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ORIGINAL RESEARCH

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Effects of docetaxel combined with radiotherapy on immune function, tumor marker indices, and suppression of gene inactivation in breast cancer patients

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Abstract

This study aimed to investigate the clinical efficacy of combining docetaxel with radiotherapy in breast cancer patients who have undergone surgery. 110 breast cancer patients admitted to our hospital were selected between January 2023 and June 2023 and randomly allocated into two groups (n = 55/group) using a randomized numerical table method. After surgery, the control group received docetaxel, while the study group received docetaxel in conjunction with X-ray intensity-modulated radiation therapy. Their clinical effectiveness, tumor markers, DNA methyltransferase (DNMT1), oncogene cripto-1 (CP-1), and CUE structural domain protein 2 (CUEDC2) expression levels and adverse events were compared before and after treatment. After treatment, we found no significant differences in T lymphocyte subpopulation indices between the two groups ($p > 0.05$), while the study group had significantly lower levels of all tumor markers, including DNMT1, CP-1 and CUEDC2, compared to the control group ($p < 0.05$). The incidence of adverse events in the study group (9.09%) was slightly higher than in the control group (5.45%), but the difference was not statistically significant ($p > 0.05$). In summary, postoperative docetaxel combined with X-ray intensity-modulated radiotherapy may lower tumor marker levels, inhibit malignant tumor cell behavior, enhance overall clinical efficacy, and extend patient survival without significantly impacting immune function or increasing the risks of adverse effects.

Keywords

Docetaxel; Radiotherapy; Breast cancer patients; Immune function; Tumor markers; Inhibiting gene inactivation

1. Introduction

Clinical data reveals a concerning trend in the increasing incidence of breast cancer in recent years, with over 160,000 new cases reported annually in China [1, 2], making it a significant threat to the physical and emotional well-being of women, especially considering that more and more younger women are being affected by this cancer.

Various clinical treatments are currently available for breast cancer, with surgery, radiotherapy and chemotherapy being the most commonly used treatment modalities [2]. Notably, surgery has proven to be clinically effective, particularly for early-stage breast cancer patients. Surgical interventions encompass radical resection and breast-conserving surgery, the latter being increasingly favored due to its favorable cosmetic outcomes, particularly among the younger population of breast cancer patients. However, clinical evidence indicates a notable recurrence rate associated with breast-conserving surgery alone in early-stage breast cancer patients. To mitigate this risk and consequently enhance patient survival and improve their quality of life, the post-surgical application of targeted radiotherapy or chemotherapy is now being implemented. Thus, we designed this present study to further investigate the clinical efficacy of combining docetaxel with radiotherapy in breast cancer surgery patients.

2. Information and methods

2.1 Clinical data

This study comprised a cohort of 110 breast cancer patients who were admitted to our hospital between January 2023 and June 2023. The study inclusion criteria were: patients had to meet the relevant criteria specified in the Guidelines and Norms for the Diagnosis and Treatment of Breast Cancer of the Chinese Anti-Cancer Association, had unilateral breast lesions, undergoing their initial treatment for breast cancer, have undergone breast-conserving surgery, had an expected survival period of at least 6 months, and had complete clinical

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data for study analysis.

The exclusion criteria for this study were: (1) contraindications to radiotherapy; (2) had recurrent breast cancer; (3) metastatic breast cancer; (4) abnormalities in heart, liver, kidney or other vital functions; (5) exhibiting severe bleeding or signs of infection; and/or (6) experiencing communication or comprehension difficulties.

The patients were randomly classified into two groups, namely the study and control groups, with each group comprising 55 cases, based on the random number table method. A comparison of clinical data between these two groups revealed no statistically significant differences (Table 1).

2.2 Treatment method

All patients underwent breast-conserving surgery, during which the local tumor lesions and the adjacent 2 cm of normal tissue were excised. Notably, preservation of the thoracic dorsal and long thoracic nerves was conducted in all patients, and the surgical procedures were performed following current guidelines [3].

In the control group, docetaxel (Shenzhen Wanle Pharmaceutical Co., Ltd.; approval number: State Drug Administration H2005206; specification: 0.5 mL:20 mg) was administered intravenously at a dose of 75 mg/m^2 once every 3 weeks, starting from 2 to 4 weeks after surgery.

The study group, in addition to docetaxel treatment, received X-ray intensity-modulated radiotherapy. The specific procedure was as follows: Patients were positioned in the supine position, and they were secured using a breast brace, with a suitable headrest provided based on individual requirements. Prior to marking for patient positioning, it was ensured that the upper extremity was maintained in an abducted position with an abduction angle exceeding 90 degrees. A Computed tomography (CT) scanner was used for patient imaging and data acquisition, which was subsequently entered into the Eclipse system for a precise treatment plan. To ensure accurate positioning and error control within a 5 mm margin, calibration was performed on the simulated positioning machine before radiation therapy.

Then, dynamic intensity-modulated treatment was administered using a linear accelerator (VARIAN 23EX, Palo Alto, California, USA) with 6MV-X-ray, delivering 2 Gy per session, five times per week.

Before assessing efficacy, both groups underwent two sessions of treatments, constituting one course lasting for 21 days.

