Association of Serum Estrone Levels with Estrogen Receptor-positive Breast Cancer Risk in Postmenopausal Japanese Women

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ABSTRACT

Purpose: Several reports have demonstrated the association between high serum estrogens levels and breast cancer risk in postmenopausal women. It is hypothesized that breast cancers arising in postmenopausal women with high serum estrogens levels are more likely to be estrogen receptor (ER)-positive. Thus, we have investigated whether or not high serum estrone (E1) levels are associated with ER-positive breast cancer risk in postmenopausal women.

Experimental Design: A case-control study was conducted on 71 cases (postmenopausal breast cancer patients) and 73 controls (postmenopausal healthy women). Serum E1 levels were examined in their association with breast cancer risk after adjustment for the various epidemiological risk factors. In addition, clinicopathological characteristics of breast cancers arising in the women with high E1 levels were investigated.

Results: Women in the high tertile of E1 levels had a significantly (P < 0.01) increased risk of breast cancer as compared with women in the low tertile [odds ratio (OR), 4.14; 95% confidence interval (CI), 1.44–11.87]. Subset analysis according to the ER status showed that women in the high tertile of E1 levels had a significantly increased risk for ER-positive breast cancer (OR, 23.79; 95% CI, 3.50–161.59) but not for ER-negative breast cancer (OR, 1.45; 95% CI, 0.41–5.15) as compared with women in the low tertile. Tumor size and lymph node status were not significantly different between women in the high tertile and those in the intermediate and low tertiles. But the frequency of low-histological-grade tumors and ER-positive tumors (88 in the intermediate and low tertiles) was significantly different between women in the high tertile and those (69 and 46%, respectively) in the intermediate and low tertiles. In addition, ER levels in ER-positive tumors were significantly (P < 0.05) higher in women in the high tertile (245.3 ± 37.1 fmol/mg protein) than those in the intermediate and low tertiles (134.0 ± 31.3 fmol/mg protein).

Conclusions: Postmenopausal women with high serum E1 levels have a significantly increased risk for ER-positive, but not ER-negative, breast cancer. Breast cancers arising in women with high E1 levels show a high ER positivity as well as high ER content. Measurement of serum E1 levels would be clinically useful in the selection of postmenopausal women who can benefit from prophylactic use of tamoxifen because tamoxifen can prevent ER-positive, but not ER-negative, breast cancer.

INTRODUCTION

Tamoxifen is the only agent cleared by the Food and Drug Administration for prophylactic use to reduce breast cancer risk. It is proposed that tamoxifen is effective in women aged 35–49 with a 5-year Gail risk of 1.67% or greater, in hysterectomized women ages 50 and older with a 5-year Gail risk of 2.5% or greater, and in the nonhysterectomized women ages 50 and older with a 5-year Gail risk of greater than 5.0% (1). The Gail model predicts the breast cancer risk on the basis of a combination of epidemiological risk factors such as age, parity, family history, age at menarche, and breast biopsy history (1).2 Gail model can predict the risk of all breast cancer but not the risk specific to ER4-positive breast cancer. However, from the viewpoint of chemoprevention, it is important to predict the risk of ER-positive breast cancer because the chemoprevention [National Surgical Adjuvant Breast and Bowel Project P-1 Study (NSABP P-1)] trial has shown that tamoxifen is effective in reducing the incidence of ER-positive, but not ER-negative, breast cancer (2). Thus, to conduct chemoprevention with tamoxifen more efficiently, we need to select the women at high risk for developing ER-positive breast cancer, and, for that purpose, a risk factor that can predict the risk of ER-positive breast cancer needs to be developed.

