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Expression and association of reproductive hormones and receptors in postmenopausal patients with breast cancer

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Summary

Background: Reproductive hormones and receptors play a crucial role in the development and progression of breast cancer. *Materials and Methods:* In the present study, the authors determined expression and association of reproductive hormones and receptors in 352 postmenopausal (PMP) patients with breast cancer. *Results:* The authors found that serum level of luteinizing hormone (LH) declined as PMP years progressed in this patient cohort. The median value of LH was significantly higher in patients within five years of PMP (23 mIU/ml) than patients with PMP beyond five years (18.32 mIU/ml, $p < 0.0001$). The numbers of strong expression of estrogen receptor (ER) and progesterone receptor (PR) were significantly higher in patients within five years of PMP (103 and 93 cases, respectively) than patients with PMP beyond five years (61 and 46 cases, $p = 0.019$ and $p = 0.0005$, respectively). While most patients either lacked (97.1%) or coexpressed (84.3%) both ER and PR, a substantial number of patients expressed either ER or PR alone. Expression of ER and PR was negatively associated with HER2 expression in PMP patients with breast cancer. The present authors observed that increased expression levels of ER and PR were associated with a decline of serum levels of LH or follicle-stimulating hormone (FSH). *Conclusion:* These results indicated that PMP-mediated decrease in LH and FSH serum level was associated with increased expression level of ER and PR, as well as a decreased expression level of HER2 in patients with breast cancer.

Key words: Breast cancer; Postmenopause; Reproductive hormones; Estrogen receptor; Progesterone receptor; HER2 receptor.

Introduction

Breast cancer is the most common cancer and leading cause of cancer death in women worldwide [1]. While the causes leading to its development are unknown, a number of risk factors associated have been identified and include age and elements related to reproductive life [2]. Among those risk factors, hormones such as estrogen and progesterone (P) play an important role in accelerating the growth of breast cancer cells. Studies have shown that cumulative exposure to hormones such as estrogen and P increases the likelihood of breast cancer development [3-5].

Breast cancer cells express estrogen receptor (ER) or progesterone receptor (PR) and about two-thirds of breast cancers are ER and/or PR positive [6]. Breast cancer cells expressing ER or PR require estrogen or progesterone to grow. In addition to ER and PR, receptor tyrosine-protein kinase erbB-2, frequently called HER2, has also been shown to play a crucial role in the progression of breast cancer [7]. Cancer cells expressing increased level of HER2 tend to grow and spread more aggressively than cancer cells lacking HER2 expression [8]. Clinically, positivity status of ER, PR or HER2 alone or in combination is critically involved in the selection of the therapeutic approaches and determines patient outcome in breast cancer [9, 10].

Like other types of cancers, the incidence of breast cancer increases with age. About two out of three invasive breast cancers are found in women 55 years or older, which means that most patients with breast cancer are under postmenopause (PMP). It is unknown whether the duration of PMP affects the levels of hormones and expression of hormone receptors. In the present study, the authors measured serum levels of reproductive hormones, such as luteinizing hormone (LH), estradiol (E2), P, testosterone (T), follicle-stimulating hormone (FSH), and prolactin (PRL) in PMP patients with breast cancer. In addition, expression level of ER, PR, and HER2 as well as p53 have been determined in this cohort of patients.

Materials and Methods

A total of 352 PMP patients diagnosed with breast cancer were included in this study. The diagnosis was performed based on pathological finding from thick-needle biopsy specimens of breast tissues and included ductal carcinoma in situ (DCIS) and invasive. The authors retrospectively extracted and analyzed data of this cohort from October 2007 to October 2010 in this patient database. These data were collected before treatment of chemotherapy, radiotherapy or hormone therapy. All patients were absent of chronic hepatitis and nephritis with a normal liver and renal function. This study was approved by the medical Ethics Committee of Zhejiang Cancer Hospital, China.

