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ENDOGENOUS ESTROGEN, TESTOSTERONE, AND PROGESTERONE LEVELS IN RELATION TO BREAST CANCER RISK

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Abstract

Multiple lines of evidence support a central role of hormones in the etiology of breast cancer. In epidemiologic studies, considerable effort has focused on delineating the role of endogenous hormones in risk of breast cancer among postmenopausal women. Recently, substantial additional data has accrued from prospective studies where endogenous hormones are measured in study subjects prior to disease diagnosis. In this review, the epidemiologic evidence linking sex steroids —estrogens, testosterone, and progesterone, specifically—with subsequent risk of breast cancer in both premenopausal and postmenopausal women is summarized. Overall, a strong positive association between breast cancer risk and circulating levels of both estrogens and testosterone has now been well confirmed among postmenopausal women; women with hormone levels in the top 20% of the distribution (versus bottom 20%) have a 2- to 3-fold higher risk of breast cancer. Evidence among premenopausal women is more limited, though increased risk associated with higher levels of testosterone is consistent. However, both positive and null associations have been observed with estrogens and progesterone and clearly more evaluation is needed.

Keywords

prospective; estrogens; testosterone; breast cancer; premenopausal; postmenopausal; epidemiology

Introduction

Sex steroids play a critical role in the etiology of breast cancer. Supporting evidence includes the known relationships between reproductive factors, such as early age at menarche, nulliparity and late age at menopause, in increasing risk [1]. Further, after menopause, adipose tissue is the major source of estrogen and obese postmenopausal women have both higher levels of endogenous estrogen and a higher risk of breast cancer [2]. Also, selective estrogen receptor modulators (e.g., tamoxifen) reduce breast cancer incidence [3] and aromatase inhibitors are used in treatment [4] and are being evaluated as chemopreventive agents.

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Mechanistically, estrogens contribute to tumor growth by promoting the proliferation of cells with existing mutations or perhaps by increasing the opportunity for mutations [5]. Androgens have been hypothesized to increase breast cancer risk either directly, by increasing cellular growth and proliferation, or indirectly, by their conversion to estrogen [6]. In experimental studies, androgens either increase or decrease cell proliferation, depending upon the model system [6].

Progesterone has strong influences on breast physiology and has been hypothesized to both decrease and increase breast cancer risk. Depending on the model system, evidence from animal and *in vitro* studies supports each hypothesis [7,8]. While postmenopausal estrogen use alone increases risk, the association is stronger with the combination of estrogen and progestin [1].

Determining the association between circulating sex steroid hormones and breast cancer risk in epidemiologic studies can provide further insight into etiology and may ultimately help identify high-risk women who would benefit from increased screening or chemoprevention. Substantial evidence that blood or urinary hormone levels are associated with breast cancer risk exists in postmenopausal women but much more limited data are available for premenopausal women. A summary of this literature is provided below. Only data from prospective studies (i.e., “nested” case-control studies) will be reviewed as, in these studies, hormone levels are measured prior to breast cancer diagnosis and hence are less susceptible to bias than are retrospective case-control studies. Due to space limitations, data on estrogen metabolites and androgens other than testosterone will not be addressed.

Methodologic considerations

Several complexities should be considered in interpreting data from epidemiologic studies of circulating hormones and breast cancer risk. In most epidemiologic studies only a single blood sample can be collected per study subject, due to both logistic and financial reasons. However, a single blood sample has been found to reflect long-term hormone levels fairly well. For example, in postmenopausal women, the correlation over a 2 to 3 year period for the steroid hormones ranged from 0.5 to 0.9 [9–12]. In premenopausal women, androgens are similarly well correlated over time (11–13) but estrogens (evaluated separately in the follicular and luteal phase) and progesterone (evaluated in the luteal phase) are more modestly correlated [13,14]. Thus, although some attenuation of RR estimates undoubtedly results from the use of a single blood measure for these hormones, this reproducibility is similar to that of blood pressure or serum cholesterol, parameters that are reasonably measured and consistent predictors of disease in epidemiologic studies [15].