The impact of estrogens on breast cancer etiology has been well established. A positive association between the breast cancer risk and the reproductive factors such as early menarche, late menopause, nulliparity, and postmenopausal obesity seems to

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The abbreviations used are: ER, estrogen receptor; HRT, hormone replacement therapy; E1, estrone; E2, estradiol; OR, odds ratio; CI, confidence interval; BMI, body mass index; SHBG, sex hormone-binding globulin.
indicate that estrogens influence breast cancer risk (3, 4). In addition, evidence linking the circulating estrogen levels to breast cancer risk have also been reported both in the retrospective and in the prospective studies (5–12). Thus, the higher levels of estrogens as well as the longer time of estrogen exposure (high-estrogen milieu) are considered associated with the higher risk of breast cancer. It is speculated that a high-estrogen milieu would stimulate the development of ER-positive breast cancer rather than ER-negative breast cancer because estrogens stimulate the carcinogenesis and development of breast cancer through ER. This speculation seems to be supported by the fact that women with HRT are more likely to develop ER-positive breast cancer (13). Thus, women with high levels of serum estrogens are speculated to be at high risk for ER-positive breast cancer. Although the several reports have demonstrated a significant association between the serum estrogens levels and breast cancer risk, no report has been available until now that has investigated the relationship between the serum estrogen levels and ER-positive breast cancer risk (5–12).

Therefore, in the present study, we have studied the association of serum E1 and E2 levels with the ER-positive breast cancer risk in postmenopausal Japanese women by a case-control study. Clinicopathological characteristics of tumors developing in women with high estrogen levels have also been investigated.

MATERIALS AND METHODS

Cases and Controls. Eligible cases were 71 Japanese postmenopausal breast cancer patients who were consecutively treated with mastectomy or breast-conserving surgery in Osaka University Hospital during the period of March 2000 to October 2001. Histological diagnosis of breast cancer was confirmed in each case (67 infiltrating ductal carcinoma and 4 noninfiltrating ductal carcinoma). Controls were 73 Japanese healthy postmenopausal women who participated in the breast cancer screening program at the affiliated institutes in Osaka and were found to be free from breast cancer by physical examination and mammography. They were consecutively recruited in this study during the period of April 2001 to March 2002. Both cases and controls were required to be older than 55 years and to have passed more than 1 year from the last menstruation. Written informed consent as to the study was obtained from each of the participants. Two cases and five controls had a history of premenopause (yes or no), menarche (12–14 years), parity (first live birth at 12–14 years), and BMI (25–30 years, or nulliparity, age at menarche, age at first live birth or nulliparity, age at menopause, and BMI). These risk factors were categorized as follows; family history of first-degree relatives (yes or no), menarche (±12, 13–14, and ±15 years), parity (first live birth at ±25, 26–29, and ≥30 years, or nulliparity), menopause (±46, 47–50, and ≥51 years), and BMI (<21.0, 21.0 to <23.1, and ≥23.1 kg/m2). The association between serum E1 levels and clinicopathological characteristics of tumors was assessed using the χ2 test. The ER concentrations in tumors were compared between the E1 high and low groups by the Mann-Whitney test. A P of <0.05 was considered significant.

RESULTS

Serum E1 Levels and Breast Cancer Risk. E1 levels were detectable in every case and control (mean, 8.32 pg/ml) but E2 levels were very low and undetectable in as many as 27 (38%) cases and 19 (26%) controls. Even in cases and controls in which E2 levels were detectable, they were mostly distributed near the detection limit (mean, 3.42 pg/ml). Thus, E1 levels were considered not reliable enough to be used for the precise comparison between cases and controls, and were eliminated in further analysis. Only E1 levels were included in the following analysis.

E1 levels (9.56 ± 0.71 pg/ml, mean ± SE) in cases were significantly (P < 0.005) higher than those (7.11 ± 0.52 pg/ml) in controls (Table 1). Subset analysis according to the ER status of tumors showed that E1 levels in cases with ER-positive tumors, but not in cases with ER-negative tumors, were significantly (P < 0.001) higher than those in controls (Table 1).

Controls were divided into the three groups according to the E1 levels [low, <5.0 pg/ml (n = 25); intermediate, 5.0 to

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Serum E1 levels in cases and controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
</tr>
<tr>
<td>Cases</td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>71</td>
</tr>
<tr>
<td>ER-positive</td>
<td>42</td>
</tr>
<tr>
<td>ER-negative</td>
<td>29</td>
</tr>
<tr>
<td>Controls</td>
<td>73</td>
</tr>
</tbody>
</table>

* Adjusted for age and BMI.

a Mean ± SE.

b Compared with controls.

