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Comparison between Pelvic IMRT and 3D-CRT in Combination with Chemotherapy via Nrf2 Expression on the High-Risk Endometrial Cancer

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ABSTRACT

This study aimed to explore the clinical efficacy of pelvic intensity-modulated radiation therapy (IMRT) and 3-dimensional conformal radiotherapy (3D-CRT) in combination with chemotherapy on high-risk endometrial cancer. The effect of these methods is evaluated via Nuclear factor (erythroid-derived 2)-like 2 (Nrf2) expression, the levels of chitinase protein 40 (YKL-40), human epididymis protein 4 (HE4), and prolactin (PRL) in serum. For this purpose, between August 2014 and July 2017, 114 endometrial cancer patients admitted to this hospital for treatment were randomized into the observation group (n=60) and control group (n=54). Following the surgery, patients in these two groups received the chemotherapy of taxol and carboplatin (TC). Based on the chemotherapy, patients in the observation underwent the IMRT, while those in the control group adopted the 3D-CRT. The Nrf2 expression was performed based on the Real-time PCR technique. The incidence rate of adverse reactions was a 3-year recurrence rate and mortality rate. Results showed that after treatment, levels of YKL-40, HE4, and PRL in the serum of patients in two groups decreased compared to those before treatment (all $P < 0.05$). In comparison, the difference between the two groups showed no statistical significance ($P > 0.05$). The evaluation of Nrf2 transcription factor expression showed significant differences started in comparisons of the Nrf2 Expression between two groups ($P > 0.05$), and this enhancement was significant in the control group after treatment. Comparison of the incidence rates of the bone marrow suppression during treatment showed no significant difference ($P > 0.05$). However, the incidence rates of radiation enteritis and radio-cystitis in the observation group were much lower than those in the control group ($P < 0.05$). During the follow-up, there were five patients in the control group and 7 in the observation group losing to the follow-up, and among the remaining subjects, no significant difference was identified in the comparison of the recurrence rate or mortality rate between the two groups (all $P > 0.05$). In general, Pelvic IMRT in combination with chemotherapy is a promising and safe candidate for high-risk endometrial cancer with mild radiation injury; besides, YKL-40, HE4, and PRL are the effective indicator for the prediction of efficacy in chemotherapy for endometrial cancer.

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Introduction

Endometrial cancer has a high prevalence taking up 20% to 30% of the genital tract tumors, and 200,000 new cases have been reported annually, with a mortality rate only secondary to ovarian cancer and cervical cancer (1, 2). Surgical resection is the major strategy, while radiotherapy dominates post-surgery treatment. However, high-risk endometrial cancer has a high risk of extra-uterus or distal metastasis, and, correspondingly, chemotherapy is advised for post-surgery treatment (3). Recently, people have increasingly noted the significance of post-surgery chemotherapy, synchronized chemotherapy and radiotherapy or a combination of them in the treatment of various cancers, while the efficacy and tolerance remain uncertain (4). According to the current guidelines for the diagnosis and treatment of endometrial cancer, auxiliary radiotherapy is not necessary for patients with low-risk endometrial cancer, or with only one high-risk factor, while for moderate-risk endometrial cancer, guided brachytherapy is more preferred than external irradiation and for high-risk endometrial cancer, and chemotherapy may work better in improving the median survival time (5-7). Pelvic external radiation is the major auxiliary method for treatment of endometrial cancer patients with high-risk pathogens, while so far, intensity-modulated radiation therapy (IMRT) is extensively applied in the treatment of prostatic cancer and head and neck tumors, gaining

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promising efficacy, while leaving no damage to the adjacent normal tissues (8). Taxel + carboplatin (TC) remains the standard protocol for the treatment of endometrial cancer that excels in extending the progression-free survival time, but there remains little information regarding the efficacy of the IMRT in combination with chemotherapy on endometrial cancer (9).