In epidemiologic studies circulating hormone levels are most often measured, yet relatively little is known about how these levels correlate with exposure in the breast tissue. Levels of 17β-estradiol are considerably higher in breast tissue than in the circulation [16] because of its conversion from steroid precursors [17]. However, the correlation between local nonmalignant tissue levels and circulating levels is not known; most studies evaluated the correlation between circulating levels and tumor tissue hormone levels or did not present correlations between circulating levels and nonmalignant tissue [18–20]. Certainly, the consistent positive associations between circulating hormone levels and risk in postmenopausal women (described below) suggest these levels are an important marker of tissue exposure.

Sex steroids and breast cancer risk in postmenopausal women

Estrogens

In the last several years, substantial prospective data have accrued on circulating estrogens and breast cancer risk in postmenopausal women. In 2002, a pooled analysis was published consisting of all 9 prospective studies available at that time [21]. The analysis included 663 breast cancer cases and 1765 controls; none of the women were using exogenous hormones at blood collection. Median time from blood collection to cancer diagnosis ranged from 2 to 12 years. Circulating estrogen levels were positively associated with breast cancer risk. The RRs (95% CI) for increasing quintiles of estradiol level, relative to the lowest quintile, were 1.4, 1.2, 1.8, and 2.0 (1.5–2.7) (Table 1). Other estrogens were similarly related to risk. The variation in RRs between studies was not statistically significant for any of the hormones and the associations did not vary significantly according to the type of laboratory assay used. Since the pooled analysis was published, a Swedish prospective study with 173 cases reported similar positive associations between circulating estrogens and postmenopausal breast cancer [22]. Further, similar positive associations with urinary estrogen levels were noted in 2 prospective studies [23,24].

More recently, findings from the large multi-country European Prospective Investigation into Cancer and Nutrition (EPIC) study were reported [25]. In EPIC, among postmenopausal women, 677 incident breast cancer cases and 1309 age and recruitment center matched controls were accrued over 6 years of follow-up; findings confirmed those of the pooled analysis of 9 studies. For example, for circulating estradiol, the RRs (95% CI) for increasing category of levels were 1.0, 1.1, 1.4, 1.7, 2.3 (1.6–3.2) (Table 1). Other estrogens again were similarly related to risk.

Updated analyses from 2 cohorts included in the 9 study pooled analysis have expanded upon the observed associations in important ways. In the NYUWHS, with up to 13 years of follow-up after blood collection, the hormone/breast cancer associations remained unchanged with the exclusion of the first 5 years of follow-up [26]. Additionally, the authors used 2 blood samples collected from a large number of women (for cases, one within 5 years of diagnosis and a second at least 5 years post diagnosis) to assess if the change in levels over time varied between cases and controls. Changes in estrogens and testosterone were comparable between the 2 groups. Thus, this study provides strong evidence that circulating hormones are truly a marker of increased risk in postmenopausal women and not simply a result of tumor-related hormone production.

Only a single detailed assessment of the association between plasma hormones and breast cancer risk by estrogen and progesterone receptor status of the tumor has been published [27]. Strong positive associations were observed for ER+/PR+ tumors, and weak or no association noted for ER+/PR− and ER−/PR− tumor types (too few ER−/PR+ tumors were available to evaluate separately). For example, for estradiol, the top versus bottom quartile RR (95% CI) was 3.3 (2.0–5.4) for ER+/PR+ tumors (p-trend<0.001), 1.0 (0.4–2.6; p-trend=0.82) for ER+/PR− tumors, and 1.0 (0.4–2.4; p-trend=0.46) for ER−/PR− tumors (p for heterogeneity<0.001).