2.3 Observation indicators

2.3.1 T lymphocyte subsets

Venous blood samples were collected from the patients, and the levels of absolute CD3 cells (CD3+), absolute CD4 cells (CD4+), and absolute CD8 cells (CD8+) were quantified using flow cytometry (BD FACSLyric™, Becton Drive Franklin Lakes, New Jersey, USA).

Flow cytometry steps:

First, 20 μL of CD3, CD4, and CD8 reagents were added

TABLE 1. Comparison of clinical data between the study and control groups.

Indicators	Study Group (n = 55)	Control Group (n = 55)	Statistical values	*p* value
Age (yr)	45.35 ± 4.15	45.38 ± 4.09	−0.046	0.963
BMI (kg/m^2)	24.15 ± 2.16	24.20 ± 2.19	−0.097	0.923
Tumor diameter (cm)	4.26 ± 0.51	4.28 ± 0.53	−0.266	0.791
Location of incidence (n, %)				
Left	23	22	0.038	0.846
Right	32	33		
Menopause status (n, %)				
Menopausal	23	24	0.037	0.847
Non-menopausal	32	31		
Clinical stage (n, %)				
Stage I	34	35	0.039	0.844
Stage II	21	20		
Axillary lymph node status (n, %)				
Positive	31	30	0.037	0.848
Negative	24	25		
Pathological type (n, %)				
Infiltrating ductal carcinoma	52	51	0.210	0.900
Medullary carcinoma	1	1		
lobular carcinoma	2	3		

BMI: Body Mass Index.

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to the bottom of the flow tube. Second, 50 μL of thoroughly mixed EDTA-K2 anticoagulated whole blood was carefully pipetted into the tube using the reverse pipetting technique, ensuring that the blood was positioned at the tube's base and away from its upper wall. Third, the tube was capped, gently mixed for approximately 3 seconds, and allowed to incubate at room temperature, shielded from light, for 15–25 minutes. Fourth, 450 μL of hemolytic agent for hematology analysis was added into the tube, which was then recapped and incubated for an additional 15 minutes at room temperature, still protected from light. Lastly, absolute counting microspheres were added, and the sample was prepared for testing on the machine, following which the data were analyzed using flow cytometry software (FlowJo v10.6.2, Becton, Dickinson, and Company (BD), Ashland, OR, USA).

2.3.2 Tumor markers

Venous blood samples were collected from patients, and the levels of chitinase-3-like protein-1 (YKL-40), glycoconjugate antigen 125 (CA125), carcinoembryonic antigen (CEA), and glycoconjugate antigen 15-3 (CA15-3) were quantified using Enzyme-Linked ImmunoSorbent Assay (ELISA).

2.3.3 Venous blood samples

Venous blood samples were collected from patients, and the expression levels of DNMT1, CP-1, and CUEDC2 were assessed using reverse transcription-polymerase chain reaction (RT-PCR). Briefly, 1 mL of TrizoL was added to serum samples to extract total DNA, followed by purity and concentration assessment. Subsequently, 2 μg of total DNA was utilized and combined with the internal reference Glyceraldehyde-3-phosphate dehydrogenase (GAPDH), as well as the target small molecules DNMT1, CP-1, and CUEDC2 reverse transcription primers. The mixture was reverse transcribed to synthesize cDNA at 42 °C for 15 minutes and 85 °C for 5 seconds. The PCR reaction conditions included pre-denaturation at 95 °C for 10 minutes, followed by a cycling pattern of 95 °C, 60 °C, 95 °C, 60 °C, 95 °C, 60 °C, and respective durations of 12 seconds, 40 seconds, 15 seconds, 30 seconds and 15 seconds (to gather fluorescence) over 40 cycles. The corresponding primer sequences were designed using Primer 5.0 software (Primer-E Ltd, Auckland, New Zealand).

2.3.4 Clinical efficacy

The clinical efficacy was assessed according to the criteria established by the International Anti-Cancer Society [4], classifying it into several categories: Complete Remission (CR), which signifies the complete disappearance of tumor tissue; Partial Remission (PR), characterized by a reduction in tumor diameter of $\geq$50%; Stable Disease (SD), indicating a reduction in tumor tissue, but not to the extent of partial remission, or a slight reduction or insignificant increase; and Disease Progression (PD), defined by a 25% increase in tumor tissue or the appearance of new tumor tissue. The total effectiveness rate of the treatment was calculated as the number of cases with CR or PR divided by the total number of cases multiplied by 100%. Patients were followed up for 1 to 3 years, and the median follow-up time was 6 months.

2.4 Statistical methods

Data analysis was conducted using SPSS 27.0 (IBM, Armonk, NY, USA). Normally distributed measurement data are presented as mean $\pm$ standard deviation ($\pm$s) and compared using the t-test. Count data are presented as cases (%) and analyzed using the χ^2 test, with significance set at $p < 0.05$. A binary logistic regression analysis model was employed for multifactor regression analysis. The R software (R 4.1.1, Copyright (C) 2023 The R Foundation for Statistical Computing, New Zealand) was used to generate column line graphs for the prediction model. The goodness-of-fit of the probability model was assessed using the Hosmer and Lemeshow test, and the predictive value of the model was evaluated by drawing the Receiver Operating Characteristic (ROC) curve using SPSS.

3. Results

3.1 Comparison of tumor markers between the two groups

Our results showed that patients from the study group had significantly lower levels of tumor marker levels compared to the control group after treatment ($p < 0.05$), as indicated in Table 2, Figs. 1,2.