Nuclear factor (erythroid-derived 2)-like 2, abbreviated NFE2L2 or Nrf2, is a transcription factor encoded in humans by the NFE2L2 gene (10). This molecule is a bispecific domain protein that regulates the expression of genes responsible for producing antioxidant proteins. These antioxidant proteins work to prevent oxidative damage to cells due to inflammation or damage. Several factors influence the regulation of Nrf2 transcription factor expression (11). Under normal circumstances, ROS levels and antioxidants are in balance; Therefore, Nrf2 has a minimal role in regulating the antioxidant system in the heart. When this balance is disturbed to increase ROS (such as radiotherapy techniques), it causes oxidative stress and increases Nrf2 expression in the cell. In this regard, many studies have been conducted to determine the relationship between radiotherapy techniques and oxidative stress products (12).

Thus, we explored the efficacy of pelvic IMRT and 3-dimensional conformal radiotherapy (3D-CRT) in combination with the chemotherapy via Nuclear factor (erythroid-derived 2)-like 2 (Nrf2) expression, the levels of chitinase protein 40 (YKL-40), human epididymis protein 4 (HE4), and prolactin (PRL) in serum.

Material and methods

General material

Between August 2014 and July 2017, 114 endometrial cancer patients who were admitted to this hospital for treatment were randomized into the observation group (n=60) and control group (n=54). This study was approved by the Ethical Committee of the hospital. Inclusion criteria: 1) Patients conforming to the diagnostic criteria of endometrial cancer (13, 14) that was further confirmed by the pathological examination after the epifascial panhysterectomy; 2) patients with no history of the chemotherapy or radiotherapy before surgery; 3) patients with a Karnofsky score ≥70 points; 4) patients that agreed to participate in this study after they were informed of the content. Exclusion criteria: 1) Patients with an anomaly in routine examinations of blood, urine or feces, or in the function test of liver, kidney or heart; 3) patients with the contraindications of chemotherapy or radiotherapy; 3) patients complicated with other malignant tumors; 4) patients who were allergic to the drugs of this study. Comparisons over the age, pathological type and clinical stage of patients between the two groups showed no significant differences (P > 0.05) (Figure 1).

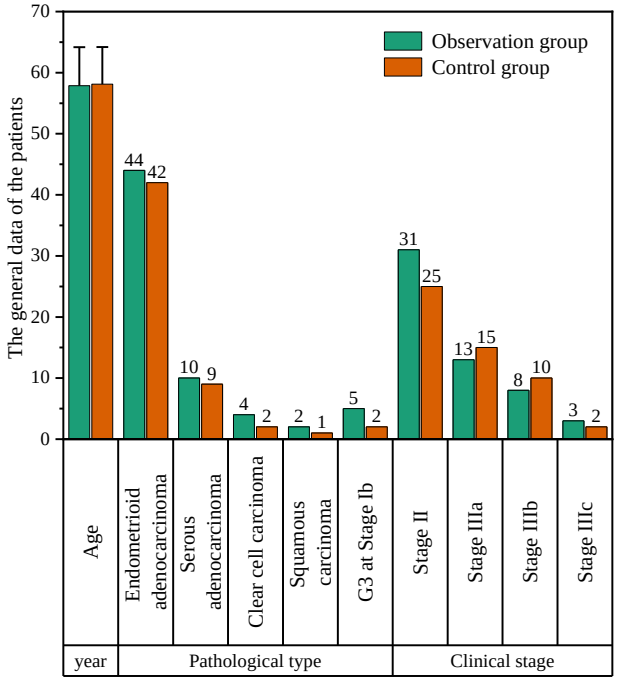

Figure 1. Comparisons of the age, pathological type, and clinical stage of patients between two groups

