatotropin, are primarily responsible for mammary growth and development (4); mammary carcinoma can be induced in male and female rodents by administration of estrogens (5); transsexual males given high doses of estrogens to induce breast development have developed breast cancer (5); several reproductive factors such as number of children (6–8) and ages at menarche (9, 10), at first full-term pregnancy (11, 12), and at natural or artifical menopause (13, 14) are associated with breast

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[1] Department of Epidemiology, School of Hygiene and Public Health, The Johns Hopkins University, Baltimore, MD.
[2] Present address: Division of Epidemiology and Surveillance, Food and Drug Administration, Rockville, MD.
[3] Present address: Queen's Physicians Building, Honolulu, HI.
Reprint requests to Dr. George W. Comstock, Training Center for Public Health Research, P.O. Box 2067, Hagerstown, MD 21742–2067.
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cancer risk; and anti-estrogen treatment has been shown to be efficacious in reducing certain malignant breast tumors (15).

Because of this evidence, investigators for the past decade and a half have studied absolute and relative levels of various sex hormones (primarily estrogen, estrone, estradiol, estriol, progesterone, androgen and metabolites, and prolactin) in the body fluids of breast cancer cases and controls (16–18) and of women at high and low risks of breast cancer (19–34). Except for one study that reported urinary hormone levels (35), none are known to have compared hormone levels in women who were subsequently diagnosed with breast cancer and those who were not. This report presents results obtained from such a prospective study.

Materials and methods

In the autumn of 1974, 15 ml of blood were donated by each of 25,620 persons, representing approximately one-third of the adult population of Washington County, Maryland. Another 182 specimens were donated in July 1975. Slightly more than half of the volunteers were women. Blood was collected in 15 ml Vacutainers (Becton-Dickenson and Co., Orangeburg, NY) and allowed to clot at room temperature for approximately three hours. It was then kept at 5 C until the serum was removed, usually within several hours. The serum, in two aliquots, was promptly frozen and stored with only trivial deviations from −73 C until it was thawed for the hormone analyses.

During the seven-year period from December 1, 1974 through November 30, 1981, 73 female participants were reported to the Washington County cancer registry as having breast cancer which had been diagnosed after their blood had been drawn. Of these cases, three said that they were taking oral contraceptives or other female hormones, and hence were excluded from the present analysis. In addition, one case diagnosed less than six months after blood was drawn was also excluded. Of the remaining 69 cases, nine were premenopausal and 60 were postmenopausal. Because of the small number of premenopausal cases, eight more such cases who were reported to the cancer registry during the next four years and who met the study criteria were added. Because laboratory A, which performed the initial hormone assays, had been closed, the last eight sets of sera from premenopausal women were examined by laboratory B.

Controls were selected from the other serum bank donors and were matched to their respective cases by race, age, and time since last menstrual period. Age-matching was done from a birth date listing of donors to the serum bank, selecting the two older and two younger women nearest in age to the case who also met the other matching criteria. Matching on time since last menstrual period was done within the following specified intervals: 0–39 days, matched to the same day; 40–365 days, matched within 30 days; and one year, 2–4 years, 5–9 years, 10–14 years, and 15 or more years, matched to the same group. Before a woman was finally accepted as a control, the records were searched to be sure that 1) she was not listed in the cancer registry; and 2) if a participant in the 1975 county health census, she stated that she had not had a diagnosis of cancer. If a selected control was found to have said at the time of donating blood that she was taking oral contraceptives or female sex hormones, a substitute control was selected in the same manner. Controls taking these hormonal preparations have been excluded from the analyses except when their inclusion is specified.

Each set, consisting of serum from one case and her matching controls, including those taking estrogens, was examined on the same day with the same reagents. Each serum specimen was identified only by a code number that was unrelated to case-control status so that laboratory personnel knew only that a set contained sera from a case and controls. They could identify neither the case nor which controls were taking estrogens. Sera were examined for es-

trone, estradiol, estriol, androstenedione, progesterone, and testosterone. Both laboratories used radioimmunoassay methods; laboratory A followed procedures described by Emment et al. (36) with antisera obtained from Miles-Yeda, Rehovot, Israel, while laboratory B used their own antisera and followed procedures typified by Abraham et al. (37). Duplicate assays were done on each specimen, and the results reported here are the mean values of these two determinations. Quality control procedures included the assay of known concentrations of hormones on each working day and of occasional "blind" replicates of serum specimens. Sera from one premenopausal and one postmenopausal control were lost, leaving one case in each of these groups with only three matched controls. In addition, among the eight premenopausal cases whose sera were examined by laboratory B, there were insufficient sera for assays of androstenedione for three controls, of progesterone for two controls, and of testosterone for one control. No set had less than three controls. In these sets, the average value of the three examined controls was used in place of the missing control value.

