Increased Igf-I/Igfbp-3 Ratios in Postmenopausal Taiwanese with Breast Cancer, Irrespective of Er and Pr Statuses and Her2 Expression in a Case–Control Study

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Background: In most research, there were positive associations between the insulin-like growth factor I (IGF-I) status, including IGF-I, insulin-like growth factor binding protein-3 (IGFBP-3), and ratio of IGF-I/IGFBP-3, and risks of breast cancer (BC), which was influenced by many factors, including hormone statuses and ethnicity. Therefore, the alterations of the IGF-I status in Taiwanese women with BC by menopausal statuses and hormone receptors were investigated. **Methods:** The levels of IGF-I and IGFBP-3 were determined by the enzyme-labeled chemiluminescent immunometric assay, and the protein expression of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor 2 (HER2) on paraffin-

embedded sections of tissues with BC were analyzed by the immunohistochemical method. **Results:** The ratios of IGF-I/IGFBP-3 were significantly higher in the women with BC than those in the controls, but not of the levels of IGF-I and IGFBP-3; furthermore, the significantly higher ratios were found only in the postmenopausal status. In addition, there was no significant difference between the IGF-I status and ER and PR statuses, and HER2 expression, respectively, in the women with BC. **Conclusions:** The ratios of IGF-I/IGFBP-3 were increased in postmenopausal Taiwanese women with BC, irrespective of their ages, ER and PR statuses, and HER2 expression. J. Clin. Lab. Anal. 30:58–64, 2016. © 2014 Wiley Periodicals, Inc.

Key words: breast cancer; IGF-I; IGF-I/IGFBP-3 ratio; IGFBP-3; menopausal status

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INTRODUCTION

Recently, obesity was shown as a major risk factor for several types of cancer including breast cancer in a review article, and four systems have been identified as potential producers of cancer in obesity: insulin, insulin-like growth factor-I (IGF-I), sex steroids, and adipokines (1). IGFs, comprising IGF-I and IGF-II which share 62% homology in their amino acid sequences, are important mitogens that can modulate cell growth, metabolism, and differentiation (2). IGF-I performs its activity after birth when the liver becomes its main source and under the control of growth hormones (GHs), which stimulates cell proliferation and inhibits cell apoptosis in both normal and neoplastic cells (3). The mitogenic and antiapoptotic bioactivities of IGF-I are mediated through an IGF-I receptor (IGF-IR) (4), and the interaction between IGF-I and IGF-IR is regulated by insulin-like growth factor binding proteins (IGFBPs) (5). In circulation, less than 1% of them are free and most of the IGFs are bound to IGFBPs. In particular, more than 90% of IGFs are bound to IGFBP-3 (6). It has been observed that IGFBP-3 not only modulates IGF-I bioactivity by competitively binding to the IGF-IR, but it also has an IGF-I independent inhibitory effect on cell growth in breast cancer (7).

Although the levels of IGF-I were positively (8–14) associated and the levels of IGFBP-3 were negatively associated with risks of breast cancer (8–13), several studies (15–18) did not support the concepts. The diverse results could be due to the influences of ages and menopausal statuses that were found to have statistically different distribution between BC subtypes of ER, PR, and HER2 on the levels of IGF-I and IGFBP-3 (19, 20).

In addition to the nongenetic factors mentioned above, the study of Rollison et al. (21) demonstrated that both the levels of IGF-I and its relationship with breast cancer were related to racial variations (22). Most previous research in breast cancer were about Western women and prospective studies, which might be different from the results in case-control studies in the Taiwanese population. Therefore, we investigated the alterations of the IGF-I status in Taiwanese women with BC by menopausal statuses and hormone receptors.

METHODS

Subjects

One hundred and forty subjects were selected and divided into two groups (the BC group and controls) in this study: 70 newly diagnosed female patients with histologically verified primary BC at the Kaohsiung Medical University Hospital between March 2005 and June 2006 (ages: 28–69 years, mean: 50.0 years) and 70 healthy females as the controls (ages: 29–70 years, mean: 50.2 years)

TABLE 1. Clinical Characteristics of the Patients with Breast Cancer and the Controls^a