Treatment methods

Postoperatively, patients in two groups were required to take the chemotherapy of taxel in combination with carboplatin (TC protocol): Taxel (Shanghai Acebright Pharmaceutics Group Co., Ltd, SFDA Approval No.: H20103297), at a dose of 135 mg/m2 resolved in 500 mL of 0.9% normal saline for intravenous infusion, D1; carboplatin (Qilu Pharmaceutical. SFDA Approval No.: H10920028), at a dose of 300 mg/m2 resolved in 500 mL of 0.9% normal saline for intravenous infusion, D2; 21 days consisted of 1 course. Following 2 courses of chemotherapy, radiotherapy was initiated. Delineation of the target zone was performed by CT-assisted simulation orientation from the upper edge of the 3rd

lumbar vertebra to the site at 5 cm to the ischial tuberosity, with a layer space of 5 mm. Patients in the control group took the 3D-CRT by 4-field radiation, while those in the observation group underwent the IMRT via 5-field radiation, at a total dose of 45 to 50 Gy, 1.8 to 2.0 Gy per time, once per day, 5 times per week. Additionally, 29 patients that had positive responses in the common iliac lymph nodes or the lymph nodes adjacent to the abdominal aorta took the extended-field radiation, at a total dose of 40 to 45 Gy. Vaginal brachytherapy: 22 patients underwent pelvic external irradiation, immediately followed by intrapelvic irradiation at 1 cm to the applicator, at a dose of 10 to 20 Gy.

Nrf2 gene expression

The endometrial biopsy was performed to evaluate the expression of Nrf2 from the uterine endometrium. After providing a sample from all patients, the total RNA was extracted by GeneJET RNA Purification Kit (Thermo Fisher Scientific, USA). After removing genomic contaminants with free-RNase DNase enzyme (Thermo Fisher Scientific, USA), the RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, USA) was used for cDNA synthesis. For this purpose, 1µl of total RNA and 1µl Oligo dT primer were used to synthesis cDNA according to the manufacturer's instructions. The GAPDH reference gene primer design was done with Primer Premier Ver. 5 software based on GAPDH gene information in the NCBI gene bank. Specific primers reported in the study by Bai *et al*. (15) were used to replicate the Nrf2 gene. The primers were then synthesized by Macrogen Inc. (Seoul, South Korea). The sequences of the primers in this study are shown in Table 1.

Table 1. The characterization of primers for GAPDH and Nrf2

Gene	Primer Sequences	Product Length	Accession No.
GAPDH	5´-GGCAAGTTCAACGGCACAG-3´	144 bp	NM_017008.4
	5´-GACGCCAGTAGACTCCACGAC-3´		
Nrf2	5´- GCTGCCATTAGTCAGTCGCTCTC-3´	104 bp	NM_031789.2
	5´- ACCGTGCCTTCAGTGTGCTTC -3´		

Real-time PCR was performed by Syber Green method by Rotor gene Corbett 6000. Real-time PCR reactions were performed at a final volume of 20µl, and each reaction was duplicated. The reaction mixture consisted of 3µl of cDNA (50 ng/microliter), 8µl of RealQ Plus 2x Master Mix Green (Amplioqon, Denmark), 0.4µl of each reciprocating primer (10 pmol), and 2.8µl of free ribonuclease water. The temperature program for Real-Time PCR includes a temperature cycle of 95°C for 13 minutes, 40 temperature cycles (95°C for denaturation for 30 seconds, 60°C for binding of Nrf2 gene primers, and 58°C for binding of GAPDH primers for 40 seconds and 72°C for extension for 30 seconds). Melting diagrams were drawn to verify the data. The expression level of the genes was measured by the $2^{-\Delta\Delta CT}$ method.

Observation indexes

Comparison of the levels of YKL-40, HE4 and PRL in serum of patients between two groups before and after treatment: Prior to and after the treatment, 3 mL of the fasting venous blood was collected and placed in the anticoagulatory-free tubes for centrifugation at 3500 r/min for 10 min to isolate the serum. The serum was then preserved in the EP tubes for the Enzyme-linked immunosorbent assay (ELISA) kit (R&D, USA). References for YKL-40, HE4 and PRL: YKL-40, 78.85µg/L; HE4, 0 to 150pmol/L; PRL, 2.58 to 18.12ng/mL for males, and 1.20 to 29.93ng/mL for females. We also compared the incidence rate of adverse reactions during the treatment as well as the 3-year recurrence rate and mortality rate of patients between two groups.

