Association between serum cytokines and progression of breast cancer in Chinese population

Haiyan Wang, MSa,*, Xianlu Yang, MSb

Abstract

The aim of this study was to investigate the roles of serum interleukin-6 (IL-6), IL-8, IL-10, squamous cell cancer antigen (SCC-Ag), and cytokeratin 21-1 fragment (CYFRA 21-1) in the metastasis and prognosis of breast cancer.

A total of 534 breast cancer patients admitted to our department between January 2011 and December 2014 were enrolled in this study. Besides, 452 matched healthy individuals received physical examination at the same period served as the normal control. Serum IL-6, IL-8, IL-10, and tumor necrosis factor-α (TNF-α) were determined using an immunoradiometric assay. SCC-Ag level was evaluated using chemiluminescent microparticle immunoassay. CYFRA 21-1 was determined using the chemiluminescence assay.

Compared with the control group, a significant increase was noticed in the serum IL-6, IL-8, and IL-10 in breast cancer patients, especially those with severe conditions (P < .01). Serum IL-6, IL-8, and IL-10 showed a significant increase in the patients with severe breast cancer compared with those with mild conditions (P < .05). For the patients with response after radiotherapy, the serum IL-6, IL-8, and IL-10 were significantly decreased compared with the baseline levels (P < .05). The median survival duration for the patients of SCC-Ag negative patients was 25 months, while that for the SCC-Ag positive group was 16 months. Significant difference was noticed in the survival of SCC-Ag negative group compared with that of SCC-Ag positive group (P < .05).

The aim of this study was to investigate the roles of serum interleukin-6 (IL-6), IL-8, and IL-10, squamous cell cancer antigen (SCC-Ag), and cytokeratin 21-1 fragment (CYFRA 21-1) in the pathogenesis and prognosis of breast cancer.

Abbreviations: APCs = antigen-presenting cells, CYFRA 21-1 = cytokeratin 21-1 fragment, IL = interleukin, KPS = Karnofsky Performance Status, SCC-Ag = squamous cell cancer antigen, TNF-α = tumor necrosis factor-α.

Keywords: breast cancer, cytokine, metastasis, serum

1. Introduction

Breast cancer is the most common cancer for women in China, with about 21,000 new cases diagnosed and 8000 deaths recorded annually.[1,2] Nowadays, radical resection has been preferred for the treatment of breast cancer. Unfortunately, the outcome of patients is not satisfactory with a 5-year survival rate of less than 30.0% due to recurrence.

Currently, the diagnosis of breast cancer is basically through the imaging techniques such as computed tomography and magnetic resonance imaging.[3] Nowadays, extensive studies have been carried out to investigate the feasibility of serum tumor markers in the diagnosis, prognosis, or clinical management of malignant diseases. Several studies have been performed to investigate the correlation between serum cytokines the pathogenesis of various cancer types.[4,5] For example, interleukin-6 (IL-6), IL-8, and IL-10 were strongly associated with an increased risk of breast cancer.[6] On the contrary, 7 common polymorphisms in genes of inflammatory cytokines including IL-6, IL-8, and IL-10 and tumor necrosis factor-α (TNF-α) were reported to show no roles in the prostate cancer.[7] Besides, studies have been conducted to investigate the feasibility of squamous cell cancer antigen (SCC-Ag), and cytokeratin 21-1 fragment (CYFRA 21-1) in cancer patients, especially those with esophageal cancer.[8] However, rare studies have been carried out to investigate the feasibility of these factors in the pathogenesis and prognosis of breast cancer.

In this study, 534 patients with breast cancer admitted to our department from January 2011 to December 2014 were included. We aim to investigate the roles of serum IL-6, IL-8 and IL-10, SCC-Ag, and CYFRA 21-1 in the pathogenesis and prognosis of breast cancer.

2. Methods

2.1. Patients

A total of 534 patients with breast cancer admitted to our department between January 2011 and December 2014 were enrolled in this study. The diagnosis of breast cancer was based on imaging technique, cytological pathological analysis as previously described. The criteria of exclusion were as follows: those who received radical resection; concurrent with other types of tumor; died from treatment-related complications; and those with a Karnofsky Performance Status (KPS) score of less than 60 showed no tolerance to radiotherapy or the combination of radiotherapy...
and chemotherapy. Patients were divided into 2 groups according to the TNM staging: group I, those with a TNM stage of I or II; group 2, those of TNM stage of III or IV. Besides, 432 matched healthy individuals received physical examination served as the normal control. Written informed consent was obtained from each patient or the guardians. The study is in line with the Declaration of Helsinki, and the protocols were approved by the Ethic Committee of Weihai Maternal and Child Hospital.

