



Effect of DC-CIK treatment on tumor markers and T cell subsets in patients with advanced ovarian cancer

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ABSTRACT

Objective: To investigate the effects of dendritic cells (DC) and cytokine induced killer cells (CIK) on tumor markers and T cell subsets in peripheral blood of patients with advanced ovarian cancer. **Methods:** A total of 100 cases of patients with advanced ovarian cancer who were proved by operation and pathology in the department of gynecologic oncology in our hospital were selected from April 2013 to April 2016, and randomly divided into experimental group and control group, the control group was treated with TC (Taxinol+Cisplat) chemotherapy alone, the experimental group was treated with DC-CIK combined with chemotherapy. Before and after treatment, the changes of CD3⁺, CD4⁺, CD8⁺, CD4⁺/CD8⁺, CD4⁺/CD25⁺, NK cells in peripheral blood and serum tumor markers (CA125, CA19-9, HE4) were detected. **Results:** Before treatment, the phenotypes of T cell subsets in the two groups were not significantly different; in the experimental group after treatment, the levels of CD3⁺, CD4⁺, CD4⁺/CD8⁺, and NK cells were increased, while the levels of CD4⁺/CD25⁺ and CD8⁺ were decreased, compared with before treatment, the differences were statistically significant; the phenotype changes of T cells were not statistically significant before and after treatment in the control group; after treatment, there were significant differences in the levels of CD4⁺, CD8⁺, CD4⁺/CD8⁺, CD4⁺/CD25⁺ and NK cells between the two groups. Before treatment, there were no significant differences in HE4 value, CA125 value and CA19-9 value between the two groups; after treatment, the tumor markers in the two groups were all decreased, and the difference was significant as compared with those before treatment; after treatment, the CA125 value, CA19-9 value and HE4 value were (73.68±79.46) U/mL, (54.32±32.85) U/mL and (69.57±39.85) pmol/L respectively, the values of three tumor markers were compared with the control group, with a statistical difference. **Conclusion:** DC-CIK treatment can improve the immune ability and antitumor activity of T lymphocytes in patients with advanced ovarian cancer.

1. Introduction

Ovarian cancer is one of the common malignant tumors in women, the mortality rate ranks first in gynecological malignant tumors. The efficacy of postoperative radiotherapy and chemotherapy is not very obvious in patients with ovarian cancer, especially in patients with advanced stage, and easy to recurrence and metastasis[1].

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Tumor immunotherapy as a new treatment is paid more and more attention[2], dendritic cells (DC) combined with cytokine induced killer cells (CIK) immunotherapy is a new method of immunotherapy for malignant tumors, studies have reported that it had the advantages of enhancing immune ability, little toxic side effects, better tolerance, improving the quality of life and so on[3-5]. This research combined with DC-CIK immunotherapy on the basis of chemotherapy in the treatment of patients with advanced ovarian cancer, the effects of cell immunotherapy on tumor markers and cellular immune function in patients were studied and evaluated.

2. Materials and methods

2.1 Clinical data

From April 2013 to April 2016, a total of 100 patients with advanced ovarian cancer were admitted in the department of gynecologic oncology in our hospital, which were all untreated, and were confirmed as epithelial ovarian cancer in III-IV stage by pathology after cytoreducing-exligating operation on tumor, divided into the experimental group and the control group according to the random number table, 50 cases in each group, in the experimental group, aged 33-69 years old, with an average (56.6±13.4) years old; the pathological types were 26 cases of serous carcinoma, 13 cases of mucinous carcinoma, 7cases of endometrioid carcinoma and 4 cases of transitional cell carcinoma; the tumor stages were 19 cases of stage III, 31cases of stage IV. In the control group, aged 35-68 years old, with an average (55.2±12.8) years old; the pathological types were 27 cases of serous carcinoma, 12 cases of mucinous carcinoma, 6 cases of endometrioid carcinoma and 5 cases of transitional cell carcinoma; the tumor stages were 22 cases of stage III, 28 cases of stage IV.

2.2 Inclusion and exclusion criteria

Inclusion criteria: (1) previously untreated, after cytoreducing-exligating operation; (2) with no cell Immunotherapy method in the past; (3) QOL>20 points, KPS 40 points; (4) the expected survival time was more than 3 months. All patients signed informed consent. Exclusion criteria: (1) with disease of heart, kidney, liver and other important organs; (2) complicated with other malignant tumor; (3) complicated with psychological or mental disease; (4) unwilling to cooperate with medical staff to carry out relevant worker; (5) complicated with acute or chronic infectious diseases.