Statistical methods

SPSS 18.0 software was applied to perform the data analysis. Measurement data were shown in mean ± standard deviation and compared using the t-test between two groups. Enumeration data were shown in rate (%)and compared using the chi-square test. $P < 0.05$ suggested the statistical significance of the difference.

Results and discussion

Comparison of Nrf2 Expression between two groups before and after treatment

Before treatment, no significant differences were shown in comparisons of the Nrf2 Expression between two groups ($P > 0.05$). After treatment, significant differences were shown in comparisons of the Nrf2 Expression between two groups ($P > 0.05$),

and this enhancement was significant in control group (Figure 2).

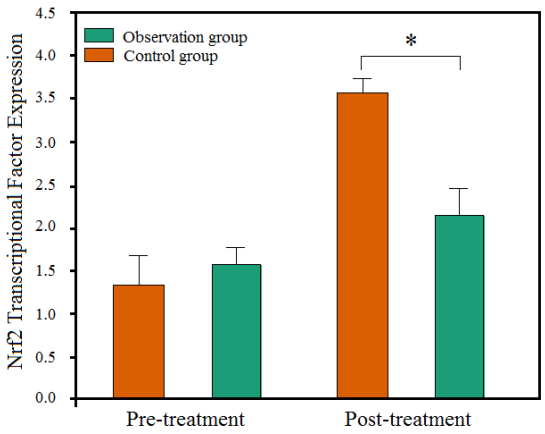

Figure 2. Comparison of Nrf2 Expression between two groups before and after treatment; * P < 0.05

Comparison of the levels of YKL-40, HE4 and PRL in serum of patients between two groups before and after treatment

Before treatment, no significant differences were shown in comparisons of the levels of YKL-40, HE4 and PRL in serum of patients between two groups (P > 0.05). After treatment, levels of YKL-40, HE4 and PRL in serum of patients in two groups were decreased when compared to those before treatment (all P < 0.05), while the difference between the two groups showed no statistical significance (P > 0.05) (Figure 3).

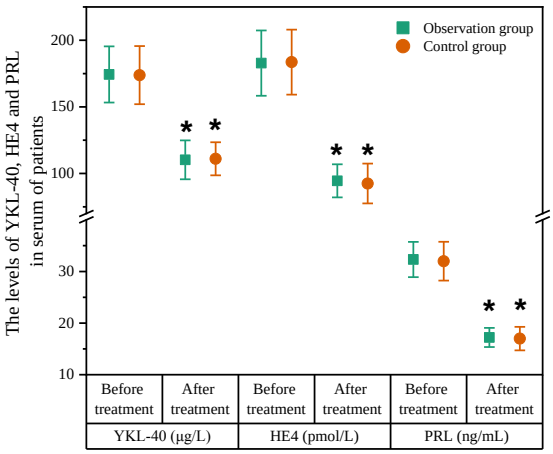

Figure 3. Comparison of the levels of YKL-40, HE4 and PRL in serum of patients between two groups before and after treatment; * P < 0.05 vs. the level before treatment

Comparison of the incidence of adverse reactions of patients between two groups

Comparison of the incidence rates of the bone marrow suppression during treatment showed no significant difference (P > 0.05). However, the incidence rates of radiation enteritis and radiocystitis in the observation group were much lower than those in the control group (P < 0.05) (Table 2).

Table 2. Comparison of the incidence of adverse reactions of patients between two groups [n (%)]

Group	Case (n)	Radiation enteritis	Radio cystitis	Bone marrow suppression
Observation group	60	6(10.0)	7(11.7)	5(8.3)
Control group	54	15(27.8)	17(31.5)	4(7.4)
□^{2}		4.76	5.53	0.03
P		0.03	0.02	0.87

Comparison of the follow-up of patients between two groups

Follow-up was terminated on July 31, 2020. During the follow-up, there were 5 patients in the control group and 7 in the observation group losing to the follow-up. The medians of follow-up of the patients in the control group and the observation group were (40.89±7.41) months and (41.08±7.25) months, respectively. Among the remaining subjects, no significant difference was identified in the comparison of the recurrence rate or mortality rate between the two groups (all P > 0.05; Table 4).

