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PLASMA SEX STEROID HORMONES AND BREAST CANCER RISK IN CHINESE WOMEN

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Estrogens regulate the growth and differentiation of mammary cells and play an important role in the development of breast cancer. High circulating levels of estrogens are associated with increased risk of breast cancer in Caucasian women. Because Asian women have low estrogens in the circulation compared with their Caucasian counterparts, the effect of estrogens on breast cancer risk in populations with low circulating estrogens remains to be elucidated. We conducted a population-based case-control study in China to evaluate the association of sex steroid hormones with breast cancer risk in Chinese women. Our study included 300 incident cases with primary breast cancer and 300 age- and menopausal status-matched healthy controls randomly selected from the general population in Shanghai. Fasting blood samples were collected from cases prior to any treatment and from their matched controls. Commercial immunoassays were used to measure plasma concentrations of estradiol, estrone, estrone sulfate, testosterone, progesterone, dehydroepiandrosteindian sulfate (DHEA-S) and steroid hormone-binding globulin (SHBG). Conditional logistic regression analysis was performed to examine the association between steroid hormones and breast cancer risk. The results showed that breast cancer risk was elevated with increasing levels of estrone and testosterone (p for trend < 0.05) but not with DHEA-S, estradiol, estrone sulfate, progesterone or SHBG. The estimated relative risks between upper and lower tertiles were 2.07 (95% confidence interval [CI] 0.97–4.41) for estrone in postmenopausal women, 2.01 (95% CI 0.96–4.21) for testosterone in premenopausal women, and 2.40 (95% CI 1.11–5.21) for testosterone in postmenopausal women, after adjusting for age at first live birth, waist-to-hip ratio, total calorie intake, a history of fibroadenoma, a family history of breast cancer and SHBG. These results, in general, are consistent with the findings in Caucasian women and indicate that high sex steroid hormones in the circulation, both androgen and estrogen, are associated with increased risk of breast cancer even in populations with relatively low sex hormones.

Key words: *steroid hormones; breast cancer; Chinese women; epidemiological study*

Sex steroid hormones, especially estrogens, play an important role in the development of breast cancer. Both *in vitro* and *in vivo* experiments demonstrate that estrogens are strong mitogens for mammary cells[1–4] and are able to stimulate the development and growth of breast tumor in animal models.[5–8] Epidemiologic studies show that early menarche, late menopause, alcohol use, overweight in postmenopausal women and hormone replacement therapy are associated with increased risk for breast cancer.[9–12] Furthermore, high circulating levels of sex steroid hormones, including both estrogens and androgens, are found in prospective cohort studies to be associated with increased risk of breast cancer in postmenopausal women.[13–19] Recently, findings from a chemoprevention trial show that women who use tamoxifen, an antiestrogen agent in the breast, have reduced risk for breast cancer compared with those who do not use the drug.[20]

Most of the epidemiologic studies on estrogen and breast cancer are conducted in Caucasian populations; studies assessing the association in other racial groups are scarce. Because circulating levels of estrogens are substantially lower in Asian women than in Caucasian women,[21–24] the association between circulating sex hormones and breast cancer risk in women with relatively low estrogens remains to be evaluated. To address this issue, we conducted a population-based case-control study of breast cancer among Chinese women in Shanghai, China.

MATERIAL AND METHODS

Study subjects

From August 1996 to March 1998, a population-based case-control study was conducted in Shanghai, China. Our study has been described in detail elsewhere.[25] Briefly, 1,459 incident breast cancer patients aged 25–64 years were enrolled in our study, along with 1,556 healthy control women who had a similar age distribution to the cases based on frequency match. During our study, newly diagnosed breast cancer patients were identified through a rapid case-ascertainment system established for the study in the Shanghai Cancer Institute, which also has hosted a population-based tumor registry in the city since 1972. The cases enrolled in our study represented 91% of the newly diagnosed breast cancer patients identified for our study during the study period. The controls were randomly selected from the general population in Shanghai, using the resident registration information provided by the Shanghai Resident Registry, which registers, under the government mandatory regulation, all permanent residents in urban Shanghai. Before random selection, we first determined the number of controls needed in each age group, a 5-year interval, based on the number of cases in the corresponding age group reported to the Shanghai Cancer Registry in recent years. Once the numbers were determined, potential controls were selected randomly using their resident registration number. After the study eligibility of the identified potential control was confirmed, an in-person interview was scheduled and conducted by a trained interviewer. Of those who were eligible for our study, 90% completed an in-person interview.