Parameters	Patients (N = 70)	Controls (N = 70)	P-value ^b
Ages (year, mean $\pm$ SD)	50.0 $\pm$ 8.5	50.2 $\pm$ 8.4	>0.05
Menopausal statuses			
Premenopausal	30 (42.9%)	30 (42.9%)	>0.05
Postmenopausal	40 (57.1%)	40 (57.1%)	>0.05
Cancer sites-			
Left breast	37 (52.9%)	-	
Right breast	32 (45.7%)	-	
Bilateral sites	1 (1.4%)	-	
Clinical stages			
In situ	3 (4.3%)	-	
Stage I	27 (38.6%)	-	
Stage II	22 (31.4%)	-	
Stage III	12 (17.2%)	-	
Stage IV	1 (1.4%)	-	
Unclassified	5 (7.1%)	-	
Estrogen receptor			
Positive	35 (56.5%)	-	
Negative	27 (43.5%)	-	
Progesterone receptor			
Positive	33 (53.2%)	-	
Negative	29 (46.8%)	-	
HER2			
Positive	31 (50%)	-	
Negative	31 (50%)	-	
IGF-I status			
IGF-I (ng/ml)	122.1 $\pm$ 331.3	121.4 $\pm$ 28.1	>0.05
IGFBP-3 (μ g/ml)	4.49 $\pm$ 1.23	4.80 $\pm$ 0.82	>0.05
IGF-I /IGFBP-3 ($\times 10^3$)	28.2 $\pm$ 7.0	25.7 $\pm$ 5.9	<0.05

^aResults were expressed as mean $\pm$ SD.

^bP-values represented the differences of parameters between the patients and controls. P-value < 0.05 was considered significant.

who matched to BC patients in ages within 5 years. The characteristics of the study subjects were shown in Table 1. Clinical stages of the 70 breast cancer cases were defined by the criteria of the American Joint Commission on Cancer (AJCC). All the patients did not receive any treatment prior to their participation in this study. This study was approved by the Ethics Committee of the Kaohsiung Medical University Hospital (KMUH-IRB-940225), and the informed consent of the subjects was obtained.

To investigate the differences of the IGF-I statuses between the BC group and controls at the premenopausal and postmenopausal statuses, respectively, we further divided each group into two subgroups by menopausal statuses. In this study, the premenopausal women were defined by their ages, which were less than or equal to 50, and the postmenopausal women were those who were more than 50 years old (23, 24).

To further investigate the IGF-I statuses between the positive and negative expressions of the ER, PR, and HER2 in the women with BC, we divided the BC patients into ER+, ER-, PR+, PR-, and HER2+ and

TABLE 2. The IGF-I Statuses in the Study Subjects^a

Menopausal status	Patients (<i>N</i> = 70)		Controls (<i>N</i> = 70)	
	Pre- (<i>N</i> = 30)	Post- (<i>N</i> = 40)	Pre- (<i>N</i> = 30)	Post- (<i>N</i> = 40)
IGF-I (ng/ml)	125.9 ± 31.3	119.3 ± 31.5	130.0 ± 25.7 ^b	115.0 ± 28.4
IGFBP-3 (μg/ml)	4.39 ± 1.17	4.57 ± 1.28	4.64 ± 0.80	4.91 ± 0.81
IGF-I/IGFBP-3 (×10 ³)	29.7 ± 7.0 ^b	27.1 ± 6.8 ^b	28.3 ± 5.2 ^b	23.7 ± 5.7

^aData were represented by mean ± standard deviation.^bValues significantly differed (*P* < 0.05) from those in the postmenopausal controls.

HER2— by the statuses of the ER, PR, and HER2 protein expressions, respectively.

IGF-I and IGFBP-3 Assays

Blood samples were collected from all participants after an 8-hr fasting period in plain tubes and centrifuged at 2500 rpm for 10 min as soon as they had been collected. The serum was separated immediately and stored at −20°C until used for assay. Stored serum samples were assayed within 1 week after sampling. The IGF-I and IGFBP-3 levels were determined by the solid-phase, enzyme-labeled chemiluminescent immunometric assay by DPC's IMMULITE 2000 (Diagnostic Products Corporation, CA). The coefficients of variation of the IGF-I assay by within-run and between-run at 196 ng/ml were 4.3% and 6.9%, respectively. The coefficients of variation of the IGFBP-3 assay by within-run and between-run at 3.59 μg/ml were 4.2% and 7.2%, respectively.

Immunohistochemistry Analyses for ER, PR, and HER2

Immunohistochemical analyses were performed on paraffin-embedded sections of tissues with BC that were incubated with the anti-human HER2 oncoprotein (DAKO M7269, Carpinteria, California) the anti-human estrogen receptor α (DAKO M7047, Carpinteria, California, USA), and the anti-human progesterone receptor (DAKO M3569), respectively. The score of the HER2 immunohistochemical stain, which was 2+ or 3+, was categorized as HER2+, otherwise as HER2-.