3.2 Comparison of DNMT1, CP-1 and CUEDC2 expressions between the two groups

Before treatment, we found that there were no significant differences in the expression of DNMT1, CP-1 and CUEDC2 between the two groups ($p > 0.05$). However, after treatment, the expression levels of DNMT1, CP-1 and CUEDC2 in the study group were lower than those in the control group, as illustrated in Table 3, Figs. 3,4.

3.3 Comparison of T-lymphocyte subpopulation indices between the two groups

The T-lymphocyte subpopulation indices in both groups were not significantly different before or after treatment ($p > 0.05$). Nevertheless, both groups showed lower levels of CD3+ and CD4+ after treatment, and this difference was statistically significant ($p < 0.05$) (Table 4, Figs. 5,6).

3.4 Comparison of adverse reactions between the two groups

In the study group, 9.09% of patients experienced adverse events, while in the control group, the incidence was 5.45%. However, this difference was not found to be statistically significant ($p > 0.05$) (Table 5).

3.5 Comparison of clinical efficacy between the two groups

The total treatment effectiveness rate in the study group was 80.00%, which was significantly greater than the 60.00% rate observed in the control group ($p < 0.05$), as demonstrated in Table 6.

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TABLE 2. Comparison of tumor markers between the two groups ($\bar{x} \pm s$).

Indicators	Study Group (n = 55)	Control Group (n = 55)	*t* value	*p* value
YKL-40 (ng/mL)				
Before Treatment	94.35 ± 8.65	94.36 ± 8.61	−0.006	0.995
After Treatment	71.25 ± 7.26	81.25 ± 8.11	−6.820	<0.001
t value	15.227	7.751	——	——
p value	<0.001	<0.001	——	——
CA125 (U/mL)				
Before Treatment	26.35 ± 2.16	26.36 ± 2.09	−0.025	0.980
After Treatment	13.24 ± 1.34	19.35 ± 1.94	−19.227	<0.001
t value	38.066	18.956	——	——
p value	<0.001	<0.001	——	——
CEA (ng/mL)				
Before Treatment	1.86 ± 0.16	1.84 ± 0.19	0.596	0.553
After Treatment	0.61 ± 0.04	1.14 ± 0.11	−33.796	<0.001
t value	59.446	23.771	——	——
p value	<0.001	<0.001	——	——
CA15-3 (U/mL)				
Before Treatment	36.35 ± 3.19	36.33 ± 3.09	0.033	0.973
After Treatment	25.96 ± 2.64	30.31 ± 2.98	−8.085	<0.001
t value	16.858	9.938	——	——
p value	<0.001	<0.001	——	——

YKL-40: chitinase-3-like protein-1; CA125: glycoconjugate antigen 125; CEA: carcinoembryonic antigen; CA15-3: glycoconjugate antigen 15-3.

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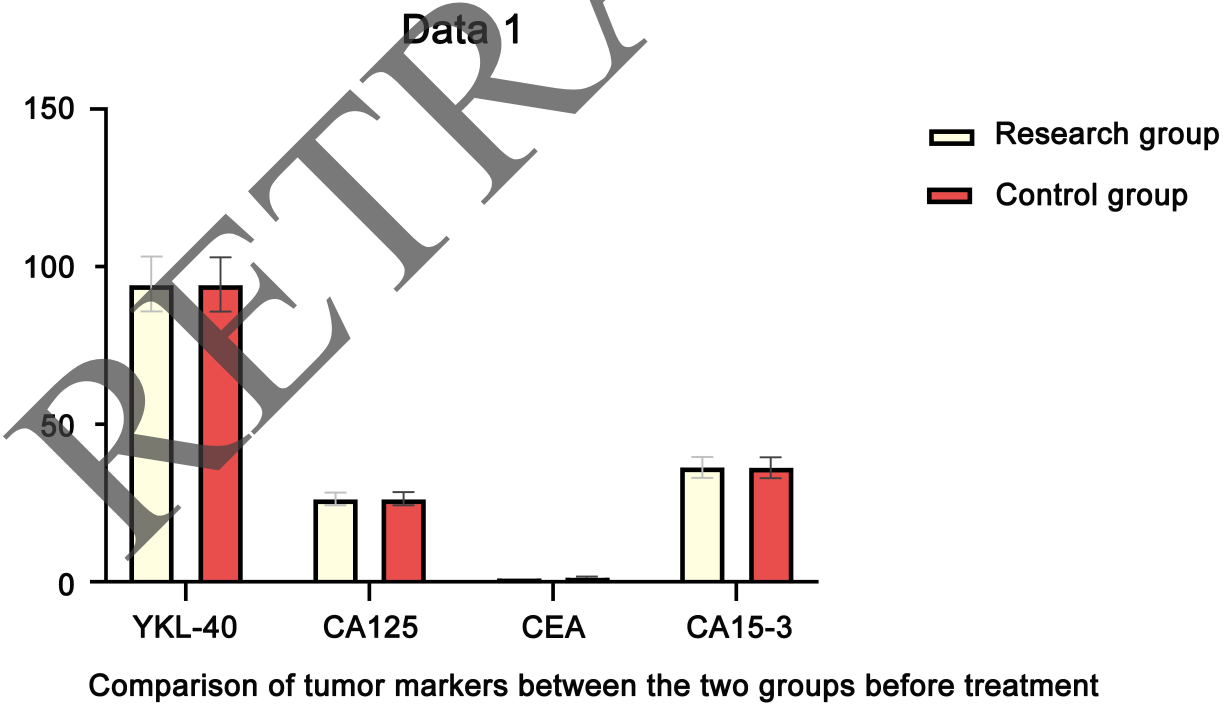


FIGURE 1. Comparison of tumor markers between the two groups before treatment. YKL-40: chitinase-3-like protein-1; CA125: glycoconjugate antigen 125; CEA: carcinoembryonic antigen; CA15-3: glycoconjugate antigen 15-3.