Whether the association between plasma estrogens and postmenopausal breast cancer is similar in women at varying levels of breast cancer risk has been evaluated in 2 recent studies. No association between plasma estradiol and breast cancer risk was observed in the high risk population of the National Surgical Adjuvant Breast and Bowel Project Cancer Prevention Trial (P-1) (top versus bottom quartile RR=0.96 {95% CI=0.5–2.0}). Here, only the 89 cases and 141 non-cases enrolled in the placebo arm of the trial were included in the analysis [28]. Within the Nurses' Health Study (NHS) cohort, with 418 cases and 817 controls [29], the

associations of plasma estradiol and estrone sulfate with breast cancer were robust across risk categories regardless of which metric was used to define risk (e.g., 5-year modified Gail score or by family history of breast cancer). For example, estradiol appeared as or more strongly associated with breast cancer in women with higher predicted risk by the Gail risk score (modified Gail score ≥2.25%: RR=4.5, 95% CI (2.1–9.5)), compared to lower risk (modified Gail score <1.66%: RR=2.1, 95% CI (1.2–3.6)), but these differences in relative risk were not statistically significant. The association between plasma estrone sulfate and breast cancer also was similar in the 2 groups. Thus evidence from this larger cohort suggests that circulating estrogens are predictive of risk in women across both low and high predicted risk of breast cancer, however, confirmation in other studies is needed.

Only 1 prospective study has addressed whether estradiol levels are associated with breast cancer risk even in women using postmenopausal hormones [30]. Modest positive associations were observed (top vs. bottom quartile RR (95% CI) for estradiol=1.3 (0.9–2.0) p-trend=0.20) which were stronger and statistically significant among women who were older, leaner and who had the longest duration of non-use of hormones since menopause. Thus, even in postmenopausal hormone users, plasma estradiol levels appear to be at least modestly associated with risk.

Testosterone

The pooled analysis of 9 prospective studies described above [21] provides a comprehensive summary of evidence on circulating testosterone levels and breast cancer risk in postmenopausal women, along with the recently published report from the EPIC study [25] (Table 1). In the pooled analysis, breast cancer risk increased with increasing testosterone levels: the relative risks (95% CI) for increasing quintile (all relative to the lowest quintile) were 1.3, 1.6, 1.6 and 2.2 (1.6–3.1). Results were similar in analyses excluding cases diagnosed within 2 years of blood collection. Extensions of these findings, with up to 12 years of follow-up after the initial blood collection, have been published for 2 of the studies included in the pooled analysis and the observed associations were very similar [26,29]. In the EPIC cohort, similar associations were observed [25]. In addition, the association of plasma testosterone levels and subsequent breast cancer risk was generally similar in women using postmenopausal hormones [30].

In each of these analyses, adjustment for estradiol in the statistical models only modestly attenuated relative risks for testosterone, suggesting some independent association of testosterone levels with breast cancer [21,25]. However, possible differences between estradiol and testosterone in assay precision, stability within woman over time, and intracellular conversion of androgens to estrogens complicate the interpretation of these epidemiologic analyses. In the NHS, the association between testosterone and breast cancer was stronger for ER+/PR+ tumors (p for heterogeneity=0.03) [27]. Specifically, the top versus bottom quartile relative risk (95% CI) was 2.0 (1.2–3.4; p-trend<0.001) for ER+/PR+ tumors, 1.9 (0.7–5.0; p-trend=0.12) for ER+/PR− tumors, and 0.7 (0.3–1.6; p-trend=0.35) for ER−/PR− tumors. In the 2 studies previously described, the association between circulating testosterone and breast cancer risk across categories of predicted risk has been addressed. No association was observed between testosterone levels and breast cancer risk in the P-1 trial with 89 cases and 141 non-cases (RR {95% CI} for top versus bottom quartile: 0.5 {0.2–1.1}) (28), although the association was noted to be quite robust in the larger NHS cohort [29].