2.2. Laboratory analysis

Venous blood was collected from each patient under fasting conditions. Serum tumor markers were determined before and 1 month after treatment. Serum IL-6, IL-8, IL-10, and TNF-α were determined using an immunoradiometric assay as previously described.[9] SCC-Ag level was evaluated using chemiluminescent microparticle immunoassay with the commercial kit purchased from Abbott Laboratories (CA). Cyfra21-1 was determined using the chemiluminescence assay with the kit purchased from Roche (MA). All the procedures were carried out at least in triplicate according to the manufacturer’s instructions.

2.3. Treatment regimen

Three hundred fifty-six patients (66.7%) received radiotherapy, and the other 168 patients (33.3%) received radiotherapy combined with chemotherapy. For the patients who received radiotherapy, the lesions at the deep of the thoracic and abdominal cavity were exposed to 3-dimensional conformal radiation therapy or intensity-modulated radiation therapy using 6-MV X-ray beams. The cervical and supravacular lesions were treated by the mixed irradiation by 6-MV X-ray beams and 9-Mev electron rays with a dosage of 1.8 to 2.0 Gy once (total dosage: 50–74 Gy). For the chemotherapy, patients received 1 to 6 cycles of platinum-based chemotherapy.

2.4. Follow-up and survival analysis

The patients were followed up once every 3 to 6 months within 3 years after the treatment. Three years after treatment, the patients were followed up once every year until January 2016 or death. The survival was defined as the duration from presentation of local recurrence to death. Besides, the survival rates were compared in the patients with negative results in the tumor marker screening and those with positivity. The normal ranges for SCC-Ag and Cyfra21-1 were defined as less than 1.5 and 4 ng/mL, respectively.

2.5. Statistical analysis

All data were presented as mean ± standard deviation. SPSS19.0 software (IBM, NY) was used for the statistical analysis. The survival rates were measured using Kaplan–Meier analysis, and were tested using Logrank analysis. Student t test was used for the comparison between groups. Chi-square test was used for the comparison among groups. Multiple logic regression analysis was utilized for the analysis of prognostic factors. P value of less than .05 was considered to be statistically significant.

3. Results

3.1. Patient characteristics

Five hundred thirty-four patients with breast cancer were included in this study. The patients were aged 39 to 75 years (median: 56 years). For the pathological staging, the number of patients of stage I, II, III, and IV was 98, 114, 126, and 196, respectively. In addition, 138 patients (25.8%) showed recurrence in single site, while 224 patients (41.9%) showed recurrence in multiple sites. Compared with the normal control, no statistical difference was noticed in the age and body weight in the patients with breast cancer (P > .05, Table 1).

3.2. Comparison of serum IL-6, IL-8, and IL-10

Compared with the normal individuals, a significant increase was noticed in the serum IL-6, IL-8, and IL-10 in patients with breast cancer of group I and II, respectively (P < .05). Compared with the serum markers in group I, a significant increase was noticed in the IL-6, IL-8, and IL-10 in patients with severe conditions (P < .05, Table 2).

In this study, we also determined the serum IL-6, IL-8, and IL-10 after radiotherapy and/or chemotherapy. Among the patients with partial response and complete response, a significant decrease was noticed in these patients compared with the baseline levels (P < .05). For the patients with stable disease or progressive disease, a remarkable increase was noticed in the IL-6, IL-8, and IL-10 compared with the baseline levels, while no statistical difference was identified in the IL-10 in the breast cancer patients compared with the baseline levels (Table 3).

### Table 1

| Characteristics | Patient number (percentage) |
|----------------|----------------------------|
| Age, y         |                            |
| <60            | 387 (72.5)                 |
| ≥60            | 147 (27.5)                 |
| TNM stage      |                            |
| I              | 98 (18.4)                  |
| II             | 114 (21.3)                 |
| III            | 126 (23.6)                 |
| IV             | 196 (36.7)                 |
| SCC-Ag level   |                            |
| Low            | 129 (24.2)                 |
| High           | 257 (47.1)                 |
| Cyfra21-1 level|                            |
| Low            | 101 (18.9)                 |
| High           | 138 (25.8)                 |
| CEA level      |                            |
| Low            | 152 (28.5)                 |
| High           | 136 (25.5)                 |

### Table 2

| Group | N  | IL-6, pg/mL | IL-8, pg/mL | IL-10, pg/mL |
|-------|----|-------------|-------------|--------------|
| Control | 7.58 ± 1.25 | 9.98 ± 0.97 | 4.92 ± 0.84 |
| Group I | 68.12 ± 7.59 | 4.46 ± 1.12 | 50.35 ± 12.46 |
| Group II | 87.19 ± 8.91 | 90.64 ± 20.67 | 78.63 ± 15.83 |

| Stage | N  | IL-6, pg/mL | IL-8, pg/mL | IL-10, pg/mL |
|-------|----|-------------|-------------|--------------|
| I     | 98 | 65.34 ± 12.45 | 35.93 ± 9.97 | 45.63 ± 8.11 |
| II    | 114 | 70.25 ± 15.39 | 50.26 ± 17.29 | 55.86 ± 11.27 |
| III   | 126 | 85.23 ± 18.34 | 83.42 ± 19.07 | 76.29 ± 19.93 |
| IV    | 106 | 129.37 ± 26.97 | 100.29 ± 32.74 | 90.47 ± 20.47 |

