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Relationship between the Levels of Serum Thyroid Hormones or Estrogen Status and the Risk of Breast Cancer Genesis in Japanese Women

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ABSTRACT

For the purpose of investigating a possible correlation between the genesis of breast cancer and the levels of serum thyroid hormones or the estrogen status, which is one of the potential risk factors for breast cancer in Japanese women, we measured the percentage of free estradiol (E_2) and the amounts of sex hormone-binding globulin (SHBG) and thyroid hormones in serum samples from Japanese patients with breast cancer ($N = 39$) and normal controls ($N = 36$). The patients were found to have significantly higher free E_2 and significantly lower SHBG than controls. Moreover, the serum levels of free triiodothyronine (FT_3) and free thyroxine (FT_4) were lower in the patients than in controls, while the serum levels of TSH and TBG in the patients were not significantly different from those in controls. The percentage of free E_2 in serum was not significantly correlated with the level of any one of FT_3, FT_4, TSH, and TBG either in the patients or in controls regardless of menstrual status. These results suggest the possibility that the reduction in the serum FT_3 and FT_4 levels, which is independent of changes in the serum level of free E_2, may be one of the risk factors for breast cancer in Japanese women.

INTRODUCTION

Since the early suggestion made by Bulbrook *et al.* that one of the reasons for the lower incidence of breast cancer in Japanese women as compared with Caucasian women might be a higher level of thyroid activity in the Japanese (1, 2), the question of whether thyroid dysfunction plays a certain role in the incidence and progress of breast cancer has been a matter of controversy (3–9).

In collaboration with investigators in England, we have been attempting to find out differences in the distribution of serum E_2[2] (10) and the serum level of FT_4 (11) between Japanese and British women. Recently, we have reported that the elevation of free E_2 in serum may be one of the potential risk factors for the genesis of breast cancer in not only British women but also Japanese women (12).

On the basis of our previous findings, we compared the serum levels of thyroid hormones in Japanese patients with breast cancer and those in normal Japanese women in the present study. Moreover, the correlation between the estrogen status, which is one of the risk factors for breast cancer, and the serum levels of each thyroid hormone in Japanese women was also examined.

SUBJECTS AND METHODS

Subjects. Thirty-nine preoperative Japanese patients with Stage II breast cancer examined at our hospital for a period of 2 years from September 1985 to June 1987, and 36 normal Japanese women (controls) were subjects of this study. All these women had not taken any contraceptive steroids and had no history of thyroid disease. The control women were selected randomly, but with written consent from the age-stratified pools of the normal women who had cleared the two breast cancer screenings performed at our department within 10–24 months before the present study, resulting in 36 control women available for the study. The background data for all subjects of this study showed no significant differences between the patient and control groups (Table 1).

A serum sample (2 ml) was collected from each subject constantly at 7:00–10:00 in the morning, quickly frozen, and stored at −20°C until analysis. The average number of patients or normal women used for blood sample collection was three patients per month, and an overall period of 2 years was needed to obtain all blood samples used. In the case of premenopausal women, serum samples were collected in the follicular phase, *i.e.*, within 10 days after the end of menstruation, to minimize as much as possible the influence of cycle changes in hormone levels. The length of the menstrual cycle of all women in the present study ranged from 26 to 35 days.

Methods. Every assay described below was carried out on a pair of approximately same numbers of serum specimens from patients and controls. Serum FT_3 and FT_4 were measured by the use of the Amerlex-M Free T_3 RIA kit and the Amerlex-M Free T_4 RIA kit (Amersham Int. plc., Buckinghamshire, UK), respectively. The intra-assay coefficient of variation was <7.2% for FT_3 and <5.4% for FT_4. Serum TSH was assayed by using the Amerwell TSH RIA kit (Amersham Int. plc.), and serum TBG was by using the TBG ^{125}I RIA kit (Midori Juji, Chiba, Japan). The amount of SHBG in serum was measured by the SHBG-IRMA (^{125}I) kit (Farmos Diagnostica, Finland). The ratio of free E_2 to total E_2 in each undiluted serum sample was determined by a centrifugal ultrafiltration dialysis technique (12, 13) in the following manner: Each sample (450 μl) was incubated with purified [^{3}H]E_2 (80,000 cpm) and [^{14}C]glucose (8,000 cpm) for 30 min at 37°C. Duplicate aliquots (200 μl each) of the incubates were then centrifuged through a dialyzing membrane at 3,000 × *g* for 60 min at 37°C. The percentage of free E_2 in the sample was estimated by dividing the ratio of [^{3}H]E_2 to [^{14}C]glucose in the ultrafiltrate by the corresponding ratio in 30 μl of the serum retained in the dialyzing membrane. The intra- and inter-assay coefficients of variation of this assay were <5.9 and <7.7%, respectively.