Progesterone

Only one large prospective study, with 270 cases, has evaluated the association of postmenopausal circulating progesterone and breast cancer risk. No association was observed either overall (top versus bottom quartile of levels: RR=0.9; 95% CI=0.6–1.5; p-trend=0.90),

when evaluated by tumor hormone receptor status or stratified by circulating estradiol levels [27].

Conclusion

The positive association between circulating estrogens and testosterone in postmenopausal women and subsequent risk of breast cancer is now well established. For both estradiol and testosterone, women in the top, versus bottom, 20% of estrogen levels have a two- to three-fold higher breast cancer risk. Although confirmation is needed, the association appears strongest for ER+ breast tumors and seems robust across groups of women at varying risk of breast cancer. Whether the association observed with testosterone is direct or indirect (through its conversion to estradiol) is unclear; both may be true.

Studies are now needed to determine if circulating steroid hormone measurements add substantially to existing breast cancer risk prediction models. Several statistical models have been developed for use as an entry criterion into breast cancer chemoprevention trials (e.g., NSABP P-1 trial), in counseling women on the potential use of chemopreventives (e.g., tamoxifen or aromatase inhibitors) and to provide general insight into a woman's individual breast cancer risk [31–34] but none of them include circulating hormone levels. Similarly, whether circulating sex steroid levels can be used to identify women who would most benefit from anti-estrogens is as yet unknown; baseline estradiol levels predicted the subsequent reduction in breast cancer risk associated with raloxifene use in the MORE trial [35] but not with tamoxifen use in the P-1 trial [28].

Sex steroid concentrations and breast cancer risk in premenopausal women

In contrast to the rapidly accumulating data on postmenopausal women, relatively few studies on circulating sex steroids levels and breast cancer have been conducted in premenopausal women. This is largely due to the variation in hormone levels, particularly estrogen levels, over the menstrual cycle thus making epidemiologic studies (that routinely depend on collecting a single blood sample from each study subject) particularly complex.

Estrogens

Seven prospective studies in premenopausal women have been published to date, although 5 of the 7 had fewer than 80 cases (range 14–79 cases) [36–40]. In none of the 5 studies were significant associations between estrogen levels and breast cancer risk noted, although as expected given their size, precision of the estimates was uniformly low. Two much larger studies have recently been published. In the largest study to date, conducted in the EPIC cohort, with 285 invasive breast cancer cases and 555 controls, a single blood sample was collected per woman and the day of collection within the menstrual cycle was recorded [41]. Controls were matched to cases on age, study center and time of day of collection, and phase of the menstrual cycle at blood collection (in 5 categories). Comparisons between case and control hormone levels were based on residuals from spline regression models; the residuals indicated how much an individual's hormone level deviated from the predicted hormone levels on that day. Overall, no association was observed for either estradiol or estrone (top to bottom quartile comparison RR = 1.0 {95% CI=0.7–1.5} for estradiol) (Table 1). Of note, because blood samples were collected across the menstrual cycle, the investigators had relatively limited ability to evaluate associations within specific parts of the cycle.

In the second large prospective study, conducted within the Nurses' Health Study II (NHSII), both early follicular (day 3–5) and mid-luteal (7–9 days prior to next cycle) samples were collected from each woman. Timing of the luteal sample collection was by backward dating from the onset of the next menstrual cycle. The analysis included 197 cases (*in situ* and invasive

combined) with 394 controls matched on age, menopausal status, ethnicity, luteal day, date and time of blood draw, and fasting status. Follicular, but not luteal, total and free estradiol were significantly associated with breast cancer risk (top to bottom quartile comparison RR = 2.1 {95% CI=1.1–4.1} for follicular total estradiol) (Table 1). Associations were stronger among the 89 ER+/PR+ cases (similar comparison RR = 2.7 {95% CI=1.2–6.0} for follicular total estradiol). No association was observed with either estrone or estrone sulfate (in either phase of the cycle).