2.3 Treatment methods

Both the experimental group and the control group were treated with TC (Taxino+Cisplat) chemotherapy: Taxino was 175 mg/m², Cisplat was 75 mg/m² for intravenous fluid infusion, the first day, 21 d as a cycle. The experimental group was treated with DC-CIK combined with TC chemotherapy: treated with DC-CIK cells cultured in peripheral blood in 1 d before conventional TC therapy. DC-CIK was prepared in the peripheral blood of patients for 50

mL in 7 d before the first cycle of treatment, centrifuge for plasma reserve, the blood was diluted by normal saline and separated by lymphocyte separation medium, collection of single nuclear cells in the interfacial layer and washed by culture medium, the cells were suspended in the medium in a certain concentration, and then cultured in incubator of 50 mL/L, temperature of 37 °C after adding the recombinant human interferon- γ , 2 d later, adding Anti-CD3 and rhIL-2 culture medium, replacing the culture medium of rhIL-2 every 3 d, culturing for about 7 d and then reinfusion in 10 times, all patients were treated with 21 d for a cycle, totally treated for 3 cycles.

2.4 Curative effect observation

2.4.1 T cell subsets Detection

3 mL peripheral venous blood was extracted from patients respectively before treatment (1 d before the first cycle of chemotherapy) and after treatment (1 d before the last cycle of chemotherapy). The flow cytometry produced by American Coulter Beckman company was used to detect, the main detection indicators were T helper cells (CD4⁺), T suppressor cells (CD8⁺), immune status (CD4⁺/CD8⁺), total T lymphocytes (CD3⁺), regulatory T cells (CD4⁺/CD25⁺) and natural killer cells (NK cells).

2.4.2 Serum tumor markers detection

The peripheral blood was extracted from patients in 1 day before the first cycle of chemotherapy and the third cycle of chemotherapy, the values of serum CA125, CA19-9 and HE4 were detected by ELISA method, the reagents were provided by Wuhan Boster Biotechnology Co., Ltd, operations were strict adherence to the instruction.

2.5 Statistical treatment

All indicators before and after treatment in the two groups were processed by SPSS 20.0 software, *t* test was used for comparison, with *P*<0.05 for the difference was statistically significant.

3. Results

3.1 Comparison of the changes of T cell subsets (Table 1)

Table 1.

Phenotypic changes of T cell subsets [($\bar{x}\pm s$),%].

Group	Time	CD3 ⁺	CD4 ⁺	CD8 ⁺	CD4 ⁺ /CD8 ⁺	CD4 ⁺ /CD25 ⁺	NK cells
Experimental (n=50)	Before treatment	60.35±5.72	30.58±6.58	31.58±5.84	1.01±0.21	12.82±2.21	12.84±5.47
	After treatment	62.78±6.43 [#]	43.01±7.98 ^{**}	24.62±6.14 ^{**}	2.35±0.96 ^{**}	7.95±2.78 ^{**}	26.33±6.28 ^{**}
Control (n=50)	Before treatment	61.14±4.21	30.84±10.24	30.92±7.25	0.98±0.16	13.21±2.56	13.18±4.77
	After treatment	62.38±5.54	27.87±9.17	32.92±6.42	0.97±0.24	12.65±3.67	14.54±5.95

Note: compared with the control group, ^{*}*P*<0.05; compared with before treatment, [#]*P*<0.05.

Before treatment, the comparison of T cell subsets phenotypes in both groups was not statistically significant ($P>0.05$); after treatment, the levels of CD3⁺, CD4⁺, CD4⁺/CD8⁺, NK increased and CD4⁺/CD25⁺, CD8⁺ decreased in the experimental group, the difference was statistically significant in comparison to before treatment ($P<0.05$); phenotypic changes of T cell subsets were not statistically significant before and after treatment in the control group ($P>0.05$); after treatment, the comparison of CD4⁺, CD8⁺, CD4⁺/CD8⁺, CD4⁺/CD25⁺, NK between the two groups was statistically significant ($P<0.05$).

3.2 Changes of serum tumor markers (Table 2)

Before treatment, the comparison of HE4 value, CA125value, CA19-9 value in both groups was not statistically significant ($P>0.05$); after treatment, the levels of tumor markers in both groups were all decreased in the experimental group, the difference was statistically significant in comparison to before treatment ($P<0.05$); after treatment, the values of CA125, CA19-9 and HE4 were (73.68±79.46) U/mL, (54.32±32.85) U/mL and (69.57±39.85) pmol/L, three tumor markers compared with the control group, the difference was statistically significant ($P<0.05$).