Endometrial cancer, as one of the most common tumors in the female reproductive system, is only secondary to cervical cancer in prevalence. A survey in recent years has shown an increasing trend of the mortality rate and morbidity rate in the world, gradually affecting the health of the younger women (5, 16). Correspondingly, the application of tumor markers has facilitated the progress of cancer surveys, screening of the high-risk population, differential diagnosis, and evaluation of clinical stage of tumor, efficacy and prognosis of treatment (17, 18). YKL-40, as a member of mammal chitinases, is critical to the proliferation, survival and infiltration of malignant tumors, and the angiogenesis, inflammation and remodeling of the extracellular matrix surrounding the tumor tissues, and, as a growth factor of tumor cells, can suppress the apoptosis of tumor cells (19, 20). HE4 is mainly distributed in the epithelium of the reproductive system, including seminiferous tubule,

epididymis, tubal epithelium and endometrium, and almost over 90% of endometrial cancer patients are positive to the HE4 expression, which, thus, making HE4 a key indicator for endometrial cancer (21, 22). PRL, mostly derived from the lactating cells in the pituitary body, is regulated by the neuroendocrine system to promote lactation, and PRL at a high level can increase the motility of cancer cells, and promote angiogenesis in tumors. Thus, regulating the generation of PRL by monitoring is conducive to delaying the progression of tumors (19).

Efficient chemotherapeutic protocol plays a positive role in decreasing the recurrence rate and increasing the long-term survival rate of high-risk endometrial cancer patients. Accumulating evidence has shown that TC protocol is more preferable to the treatment of high-risk endometrial cancer, excelling the protocol of doxorubicin and cisplatin in efficacy, but showing less toxicity and better tolerance for patients (13, 23). However, it is reported that single chemotherapy for high-risk endometrial cancer patients fails in control of local recurrence when compared to those receiving radiotherapy (24). A single application of radiotherapy, though decreasing the recurrence rate, fails to reduce the distal metastatic rate, or prolong the median survival time (25). The latest evidence has shown that sequential chemotherapy and radiotherapy works better in reducing the long-term recurrence rate, shortening the treatment time, and mitigating the adverse reaction, thereby benefiting the patients (26). Thus, a combined strategy of chemotherapy and radiotherapy is critical to high-risk endometrial cancer.

IMRT, developed from the 3D-CRT, can align the high-dose rays in 3D space according to the tumor shape by adjusting the dose rate and strength, thereby protecting the surrounding organs, increasing the dose in the target zone and the control rate. As compared to the previous 3D-CRT, IMRT can not only attain the more optimal pattern of dose distribution in the target zone but also mitigate the injury to the surrounding tissues (27, 28). In this study, we found that after treatment, levels of YKL-40, HE4 and PRL in serum of patients in two groups were decreased when compared to those before treatment (all $P < 0.05$), while the difference between the two groups showed no statistical significance ($P > 0.05$).

Hence, IMRT shows efficacy similar to the 3D-CRT. However, a comparison of the incidence rates of the bone marrow suppression during treatment showed no significant difference ($P > 0.05$). The incidence rates of radiation enteritis and radiocystitis in the observation group were much lower than those in the control group ($P < 0.05$). Analysis revealed that the difference may derive from the capability of IMRT in protecting the surrounding tissues via aligning the doses in the target zone efficiently. On other hand, the evaluation of Nrf2 transcription factor expression showed that significant differences were shown in comparisons of the Nrf2 Expression between two groups ($P > 0.05$), and this enhancement was significant in the control group after treatment, which means that the amounts of ROS and antioxidants in patients who were treated with the pelvic IMRT method were less than the 3D-CRT method, so Nrf2 had a minimal role in regulating the antioxidant system in this method.

In conclusion, pelvic IMRT in combination with chemotherapy is a promising and safe candidate for high-risk endometrial cancer, with mild radiation injury; besides, YKL-40, HE4 and PRL are the effective indicators for prediction of efficacy in chemotherapy for endometrial cancer.

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None.

Interest conflict

None.

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