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Effect of combination of radiotherapy and docetaxel on Cripto-1, β-catenin and DBC1 expression in breast cancer patients

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Among all cancer types, blood cancer tops in total number of estimated cases and ranks fourth in fatalities caused by cancer. Early diagnosis and treatment, particularly for breast cancer, may prevent such fatalities to some extent. In this context, here, we have explored the effect of docetaxel combined with radiotherapy on cripto-1, β-catenin and DBC1 expression in breast cancer patients. In this study, a total of 80 breast cancer patients treated in our hospital from May 2017 to May 2020 were selected and divided into 40 patients each in radiotherapy treatment group and docetaxel combination group according to complete randomization method. Patients in both groups were treated with conventional basic therapy, patients with breast cancer were treated with radiotherapy in the radiotherapy treatment group, and patients with breast cancer were treated with docetaxel and radiotherapy in the docetaxel combination group. Serum samples were analyzed by ELISA for tumor markers: carbohydrate antigen CA15-3 (CA15-3), glycoprotein CA125 (CA125), carcinoembryonic antigen (CEA); the protein Cripto-1 (Cripto-1), β-catenin, deletion gene 1 (DBC1) were detected by fluorescence quantitative PCR method (qPCR); clinical efficacy as well as adverse effects were evaluated and the cycle of breast cancer patient survival was counted. Compared with radiotherapy treatment group, the levels of CA15-3, CA125, CEA, Cripto-1, β-catenin and DBC1 in docetaxel combination group were significantly decreased, with statistically significant difference (P <0.05). The overall response rate for breast cancer treatment was higher in the docetaxel combination group compared with the radiotherapy treatment group alone (P <0.05). Median overall survival and survival rate were higher in the docetaxel combination group compared with the radiotherapy treatment group alone (P <0.05). The combination of docetaxel and radiotherapy significantly improved the clinical symptoms of breast cancer patient, the expression level of Cripto-1, β-catenin and DBC1 in patients, which promote the physical recovery of breast cancer patients, prolong the survival cycle, reduce the mortality rate, and have a better effect.

Keywords: Carbohydrate antigen CA15-3, Deletion gene 1, Tumor

Breast cancer, more common in women, may begin in different parts of the breast, such as the ducts, lobules or the connective tissue. Globally, breast cancer ranks among the primary contributors to both the incidence and fatality of all types of cancers. Based on the GLOBOCAN 2020 report on cancer statistics, it stood as the most common diagnosed malignancy, constituting over 11.7% of all cases in females. With 2261419 estimated cases all over the World, breast cancer ranks 1st, and 684996 total number of deaths, ranks 4th among various types of cancers[1,2]. It induces a substantial public health burden, leading to a loss of 14.8 million Disability Adjusted Life Years (DALYs)[3]. In breast cancer, the term breast refers to the body's mammary glands, skin, fibrous tissue and fat together. Breast masses, nipple changes and a series of changes, distant metastasis will occur in the late stage of breast cancer and there will be a great possibility of multi organ changes[4]. Some patients may have recurrence after treatment. Monitoring the physical changes and adjust the lifestyle may reduce the recurrence rate[5].

Breast cancer can be classified pathologically and can be classified into three types, namely, non-invasive, invasive as well as other rare cancers, of which non-invasive carcinoma is classified as early-stage and includes lobular carcinoma *in situ*, intraductal, papillary eczema like breast cancer; invasive carcinoma includes two In the early stage of breast cancer, the clinical symptoms are mainly areolar changes, among which invasive special carcinoma includes adenoid cystic carcinoma, medullary carcinoma and apocrine carcinoma, etc., and invasive non-special carcinoma includes scirrhous carcinoma, medullary carcinoma and adenocarcinoma, etc.[6].