IL = interleukin.  
* P < .05 vs control.  
† P < .05 vs Group I.
3.3. Comparison of SCC-Ag and CYFR21-1

Significant elevation was noticed in the SCC-Ag and CYFR21-1 in patients compared with those of the normal individuals ($P < .05$, Table 4). Besides, the positive rates of SCC-Ag and CYFR21-1 in the patients were 67.7% and 69.4%, which were higher than that of the CEA (16.1%), respectively ($P < .05$, Table 2). Compared with the SCC-Ag, no statistical difference was observed in the positive rate of CYFR21-1 in the patients.

3.4. Survival analysis

The median survival duration was 12 months after radiotherapy (95% confidence interval (95% CI): 6.98–10.93) or a combination of chemotherapy and radiotherapy (95% CI: 13.07–17.59). The survival rates at 1 year, 2, 3, and 5 years were 44.7%, 18.6%, 13.3%, and 7.8%, respectively.

The median survival duration for the patients of SCC-Ag negative patients was 25 months, while that for the SCC-Ag positive group was 16 months. Significant difference was noticed in the survival of SCC-Ag negative group compared with that of SCC-Ag positive group ($P < .05$). The median survival duration for the patients of CYFR21-1 negative group was 14 months, which was superior to the serum CYFR21-1 positive group with a median survival duration of 9 months ($P < .05$).

3.5. Risk factors for overall survival in breast cancer

In this study, multivariate Cox regression analysis was performed to identify the risk factors for overall survival in patients, including age, gender, pathological stage, tumor lesions, IL-6, IL-8, IL-10, SCC-Ag, CYFR21-1, and serum cytokines. Our data indicated that age, gender, tumor lesions, TNM stage, SCC-Ag, and CYFRA 21-1 were not the risk factors for the survival of breast cancer patients (Table 5).

### Table 3

Comparison of serum IL-6, IL-8, and IL-10 in patients before and after treatment.

|       | Group I          | Group II         |
|-------|------------------|------------------|
|       | CR + PR SD + PD | CR + PR SD + PD |
| IL-6  | 30.21 ± 9.88     | 67.52 ± 8.83     |
|       | 33.68 ± 4.61     | 85.51 ± 10.98    |
| IL-8  | 22.74 ± 8.12     | 56.24 ± 10.37    |
|       | 42.19 ± 9.83     | 110.54 ± 17.32   |
| IL-10 | 30.55 ± 9.79     | 50.05 ± 9.98     |
|       | 30.95 ± 6.11     | 77.25 ± 10.94    |

### Table 4

Expression of serum SCC-Ag, CYFR21-1, and CEA in esophageal carcinoma patients with postoperative recurrence.

| Group              | Level, ng/mL | Positive rate (%) | Level, ng/mL | Positive rate (%) |
|--------------------|--------------|-------------------|--------------|-------------------|
| Normal control     | 0.96 ± 0.37  | 0                 | 2.46 ± 1.23  | 0                 |
| Patients           | 3.15 ± 3.43  | 42 (67.7%)        | 6.67 ± 3.83  | 43 (69.4%)        |

CYFRA 21-1 = cytokeratin 21-1 fragment, SCC-Ag = squamous cell cancer antigen.

| $P < .05$, compared with normal control. |

| $P < .05$, compared with serum CEA in recurrence group. |

### Table 5

Multivariate Cox regression analysis for overall survival in patients with breast cancer.

| Characteristics | B       | OR 95% CI | $P$   |
|-----------------|---------|-----------|-------|
| Age, y          |         |           |       |
| <60 vs >60      | −0.432  | 0.649     | 0.344–1.225 | .182 |
| Tumor lesion(s) |         |           |       |
| 1 vs >1         | 0.178   | 1.195     | 0.611–2.337 | .603 |
| TNM stage       |         |           |       |
| I–II vs III–IV  | −0.347  | 0.706     | 0.374–1.335 | .284 |
| SCC-Ag level    |         |           |       |
| <1.5 vs >1.5 ng/mL | −0.688 | 0.503    | 0.235–1.076 | .077 |
| Cyfra21-1 level |         |           |       |
| <4.0 vs >4.0 ng/mL | −0.030 | 0.971    | 0.465–2.027 | .937 |

95% CI = 95% confidence interval, OR = odds ratio.