Testosterone

As with estrogens, few prospective studies have evaluated the association between circulating testosterone and breast cancer. Of the 5 prospective studies published to date, 3 had 65 or fewer cases; in these studies non-significant positive [42] or null [36,39] associations with testosterone were reported. Again, confidence intervals were wide.

In the large EPIC cohort, with 370 invasive breast cancer cases and 726 controls, significant positive associations were observed between circulating levels of testosterone and risk of breast cancer [41]. The RRs (95% CI) with increasing testosterone level (in quartile categories) were 1.0, 1.4 (1.0–2.1), 1.4 (0.9–2.0), and 1.7 (1.2–2.6) (p-trend=0.01) (Table 2).

In the NHSII, with 197 cases (including both *in situ* and invasive disease) and 394 controls, modest, but not statistically significant, positive associations were observed for testosterone (in both the follicular and luteal phase); the associations, particularly for follicular testosterone, did not appear entirely linear. The associations were stronger and statistically significant when restricting to invasive (comparable case group to EPIC study) or ER+/PR+ tumors. For example, in the luteal phase, women in the top (versus bottom) 25% of testosterone levels had a twofold increased risk of invasive cancer (RR=2.0 {95% CI 1.1–3.6}, p-trend=0.05) and a threefold higher risk of an ER+/PR+ tumor (RR=2.9 {95% CI 1.4–6.0}, p-trend=0.02). Findings for free testosterone generally mirrored those for total testosterone.

Progesterone

To date, only 6 prospective studies have examined progesterone levels and breast cancer risk in premenopausal women, with 4 of the 6 studies including 65 or fewer cases [36,37,42,43]. Non-significant inverse associations were observed in 3 of the smaller studies [36,37,39,42], and a non-significant positive association was observed in the fourth [37].

In the large EPIC cohort study, with 285 cases and 555 controls, a significant inverse association was observed between progesterone levels (residuals from spline regression model) and breast cancer risk (top to bottom quartile comparison RR=0.6 {95% CI 0.4–1.0}) [41]. This association was driven by women with samples drawn in the luteal phase, and was only apparent among cases and controls matched by forward dating, not among those matched by the more accurate backward approach. In the second large study, utilizing backward dating with 197 cases and 394 controls, no association was observed between luteal progesterone levels and risk [29].

Conclusion

Although there are few prospective studies of premenopausal testosterone and brest cancer risk, a positive association has been observed consistently with approximate twofold increases in invasive breast cancer risk among women with high levels. The associations between estrogen and progesterone levels in premenopausal women and breast cancer risk have not been consistent, and further assessments are needed. In the only study to detect a significant association with estrogen, follicular, but not luteal, estradiol levels were associated with risk.

It is possible that follicular levels better reflect breast tissue estrogen exposure [44–46] or that estradiol has a greater impact in the low-progesterone environment of the follicular phase [47–51]. This finding was not consistent across estrogens, as no associations were observed with estrone or estrone sulfate. The stronger associations observed with ER+/PR+ tumors is consistent with findings among postmenopausal women, although again this needs to be replicated in future studies. The 2 largest studies also had conflicting findings for progesterone. The importance of timing within the menstrual cycle needs to be resolved since the association was only apparent in EPIC when the less accurate form of menstrual cycle timing was utilized. Thus, while evidence is beginning to accumulate supporting an association between premenopausal sex steroid hormones and breast cancer risk, the nature and magnitude of the associations require further study.

Summary

As cumulative indirect evidence has suggested, sex steroid hormones are important in the etiology of breast cancer. Among postmenopausal women, the associations between estrogens, testosterone and breast cancer risk are consistent and well established. Recent work has helped identify subgroups of women in whom hormone levels appear particularly important (e.g., those with ER+/PR+ tumors) and hormone levels may improve current models used to predict a woman's risk of breast cancer. Premenopausal hormones also appear to play an important role in breast cancer although evidence is not as plentiful nor as consistent hence further research is necessary to elucidate these relationships.