Cripto-1 is a protein that plays a role in embryonic development and has been implicated in various cancers, including breast cancer. Its expression is associated with tumor progression and poor prognosis. β-catenin is a protein involved in cell adhesion and the Wnt signaling pathway. Dysregulation of β-catenin is associated with several cancers, including breast cancer. Abnormal activation of the Wnt pathway can contribute to tumor development. DBC1 is a protein that has been studied for its potential role in breast cancer. It is thought to function as a tumor suppressor, and its down regulation has been associated with breast cancer development.

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Docetaxel is a chemotherapy drug used in the treatment of breast cancer. It belongs to a class of drugs called taxanes and works by interfering with the microtubule structures in cancer cells, preventing their normal breakdown and division. In addition, radiotherapy uses high doses of radiation to target and kill cancer cells. It is often employed in breast cancer treatment, either as part of the initial treatment plan or after surgery to eliminate any remaining cancer cells. Combination of docetaxel and radiotherapy may enhance locoregional control, and thereby help to target and control cancer cells in the breast and nearby lymph nodes more effectively. Also, the combination therapy may decrease the risk of cancer recurrence, especially when used in adjuvant settings after surgery. Adjuvant therapy aims to eliminate any remaining cancer cells and reduce the risk of the cancer coming back. Hence, in this study, we applied both these treatments in combination to get the desired results.

Subjects and Methods

Patients

Eighty cervical cancer patients who were treated in our hospital from May 2017 to May 2020 were selected as the study subjects, and all breast cancer patients were randomly divided into 40 cases each of radiotherapy treatment group and docetaxel combination group by random method, aged 36-67 years, with mean age (43.42 ± 13.78) years, TNM stage: stage III: 16 cases, stage IV: 24 cases; years (range, 38-69), mean age (43.42 ± 12.83) in the docetaxel combination group, TNM stage: stage III: 18 patients, stage IV: 22 patients; the two groups of breast cancer patients were comparable in the above mentioned data sets, and the differences were not statistically significant (P >0.05). After research on breast cancer disease is approved by the Ethics Committee of our hospital, all breast cancer patients and family members of breast cancer patients gave written informed consent.

Diagnostic criteria: We followed the criteria of breast cancer diagnosis and treatment of "Guidelines and Guidelines for the Diagnosis and Treatment of Breast Cancer of the Chinese Anti-Cancer Association (2019 Edition)"[7].

Exclusion criteria: Concomitant with other medical diseases, second recurrence of breast cancer, having other tumors in the body, scalding chest, and drug allergy.

Drug source: docetaxel (Shenzhen Main Luck Pharmaceuticals Inc., SFDA Approval No.: H20052067), specification: 20 mg/nos.

Treatment methods

In the radiotherapy treatment group, the breast cancer patients were treated with radiotherapy: the breast cancer patients were given epirubicin by adding sodium chloride solution diluted 150 mL, intravenously dripping for 1 h over a period of 1-3 days, and 21 days are 1 cycle. A total of 42-days cycle is adopted for chemotherapy. 6 MV was used for X-ray irradiation in the supraclavicular area of the patients, 50Gy in 25 fractions over a period of 35 days, and note that the axillary and inner breast area must not be irradiated. The docetaxel combined with radiotherapy was administered to patients with breast cancer in the docetaxel combination group: 2-3 days before radiotherapy, the patients were given an injection of docetaxel. Once a day, 500 ml of 0.9% saline intravenously was added in 250 mg of docetaxel injection and a skin test was administered before injection and radiotherapy was the same as that of the radiotherapy treatment group.

Tumor markers CA15-3, CA125 and CEA were detected and 4.79 mL fasting venous blood was extracted from all subjects in the morning, centrifuged at a centrifugation radius of 5 cm and a rotation speed of 3000 rpm for 10 min, and the upper serum was separated and stored at –49°C for test. ELISA was used to measure tumor markers CA15-3, CA125, CEA in serum to make standards. Samples were diluted in 1-2 dilutions, standards 100 μL/hole diluted were added to the reaction wells, wet incubation for 2 h and one second in an incubator with constant temperature at 37.04°C. After washing the reaction plate thrice with wash solution, 1:100 fold diluted antibody working solution was added in 100 μL/well and incubated wet for 47.19 min in an incubator with constant temperature at 37.04°C. After continuing to wash the reaction plate twice, TMB solution 100 μL/well was added to the reaction wells and placed again in incubator at the same constant temperature for 47.19 min before adding stop solution 100 μL/well in the reaction well to terminate the reaction. Determine absorbance at 451 nm wavelength, colour reaction deepness is proportional to CA15-3, CA125 and CEA, and CA15-3, CA125 and CEA were calculated by plotting standard curve.