The in-person interview was done with the use of a structured questionnaire, which elicited information on demographic features, menstrual and reproductive history, use of sex steroid hormones, medical history, physical activity, alcohol and tobacco use,

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dietary habits and family history of cancer. Of the 1,459 cases and 1,556 controls, morning fasting blood samples were collected from 1,193 cases (82%) and 1,310 controls (84%). The blood samples were processed to separate plasma within 6 hr of collection, and the plasma specimens were immediately stored at −70°C.

For our current study, we used an individually matched study design to enhance the comparability between cases and controls as well as to minimize the variability of laboratory testing from batch to batch (between-assay variation). Of the breast cancer patients whose blood samples were collected before any cancer treatment, successful matches on age (+5 years), date of blood collection (+30 days) and menopausal status (except for 14 pairs) were achieved for 300 case-control pairs, and these subjects were included in our study. Furthermore, for premenopausal women, the cases and controls were also matched individually on their menstrual cycle, which was either within the first 10 days of the menstrual cycle, matching mainly on follicular phase, or within 3 days after the first 10 days, matching either on follicular phase or luteal phase. To evaluate the resemblance of our current study to the parent study, we compared several characteristics of the study subjects between the 2 studies, which included age at first live birth, body mass index (BMI), waist-to-hip ratio (WHR), total energy take, age at menarche, age at menopause, education, family income, parity, history of fibroadenoma and family history of breast cancer. Overall, the 2 study populations were not different in the distributions of these variables, except for the age at menopause, which suggested an improved matching between cases and controls on this variable in the current study. Of the 600 study subjects, 1 plasma sample was not available for our study.

Specimen measurement

Plasma concentrations of testosterone, estradiol, estrone, estrone sulfate, DHEA-S and progesterone were measured directly without extraction. The direct methods have been evaluated recently by Rinaldi *et al.* in comparison with indirect methods with extraction procedures. Findings of the study suggest that the direct methods are reliable and reproducible for epidemiologic studies.[26] Measurement of steroids and SHBG in our study was performed in a reference laboratory at Diagnosed Systems Laboratory Inc. (DSL, Webster, TX). The reference laboratory specializes in *in vitro* diagnostic testing of endocrine markers and is certified by Clinical Laboratory Improvement Amendments (CLIA) and the International Standard ISO 9002. Each sample was tested in duplicate. To reduce measurement variation from batch to batch, samples from matched cases and controls were assayed in the same batch. Technicians who performed the tests did not know the source of the specimens.

Commercial radioimmunoassays (RIA) from DSL were used for the measurement of steroids; an immunoradiometric assay (IRMA) from DSL was used for SHBG. The method intra- and interassay precisions expressed as coefficient of variation (CV) were 6.7–8.1% and 5.7–10.5%, respectively, for testosterone; 3.2–5.3% and 8.1–9.3% for estradiol; 4.4–9.4% and 6.0–11.1% for estrone; 4.6–9.2% and 5.1–8.8% for estrone sulfate; 1.8–5.2% and 4.8–5.3% for DHEA-S; 4.8–8.0% and 9.2–13.1% for progesterone; and 1.1–3.7% and 8.7–11.5% for SHBG. The assay's standard range and sensitivity (in bracket) were 0.1–25 ng/ml (0.05 ng/ml) for testosterone; 20–6,000 pg/ml (4.7 pg/ml) for estradiol; 15–2,000 pg/ml (12 pg/ml) for estrone; 0.025–15 ng/ml (0.01 ng/ml) for estrone sulfate; 50–8,000 ng/ml (14 ng/ml) for DHEA-S; 0.3–60 ng/ml (0.1 ng/ml) for progesterone; and 5–300 nmol/l (3 nmol/l) for SHBG.

Statistical analysis

For the samples that had concentrations below the assay detection limit, we estimated the values using a recommended formula,[27] in which the lowest value detectable by a specific method was divided by the square root of 2. For testosterone, 13% of case samples and 12.8% of control samples were below the detection limit; these percentages were not substantially different ($p = 0.928$). The samples with undetectable estradiol were 29.7% in cases and 29.2% in controls ($p = 0.899$), and the majority of the undetected samples, 78%, were from postmenopausal women. For estrone, the numbers were 19.2% and 15.9%, respectively ($p = 0.289$). The undetected samples were 0.3% and 0% for estrone sulfate, 1.0% and 0.3% for DHEA-S ($p = 0.624$), and 1.0% and 2.0% for SHBG ($p = 0.505$). Since progesterone was measured only in premenopausal women, no samples were found having values below the detection limit.