After sera from all but 21 postmenopausal cases and their controls had been assayed, the results were examined. Because addition of data from these 21 cases seemed unlikely to alter the findings appreciably and because the hormone determinations used up all the stored serum for each subject, a decision was made to discontinue the serum assays, thereby saving sera from 105 women for possible future use.

Percentage differences in hormone levels between cases and controls were calculated by subtracting the mean level for the controls from the level for the cases, dividing the result by the control serum level, and multiplying by 100. This was done separately by laboratory and by menopausal status. Tests of statistical significance of the mean differences between case and control levels were performed by paired *t* tests.

Results

Characteristics of the cases with respect to age, time since last menstrual period, and months from initial blood donation to diagnosis are summarized in table 1. One case and her four controls were black. All other study participants were white. Matching of controls to cases by exact year of age was achieved in 82 per cent of the attempts. Among the remainder, the mean deviation (ignoring sign) was only 2.0 years. Premenopausal cases and controls were matched to the exact day since last menstrual period. All postmenopausal subjects were at least two years past their last menstrual period; these cases and controls were all matched within the following time periods: 2–4, 5–9, 10–14, and 15 or more years since last menstrual period.

Mean serum levels of estrone, estradiol, estriol, androstenedione, progesterone, and testosterone among cases and matched controls are shown in table 2. Among postmenopausal women, serum levels of all six hor-

TABLE 1

Characteristics of breast cancer cases, by menopausal status and laboratory, Washington County, MD, 1975–1985

Menstrual status and laboratory	No. of subjects	Age (years)		Time since last menstrual period		Months prior to diagnosis	
		Mean	Range	Mean	Range	Mean	Range
Premenopausal							
Laboratory A	9	41	(25–49)	16 days	(2–28)	34	(16–72)
Laboratory B	8	43	(32–50)	8 days	(0–18)	50	(8–132)
Postmenopausal*	39	61	(36–90)	14.4 years	(2–15+)	28	(6–72)

* All assays were done in laboratory A.

TABLE 2

Mean serum levels of selected hormones among breast cancer cases diagnosed after having blood drawn in 1974 and their matched controls, by menopausal status and laboratory, Washington County, MD, 1975–1985

Hormone	Mean level (pg/ml)		
	Cases	Controls	% difference
	Premenopausal		
Laboratory A (9 cases, 36 controls)			
Estrone	77	111	−31
Estradiol	100	135	−26
Estriol	376	369	2
Androstenedione	2,191	2,389	−8
Progesterone	1,810	2,278	−21
Testosterone	309	315	−2
Laboratory B (8 cases, 31 controls)			
Estrone	66	68	−3
Estradiol	110	116	−5
Estriol	18	18	0
Androstenedione*	890	986	−10
Progesterone†	1,081	1,707	−37
Testosterone‡	305	299	2
	Postmenopausal		
Laboratory A (39 cases, 155 controls)			
Estrone	59	62	−5
Estradiol	51	52	−2
Estriol	359	361	−1
Androstenedione	1,795	1,677	7
Progesterone	542	558	−3
Testosterone	304	274	11

* Eight cases and 28 controls.
† Eight cases and 29 controls.
‡ Eight cases and 30 controls.

mones were virtually the same for cases and controls. Among premenopausal women, differences between cases and controls were similar in direction for the groups examined by the two laboratories. Weighted average per cent differences between cases and controls for the 17 premenopausal sets are as follows: estrone, −18; estradiol, −16; estriol, +1; androstenedione, −9; progesterone, −29; and testosterone, 0. None of the observed case-control differences achieved statistical significance at the 0.05 level.

Dividing cases into those diagnosed early and those diagnosed late in the 11-year observation period gave no consistent or significant indications that case-control differences might be related to time of diagnosis of the case and hence to the influence of unrecognized cancers at the time of donating blood (table 3).