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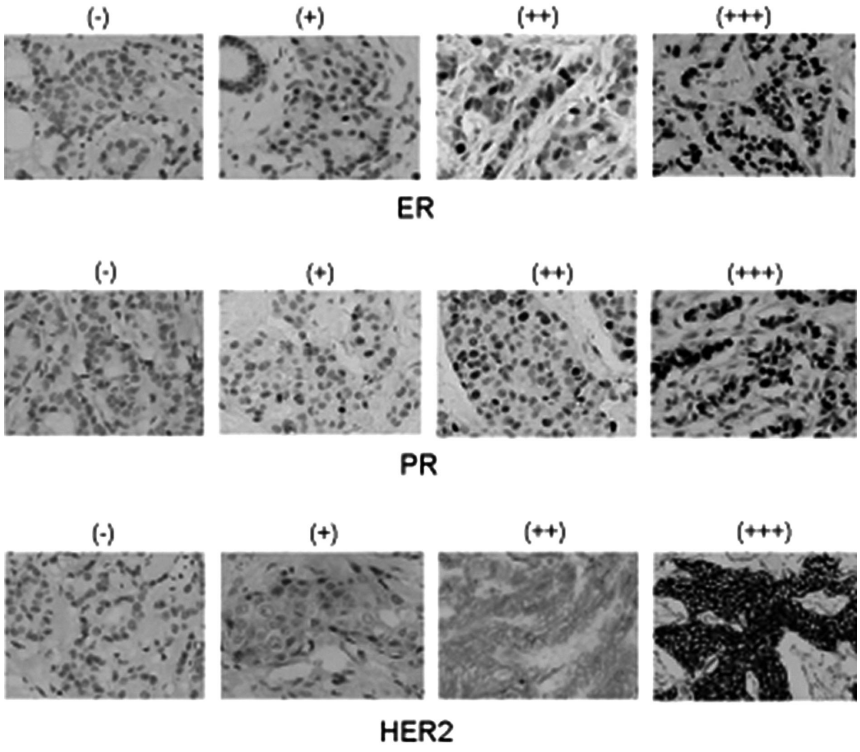

Figure 1. — Expression of ER, PR, and HER2 in PMP patients with breast cancer. Expression levels of ER, PR, and HER2 were determined by immunohistochemistry and assessed by staining intensity from no expression (-) to weak (+), intermediate (++), and strong (+++) staining.

The serum concentration of six hormones (LH, E2, T, P, FSH, and PLT) were measured using chemiluminescence immunoassay kit following the manufacturer instruction for routine laboratory tests.

Tumor specimens less than half hour after surgery were fixed with 4% neutral formalin and embedded in paraffin blocks. The block was sliced into sections of four-μm thick. Immunohistochemistry was performed using Universal DAB Detection kit following the manufacturer instructions. Anti-ER (clone No. SPl) and anti-PR (clone No. PgR636) monoclonal antibodies, and anti-HER2 monoclonal antibody (clone No. 4B5) were utilized.

ER and PR staining was assessed according to the instructions in a reference article "Immunohistochemistry guide for the staining of estrogen and progesterone receptor in breast cancer (2015 edition in Chinese)" with a slight modification (Figure 1). The positivity or negativity of ER or PR expression was defined by percentage of nuclear-stained cells over all tumor cells in entire section. When the number was greater than 10%, the case was defined as positive. If nuclear staining was absent or the percentage of nuclear-staining cells was less than 10%, the case was defined as negative. Positive cases for ER or PR were further divided into three groups based on nuclear staining intensity with pale-yellow as + (weak), brown-yellow as ++ (middle), and dark-brown as +++ (strong).

HER2 staining was assessed according to the instructions in a reference article "Testing guide for the staining of HER2 in breast cancer (2014 edition in Chinese)". HER2 staining in each case was given a score of 0, +, ++ or +++ (Figure 1). The score (0) indicated no staining or an incomplete and weak membrane staining in ≤ 10% of cancer cells. The score (+) indicated an incomplete and weak membrane staining in >10% of cancer cells. The score (++) was given when an incomplete and/or weak to moderate membrane staining was seen in > 10% of cancer cells or if strong and complete membrane staining in ≤ 10% of cancer cells. When a strong and complete membrane staining was found in > 10% of cancer cells, the score was (+++). HER2 was considered negative or positive when the score was (0)/(+) and (+++), respectively. When scored as (++), HER2 positivity was considered as uncertain and further tests such as ISH or genotype were needed to confirm its positivity.

Serum levels of hormones were expressed as median ± standard deviation. Statistical analysis for hormone serum levels was performed using the Student *t*-test. For receptor expression levels, statistical analysis was performed using Chi-square test. The difference was considered to be statistically significant at $p < 0.05$.