Serum E1 and E2 Analysis. All of the blood samples were obtained at fasting early in the morning, immediately before surgery in cases or before breast examination in controls. The serum was immediately separated by centrifugation and stored at −20°C until use. Serum E1 and E2 levels were measured by radioimmunoassay without a prior separation step. Total E1 and E2 levels were assayed by using the kits provided by Diagnostic Systems Laboratories (DSL-8700; Webster, TX) and by CIS Biointernational (ESTR-US-CT; Cedex, France), respectively, following each manufacturer’s protocol. These assays were a two-step radioimmunoassay using a rabbit polyclonal antibody that is highly specific to E1, or E2. The cross-reactivity of E1 assay with E2 was 1.25% and that of E2 assay with E1 was 0.97%. All of the case and control samples were assayed in duplicate, and the mean value was used for the estrogens levels. The detection limits of E1 and E2 were 1.2 pg/ml and 1.5 pg/ml, respectively.

ER Assay. ER levels in breast cancers were measured by enzyme immunoassay using the kit provided by Abbott Research Laboratories (Chicago, IL). The cutoff value for ER was defined as 13 fmol/mg protein according to the manufacturer’s instructions.

Statistical Analysis. E1 and E2 levels in cases and controls were assessed by the Mann-Whitney test. The relationship between the serum estrogen levels and breast cancer risk was determined using a logistic regression method to obtain the OR and 95% CI, being adjusted for the other epidemiological risk factors such as age, family history, age at menarche, age at first live birth or nulliparity, age at menopause, and BMI. These risk factors were categorized as follows; family history of first-degree relatives (yes or no), menarche (±12, 13–14, and ±15 years), parity (±15 years), parity (±25, 26–29, and ≥30 years, or nulliparity), menopause (±51 years), and BMI (<21.0, 21.0 to <23.1, and ≥23.1 kg/m2). The association between serum E1 levels and clinicopathological characteristics of tumors was assessed using the χ2 test. The ER concentrations in tumors were compared between the E1 high and low groups by the Mann-Whitney test. A P of <0.05 was considered significant.
frequency of tumors was significantly different between women in the high tertile and those in the low tertile, women in the low and the intermediate did not show a risk that was significantly higher than that of women in the low tertile when ER-positive cases were considered. Results of the case-control study on the relationship between E1 levels and breast cancer risk are shown in Table 2 in which ORs were calculated after adjustment for the epidemiological risk factors including BMI. These results strongly suggest that serum E1 levels would be useful as a risk factor for ER-positive breast cancer. In the present study we have identified postmenopausal women with high serum E1 levels as a risk for ER-positive breast cancer because the breast cancer prevention trial (P-1 trial) has demonstrated that tamoxifen reduces the risk of ER-positive, but not ER-negative, breast cancer (2). Although the conventional epidemiological risk factors, such as family history, age at menarche, parity, age at menopause, and BMI, are usually used in the assessment of breast cancer risk, these factors are associated with the risk for breast cancer but not the risk for ER-positive breast cancer. In addition, ER levels in ER-positive tumors were significantly higher in women in the high tertile (24.5 ± 37.1 fmol/mg protein) than in those in the intermediate and low tertiles (134 ± 31.3 fmol/mg protein; Fig. 1).

Table 2  Relationship between serum E1 levels and breast cancer risk

<table>
<thead>
<tr>
<th>E1 level (pg/ml)</th>
<th>Cases (71)</th>
<th>Controls (73)</th>
<th>OR (95% CI)a</th>
<th>OR (95% CI)b</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;5.0</td>
<td>12 (17)</td>
<td>25 (34)</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>≥5.0 to &lt;8.0</td>
<td>16 (23)</td>
<td>24 (33)</td>
<td>1.49 (0.56–3.96)</td>
<td>1.69 (0.55–5.17)</td>
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<tr>
<td>≥8.0</td>
<td>43 (60)</td>
<td>24 (33)</td>
<td>3.70 (1.52–9.02)</td>
<td>4.14 (1.44–11.87)</td>
</tr>
</tbody>
</table>