Cripto-1, β-catenin and DBC1 expression detection Cripto-1, β-catenin and DBC1 were measured by fluorescence quantitative PCR in all breast cancer patients, and blood was collected in tubes without anticoagulant. Plasma was aspirated later by placing blood in tubes of EDTA. Immediately after the collection was completed, the drawn blood was put

into the blood sampler after adding buffer, and after mixing for 61 s, the reagent plate was dropped with three drops of the mixture and the inserted and waited 185 s, so the detection results of HAb18G are obtained. Reaction system: 8 μL cDNA template, 10 μL SYBRP Green mix, 2 μL PCR Primer mix. Reaction conditions: 95°C for 10 min; 95°C for 10s; 60°C for 1 min; 40 cycles. β-actin was used as internal reference, and the 2-△△Ct method was used to calculate Cripto-1, β-catenin and DBC1 expression. Cripto-1 primer sequences: upstream primer: CCCAGTCACGACGTTG TAAAACG, down stream primer: AGCGGATAAC AATTTCACACAGG; β-catenin primer sequences: upstream primer: CATTCTGCACGCTTCAAAAG downstream primer: GCTGTCCTCTAAGCGTCACC; DBC1 primer sequences: upstream primer: AACTCGT ACTTTGAACAGGC downstream primer: CAT CGCCTAAAAGGAGCAAG. Internal reference primer sequences: 5'-GAGGATCTTCATGAGGTAGTCA GT-3' and downstream primer: 5'-CTGGCACCACA CCTTCTACAATGAGC-3'.

Evaluation of clinical efficacy

According to WHO, clinical efficacy is classified as complete remission (complete disappearance of breast cancer tumor); partial remission (more than half tumor shrinkage in breast cancer); stable and progression (tumor shrinkage or enlargement in breast cancer no greater than 25% of the tumor itself with/without appearance of new lesions, respectively) criteria for objective response assessment in solid tumors.

Adverse reactions and survival cycle

The patients experienced adverse reactions, such as skin redness and swelling, general fatigue and decreased appetite, during the treatment.

The median survival and survival rate were calculated from the 12-month follow-up of the behavioral phase after the end of treatment for breast cancer patients.

Statistical processing

SPSS20.0 statistical software was used for analysis and processing. The mean ± standard deviation ($\bar{x} \pm s$) of the metrology data was described using the independent samples t-test; the count data were expressed as % frequency, and the χ^2 test was used, with P <0.05 considered statistically significant.

Results and Discussion

Comparison of tumor markers CA15-3, CA125 and CEA

As shown in Table 1, there was no significant difference in CA15-3, CA125 and CEA between the two groups before treatment (P >0.05); CA15-3, CA125 and CEA were lower after treatment than those before treatment, and CA15-3, CA125 and CEA were lower in the docetaxel combination group than those in the radiotherapy treatment group (P <0.05).

Cripto-1, β-catenin and DBC1 comparison

As shown in Table 2, for comparison of Cripto-1, β-catenin and DBC1 levels in the two groups before treatment, there was no significant difference (P >0.05); after treatment, Cripto-1, β-catenin and DBC1 were lower than before treatment and in the docetaxel combination group, Cripto-1, β-catenin and DBC1 were lower than those in the radiotherapy treatment group, all differences were statistically significant (P <0.05).

Comparison of clinical efficacy between breast cancer patients

As shown in Table 3, the overall response rate was higher in the docetaxel combination group than that in the radiotherapy treatment group, with statistically significant differences (P <0.05).