Results

A cohort of 352 PMP patients with breast cancer were enrolled in the study and the characteristics of the patients are summarized in Table 1. The median age of this cohort was 57 years, with the majority of patients aged from 50-59 years (58.81%). The numbers of patients aged below 50 years or above 70 years were 1.99% and 11.36%, respectively. Pathologically, 278 of 352 (78.98%) patients were invasive ductal carcinoma, a dominant type of breast cancer, in the present study subject. In this cohort, 58.24% and 48.01% of patients express ER and PR, respectively. The authors observed that the majority of patients (76.7%) were HER2 positive.

The authors first measured serum levels of six hormones (LH, E2, T, P, FSH, and PLT) in PMP patients with breast cancer using CLIA. They arbitrarily divided this patient cohort into four groups based on years of PMP (1-5, 6-10, 11-20, and 21 above). The median value and range of each

Table 1. — *Patient characteristics.*

		n	%
Age (years)	40-49	7	1.99
	50-59	207	58.81
	60-69	98	27.84
	70 above	40	11.36
Pathology	Invasive ductal	278	78.98
	Others	74	21.02
ER expression	+++	124	35.23
	++	40	11.36
	+	30	8.52
	-	147	41.76
PR expression	+++	63	17.90
	++	54	15.34
	+	41	11.65
	-	183	51.99
HER2 expression	+++	77	21.88
	++	100	28.41
	+	72	20.45
	-	82	23.30

Table 3. — *Association between PMP and hormone receptors in patients with breast cancer.*

Hormone receptors		PMP (years) 1-5 (n)	6 above (n)	*p*
ER expression	Negative	74	73	*0.019
	Weak positive	19	11	
	Strong positive	61	103	
PR expression	Negative	89	94	*0.005
	Weak positive	19	22	
	Strong positive	46	93	
HER2 expression	Negative	32	50	0.091
	Weak positive	30	42	
	Strong positive	89	88	
p53 expression	Negative	56	78	0.542
	Weak positive	37	42	
	Strong positive	44	52	
ToPo II expression	Negative	16	32	0.284
	Weak positive	47	51	
	Strong positive	6	6	

* Comparison between group of negative and group of strong positive.

hormone are listed in Table 2. As can be seen, all hormones but E2 had a median serum level within normal range with a nearly zero median value of serum level of P in PMP patients with breast cancer. Consistent with this result, E2 and P had serum level lower than ten pg/ml/ml and 0.1 ng/ml, values as undetectable for respective hormone, in 66.9% (228 of 341) and 22.6% (77 of 340) patients, respectively.

Among six hormones, serum level of LH declined as PMP years progressed in this patient cohort. The median value of LH was significantly higher in patients within five years of PMP (23 mIU/ml) than patients with PMP beyond five years (18.32 mIU/ml, $p < 0.0001$). The statistical difference was also significant when comparing group of PMP 1-5 to either group of PMP 11-20 or 21 above. While the authors did not see a significant difference of serum levels of other hormones in patients with different PMP years, these hormones tended to decline as PMP years progressed.

The authors then determined expression of hormone receptors (ER, PR, and HER2) in this cohort of patients using immunohistochemistry. As shown in Figure 1, expression levels of these hormones receptors varied among patients with expression level from negative to weak positive (+) and strong positive (++ and +++ combined). To simplify the analysis, they arbitrarily divided this patient cohort into two groups based on PMP years (1-5 and 6 above). The numbers of patients expressing variable level of hormone receptors are listed in Table 3. As can be seen, the numbers of strong expression of ER and PR were significantly higher in patients within five years of PMP (103 and 93 cases, respectively) than patients with PMP beyond five

Table 2. — *Association between PMP and hormones in patients with breast cancer.*