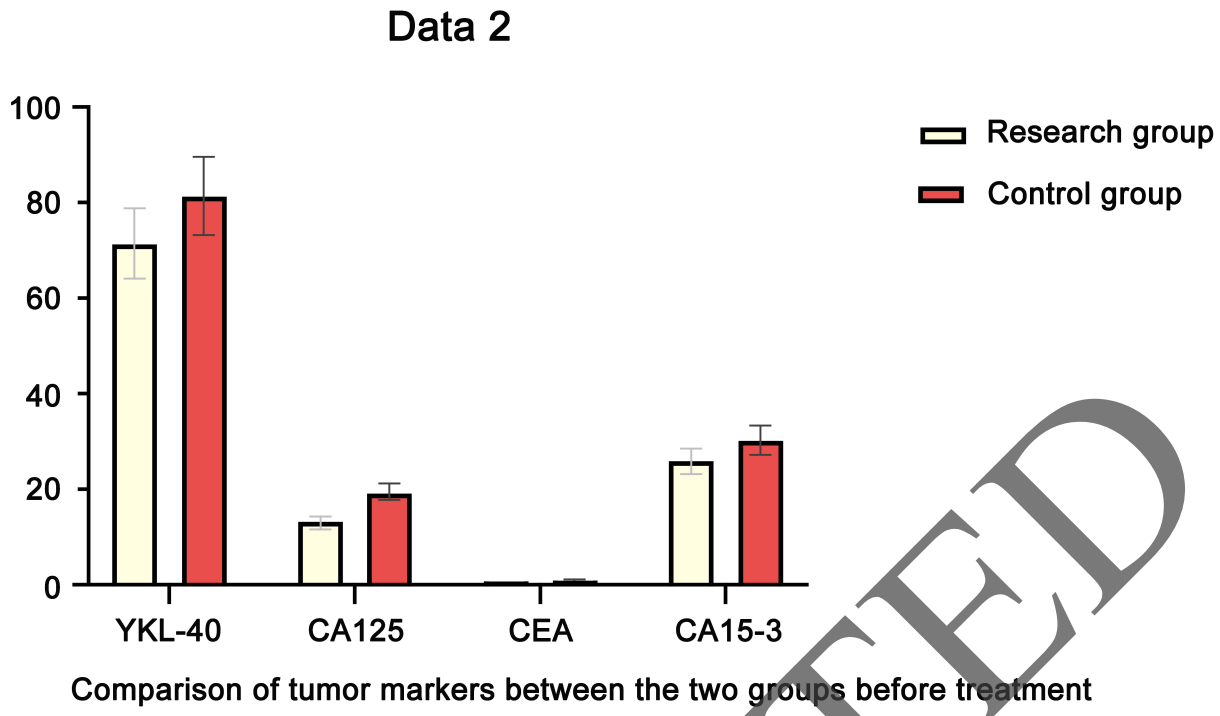


FIGURE 2. Comparison of tumor markers between the two groups after treatment. YKL-40: chitinase-3-like protein-1; CA125: glycoconjugate antigen 125; CEA: carcinoembryonic antigen; CA15-3: glycoconjugate antigen 15-3.

TABLE 3. Comparison of DNMT1, CP-1 and CUEDC2 expressions between the two groups ($\bar{x} \pm s$).

Indicators	Study Group (n = 55)	Control Group (n = 55)	*t* value	*p* value
DNMT1				
Before Treatment	2.56 ± 0.21	2.57 ± 0.22	−0.495	0.622
After Treatment	0.41 ± 0.04	1.09 ± 0.16	−29.493	<0.001
t value	73.888	43.388	——	——
p value	<0.001	<0.001	——	——
CP-1				
Before Treatment	2.16 ± 0.21	2.14 ± 0.19	0.353	0.725
After Treatment	0.54 ± 0.04	1.24 ± 0.16	−30.402	<0.001
t value	55.231	25.538	——	——
p value	<0.001	<0.001	——	——
CUEDC2				
Before Treatment	1.69 ± 0.16	1.68 ± 0.15	0.542	0.589
After Treatment	0.24 ± 0.03	0.86 ± 0.08	−56.554	<0.001
t value	68.036	36.195	——	——
p value	<0.001	<0.001	——	——

DNMT1: DNA methyltransferase; CP-1: oncogene cripto-1; CUEDC2: CUE structural domain protein 2.