Given that most steroids had a skewed distribution, median concentrations were compared between cases and controls using the Wilcoxon sign rank test. The Spearman correlation coefficients were calculated to evaluate the correlation between the steroids. The associations of steroids with breast cancer were examined by computing odds ratios (ORs) and their 95% confidence intervals (CI) using a conditional logistic regression model. In the regression analysis, steroids were analyzed as categorical variables. Concentrations of steroids, except for estradiol in postmenopausal women, were grouped into 3 categories (low, medium and high) based on the tertile distribution in the control group. Different cutoff values were used for pre- and postmenopausal women. For testosterone, the cutoffs were 152 and 230 pg/ml for premenopausal women and 129 and 185 pg/ml for postmenopausal women; for estrone, the cutoffs were 23.5 and 38.1 for pre- and 15.8 and 19.5 pg/ml for postmenopausal women. The cutoffs were 703 and 2,499 pg/ml and 630 and 2,548 pg/ml, respectively, for estrone sulfate, 673 and 995 ng/ml and 511 and 746 ng/ml, respectively, for DHEA sulfate, and 58 and 100 nmol/ml and 61 and 93 nmol/ml, respectively, for SHBG. For progesterone and estradiol, only premenopausal women were classified into the tertile groups; the cutoffs were 700 and 3,300 pg/ml and 32.8 and 63.1 pg/ml, respectively. Estradiol values in postmenopausal women were grouped into 2 categories based on the level of detection (under- *vs.* over-detection limit). Previously known breast cancer risk factors were adjusted as potential confounding variables in the logistic regression analysis, which included age at first live birth (year), total calorie intake (kcal), WHR, a fibroadenoma history (yes *vs.* no) and a family history of breast cancer (yes *vs.* no). All statistic tests were based on 2-sided probabilities.

RESULTS

The age distributions were similar between cases (mean 48.5 years, SD = 8.3, range 28–64) and controls (mean 48.5 years, SD = 8.3, range 29–64) ($p = 0.139$), as the 2 groups were matched on age. Postmenopausal women accounted for more than 40% of the study population, 42.8% in cases and 42.9% in controls ($p = 0.804$). Table I shows the comparison of certain characteristics of the study subjects between cases and controls. Cases were slightly older than controls when they gave birth to their first child, 26.6 *vs.* 25.8 years ($p = 0.005$). The cases also had higher BMI, 23.6 *vs.* 23.0 ($p = 0.048$), and higher WHR, 0.81 *vs.* 0.80 ($p = 0.048$), than the controls. More cases than controls had a history of fibroadenoma (9.7% *vs.* 4.0%, $p = 0.006$) and a family history of breast cancer (3.7% *vs.* 1.3%, $p = 0.067$). Also, more cases than controls had early menarche (27.1% *vs.* 23.7%) and late menopause (55.5% *vs.* 50.4%), although the differences were not statistically significant (Table I). The 2 groups did not differ in education, family income, total calorie intake and number of parity.

Table II shows the range and median level of steroids by menopausal status in the study subjects. Breast cancer patients had significantly higher plasma concentrations of testosterone, estrone and DHEA-S than controls ($p < 0.05$); plasma levels of estradiol, estrone sulfate and SHBG were not significantly different between the 2 groups. These findings were consistent in pre- and postmenopausal women. In premenopausal women, progesterone levels were not different between cases and controls ($p = 0.489$). Testosterone was positively correlated with estrone and DHEA-S both in pre- and postmenopausal women and with estradiol only in premenopausal women but not with estrone-S, progesterone, or

TABLE I – COMPARISON OF CHARACTERISTICS BETWEEN CASES AND CONTROLS

Variable	Case (n = 300)	Control (n = 300)	P-value[1]
Age at first live birth (year)[2] (mean [SD])	26.6 (4.2)	25.8 (4.1)	0.005
BMI(kg/m^2) (mean [SD])	23.6 (3.3)	23.0 (3.5)	0.048
Waist-to-hip ratio (mean [SD])	0.81 (0.05)	0.80 (0.06)	0.048
Total energy intake (calorie) (mean [SD])	2,277 (507)	2,234 (474)	0.275
Age at menarche (year) (%)			
<14	27.1	23.7	
14–15	39.8	40.3	
≥16	33.1	36.0	0.587
Age at menopause (year)[3] (%)			
<49	44.5	49.6	
≥49	55.5	50.4	0.415
Education (%)			
Elementary or less	15.0	17.0	
Middle school	42.7	41.3	
High school	29.3	30.0	
College or higher	13.0	11.7	0.879
Family income (%)			
Low	32.7	29.0	
Middle	29.7	32.7	
High	37.7	38.3	0.576
Number of parity (%)			
1	61.1	59.0	
2	25.6	27.8	
3+	13.3	13.2	0.839
Fibroadenoma (%)			
No	90.3	96.0	
Yes	9.7	4.0	0.006
Family history (%)			
No	96.3	98.7	
Yes	3.7	1.3	0.067