Table 3  Relationship between serum E1 levels and ER-positive or -negative breast cancer risk

<table>
<thead>
<tr>
<th>E1 level (pg/ml)</th>
<th>Cases</th>
<th>Controls</th>
<th>OR (95% CI)a</th>
<th>OR (95% CI)b</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER-positive</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;5.0</td>
<td>4 (10)</td>
<td>25 (34)</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>≥5.0 to &lt;8.0</td>
<td>9 (21)</td>
<td>24 (33)</td>
<td>2.89 (0.72–11.67)</td>
<td>5.62 (0.80–39.70)</td>
</tr>
<tr>
<td>≥8.0</td>
<td>29 (69)</td>
<td>24 (33)</td>
<td>8.90 (2.51–31.60)</td>
<td>23.79 (3.50–161.59)</td>
</tr>
<tr>
<td>ER-negative</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;5.0</td>
<td>8 (28)</td>
<td>25 (34)</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>≥5.0 to &lt;8.0</td>
<td>7 (24)</td>
<td>24 (33)</td>
<td>1.00 (0.30–3.29)</td>
<td>0.97 (0.25–3.85)</td>
</tr>
<tr>
<td>≥8.0</td>
<td>14 (48)</td>
<td>24 (33)</td>
<td>1.86 (0.64–5.45)</td>
<td>1.45 (0.41–5.15)</td>
</tr>
</tbody>
</table>

<8.0 pg/ml (n = 24); high, ≥8.0 pg/ml (n = 24)). Cases were also divided into the three groups using the same criteria for E1 levels [low, <5.0 pg/ml (n = 12); intermediate, 5.0 to <8.0 pg/ml (n = 16); high, ≥8.0 pg/ml (n = 43)]. Results of the case-control study on the relationship between E1 levels and breast cancer risk were shown in Table 3 in which ORs were calculated after adjustment for the epidemiological risk factors such as age, family history, age at menarche, parity, age at menopause, and BMI. Women in the high tertile showed a significantly (P < 0.01) increased risk of breast cancer as compared with women in the low tertile (OR, 4.14; 95% CI, 1.44–11.87). In addition, we have studied the relationship between E1 levels and ER-positive or ER-negative breast cancer risk (Table 3). Women in the high tertile showed a significantly (P < 0.005) increased risk as compared with women in the low tertile when ER-positive cases were considered (OR, 23.79; 95% CI, 3.50–161.59) but such a significant association was not observed when ER-negative cases were considered.

Serum E1 Levels and Clinicopathological Characteristics of Tumors. Because women in the intermediate E1 tertile did not show a risk that was significantly higher than that of women in the low tertile, women in the low and the intermediate tertiles were combined and then were compared with those in the high tertile in terms of various clinicopathological characteristics. Tumor size and lymph node status were not significantly different between women in the high tertile and those in the intermediate and low tertiles (Table 4). Frequency of tumors with low histological grade (1 + 2) was higher (P = 0.06) in women (88%) in the high tertile than in those (69%) in the intermediate and low tertiles. ER positivity was marginally significantly (P = 0.07) increased in women (67%) in the high tertile than in those (46%) in the intermediate and low tertiles. In addition, ER levels in ER-positive tumors were significantly (P < 0.05) higher in women in the high tertile (24.5 ± 37.1 fmol/mg protein) than in those in the intermediate and low tertiles (134 ± 31.3 fmol/mg protein; Fig. 1).