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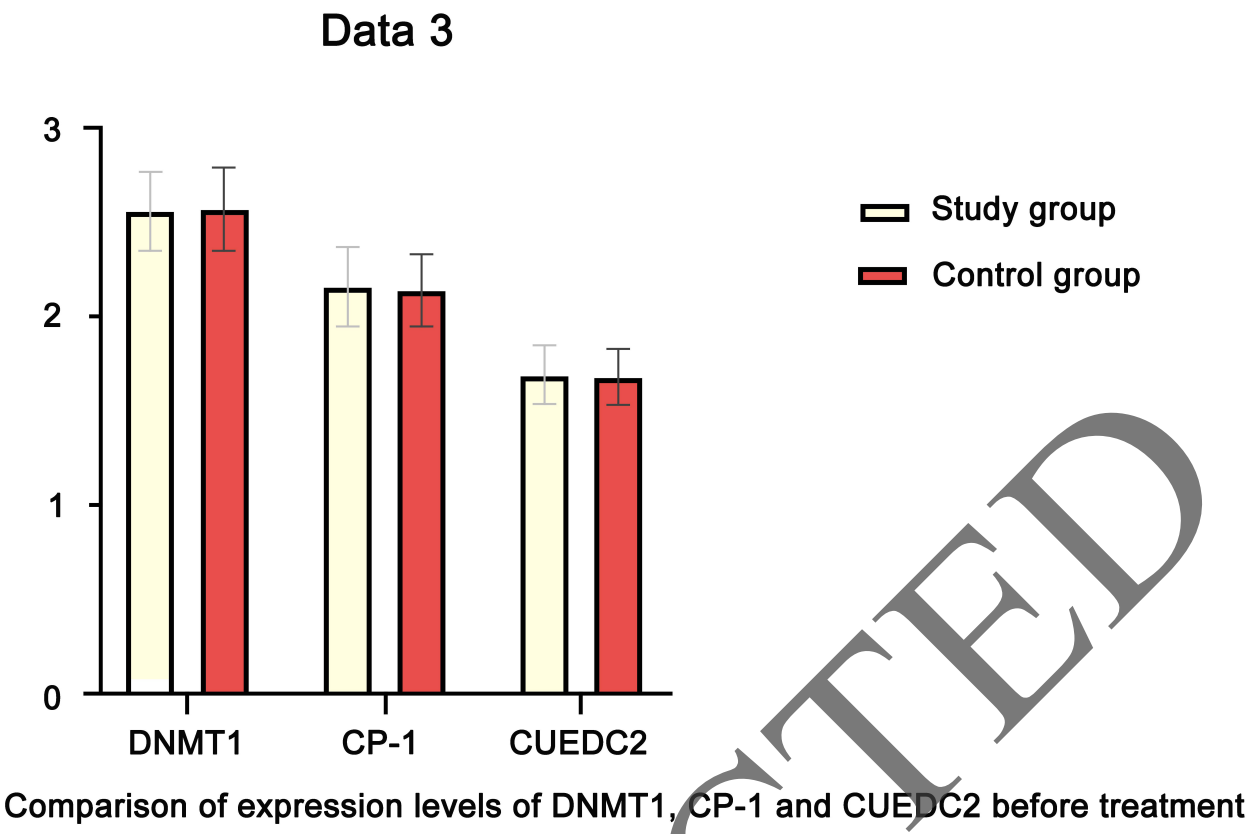


FIGURE 3. Comparison of DNMT1, CP-1, and CUEDC2 expressions between the two groups before treatment. DNMT1: DNA methyltransferase; CP-1: oncogene cripto-1; CUEDC2: CUE structural domain protein 2.