[1]Age at first live birth; BMI, waist-to-hip ratio and total energy intake are from paired Student's t test. Age at menarche, age at menopause, education, family income, number of parity, fibroadenoma and family history are from χ^2 test. –[2]Parous women among those with natural menopause.

TABLE II – COMPARISON OF STEROID HORMONES AND SHBG BETWEEN CASES AND CONTROLS

Variable	Case		Control		p*
	No.	Median (range)	No.	Median (range)	
Premenopausal women					
Testosterone (pg/ml)	171	209 (35–655)	170	189 (35–591)	0.022
Estradiol (pg/ml)	171	41.1 (3.3–1,023.9)	170	45.3 (3.3–339.3)	0.969
Estrone (pg/ml)	170	32.8 (0.1–706.3)	169	28.9 (0.4–157.9)	0.016
Estrone-S (pg/ml)	171	1412 (51–9,900)	170	1408 (54–9,921)	0.336
DHEA-S (ng/ml)	171	907 (30–2,687)	170	826 (10–2,788)	0.019
SHBG (nmol/L)	170	71 (1.4–390)	168	74 (1.4–247)	0.488
Progesterone (pg/ml)	169	1,200 (300–87,400)	169	1,200 (300–28,500)	0.489
Postmenopausal women					
Testosterone (pg/ml)	126	198 (24–3,550)	128	161 (35–3,250)	0.002
Estradiol (pg/ml)	126	5.7 (1.3–84.2)	128	5.7 (3.3–54.6)	0.950
Estrone (pg/ml)	125	19.6 (0.8–215.4)	126	17.6 (0.8–325.5)	0.003
Estrone-S (pg/ml)	125	1145 (68–8,012)	128	978 (7–17,600)	0.439
DHEA-S (ng/ml)	126	682 (10–3,385)	128	611 (10–1,839)	0.025
SHBG (nmol/L)	128	73.4 (1.9–573)	128	75 (2.7–269)	0.908

*Wilcoxon signed rank test.

SHBG (data not shown). Positive correlations were also found between estradiol and estrone both in pre- and postmenopausal women and between estradiol and estrone sulfate and progesterone in premenopausal women (data not shown).

The associations between steroid hormones and breast cancer risk are shown in Table III. Plasma levels of testosterone were significantly associated with breast cancer risk both in pre- and postmenopausal women. With increasing testosterone levels in the circulation, there was a significant trend for an increase in breast cancer risk. The disease risk was doubled between women with upper and lower tertiles of testosterone, and the significant association was sustained when other risk factors or co-variables, including WHR, age at first live birth, total calorie intake, a fibroadenoma history and SHBG, were adjusted in the analysis. Similar association was also found for estrone, although it was only in postmenopausal women. With increasing circulating estrone, breast cancer risk was elevated significantly (p for trend $<$ 0.05). A 2-fold increase in relative risk was observed among women with high estrone (top tertile) compared with those with low estrone (bottom tertile). However, our study showed no associations for estradiol, estrone-S, DHEA-S and SHBG either in pre- or postmenopausal women (Table III). Also, no association was found for progesterone in premenopausal women (Table III).

DISCUSSION

Estrogens are the major sex steroids responsible for the growth and differentiation of female mammary gland; these hormones are