4. Discussion

Ovarian cancer is a common malignant tumor in female genital system, in 2012, about 230 000 new cases in the world, of which about 140 000 patients died [7]. Early symptoms of ovarian cancer is not obvious, about 3/4 of patients have been in the middle or late stage at the time of care, so the mortality rate ranks first in the mortality of female genital system malignant tumors[8]. After cytoreducing-exligating operation on ovarian cancer tumor cells and including platinum based standard chemotherapy, ovarian cancer patients can obtain clinical remission, but most of the patients soon relapse, the 5 year survival rate is only about 30%[9]. In the past twenty years, along with the research on the immune mechanism of ovarian cancer, the door of biological immunotherapy for ovarian cancer is gradually opened. DC-CIK is a new method of adoptive immunotherapy, in the comprehensive treatment of lung cancer, gastric cancer, ovarian cancer and other tumors, improving the immune function of patients, extending the survival time of patients, and achieving good therapeutic effect[10-12].

DC can induce immune response by activating T lymphocytes, it is the important antigen presenting cells in vivo to initiate the immune function, and exerts a strong anti-tumor immune effect[13]. CIK is a kind of cytotoxic T cells, which has a strong tumor killing effect. The maximum value of DC-CIK immunotherapy lies in the regulation of immune function, Dendritic cells can play the role of antigen presenting cells to induce and enhance the function of cellular immune function and combine with CIK through the application of cytotoxic T cell function to killing tumor cells[14]. Cellular immunity is the main immune response mechanism of anti tumor in vivo, and it plays a role in immune regulation through different T lymphocytes[15].

CA125, CA19-9 and HE4 are three commonly used serum tumor markers in ovarian cancer[16]. CA125 is derived from antigen glycoprotein in epithelial ovarian cancer, which is of great clinical significance for the diagnosis of ovarian cancer[17], for serous ovarian cancer, the sensitivity is more than 80%[18]. At the same time, studies showed that CA125 has great value in the postoperative recurrence and metastasis of ovarian cancer, and to predict the sensitivity of chemotherapy drugs [19]. HE4 is a serum tumor marker of ovarian cancer founded in recent years, current researches considered that HE4 combined with CA125 can increase the diagnostic rate of ovarian cancer in women with high risk[20]. CA19-9 is a kind of tumor related markers, such as gastric cancer, pancreatic cancer, and so on, studies have found that CA19-9 is highly sensitive in the diagnosis of ovarian mucinous cancer, but it is less sensitive to serous cancer, combined detection with CA125 can improve the accuracy of ROMA index evaluation of the risk of ovarian cancer[21].

The progression of tumor is a multi-factor and multi-step process, which is closely related to the immune state of the human body, and the patients with malignant tumor are in a state of immune suppression for a long time, with low immunity. Cellular immunity plays the role of immune regulation through different T lymphocytes, and it is the main immune response mechanism of anti-tumor in vivo. The greatest value of DC-CIK immunotherapy is to regulate the immune function of the body, antigen presenting cells of dendritic cells play roles in inducing and enhancing cellular immune function of organism, CIK plays cytotoxic T cell function by killing tumor cells. CD4⁺ T cells and CD8⁺ T cells were two different types of T lymphocyte subsets after positive selection and negative selection, the former is a kind of important T helper cells, which enhances the cellular immune response; the latter is an important inhibitory T cell, which can inhibit the cellular immune response. The results

Table 2.
Changes of tumor markers.

Group	Time	CA125 (U/mL)	CA19-9 (U/mL)	HE4 (pmol/L)
Experimental (n=50)	Before treatment	387.86±149.32	305.58±95.51	254.85±158.52
	After treatment	73.68±79.46 [#]	54.32±32.85 [#]	69.57±39.85 [#]
Control (n=50)	Before treatment	376.75±148.68	298.79±89.47	258.42±163.72
	After treatment	219.59±97.59 [#]	179.25±52.86 [#]	103.87±57.69 [#]

Note: compared with the control group, [#] $P<0.05$; compared with before treatment, [#] $P<0.05$.

of this study showed that the levels of CD4⁺, CD4⁺/CD8⁺ and NK cells in the experimental group was significantly higher than those in the control group, and CD8⁺, CD4⁺/CD25⁺ cells were lower than those in the control group. After treatment, the levels of CD3⁺, CD4⁺, CD4⁺/CD8⁺, NK were significantly improved in the experimental group, which indicated that the immune therapy can regulate the immune function of the body and increase T cell immune efficacy, thus improving the immune status of patients with advanced ovarian cancer, which was beneficial to strengthen the ability of one's resistance to tumor.

In conclusion, DC-CIK combined with chemotherapy can improve the immune function of T lymphocytes in patients with advanced ovarian cancer and reduce the concentration of serum tumor markers, showing its good anti-tumor effect.

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