Comparison of occurrence of adverse effects among patients during treatment of breast cancer

As shown in Table 4, the incidence of total adverse events was higher in the docetaxel combination group

Table 1 — Comparison of tumor markers CA15-3, CA125 and CEA before and after treatment ($\bar{x} \pm s$)

Group	No. of cases	CA15-3(U/mL)		CA125(U/mL)		CEA (ng/mL)	
		Before	After	Before	After	Before	After
Radiotherapy treatment	40	10.08±5.02	8.21±4.34	11.98±8.67	10.78±7.85	14.97±7.59	12.01±5.09
Docetaxel combination	40	10.06±5.01	6.19±2.07	11.96±8.64	7.85±4.03	14.95±7.57	10.09±3.30
t		0.017	2.657	0.010	2.100	0.011	2.002
P		0.985	0.009	0.991	0.038	0.990	0.048

Table 2 — Cripto-1, β-catenin and DBC1 comparison ($\bar{x} \pm s$)

Group	No. of cases	Cripto-1		β-catenin		DBC1	
		Before	After	Before	After	Before	After
Radiotherapy treatment	40	2.09±1.87	1.74±0.89	5.65±2.19	3.58±1.66	6.94±3.75	5.69±3.88
Docetaxel combination	40	2.08±1.86	0.12±0.06	5.66±2.20	1.45±0.08	6.97±3.78	3.71±1.05
t		0.023	11.490	0.020	8.106	0.035	3.115
P		0.981	0.001	0.983	0.005	0.971	0.002

Table 3 — Comparison of clinical efficacy between breast cancer patients [n (%)]

Group	No.of cases	Complete remission	Partial remission	Stable	Disease progression	Overall response rate
Radiotherapy treatment	40	20	8	6	6	28 (70.00)
Docetaxel combination	40	29	9	1	1	38 (95.00)
x^2						8.658
P						0.033

Table 4 — Comparison of patient adverse events during breast cancer treatment [n (%)]

Group	No.of cases	Skin redness and swelling	Malaise	Decreased appetite	Overall adverse effect rate
Radiotherapy treatment	40	2	1	1	3 (7.50)
Docetaxel combination	40	3	3	4	10 (25.00)
x^2					4.500
P					0.033

Table 5 — Comparison of survival cycles of breast cancer patients

Group	No. of cases	Median survival (months)	Survival rate
Radiotherapy treatment	40	7.22±3.20	32 (80.00)
Docetaxel combination	40	9.26±5.59	38 (95.00)
t/x^2		2.003	4.114
P		0.048	0.042

than that in the radiotherapy treatment group, with statistically significant differences (P <0.05).

Comparison of survival cycles of breast cancer patients

As shown in Table 5 and Fig. 1, compared with the radiotherapy treatment group, the median survival and survival rate were higher in the docetaxel combination group, with statistically significant differences (P <0.05).

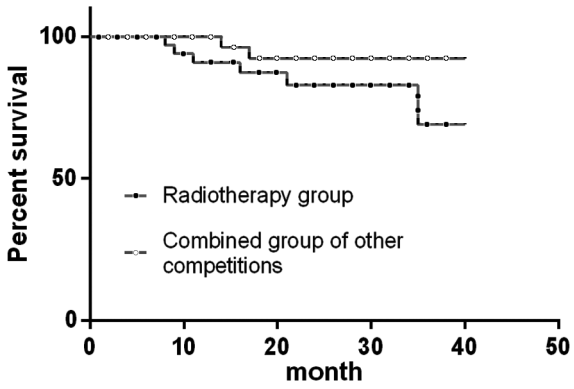

Fig. 1 — Patient survival cycles in the Radiotherapy treatment and Docetaxel combination two groups