Wilcoxon's nonparametric rank-sum test was used to examine the statistical significance of a difference between two mean values (algebraic and geometric means) regarding $P < 0.05$ as being significant. The correlation between two data groups was assessed by the linear correlation and regression analysis. As no essential difference was observed between the algebraic and geometric means in the result of statistical significance tests, all data obtained were expressed as the algebraic mean ± SD.

RESULTS

In confirmation of our previous finding (12), the mean percentage of free E_2 in serum was found to be significantly ($P < 0.00003$) higher in patients with breast cancer than that in normal controls, and this difference was independent of menstrual status (Table 2).

The mean concentration of SHBG was significantly ($P < 0.00003$) lower not only in the group of all patients but also in premenopausal and postmenopausal patient groups than in

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[2] The abbreviations used are: E_2, estradiol; FT_4, free thyroxine; FT_3, free triiodothyronine; TSH, thyrotropin; SHBG, sex hormone binding globulin; TBG, thyroxine-binding globulin.

Table 1 *Background data of normal controls and patients with breast cancer*

	Premenopausal		Postmenopausal	
	Controls	Patients	Controls	Patients
No. of subjects	23	25	13	14
Age (years)	42.9 ± 4.9	40.7 ± 6.1	64.3 ± 8.6	62.1 ± 6.2
Height (cm)	153.1 ± 5.2	155.0 ± 3.0	149.0 ± 8.0	149.8 ± 5.0
Weight (kg)	48.9 ± 5.9	51.5 ± 6.1	47.8 ± 10.3	50.2 ± 5.8
Obesity (%)[a]	105.0 ± 17.7	106.0 ± 12.1	108.5 ± 17.0	110.8 ± 11.6

[a] Obesity (%) = $\frac{\text{Weight (kg)}}{[\text{Height (cm)} - 100] \times 0.9} \times 100$.

corresponding control groups (Table 2).

As shown in Fig. 1, there was a significant negative correlation between the percentage of free E_2 and the amount of SHBG in either the patient group or the control group at all of menstrual stages.

The mean level of serum FT_3 was consistently lower in patients regardless of menstrual status as shown in Table 2, namely, 6.7 ± 1.0 pg/ml for all controls ($N = 36$) and a significantly ($P < 0.00003$) lower value of 5.6 ± 1.0 pg/ml for all patients ($N = 39$); 6.7 ± 0.7 pg/ml for premenopausal controls ($N = 23$) and 5.6 ± 1.0 pg/ml ($P < 0.00015$) for premenopausal patients ($N = 25$); and 6.5 ± 1.5 pg/ml for postmenopausal controls ($N = 13$) and 5.4 ± 1.0 pg/ml ($P < 0.025$) for postmenopausal patients ($N = 14$).

The mean levels of serum FT_4 in the all patient groups and in premenopausal and postmenopausal patient groups were found to be significantly ($P < 0.0021$, $P < 0.037$, and $P < 0.05$, respectively) lower than those in corresponding controls (Table 2).

The mean serum levels of TSH and TBG were not significantly different between the patient group and the control group without regard to menopausal status (Table 2). The percentage of free E_2 was not significantly correlated with FT_3, FT_4, TSH, or TBG either in the patient group or in the control group regardless of menstrual status (Table 3).

DISCUSSION

The ratio of urinary androsterone (5α) to urinary etiocholanolone (5β) is a sensitive biological marker for thyroid activity (14). According to the results of a urinary steroid assay by Thomas *et al.* (15), the $5\alpha/5\beta$ ratio in normal Japanese women (with a low risk of breast cancer) is significantly greater than that in British women (with a high risk of breast cancer). It must be emphasized, however, that the urinary $5\alpha/5\beta$ ratio and the thyroid function index are not necessarily the measures of the same aspect of thyroid functions (15). To investigate pathological changes in thyroid hormones in the serum of patients with breast cancer, the full biochemical spectra of thyroid functions must be examined using very sensitive assay methods such as radioimmunoassay.