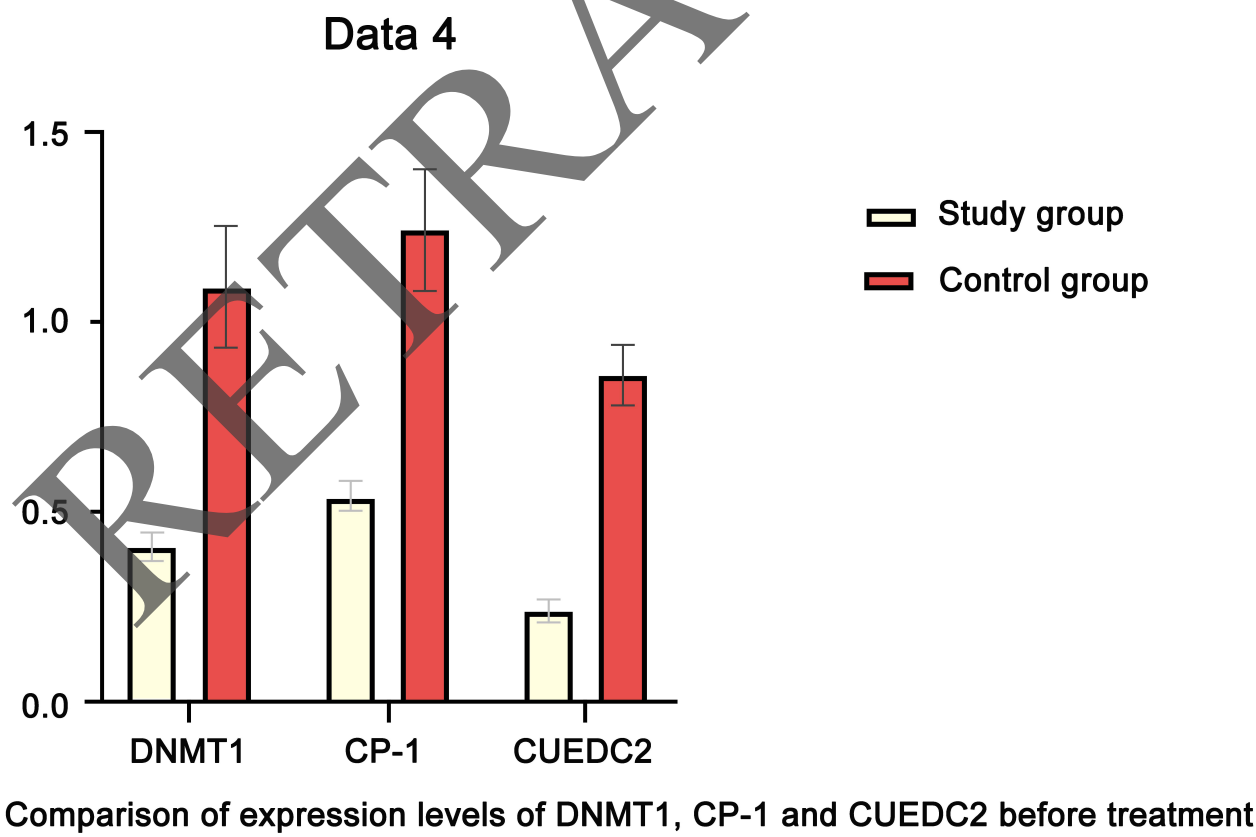


FIGURE 4. Comparison of DNMT1, CP-1 and CUEDC2 expressions between the two groups after treatment. DNMT1: DNA methyltransferase; CP-1: oncogene cripto-1; CUEDC2: CUE structural domain protein 2.

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TABLE 4. Comparison of DNMT1, CP-1 and CUEDC2 expressions between the two groups ($\bar{x} \pm s$).

Indicators	Study Group (n = 55)	Control Group (n = 55)	*t* value	*p* value
CD3+				
Before Treatment	67.25 ± 6.16	67.29 ± 6.09	−0.034	0.973
After Treatment	60.35 ± 6.15	60.29 ± 6.11	0.051	0.959
t value	5.712	5.495	——	——
p value	<0.001	<0.001	——	——
CD4+				
Before Treatment	44.15 ± 4.06	44.19 ± 4.09	−0.064	0.949
After Treatment	37.26 ± 3.62	37.24 ± 3.69	0.029	0.977
t value	9.648	9.059	——	——
p value	<0.001	<0.001	——	——
CD8+				
Before Treatment	36.25 ± 3.16	36.31 ± 3.09	−0.101	0.920
After Treatment	35.26 ± 3.06	35.19 ± 3.11	0.119	0.905
t value	1.683	1.703	——	——
p value	0.098	0.094	——	——

CD: Cluster of Differentiation.

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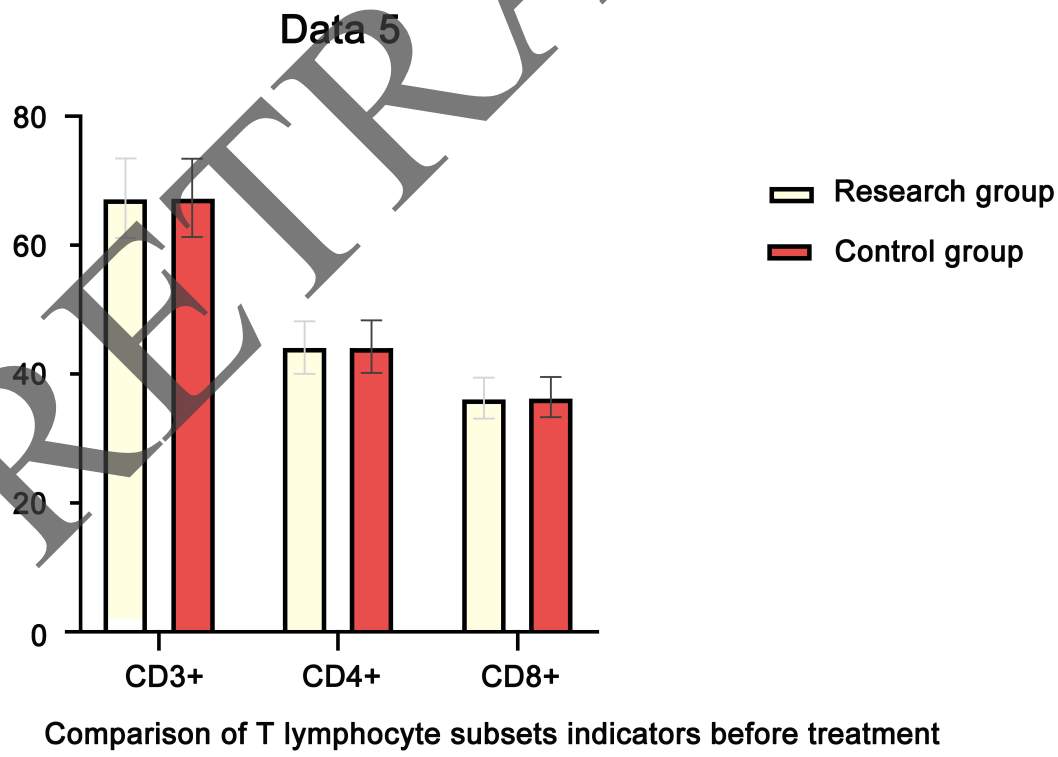


FIGURE 5. Comparison of T-lymphocyte subpopulation indices between the two groups before treatment. CD: Cluster of Differentiation.