In addition to the 39 case-control sets of sera from postmenopausal women that were analyzed for the six hormones, there were one case and 23 controls whose sera were also analyzed but who were excluded from the previous tables because they were taking estrogens when they gave blood. These 23 controls came from 17 matched sets of controls. Including the set in which only the case was taking estrogens would have made trivial changes in the results in tables 2 and 3 since case-control differences in this set were similar to those in the other sets. However, if the 23 controls taking estrogens had been included in the case-control comparisons, there would have been some changes, as can be surmised from the

TABLE 3

Differences in mean serum levels of selected hormones among cases diagnosed early and late after having blood drawn in 1974 and their matched controls, by menopausal status and laboratory, Washington County, MD, 1975–1985

Laboratory and hormone	Months from giving blood to diagnosis					
	In pg/ml			As % of control value		
	6–23	24–72	24–132	6–23	24–72	24–132
	Premenopausal					
Laboratory A						
No. of cases	3	6				
Estrone	−20	−41		−18	−37	
Estradiol	−28	−38		−21	−28	
Estriol	−15	18		−4	5	
Androstenedione	−256	−169		−11	−7	
Progesterone	291	−848		13	−37	
Testosterone	23	−20		7	−6	
Laboratory B						
No. of cases	2		6			
Estrone	12		−7	17		−10
Estradiol	98		−40	89		−34
Estriol	−3		0	−14		0
Androstenedione	20		−19	24		−19
Progesterone	−71		−813	−41		−37
Testosterone	65		−14	21		−5
	Postmenopausal					
Laboratory A						
No. of cases	12	27				
Estrone	−2	−3		−3	−5	
Estradiol	−2	−1		−4	−2	
Estriol	−40	15		−11	4	
Androstenedione	134	111		8	7	
Progesterone	−60	4		−11	1	
Testosterone	−11	48		−4	18	

findings in table 4. The two groups of controls in the table are matched within each set for the same characteristics used in selecting matched controls for cases, namely sex, race, age, and time since last menstrual period. For each hormone, the mean values within each set for controls taking estrogens and those not taking estrogens were averaged, thus allowing for the unequal numbers of controls on estrogens in the 17 sets. Controls not taking estrogens had considerably lower levels of estrone, somewhat lower levels of estradiol, similar levels of estriol and androstenedione, and slightly higher levels of progesterone and testosterone than did those on estrogens. Had the controls taking estrogens been included in the comparisons, the case-control differences for estrone and estradiol would have become more strongly negative, those for estriol, androstenedione, and progesterone would have remained close to zero, and the difference for testosterone would have become somewhat larger in the positive direction.

DISCUSSION

The study of normal women by Bulbrook et al. (35) most nearly resembles the present investigation. In 1971, they published the results of a 10-year follow-up study of 5,000 British women aged 15–55 years who resided on the Isle of Guernsey. During the

TABLE 4

Serum hormone levels among 67 postmenopausal controls who were not taking estrogens and 23 postmenopausal controls who were taking estrogens, Washington County, MD, 1975–1985

	Postmenopausal controls		Ratio (group not taking estrogens/group taking estrogens)
	Not taking estrogens	Taking estrogens	
No. of subjects	67	23	
Weighted mean hormone levels (pg/ml)*			
Estrone	65	255	0.25
Estradiol	53	91	0.58
Estriol	339	340	1.00
Androstenedione	1,773	1,694	1.05
Progesterone	285	249	1.14
Testosterone	552	474	1.16

* See text for method of calculation.

follow-up period, 27 women developed breast cancer. Each case was matched with between four and 13 controls on age, height, weight, parity, menopausal status, and day of menstrual cycle. Their 24-hour urine specimens, which had been collected at the beginning of follow-up, were assayed for various androgen metabolites. The investigators found that the mean excretion levels of androsterone and etiocholanolone in women subsequently diagnosed with breast cancer were significantly lower than those in controls, indicating a general deficiency of androgen metabolite excretion.

Using historical information and urine assay results from the 1,485 women in that study, a model was developed that enabled five levels of risk of developing breast cancer to be delineated (38). Between 1966 and 1976, blood was collected from 386 of the 1,485 women in the original study (30). Plasma estradiol levels showed no consistent relation among either pre- or postmenopausal women with the risk of breast cancer calculated from that model. Plasma progesterone levels among premenopausal women during the luteal phase of the menstrual cycle showed a significant negative association with breast cancer risk. No similar association was observed among women whose blood was drawn during the follicular phase nor among postmenopausal women.