Although the levels of FT_4 in the serum of breast cancer patients have been measured by several investigators, the results reported so far were various, namely normal (3–6), lower (7, 8), or higher (9) than the control level, and no unanimous conclusion has yet been reached about this inconsistency. In this connection, other factors affecting the serum level of thyroid hormones, including concerns about assay procedures as described above, might be taken into account. It should be noted in particular that even if the serum levels of thyroid hormones are increased or decreased in breast cancer patients (7–9), these changes are still within normal ranges. In addition, serum specimens from patients and controls should be assayed simultaneously because of possible intra-assay variations, and laboratory workers must be blinded as to the study status of all specimens. Brinckmeyer *et al.* (16) have reported the elevated level of FT_4 in patients with Stage VIB malignant lymphoma. It is also well known that thyroid hormone levels may change during a preoperative period and on the first postoperative day. In the present study, serum sample collection from patients was carried out immediately after their admission to our hospital, namely preoperatively, and control serum samples were collected from the normal women living in the same geographic region, namely nearby our hospital, on the first visit for cancer screening. Therefore, both breast cancer patients and normal women studied in the present study were judged not to be under such unusual conditions as to free steroid hormones as mentioned above.

The mean levels of FT_3 and FT_4 in the serum of Japanese patients with breast cancer were found to be significantly lower than those in corresponding age-matched controls in the present study. Thomas *et al.* (11) have recently found in seven different ethnic groups that the serum level of FT_4 is significantly and inversely correlated with the risk of breast cancer. It has also been reported that women with early breast cancer are associated with reduced thyroid function (7, 8), and that the mean level of serum FT_4 is significantly higher in mainland Japanese (a relatively low risk group) than in Hawaiian Japanese (a relatively high risk group) (11). On the other hand, Vorherr (17) has stated that thyroid hormones may influence metabolism of estrogen and carcinogens, and hyperthyroidism may be protective against breast cancer genesis. Our present results and the discussion made by Thomas *et al.* (11) and Vorherr (17) strongly suggest that the levels of free thyroid hormones in serum may be crucial for the risk of breast cancer, and the high serum levels of free thyroid hormones may be protective against carcinogenesis.

Elevated levels of serum TSH have been reported in breast cancer patients by several investigators (3–5, 7, 18–20). However, no significant difference in the serum level of TSH was found between the patient and control groups in the present study. This difference may be due to the fact that the levels of

Table 2 *Free E_2, SHBG, and thyroid functions in normal controls and patients with breast cancer*

	All subjects			Premenopausal			Postmenopausal		
Specification	Controls ($N = 36$)	Patients ($N = 39$)	P[a]	Controls ($N = 23$)	Patients ($N = 25$)	P	Controls ($N = 13$)	Patients ($N = 14$)	P
Free E_2 (%)	1.85 ± 0.26[b]	2.33 ± 0.32	<0.00003	1.83 ± 0.27	2.32 ± 0.34	<0.00003	1.89 ± 0.26	2.35 ± 0.28	<0.005
SHBG (nmol/liter)	66.4 ± 20.5	44.5 ± 15.5	<0.00003	68.2 ± 21.7	46.8 ± 17.0	<0.001	63.9 ± 18.4	41.8 ± 11.9	<0.005
Free T_3 (pg/ml)	6.7 ± 1.0	5.6 ± 1.0	<0.00003	6.7 ± 0.7	5.6 ± 1.0	<0.00015	6.5 ± 1.5	5.4 ± 1.0	<0.025
Free T_4 (ng/dl)	1.19 ± 0.23	1.04 ± 0.16	<0.0021	1.19 ± 0.27	1.04 ± 0.15	<0.037	1.18 ± 0.15	1.04 ± 0.19	<0.05
TSH (μunits/ml)	1.05 ± 0.71	1.13 ± 0.76	NS[c]	0.88 ± 0.65	1.14 ± 0.79	NS	1.22 ± 0.71	1.14 ± 0.61	NS
TBG (μg/ml)	20.0 ± 3.9	18.3 ± 3.3	NS	19.5 ± 3.9	18.0 ± 2.4	NS	20.8 ± 3.8	18.8 ± 4.3	NS

[a] Nonparametric Wilcoxon rank-sum test was used to examine the statistical significance of a difference between two data groups.
[b] Mean ± SD.
[c] Not significant ($P \geq 0.05$).