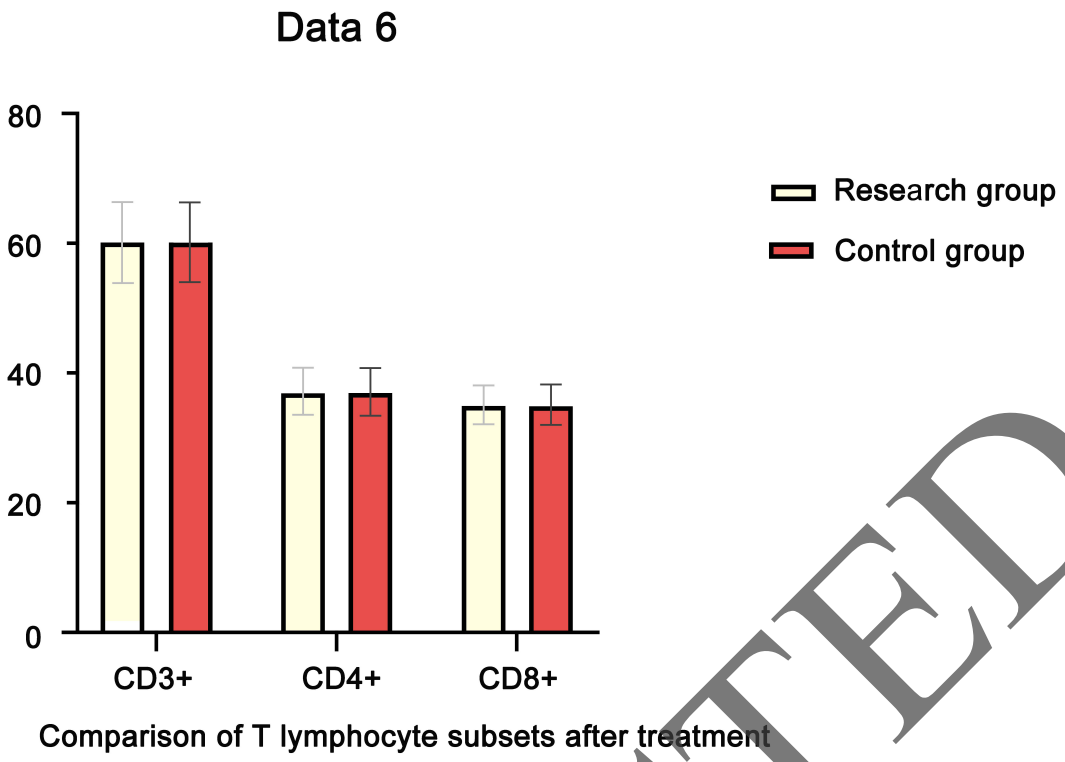


FIGURE 6. Comparison of T-lymphocyte subpopulation indices between the two groups after treatment. CD: Cluster of Differentiation.

TABLE 5. Comparison of the incidence of adverse reactions (n, %).

Groups	Number of cases	Cardiac injury	Radiation poisoning	Peripheral nerve injury	Total adverse reactions
Study Group	55	2, 3.64	1, 1.82	2, 3.64	5, 9.09
Control Group	55	1, 1.82	1, 1.82	1, 1.82	3, 5.45
χ^2 value	——		——		0.539
p value	——		——		0.463

TABLE 6. Comparison of clinical efficacy between the two groups (n, %).

Groups	Number of cases	Complete remission	Partial remission	Stable	Progression	Total effective
Study Group	55	26, 47.27	18, 32.73	6, 10.91	5, 9.09	44, 80.00
Control Group	55	22, 40.00	11, 20.00	6, 10.91	16, 29.09	33, 60.00
χ^2 value	——		——			5.238
p value	——		——			0.022

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4. Discussion

Breast cancer has remained a pervasive health concern in China, evolving into a significant public health issue with profound implications for women's well-being [5, 6]. Its treatment remains challenging as it is typically characterized by multiple lesions, limited early symptoms, and increased risks for metastasis and recurrence. Surgical interventions represent the cornerstone of breast cancer treatment, and postoperative measures, including radiotherapy, are crucial to diminish the risk of recurrence and metastasis and serve to extend patients' survival and enhance their overall quality of life.

Chemotherapy has become an effective treatment approach in the comprehensive management of numerous cancers, offering notable benefits and outcomes in the postoperative management of breast cancer. Chemotherapeutic modalities aim at eliminating subclinical or microscopic lesions that may be present in distant organs and the lymph node system of breast

cancer patients, thereby helping mitigate the risks associated with local recurrence following surgery and the potential for postoperative metastasis.

Docetaxel, as a chemotherapeutic agent, exerts a potent influence on the mitotic process of tumor cells and possesses distinct advantages in inhibiting tumor cell replication, as well as in preventing tumor cell invasion and metastasis [7]. Clinical reports suggest that patients undergoing docetaxel treatment exhibit favorable resistance profiles. Furthermore, with advancements in therapeutic medical equipment, significant progress has been achieved in radiation therapies [8]. In recent years, intensity-modulated radiation therapy has been found to have distinct advantages in the clinical management of postoperative breast cancer patients [9], such as enabling effective treatment targeting specific tissues while simultaneously safeguarding vital organs like the heart and lungs from radiation damage. The combination of these two modalities has been integrated into the clinical treatment protocols at our hospital for postoperative breast cancer patients, yielding improved outcomes [10].