HMs	Normal range	PMP (years) 1-5 (n=154)			6-10 (n=77)			11-20 (n=81)			21 above (n=29)			*p*
		Median	Range	n	Median	Range	n	Median	Range	n	Median	Range	n	
LH	10.87-58.64 mIU/ml	23	3.56-57.8	154	19.48	6.13-50.2	77	17.36	1.16-46.1	81	16.87	8.01-32.4	29	*0.0001
E2	20-40 pg/ml	13.5	11-120	55	12	11-22	26	13	11-36	26	10.5	11-17	6	N.S
P	0.00-0.78 ng/ml	0.1	0.1-1.3	123	0.1	0.1-0.6	62	0.1	0.1-0.7	59	0.1	0.1-0.8	20	N.S
T	0.00-0.75 ng/ml	0.44	0.08-4.7	152	0.42	0.13-1.2	77	0.46	0.08-1.4	81	0.39	0.08-1.15	29	N.S
FSH	16.74-113.59 mIU/ml	54.98	3.2-138	154	54.7	0.86-150	77	50.97	5.6-150	81	54.38	27.92-150	29	N.S
PRL	2.74-19.64 ng/ml	13.85	3.52-89.6	153	12.23	4.51-114	77	11.84	0.03-161	81	12.67	6.3-34.32	29	N.S

PMP: postmenopause. HMs: hormones. *Comparison between group of PMP 1-5 and group of PMP 6 above.

Table 4. — *Association between hormones and hormone receptors.*

HMs	ER expression				PR expression				HER2 expression				p53 expression			
	Negative n=151	Weak n=31	Strong n=170	*p*	Negative n=188	Weak n=43	Strong n=121	*p*	Negative n=86	Weak n=75	Strong n=182	*p*	Negative n=140	Weak n=83	Strong n=96	*p*
LH	21.7	21	18.63	0.001	20.99	20.03	19.055	0.008	18.33	17.59	22.05	0.011	20.08	18.73	20.79	0.20
E2	13	15	12	0.99	10	10	10	0.52	14	13	12	0.011	13	12	12	0.87
P	0.1	0.2	0.1	0.38	0.1	0.2	0.1	0.72	0.1	0.1	0.1	0.99	0.1	0.1	0.1	0.80
T	0.41	0.42	0.44	0.06	0.405	0.37	0.49	0.015	0.44	0.37	0.43	0.93	0.425	0.49	0.4	0.58
FSH	55.6	54.84	52.415	0.03	53.9	13.00	51.39	0.031	51.25	49.53	58.44	0.07	54.34	51.86	53.82	0.19
LPR	13.42	14.17	12.6	0.37	13.54	14.71	11.55	0.65	11.62	13.32	13.525	0.18	13.42	12.23	12.32	0.79

p: Comparison between group of negative and group of strong positive.

Table 5. — *Association between hormone receptors.*

		ER				
		-	+	++	+++	*p*
PR	-	168	13	11	16	*0.001
	+	5	10	9	20	
	++	0	5	16	36	#0.001
	+++	0	3	7	54	

		ER				
		-	+	++	+++	*p*
HER2	-	28	11	7	40	*0.001
	+	28	6	8	33	
	++	38	7	20	39	#0.022
	+++	52	7	7	13	

		PR				
		-	+	++	+++	*p*
HER2	-	34	12	16	24	*0.001
	+	36	11	11	16	
	++	53	11	21	17	#0.001
	+++	59	8	7	4	

* Comparison between (-) group and (+++) group.
\# Comparison between (-) group and combined (++/+++) group.

years (61 and 46 cases, $p = 0.019$ and $p = 0.0005$, respectively). The authors did not see a difference of HER2 expression level in breast cancer patients with different PMP years.

In addition to hormone receptors, the authors also determined expression of tumor protein p53 and type II topoisomerase in this patient cohort. After analysis, they did not find a difference of expression of these two molecules in PMP patients with breast cancer.

Next, the authors analyzed whether there was an association between hormones and hormone receptors in PMP patients with breast cancer. As shown in Table 4, patients that had strong ER or PR expression (++ and +++ combined) in tumor displayed significantly reduced serum levels of LH and FSH (18.6 and 19.1 mIU/ml, respectively) when compared to patients that were absent of ER or PR expression (21.7 and 21 mIU/ml, $p = 0.001$ and 0.008, respectively) in tumor. In contrast, they found that patients that had strong HER2 expression in tumor displayed significantly elevated serum levels of LH (22.1 mIU/ml) when compared to patients that were absent of HER2 expression (18.3 mIU/ml, $p = 0.011$) in tumor. They did not find an association between p53 expression and serum levels of any hormone in PMP patients with breast cancer.