**DISCUSSION**  
To conduct chemoprevention with tamoxifen efficiently, it seems important to identify the risk factors for ER-positive breast cancer, because the breast cancer prevention trial (P-1 trial) has demonstrated that tamoxifen reduces the risk of ER-positive, but not ER-negative, breast cancer (2). Although the conventional epidemiological risk factors, such as family history, age at menarche, parity, age at menopause, and BMI, are usually used in the assessment of breast cancer risk, these factors are associated with the risk for breast cancer but not the risk for ER-positive breast cancer. In the present study we have identified postmenopausal women with high serum E1 levels as significantly associated with an increased risk for ER-positive, but not ER-negative, breast cancer. Although serum estrogens levels have been shown to be positively correlated with BMI, the association of E1 levels with ER-positive breast cancer risk was consistent after adjustment for the epidemiological risk factors including BMI. These results strongly suggest that serum E1 levels would be useful as a risk factor for ER-positive breast cancer in postmenopausal women. It has been reported that a long-term culture of estrogen-dependent MCF-7 cells and of
T47D cells in the absence of estrogen results in the development of estrogen-independent subclone (14) as well as the disappearance of both ER mRNA and ER protein, whereas estrogen exposure causes an increase in the steady-state ER mRNA levels and a maintenance of the ER protein levels (15). These in vitro experimental data seem compatible with our present findings that high estrogen levels are associated with ER-positive breast cancer risk, i.e., estrogens stimulate the development of ER-positive, but not of ER-negative, breast cancer.

In addition to E1 levels, E2 levels have also been reported to be associated with an increased risk for breast cancer by several investigations in Western countries (10, 12, 16). In the present study, we attempted to measure the serum E2 levels and to study their association with breast cancer risk, but the E2 levels were very low and undetectable in as many as approximately one-third of cases and controls. Our results on the serum E2 levels are essentially consistent with the report of Shimizu et al. (17) that serum E2 levels in postmenopausal Japanese women were significantly lower than those in Caucasian women. It is currently unknown, therefore, whether or not serum E2 levels are associated with ER-positive breast cancer risk, but this important issue should be investigated using a more sensitive method for an E2 assay in the future. We measured total E1 levels in the present study because all of the previous studies had measured total, but not free, E1 levels and had been able to show a significant association between total E1 levels and breast cancer risk (12, 16). Ideally, total E1 and E2 levels and free E1 and E2 levels, as well as SHBG levels, should have been measured in the present study but, because of the limited sample volume from each subject, all of these parameters were not measured and only total E1 and E2 levels were measured. Thus, the relationship of free E1 and SHBG levels with breast cancer risk in Japanese women also still remains to be established.

The Multiple Outcomes ofRaloxifene Evaluation (MORE) Study has clearly revealed that women in the higher E2 levels have a significantly greater risk reduction rate with raloxifene, which is one of the selective estrogen receptor modulators (SERMs), compared with women in the lower E2 levels (18). This observation is consistent with our present findings that women with high E1 levels are at an increased risk for developing ER-positive breast cancers, which can be prevented effectively by raloxifene. Thus, it is expected that tamoxifen also confers a greater benefit on women with high serum estrogens levels in terms of a risk reduction of breast cancer, suggesting the possibility that the serum estrogen level will be a clinically useful marker in the selection of women who can benefit from chemoprevention with tamoxifen.

The limitation of the present study lies in that this is not a prospective study and serum samples were obtained from cases with breast cancer. It is very unlikely, however, that higher E1 levels in breast cancer patients than controls are attributable to the enhanced estrogen production in tumor tissue because most cases had small tumors (stage I, 41%; stage II, 55%) and serum E1 levels were not associated with tumor size. It is also reported that the results of the retrospective studies and the prospective studies conducted in the Western countries are fairly consistent, suggesting that estrogen production in tumor tissue, if any, is very unlikely to influence the serum estrogen level. A study on the comparison of the serum estrogen levels in two samples from the same postmenopausal woman collected at least 1 year apart has shown that the estrogens levels are fairly stable over a long period (19). These results seem to agree with the fact that results of the retrospective studies are mostly consistent with those of the prospective studies.

In conclusion, we have demonstrated that postmenopausal women with high serum E1 levels are significantly associated with an increased risk for ER-positive, but not ER-negative, breast cancer, and that breast cancers arising in women with high E1 levels show a high ER positivity as well as high ER content. These results seem to suggest a possibility that measurement of serum E1 levels would be clinically useful in the selection of postmenopausal women who can benefit from prophylactic use of tamoxifen.

**REFERENCES**


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