TABLE III – ASSOCIATIONS OF BREAST CANCER WITH STEROID HORMONES IN CHINESE WOMEN BY MENOPAUSAL STATUS

Variable	Premenopausal women			Postmenopausal women		
	No. Control/ case	Unadjusted OR[2] (95% CI[3])	Adjusted OR[2] (95% CI[3])	No. Control/ case	Unadjusted OR[2] (95% CI[3])	Adjusted[1] OR[2] (95% CI[3])
Testosterone (tertile)						
Low	57/47	1.00	1.00	44/37	1.00	1.00
Medium	57/52	1.21 (0.66–2.22)	1.21 (0.62–2.38)	43/20	0.56 (0.26–1.23)	0.58 (0.26–1.36)
High	56/72	1.92 (1.00–3.65)**	2.01 (0.96–4.21)*	43/70	2.08 (1.06–4.09)**	2.40 (1.11–5.21)**
Estradiol (tertile)						
Low[4]	57/66	1.00	1.00	73/68	1.00	1.00
Medium[5]	57/50	0.78 (0.47–1.30)	0.74 (0.42–1.32)	57/59	1.20 (0.60–2.38)	1.43 (0.65–3.13)
High	56/55	0.82 (0.49–1.38)	0.84 (0.47–1.50)			
Estrone (tertile)						
Low	57/43	1.00	1.00	43/39	1.00	1.00
Medium	56/61	1.46 (0.80–2.65)	1.69 (0.88–3.27)	43/23	0.71 (0.33–1.51)	0.65 (0.28–1.52)
High	56/66	1.50 (0.84–2.66)	1.53 (0.82–2.86)	42/64	1.93 (1.01–3.72)**	2.07 (0.97–4.41)**
Estrone sulfate (tertile)						
Low	57/54	1.00	1.00	44/41	1.00	1.00
Medium	57/59	1.14 (0.65–1.99)	1.05 (0.56–1.99)	43/43	1.00 (0.53–1.87)	0.93 (0.47–1.87)
High	56/58	1.19 (0.69–2.07)	0.97 (0.52–1.81)	43/42	1.00 (0.47–2.12)	0.80 (0.34–1.89)
DHEA-S (tertile)						
Low	57/48	1.00	1.00	44/35	1.00	1.00
Medium	57/56	1.09 (0.63–1.87)	1.16 (0.64–2.09)	43/39	1.01 (0.56–1.84)	0.97 (0.50–1.90)
High	56/67	1.51 (0.86–2.66)	1.20 (0.65–2.25)	43/53	1.68 (0.88–3.18)	1.76 (0.87–3.59)
SHBG (tertile)						
Low	56/65	1.00		44/44	1.00	
Medium	56/48	0.74 (0.41–1.32)		43/42	1.02 (0.52–2.00)	
High	56/57	0.83 (0.44–1.57)		43/43	0.98 (0.49–1.93)	
Progesterone (tertile)						
Low	54/43	1.00	1.00			
Medium	58/79	1.77 (0.99–3.16)	2.06 (1.05–4.03)			
High	57/47	0.99 (0.54–1.82)	1.03 (0.52–2.02)			

[1]Adjusted for WHR, age at first life birth, total calorie intake, fibroadenoma and SHBG.–[2]Odds ratio.–[3]95% confidence interval.–[4]For postmenopausal women, this category refers to samples with estradiol levels under detection limit.– [5]For postmenopausal women, this category refers to samples with detectable levels of estradiol.–*$p = 0.05$ for trend test; **$p < 0.05$ for trend test.

synthesized through complex metabolic pathways that involve a large number of metabolic enzymes, regulatory molecules, and other steroid hormones.[28–30] A large body of evidence suggests that estrogens play a pivotal role in breast cancer development.[31] Estrogens have potent mitogenic effects on mammary cells and stimulate the growth of breast tumor.[1–8] High levels of estrogens in the circulation are associated with increased risk of breast cancer;[13–19] blocking the effect of estrogens can reduce the risk of the disease.[20] In our case-control study, we found evidence that estrone was associated with breast cancer risk in Chinese women. Besides estrone, our study also showed an association of the disease with testosterone. These results were consistent with the findings of several cohort studies in Caucasian populations, indicating that the link between sex steroid hormones and breast cancer risk may be similar in both Chinese and Caucasian women, despite that Chinese women have lower estrogens in the circulation than their Caucasian counterparts.

A number of cohort studies have examined sex steroid hormones in relation to breast cancer risk in Caucasian women. Four smaller studies conducted earlier failed to identify any significant associations,[32–35] but 7 more recent studies involving relatively larger numbers of patients did find evidence that sex steroids, including both androgens and estrogens, were associated with breast cancer risk, although the results for specific steroids were not entirely consistent across the studies.[13–19] All 7 large cohort studies found that high circulating estradiol was associated with increased risk of breast cancer. Three of the 4 studies and 2 of the 3 studies that assessed estrone and estrone sulfate, respectively, also found that these estrogens were associated with breast cancer risk. Our study found similar results for estrone but not for estradiol and estrone sulfate. A prospective cohort study conducted in Japanese women found circulating estradiol being associated with breast cancer risk, but the association was seen only with bioavailable estradiol. Total estradiol was not significantly associated with the disease either in pre- or postmenopausal women.[36] This inconsistent finding can be explained by many reasons including those related to the study or study population. It is still unclear whether there is a racial discrepancy between Asian and Caucasian women with respect to their breast cancer risk to be associated with a specific form of estrogen. Further investigation of this issue may help to elucidate the role of estrogen in the disease.