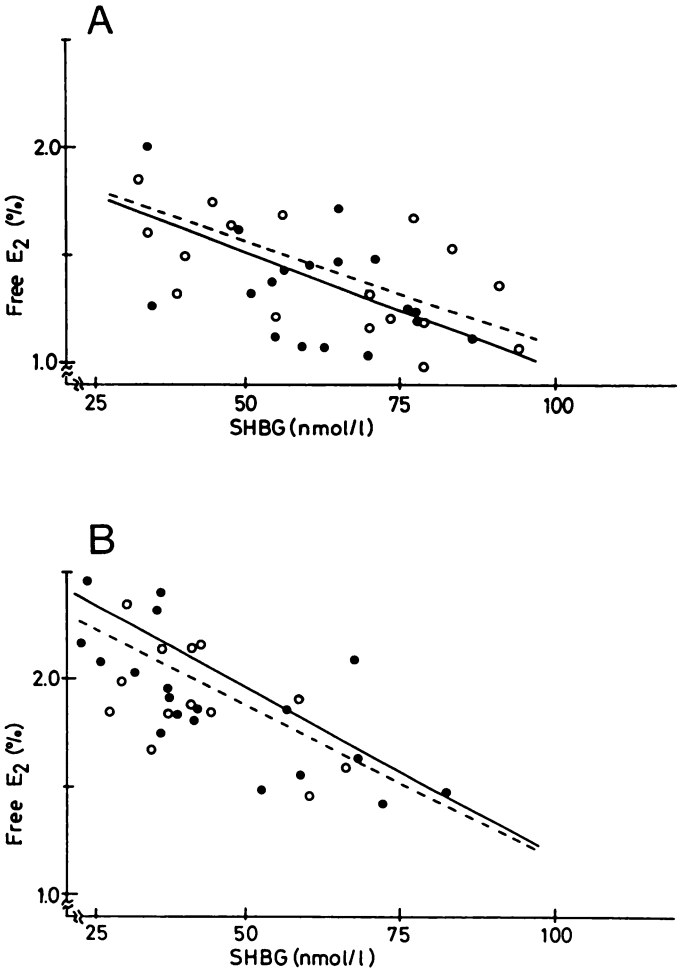

Fig. 1. Relationships between the percentage of free E_2 and the amount of SHBG in the serum of normal controls (*A*) and patients with breast cancer (*B*). ——●——, ——, premenopausal; - - - O - - -, postmenopausal. In *A*, premenopausal ($r = -0.486$, $P < 0.05$), postmenopausal ($r = -0.549$, $P < 0.05$). In *B*, premenopausal ($r = -0.709$, $P < 0.001$), postmenopausal ($r = -0.555$, $P < 0.05$).

Table 3 *Correlations between percentage of free E_2 and thyroid hormones in patients with breast cancer and normal controls*

	Controls				Patients			
	Premenopausal		Postmenopausal		Premenopausal		Postmenopausal	
Hormones	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*
Free T_3	−0.147	0.051	0.512	0.061	0.312	0.147	−0.404	0.135
Free T_4	−0.332	0.122	0.245	0.399	0.228	0.295	−0.192	0.493
TSH	0.100	0.650	0.063	0.830	−0.187	0.353	0.120	0.670
TBG	−0.001	0.999	−0.325	0.257	−0.411	0.076	−0.442	0.097

FT_3 and FT_4 in breast cancer patients were elevated but within the normal ranges. Observed ranges were 4.6–7.1 pg/ml for FT_3 and 0.75–2.10 ng/dl for FT_4.

Recently, we have reported that Japanese patients with breast cancer show significantly higher free E_2 and free testosterone levels than corresponding controls, and the SHBG binding capacity is significantly lower in postmenopausal, but not premenopausal, patients with breast cancer (12). The increase of free E_2 in serum in breast cancer patients was reconfirmed in the present study. However, the amount of serum SHBG was found to be decreased both in premenopausal and postmenopausal patients with breast cancer in the present study. This discrepancy is due to a methodological difference. In the present study, SHBG was assayed by a very sensitive method using a SHBG-IRMA kit, while the SHBG binding capacity was measured in the previous study. This new method also presented not only a better defined difference in the SHBG level between the patient and control groups but also a significant correlation between the percentage of free E_2 and the amount of SHBG in either the patient group or control group.

Since the amount of SHBG in serum is known to be considerably influenced by changes in thyroid function (21–23), we investigated whether the amounts of SHBG in breast cancer patients are correlated with changes in the thyroid function reflected by the serum levels of FT_3, FT_4, TSH, and TBG. No significant correlation was observed (data not shown). This lack of a correlation probably suggests that the changes of FT_3 and FT_4 are within the normal range and are too small for the levels of SHBG to be influenced. The percentage of free E_2 was also not significantly correlated with FT_3, FT_4, TSH, and TBG either in patients or in controls (Table 3). It is inferable from these findings that if thyroid hormones can serve as a determinant of the risk of breast cancer, they might act independently of changes in the free E_2 level. In other words, the thyroid function may have no direct relationship to the estrogen status which is one of the potential risk factors for the genesis of breast cancer, though small changes in the thyroid status may reflect or affect other changes in the endocrine function.

In conclusion, the results of the present study suggest that the condition in which the thyroid function, reflected by the serum levels of FT_3 and FT_4, is lowered but within the normal range may be an important determinant for the risk of breast cancer genesis, at least in Japanese women.

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