The authors performed an analysis to determine whether there was an association between these three hormone receptors in PMP patients with breast cancer. Using a Chi-square test, they found that there was a strong positive association between expression of ER and PR in this patient cohort. As shown in Table 5, the numbers of patients with ER and PR double negative or positive were significantly higher than patients with single negative or positive of ER or PR. Out of 173 patients that lacked ER expression, 168 (97.1%) cases also lacked PR expression. On the other hand, 84.3% (54 of 64) patients that expressed strong PR (+++) also expressed strong ER.

In contrast, the authors found that there was a negative association between expression of HER2 and ER or PR in PMP patients with breast cancer. Patients that lacked ER or PR expression tended to have strong HER2 expression and vice versa. Out of 146 and 182 patients that lacked ER and PR expression, 90 (61.6%) and 112 (61.5%) cases expressed strong ER (++/+++), respectively. On the other hand, out of 79 patients that expressed strong HER2 (+++), only 13 (16.5) and 4 (5.1%) cases expressed strong ER or PR, respectively.

Discussion

Circulating reproductive hormones play an important role in the development and progression of breast cancer. Long-term exposure to high amounts of estrogen in the blood increases risk for women to develop breast cancer [11, 12]. Binding to their receptors, hormones accelerates breast cell proliferation. In the present study, the authors focused on PMP patients to explore association between hormone and hormone receptors. The advantage is that the menstrual cycle-mediated variation of serum hormone levels mini-

mizes in PMP patients. To the best of the present authors' knowledge, this is the first study to comprehensively measure reproductive hormones and receptors in PMP patients with breast cancer in a relatively large cohort.

LH, FSH, and PRL hormones are produced by the pituitary and have effect on ovaries. FSH stimulates ovarian follicles to produce estrogen and LH stimulates the corpus luteum to secret P in premenopausal women. With ovarian atrophy, production of estrogen and P drops over the PMP course. The authors found that serum levels of LH, FSH, and PRL decreased over years after menopause, although the levels of these hormones remain stable in patients with PMP more than 21 years. While some PMP patients remained a normal level of E2 and T, a significant number of patients (66.9% for E2 and 22.6% for T) had undetectable concentration of these hormones, suggesting a clinical indication of estrogen therapy for the patient population.

ER and PR are the most widely studied markers in breast cancer [13] and their expression levels are used as predictive markers of response to endocrine therapy in breast cancer [14]. In this study, the authors determined whether PMP has effect on expression of ER and PR in breast cancer. They found that expression of ER or PR was significantly associated with PMP years in patients with breast cancer. Given an inhibitory effect of the hormones on expression of ER and PR, enhanced expression of ER and PR may be the result of a decline of estrogen and P level in PMP patients.

An interesting finding in the study was that expression of ER and PR was significantly correlated in PMP patients with breast cancer. The numbers of patients with ER and PR double negative or positive were significantly higher than patients with ER and PR single negative. The authors did see a substantial number of patients with a single positive of ER or PR. Nadji *et al.* analyzed a total of 5,993 patients with breast cancer and found no case that was ER negative but PR positive [15]. However, a number of studies have found that expression of ER and PR can be independent each other [16, 17], which is consistent with the present findings.

It is unclear whether hormone levels correlate with ER or PR expression in breast cancer patients. In this cohort, the authors observed a positive correlation between serum hormone levels and ER or PR expression, which is consistent with the finding that estrogen has an inhibitory effect on ER or PR expression [18]. In contrast, hormone levels were higher in patients with HER2 positive than negative, supporting the present finding that expression of ER or PR was reversely associated with HER2 expression.

In summary, in the present study, the authors found that serum levels of hormones decreased over the PMP course in patients with breast cancer. The number of patients expressing strong ER and PR or negative HER2 increased with late years compared to early years of PMP. While most patients either lacked or coexpressed ER and PR, a substantial number of patients expressed either ER or PR alone. Expression of ER and PR was negatively associated with HER2 expression in PMP patients with breast cancer. Increased expression levels of ER and PR were associated with a decline of serum levels of LH or FSH. These results indicated that PMP-mediated decrease in LH and FSH serum level was associated with increased expression level of ER and PR, as well as a decreased expression level of HER2 in patients with breast cancer. Overall, the present findings provided a better understanding about association of hormones and receptors in PMP patients with breast cancer.

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