Most of the previous cohort studies were conducted in postmenopausal women; only a few were done in premenopausal women. Thomas *et al.* reported a study in premenopausal women.[37] The study found breast cancer patients having higher estradiol than the controls, but the difference was not statistically significant. The results of our study showed that estrone was significantly associated with breast cancer only in postmenopausal women; no significant association was found for estradiol in either pre- or postmenopausal women. Since circulating levels of estrogen in premenopausal women not only differ significantly from person to person but also vary substantially through the menstrual cycle, our small sample size and less-stringent matching condition on menstruation days may hamper our ability to detect any significant differences in estrogen levels between the study groups. However, we assessed our matching condition among premenopausal women by comparing their progesterone levels and found no significant difference between cases and controls.

Interestingly, all 6 prospective studies, which examined the association of breast cancer risk with androgen, found high circulating testosterone to be associated with increased risk of breast cancer, although 1 study suggested that this association was not independent from estrogens. In our study, we also found a similar association between testosterone and breast cancer risk. Furthermore, the association was observed both in pre- and postmenopausal women and was sustained after other risk factors including estradiol and estrone sulfate (data not shown) were adjusted. The association did become less evident when estrone was adjusted in the analysis (data not shown). Given that testosterone is strongly

correlated with estrogens, interpretation of these findings needs to be cautious. However, no matter whether the association of testosterone with breast cancer is independent from or dependent on estrogens, androgens have been linked to the disease both directly and indirectly in laboratory studies. As a precursor of estrogen, androgens play a critical role in estrogen synthesis;[38] androgens are able to induce mammary cell transformation and to stimulate the growth of breast tumor in animal models.[39,40]

Our study results indicated that DHEA-S levels were higher in breast cancer patients than in controls; increased risk of breast cancer was suggested in both pre- and postmenopausal women with high DHEA-S, although the associations were not statistically significant. The possible link between DHEA-S and breast cancer risk has been observed in several previous studies. Of the 5 cohort studies that examined DHEA-S,[14–16,18,19] 3 found a positive association between circulating DHEA-S and breast cancer risk. Thus, our finding appears to be in agreement with most previous studies, suggesting that high DHEA-S may also be a risk factor for breast cancer in Chinese women. DHEA-S is one of the testosterone precursors and is produced mainly by the adrenal gland. The level of DHEA-S in the blood is used as an indicator of adrenal function with respect to androgen synthesis.[41] The association between DHEA-S and breast cancer risk may provide further support to the link between testosterone and breast cancer risk. Experimental studies showed that DHEA was able to increase the activity of estrogen response element[42] and to stimulate the proliferation of breast cancer cells after being converted to estradiol.[43]

The primary concern of our study was the collection of blood samples from cases after cancer diagnosis. Changes in lifestyle and the presence of breast cancer could affect the level of circulating steroids, which in turn limited our ability to determine the temporal relationship. In our study, the controls were not matched exactly to cases on their menstruation day; this could further limit our ability to detect an association between estradiol and breast cancer risk. To minimize the potential influence of breast cancer on the level of biomarkers, we used blood samples from the patients who had not received any cancer treatment; for most of the patients the samples were collected very soon after diagnosis. Therefore, the potential influence of lifestyle changes on steroid hormones after cancer diagnosis is likely to be small. Furthermore, most cases in our study had an early stage of the disease. The presence of smaller cancer may also have a less significant impact on the level of circulating steroids. Finally, we have used the same samples to evaluate other biomarkers as risk factors for breast cancer, and most of our findings were consistent with existing knowledge or etiologic hypothesis.[44,45] This provides additional assurance for the validity of our study.

In summary, we found evidence in this population-based, case-control study that sex steroid hormones were associated with breast cancer risk in Chinese women. Specifically, high circulating levels of testosterone and estrone were associated with increased risk of breast cancer. These findings, in general, were in agreement with the results of prospective cohort studies in Caucasian populations, suggesting that the role of endogenous sex steroid hormones in breast cancer is same in Chinese women as in Caucasian women even though Chinese have relatively lower sex hormones than Caucasians.

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