Statistical Analysis

Data were expressed as mean ± standard deviation (M ± SD). Statistical significance of the numerical parameters between the groups or subgroups was assessed by *t*-test. The associations between the numerical parameters and ages were assessed by using simple regression. Values of *P* < 0.05 were considered significant.

RESULTS

Comparison of the IGF-I Statuses Between the Women with BC and Controls Regardless of their Menopausal Statuses (Table 1)

Levels of both IGF-I and IGFBP-3 in the women with BC were not significantly different from those in the controls. However, the ratios of IGF-I/IGFBP-3 in the women with BC were significantly (*P* < 0.05) higher than those in the controls.

Stratified Analyses of the IGF-I Statuses in all of the Subjects by their Menopausal Statuses (Table 2)

In the controls, there were higher levels of IGF-I and ratios of IGF-I/IGFBP-3 in the premenopausal group than in the postmenopausal one, but there was no significant difference in the levels of IGFBP-3. However, IGF-I statuses were not significantly different in the premenopausal BC patients compared with the postmenopausal ones. In the postmenopausal women, the ratios of IGF-I/IGFBP-3 in the BC groups were significantly higher than those in the controls, but not in the premenopausal women. The ratios of IGF-I/IGFBP-3 in the premenopausal BC groups were significantly higher than those in the postmenopausal controls. Neither the levels of IGF-I nor the levels of IGFBP-3 were significantly different between the women with BC and controls in their premenopausal or postmenopausal statuses.

Stratified Analyses of the IGF-I Statuses by their ER and PR Statuses, and their Protein Expression of HER2 in the Women with BC (Table 3)

Because there were no significant differences in the IGF-I statuses between the BC women with premenopausal or postmenopausal statuses, the menopausal status was not taken into consideration when the stratified analysis of the IGF-I statuses by the ER and PR statuses, and the expression of HER2 was conducted. Table 3 showed that the IGF-I status was not significantly different between

TABLE 3. The IGF-I Statuses in the Women with BC by ER and PR Statuses, and Expression of HER2 on Paraffin-Embedded Sections of Tissues with BC

Protein expression	N	IGF-I (ng/ml)	IGFBP-3 ($\mu\text{g/ml}$)	IGF-I/IGFBP-3 ($\times 10^3$)
ER positive	35	123.2 $\pm$ 28.5	4.55 $\pm$ 1.26	28.4 $\pm$ 7.5
ER negative	27	126.9 $\pm$ 36.4	4.63 $\pm$ 1.26	28.0 $\pm$ 6.6
PR positive	33	121.9 $\pm$ 28.0	4.49 $\pm$ 1.23	28.6 $\pm$ 7.9
PR negative	29	128.0 $\pm$ 36.1	4.70 $\pm$ 1.28	27.8 $\pm$ 6.1
HER2 positive	31	124.3 $\pm$ 32.3	4.56 $\pm$ 1.02	28.1 $\pm$ 7.0
HER2 negative	31	125.3 $\pm$ 32.1	4.61 $\pm$ 1.46	28.4 $\pm$ 7.1

the patients with ER+ and ER–, PR+ and PR–, and HER2+ and HER2– BC, respectively.

Correlation Studies between the IGF-I Status and Ages (Fig. 1)

The levels of IGF-I and ratios of IGF-I/IGFBP-3 were negatively correlated with their ages in the controls, but not in the BC patients. However, there was no association between the levels of IGFBP-3 and the ages in either the controls or the BC patients. When we divided the controls and patients into two groups by their menopausal statuses,

we found that the levels of IGF-I and IGFBP-3 were negatively associated with their ages in the postmenopausal controls, but not in the premenopausal controls; however, there was no significant association between the IGF-I status and ages in the women with BC.

DISCUSSION

Based on the mitogenic and antiapoptotic effects of IGF-I on breast cancer cells (21), several epidemiologic studies indicated that a high level of IGF-I increased risks of breast cancer (8–14,25), especially among

Fig. 1. Negative correlation of IGF-I levels (A), IGF-I/IGFBP-3 ratios (B) with ages in the controls; IGF-I levels (C), IGFBP-3 levels (D) with ages in the postmenopausal controls.