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Table 1

Circulating levels of estradiol and risk of breast cancer: Prospective studies in postmenopausal and premenopausal women.

		RR (95% CI) by category of circulating hormone levels*				
Study	**Cases/Controls**	**1**	**2**	**3**	**4**	**5**
Postmenopausal Women						
EHBCCG¶, 2002	663/1765	1.0	1.4 (1.0–2.0)	1.2 (0.9–1.7)	1.8 (1.3–2.4)	2.0 (1.5–2.7)
Zeleniuch-Jacquotte, 2004**	297/563	1.0	1.6 (1.0–2.7)	1.2 (0.7–1.9)	1.7 (1.0–2.8)	2.5 (1.5–4.2)
Kaaks, 2005	677/1309	1.0	1.1 (0.8–1.5)	1.4 (1.0–2.0)	1.7 (1.2–2.4)	2.3 (1.6–3.2)
Missmer, 2004***	322/643	1.0	1.3 (0.9–1.9)	1.1 (0.7–1.7)	2.1 (1.5–3.2)	
Manjer, 2003	173/438	1.0	1.7 (0.7–1.7)			
Premenopausal Women						
Kaaks, 2005	285/555	1.0	1.0 (0.6–1.5)	1.0 (0.7–1.6)	1.0 (0.7–1.5)	
Eliassen, 2006	185/368	1.0	2.0 (1.1–3.6)	1.7 (1.0–3.2)	2.1 (1.1–4.1)	
Follicular, Luteal	175/349	1.0	1.2 (0.7–2.3)	1.8 (1.0–3.3)	1.0 (0.5–1.9)	

* Hormone data presented in quartiles or quintiles depending on the study, with the exception of estradiol in Manjer et al, where women in approximately the top 20% of estradiol levels were compared to women in the bottom 80% of levels.

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** Extension of study included in EHBCCG analysis; 168 new cases and 316 new controls included here.

*** Extension of study included in EHBCCG analysis; 167 new cases and 333 new controls included here.

Table 2

Circulating levels of testosterone and risk of breast cancer: Prospective studies in postmenopausal and premenopausal women.

		RR (95% CI) by category of circulating hormone levels*				
Study	**Cases/Controls**	**1**	**2**	**3**	**4**	**5**
Postmenopausal Women						
EHBCCG, 2002	585/1574	1.0	1.3 (1.0–1.9)	1.6 (1.2–2.2)	1.6 (1.1–2.2)	2.2 (1.6–3.1)
Zeleniuch-Jacquotte, 2004**	297/562	1.0	1.7 (1.0–2.8)	1.6 (0.9–2.6)	1.9 (1.2–3.2)	2.4 (1.4–4.0)
Kaaks, 2005	668/1280	1.0	1.1 (0.8–1.6)	1.3 (1.0–1.8)	1.6 (1.1–2.2)	1.9 (1.3–2.6)
Missmer, 2004***	312/628	1.0	0.9 (0.6–1.4)	1.5 (1.0–2.2)	1.6 (1.0–2.4)	
Manjer, 2003	154/417	1.0	1.2 (0.7–2.2)	1.3 (0.7–2.3)	1.9 (1.1–3.3)	
Premenopausal Women						
Micheli, 2004	40/108	1.0	1.1 (0.4–3.0)	2.2 (0.6–7.6)		
Kaaks, 2005	370/726	1.0	1.4 (1.0–2.1)	1.4 (0.9–2.0)	1.7 (1.2–2.6)	
Eliassen, 2006	190/374	1.0	1.3 (0.8–2.2)	1.4 (0.8–2.3)	1.3 (0.8–2.4)	
Follicular, Luteal	192/390	1.0	1.3 (08–2.3)	1.4 (0.8–2.3)	1.6 (0.9–2.8)	

*Hormone data presented in quartiles or quintiles depending on the study, with the exception of estradiol in Manjer et al, where women in approximately the top 20% of estradiol levels were compared to women in the bottom 80% of levels.

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