Breast cancer is a multiple incidence in women, belongs to malignant tumors. The prognosis of breast cancer is inextricably linked with the clinic, and early diagnosis helps in better treatment[8]. The comprehensive treatment methods for breast cancer are adjuvant chemotherapy, targeted therapy, endocrine therapy in addition to surgical treatment. Removal of the affected breast by surgery will impact the breast-feeding function of the patient, and may lead to sexual dysfunction[9,10]. Cancer cells lose their properties, and form a tendency of metastasis, resulting in pain and discomfort, etc. Early diagnosis helps in targeted treatment. The breast cancer has a certain recurrence, and it is always necessary to monitor patients' own physical changes constantly to avoid the incidence of recurrence. Breast cancer also has a certain local extension as well as lymphatic metastasis and revascularization, in which local extension refers to the cancer cells will invade the skin or ligaments, and lymphatic metastasis is to invade lymph nodes and then invade venous blood flow to metastasize far away. Revascularization refers to the fact that cancer cells can directly invade the blood circulation and then metastasize to distant sites, most commonly to the liver, lung and bone[11]. However, lack of effective clinical treatment for breast cancer keeps the search on.

Radiotherapy is a method that can directly treat cancer, as well as the most common adjuvant treatment for cancer, different tumor tissues, tissues and organs will show different changes after being irradiated by radiotherapy, and different tumor tissues, tissues and organs have different degree of reaction[12]. The less sensitive the cell will be to radiotherapy if it is more differentiated, and the more sensitive the cell will be to radiotherapy if it is less differentiated, but there will be some side effects associated with radiotherapy, such as some local reactions: skin desquamation, ulceration, and respiratory reactions: vomiting, nausea and malaise etc., some patients will also experience damage phenomena from encephalitis, such as flaky hair loss and alopecia[13]. Docetaxel is an antineoplastic agent, most commonly used in the case of chemotherapy failure or breast cancer treatment, the main component of which is docetaxel, a yellow, clear and

yellowish liquid that is viscous[14]. However, it is important to note that patients with breast cancer with concomitant liver impairment cannot use this drug, because it increases mortality and has extremely poor treatment outcomes, but this drug can leave breast cancer patients with a range of adverse effects symptoms, mainly chest tightness, pruritus, erythema, chills, dyspnea, edema, alopecia, and many other adverse effects[15,16]. In this study, we found that compared with breast cancer patients treated with radiotherapy alone, breast cancer patients treated with the combination of docetaxel and radiotherapy had a higher treatment effect than those treated with radiotherapy alone.

There are changes in the level of abnormal expression in breast cancer: carbohydrate antigen CA15-3 (CA15-3), glycoprotein CA125 (CA125), carcino-embryonic antigen (CEA), protein Cripto-1 (Cripto-1), β-catenin, deleted gene 1 (DBC1), all aberrantly expressed at variable levels in breast cancer, can be new tumor markers and are diagnostic criteria for breast cancer[17,18]. The carbohydrate antigen CA15-3 (CA15-3) together with glycoprotein CA125 (CA125), a tumor marker for breast cancer, would show elevated aberrant expression in breast cancer, gastric cancer, ovarian cancer, decreased CA15-3 and CA125 levels in patients after receiving the right treatment[19,20]. Carcino-embryonic antigen (CEA) can react to the presence of a variety of tumors, but the sensitivity is not high enough and not strong enough, but it can be used as a diagnosis for tumors, and it has some judgment in breast, colorectal, and lung cancers[21]. The protein Cripto-1 (Cripto-1), β-catenin and deleted gene 1 (DBC1) are associated with prognosis of breast cancer patients, and Cripto-1, β-catenin and DBC1 can be used as an indicator of prognosis in breast cancer patients[22-24]. The results of this study show that the combination of docetaxel and radiotherapy can reduce the expression of CA15-3, CA125, CEA, Cripto-1, β-catenin and DBC1 levels, which can be used as a test for breast cancer and provide high value for clinical use.

Conclusion

The above results have demonstrated that combination of docetaxel and radiotherapy significantly improved Cripto-1, β-catenin and DBC1 levels in breast cancer patients, and also improved the clinical symptoms of breast cancer patients.

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