4. Discussion

Cytokines have been reported to play crucial roles in the immune response to cancer cells. In this study, we aim to investigate the roles of serum IL-6, IL-8, IL-10 and TNF-α, SCC-Ag, and CYFRA 21-1 in the pathogenesis and prognosis of breast cancer. Besides, we also determined their alternations before and after treatment.

IL-6, a pleiotropic cytokine that was initially identified as a T cell derived lymphokine, has been reported to play crucial roles in the immune system and acute-phase responses. To our best knowledge, it is closely involved in several biological processes, including regulating cell growth, apoptosis, and proliferation. For example, it acts as an autocrine growth factor in the pathogenesis of malignancy. Besides, it could regulate the proliferation and secretion of matrix metalloproteinases in ovarian carcinoma SKOV-3 cell line. Moreover, it has been reported to activate several intracellular signaling pathways. For instance, the binding of IL-6 to its receptor could activate the Janus family of kinases bound to the cytoplasmic domain of gp130, which could promote its nuclear transfer and transcriptional function. In this study, elevation of IL-6 was noticed in breast cancer patients, especially in patients of advanced stages. Our results indicated that patients with metastasis showed higher serum IL-6 than those without. Patients with response to treatment showed remarkable decrease of serum IL-6 compared with the baseline levels.

IL-8, a cytokine initially discovered by Yoshimura, involves in inflammation and immunity, and shows tumorigenic and proangiogenic properties. Increased expression of IL-8 and its receptors has been reported in various cancer cells. In a previous study based on primary breast cancer tissues, a high expression of IL-8 was correlated with the angiogenesis process that was essential for tumor growth and progression. Besides, IL-8 was correlated with the increased proliferation of cancer cells. The role of IL-8 in cancer progression was reported to be associated with lymph node positive status, higher TNM staging, as well as the hormone receptors. In this study, IL-8 expression was correlated with the angiogenesis process that was essential for tumor growth and progression. Besides, IL-8 was correlated with the increased proliferation of cancer cells.
tumors. It has been considered as a cytokine of Th2 response, and is able to suppress the antigen-presenting cells (APCs). In cancer cells, the expression of IL-10 was elevated, which can down-regulate the cytokines of cancer and finally attenuate the phagocytosis of the macrophages. Furthermore, IL-10 could attenuate the apoptosis of small cell lung cancer.\(^{[20]}\) Our results showed that the concentration of serum IL-10 was significantly higher in breast cancer patients with severe conditions than the normal control and patients with moderate conditions. Besides, the expression of IL-10 showed reduction in patients with responses after treatment.

Serum cancer biomarkers levels were reported to be related to the postoperative pathological stage. Simultaneously, the levels of serum SCC-Ag and CYFRA21-1 in late-stage patients were higher than those of the early-stage patients.\(^{[21]}\) To our best knowledge, a higher recurrence has been frequently reported in patients of late stage, especially those received no chemotherapy and/or radiotherapy. To date, there are still disputes on the correlation between serum cancer markers and the pathological stages, as well as the patients' survival.\(^{[22]}\) For example, there was a tendency for higher serum CYFRA 21-1 concentrations in advanced T-stage rather than N or M stages in cancer patients.\(^{[23]}\)

In addition, the positive rate of CYFRA21-1 increased with the progression of esophageal cancer with a ratio of 22.2% of pTNM stage 0-IIA and 77.8% pTNM stage IIIB/III; however, SCC-Ag and CEA rates were not correlated to the pTNM stages. On this basis, the authors concluded that CYFRA21-1 elevation contributed to the diagnosis of recurrence in the absence of clinical data and imaging monitoring. Furthermore, Mao et al.\(^{[24]}\) revealed the serum SCC-Ag and CYFRA21-1 were closely related to the TNM staging in the esophageal carcinoma patients (1). According to our data, serum SCC-Ag and CYFRA21-1 were correlated to the TNM staging in breast cancer patients. To be exact, the serum SCC-Ag and CYFRA21-1 in the stage III patients were obviously higher than those in patients of stage I. Similarly, the serum CYFRA21-1 in stage III patients was also higher than that in stage II patients. Our results indicate the monitoring of serum SCC-Ag and CYFRA21-1 is of prime importance in the advanced esophageal cancer patients after radical resection, as the elevation of these markers contributes to the diagnosis of recurrence.

In this study, we explored the roles of serum IL-6, IL-8 and IL-10, SCC-Ag, and CYFRA21-1 in the pathogenesis and prognosis of breast cancer. Our data demonstrated that serum IL-6, IL-8, IL-10, SCC-Ag, and CYFRA 21-1 may serve as potential markers for predicting metastasis and prognosis of breast cancer. Our study may contribute to development of cancer markers for the evaluation of breast cancer metastasis and prognosis.

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