Our study results revealed that after treatment, both the study and control groups exhibited a significant reduction in tumor markers compared to their pre-treatment levels ($p < 0.05$). Notably, the reduction observed in the study group was more substantial than that in the control group, and this difference was statistically significant ($p < 0.05$). YKL-40 is a prominent member of the mammalian 18-glycosyl hydrolase family and serves as an important marker in various cancers, including colorectal, thyroid and breast cancers [11]. CA125 is a type of glycoprotein and can be effectively detected using the monoclonal antibody Ovarian Cancer 125 (OC125). CEA is closely associated with the tumor's malignancy and stage and was found to play a pivotal role in evaluating treatment efficacy, prognosis, and the clinical diagnosis of breast cancer [12].

CA15-3, a variant of glycoprotein, is abundant on the cell surfaces of the breast epithelium and constitutes a significant component of breast cancer antigens. CA15-3 has distinct advantages in evaluating the status of breast cancer patients and detecting metastasis and recurrence [13].

According to our study's findings, DNMT1, CP-1, and CUEDC2 levels were significantly lower in the study group compared to the levels observed in the control group ($p < 0.05$). DNMT1, a protease responsible for maintaining the methylation of malignant tumor suppressor genes, is found in various malignant tumor cells, and its abnormal elevation has been strongly associated with poor prognosis [14]. CP-1 can promote the inactivation of suppressor genes and plays a crucial role in the division of malignant tumor cells, facilitating their proliferation. Comparatively, CUEDC2 contributes significantly to breast carcinogenesis and enhances tumor cell infiltration and invasion [15]. Acting as a malignant oncogene in breast carcinogenesis, CUEDC2 plays a pivotal role in promoting the malignant progression of tumor cells [16–18].

In terms of adverse events, the study group had an incidence rate of 9.09%, slightly higher than the rate of 5.45% observed in the control group; however, this difference did not reach statistical significance ($p > 0.05$), suggesting that the combination treatment of X-ray intensity-modulated radiotherapy and docetaxel did not significantly exacerbate immune function impairment in patients. We hypothesized that this could be primarily attributed to the protective effects on normal tissues during intensity-modulated radiotherapy, effectively safeguarding the patients' heart, lungs, and other vital functions, thereby enabling effective control of adverse reactions.

The findings of this study indicate that the total treatment effectiveness rate in the study group reached 80.00%, significantly surpassing the rate of 60.00% observed in the control group ($p < 0.05$), which highlights the effectiveness of combining docetaxel with X-ray intensity-modulated radiotherapy in effectively restraining the malignant behavior of breast cancer cells, thereby influencing disease progression positively and improving the clinical prognosis.

Additionally, we also observed no significant differences between the two groups in terms of T lymphocyte subpopulation indices both before and after treatment ($p > 0.05$). Notably, certain published data suggest that docetaxel may have an immune-stimulating effect that is favorable for triggering an anti-tumor immune response. However, in our present study, no similar conclusions can be drawn, potentially due to factors such as our relatively smaller sample size.

The study group exhibited an adverse reaction incidence of 9.09%, slightly higher than the 5.45% rate observed in the control group; however, this difference did not reach statistical significance ($p > 0.05$), which implies that the combination treatment of X-ray intensity-modulated radiotherapy and docetaxel did not significantly exacerbate immune function impairment. This might be primarily attributed to the protective effect on patients' normal tissues during intensity-modulated radiotherapy, effectively safeguarding the functions of vital organs such as the heart and lungs and thereby allowing for effective control of adverse reactions.

Furthermore, our present results also demonstrate that the total treatment effectiveness rate in the study group reached 80.00%, surpassing the rate of 60.00% observed in the control group ($p < 0.05$). These findings further confirm the potential efficacy of combining docetaxel with X-ray intensity-modulated radiotherapy in effectively mitigating the malignant behavior of breast cancer cells, which positively influenced disease progression and improved clinical prognosis.

5. Conclusions

In conclusion, based on the study's findings, it can be deduced that the combination of docetaxel and X-ray intensity-modulated radiation therapy has a significant positive impact on the clinical outcomes of breast cancer patients who have undergone surgery. However, it is also important to acknowledge the limitations such as the small sample size and retrospective data in this study. Therefore, future research is needed to broaden the scope and content of the investigation to derive more comprehensive and objective conclusions to further enhance the clinical management of breast cancer patients.

AVAILABILITY OF DATA AND MATERIALS

The authors declare that all data supporting the findings of this study are available within the paper and any raw data can be

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obtained from the corresponding author upon request.

AUTHOR CONTRIBUTIONS

YY, CL, LD, and GZ—designed the study and carried it out; supervised the data collection, analyzed the data, and interpreted the data; prepared the manuscript for publication and reviewed the draft. All authors have read and approved the manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Ethical approval was obtained from the Ethics Committee of Jingjiang People's Hospital (Approval no. 2022-KY-002-01). Written informed consent was obtained from a legally authorized representative(s) for anonymized patient information to be published in this article.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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