The results among postmenopausal women in our study agree with those of Bulbrook et al. (30) in failing to find an association of risk with either serum estradiol or progesterone. However, findings among our small series of premenopausal cases were not similar. Among premenopausal women whose times from last menstrual period were 1–14 days (an approximation of the follicular phase of Bulbrook et al.), cases in eight of the nine sets had levels of estradiol that were lower than the averages of their matching controls, and in seven of nine sets, case levels of progesterone were lower than the averages of their matching controls. Among premenopausal women with times from last menstrual period of 15–28 days (an approximation of the luteal phase), there was no evidence of differences between cases and controls in levels of either of these two hormones.

Lemon (39) hypothesized in 1969 that the relative levels of individual estrogen fractions (estrone, estradiol, and estriol) might be important breast cancer predictors. Specifically, he suggested that a low urinary estriol excretion ratio might facilitate cancer development. His hypothesis was modified by Cole and MacMahon (40), who suggested that a low estriol ratio in the first decade or so after puberty was an important determinant of a woman's lifetime risk of breast cancer. Since then,

doubts have been raised about some of the assumptions on which the estriol ratio hypothesis was based (2, 3, 41). Questions have arisen about the use of urine as opposed to blood as an indicator of biologically active estrogen (42) and about the possibility that other hormones such as prolactin (27), progesterone (43), and androstenedione (33, 34, 44, 45) may play a role in breast cancer etiology. Investigators have focused more on absolute rather than relative levels of estrogen fractions in cross-sectional analyses of women at high and low risks of breast cancer (24, 25, 27, 30–32).

Results of the present study do not support any of these hypotheses. Whether estriol ratios are based on denominators using the sum of estrone and estradiol or the sum of all three estrogens, they are slightly higher among cases than among controls. While the lower level of progesterone among premenopausal cases is in the direction suggested by the findings of Cowan et al. (43) that progesterone-deficient women had an increased risk of breast cancer, the difference between levels for cases and controls could easily have occurred by chance. Unfortunately, we were able to look at only six hormones. Important omissions in view of current thinking were prolactin and levels of bound and unbound estrogens.

The prospective nature of the present study, its general population base, the use of serum rather than urine specimens, and similar, blinded hormone assays for each case-control set are important strengths. The major weakness of the study is lack of power. The decision not to analyze the last 21 postmenopausal case-control sets was not an easy one. While their inclusion would have resulted in some added power, this would not have been great. To change the present findings among postmenopausal cases to an interesting degree would have required the results of the case-control comparisons in the 21 sets whose sera were not assayed to be markedly different from those in the 39 sets whose hormonal analyses are reported here, an outcome that seems highly unlikely. Another problem is the markedly different levels of estriol reported by the two laboratories and the less marked differences in androstenedione levels. While changes associated with increased storage time for the specimens examined by laboratory B could be the explanation, this does not seem likely. For five of the six hormones, the levels reported here are close to or slightly above those each laboratory considered normal for women. The exception is estriol which averaged 19 pg/ml in laboratory B, where the normal level for nonpregnant females is stated to be less than 10 pg/ml. However, there have been no indications of evaporative losses from the stored sera. Discussions with laboratory personnel have failed to shed any light on reasons for the different levels. Regardless of the interlaboratory differences, the important feature is that the examination procedures were similar for each set of a case and four controls and independent of case-control status, so that comparisons of cases and controls are valid regardless of laboratory differences.

With respect to prediagnostic serum levels of hormones, this study leaves the situation regarding premenopausal cases less clear than one would like. The numbers of cases are not large. Matching of cases and controls by time since last menstrual period is only an approximation to matching on phase of the menstrual cycle so that control values may provide a less than ideal approximation to expected values among cases. In spite of these problems and the unexplainable laboratory differences, it seems unlikely that major case-control differences could be missed. The situation for postmenopausal breast cancer is clearer. The differences between postmenopausal cases and controls are so slight that even with the modest number of cases in this study it is not unreasonable to suggest that prediagnostic serum levels of the six hormones do not play a meaningful role in the pathogenesis of breast cancer in this age group. Furthermore, there is no support for earlier fears that high levels of estrogens

are associated with an increased risk of breast cancer.

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