premenopausal women (9–14, 25). However, these research were prospective studies, which might be different from the results in case–control studies. Since the presence of a malignant disease itself might affect the IGF-I status, assessment of the IGF-I status from case–control studies might reflect the alteration of the IGF-I status in BC patients rather than the risk assessment of BC (10). In this case–control study, we found that levels of both IGF-I and IGFBP-3 in the women with BC were not significantly different from those in the controls no matter what the menopausal status was, which was consistent with previous studies (16–18, 26–28). In the present study, the ratios of IGF-I/IGFBP-3 were significantly higher in the women with BC than those in the controls regardless of their menopausal statuses. It is consistent with the result of Li (29), which revealed that the high ratio of IGF-I/IGFBP-3 was significantly associated with risks of breast cancer. Recently, a higher IGF-I/IGFBP-3 ratio was found in the women with dense breasts than those with nondense breasts, which inferred that a high IGF-I/IGFBP-3 ratio might increase risks of breast cancer by increasing mammographic density (30). In our results, the ratios of IGF-I/IGFBP-3 in the postmenopausal BC patients were significantly higher than those in the postmenopausal controls, but not in the premenopausal women. About 20% of all cancers are caused by excess weight that is mostly found in postmenopausal women (31); moreover, there is a strong association between obesity and breast cancer. A previous study (32) showed that breast cancer risks were decreased with increasing BMI in premenopausal women, but the risks were increased with increasing BMI in postmenopausal women who did not receive hormone replacement therapy. Additionally, the association of obesity with breast cancer was particularly strong in Asia-Pacific populations (33). Furthermore, visceral fat accumulation leads to lower IGFBP-3 plasma levels (34), and lower IGFBP-3 concentrations lead to a higher IGF-I/IGFBP-3 ratio. In our finding, the ratios of IGF-I/IGFBP-3 in the women with BC were significantly higher than those in the controls, especially in the postmenopausal women with BC.

The IGF-I status could be activated by the complex of estrogen and ER α (35); therefore, estrogen enhanced the mitogenic activity of IGF-I in ER α -positive breast cancer cells (36). Although the positive association between the level of IGF-I and breast cancer risks was confined to ER (+) tumors (13), our result showed that the IGF-I status was not significantly different between the BC patients with different ER statuses, which was consistent with other studies (16, 18). A previous case–control study in Taiwan (37) indicated that the combination of elevated IGF-I levels and prolonged intervals between the age of menarche and age of first full-term pregnancy were associated with significantly increased risks of breast cancer

with ER(+). In another case–control study (38), the levels of IGF-I were not significantly different between HER2+ and HER2– breast cancer cases, but the levels of IGFBP-3 in HER2+ breast cancer cases were significantly higher than those in HER2– breast cancer cases. Our results were consistent with the former but inconsistent with the latter.

Circulating IGF-I, mainly secreted by the liver, reaches the peaks at puberty and declines with age, which may be due to the decreased secretion and sensitivity of growth hormone after puberty (39). Regarding the association of ages and the IGF-I status, we found that there were significantly negative associations between ages and IGF-I levels and ratios of IGF-I/IGFBP-3, respectively, in the controls regardless of their menopausal statuses. Meanwhile, the IGF-I levels and IGF-I/IGFBP-3 ratios were significantly different between the premenopausal and postmenopausal controls. Therefore, menopausal statuses might be considered in the clinical application of the IGF-I levels and IGF-I/IGFBP-3 ratios. In our study, the ratios of IGF-I/IGFBP-3 in the premenopausal BC groups were significantly higher than those in the postmenopausal controls. It may be the outcomes of two causes, namely, breast tumors and the menopausal status. Furthermore, in the stratified analysis, the levels of IGF-I or IGFBP-3 were significantly and negatively correlated with ages in the postmenopausal controls, which was consistent with other previous studies (8, 19), but not in the premenopausal controls. It might be due to the fact that estrogen could induce the expression of IGF-I by acting through the estrogen receptor (40), and the IGF-I bioactivity was modulated by IGFBP-3 (7) in the premenopausal controls.

In conclusion, in the postmenopausal controls, there were significantly negative correlations between ages and the levels of IGF-I or IGFBP-3, but not in the premenopausal controls. However, there was no significant association between ages and the levels of IGF-I or IGFBP-3 in the women with BC regardless of their menopausal statuses. In addition, the ratios of IGF-I/IGFBP-3 in the postmenopausal patients with BC were significantly higher than those in the postmenopausal controls. The IGF-I status was not significantly different between the BC patients with ER+ and ER–, PR+ and PR–, and HER2+ and HER2–, respectively. Therefore, the increased ratios of IGF-I/IGFBP-3 were shown in postmenopausal women with BC irrespective of their ages, ER and PR statuses, and HER2 expression in Taiwan.

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