

# Effect of radiotherapy on tumor markers and serum immune-associated cells in patients with esophageal cancer\*

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## Abstract

**Objective** This study aimed to investigate the effect of radiotherapy on serum immune-associated cells and tumor markers in patients with esophageal cancer.

**Methods** A total of 87 patients with esophageal cancer admitted to our hospital between October 2016 and July 2020 were selected as the observation group, and all patients received radiotherapy. A total of 87 healthy volunteers who underwent physical examination at our hospital during the same period were selected as the control group in order to compare the changes in serum immune-associated cells and tumor markers between the two groups.

**Results** The levels of carcinoembryonic antigen (CEA), cancer antigen (CA) 125, CA72-4, C-terminus of cytokeratin (CYFRA) 21-1, and squamous cell carcinoma (SCC) antigen in the observation group before radiotherapy were higher than those in the control group, and the differences were significant ( $P < 0.05$ ). The levels of CEA, CA125, CA72-4, CYFRA21-1, and SCC antigen in the research group after radiotherapy were significantly lower than those before radiotherapy, but were still significantly higher than those in the control group ( $P < 0.05$ ). The levels of CD3<sup>+</sup>, CD4<sup>+</sup>, CD4<sup>+</sup>/CD8<sup>+</sup>, and natural killer cells in the research group before and after radiotherapy were significantly lower, while the levels of Treg and CD8<sup>+</sup> cells were significantly higher than those in the control group ( $P < 0.05$ ). The levels of CD3<sup>+</sup>, CD4<sup>+</sup>, and CD4<sup>+</sup>/CD8<sup>+</sup> cells in the observation group after radiotherapy were lower, while the levels of CD8<sup>+</sup> cells were significantly higher than those before radiotherapy ( $P < 0.05$ ).

**Conclusion** Radiotherapy can effectively reduce the level of serum tumor markers in patients with esophageal cancer; these antigens and cells can be used as tumor markers of esophageal cancer in order to determine its prognosis. However, radiotherapy has adverse effects on the immune function of the body. The reasons behind this need to be further studied and analyzed.

**Key words:** radiotherapy; esophageal cancer; tumor markers, immune-associated cells

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Esophageal cancer is a common malignant tumor of the digestive system, which gravely threatens human health and safety. Most esophageal cancer patients are already in the middle or late stages of the disease by the time they seek treatment and are no longer suitable to undergo surgery, and have a 5-year survival rate of less than 20%<sup>[1]</sup>. Radiotherapy has become an important method of treatment for middle and advanced esophageal cancer<sup>[2–3]</sup>; however, due to the different sensitivities of

each individual to radiotherapy, identification of effective indicators is needed to determine the therapeutic effect of radiotherapy. Serum tumor markers are rapid, simple, and less invasive detection indicators. Radiotherapy can treat esophageal cancer, but may also affect the normal immune function of the body. To further understand the clinical effect of radiotherapy on esophageal cancer and its influence on the immune function, changes in serum tumor markers and T cell subsets in patients with

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esophageal cancer were determined before and after radiotherapy and analyzed in this study.

## Materials and methods

### Data

A total of 87 patients with esophageal cancer admitted in our hospital between October 2016 and July 2020 were selected as the observation group, based on the following inclusion and exclusion criteria.

(1)Inclusion criteria: Patients who underwent endoscopic examination, ultrasound examination, and pathological examination to confirm the presence of TNM stage III; who had esophageal cancer metastasis; who refused to undergo surgical treatment or were unable to undergo surgery prior to radiotherapy; with no history of radiation or chemotherapy; with no contraindications to radiotherapy or chemotherapy; who had an expected survival time of not less than 6 months; who had a quality of life score of > 60 points; and who signed an informed consent, were included in the study.

(2)Exclusion criteria: Patients with severe heart, liver, and renal insufficiencies; mental disorders; and immune system diseases were excluded.

The observation group comprised 55 men and 32 women, with ages ranging from 43 to 82 years (mean age:  $61.7 \pm 8.2$  years); with regard to the TNM stage, 37 patients had stage III disease, while 50 had stage IV disease. In terms of the pathological type, 73 patients had squamous cell carcinoma (SCC), while 14 had adenocarcinoma. Ninety healthy volunteers who underwent physical examination in our hospital during the same period were selected as the control group, which included 53 men and 34 women. Their ages ranged from 41 to 83 years (mean age:  $62.4 \pm 9.2$  years). No significant difference was observed in the sex, age, and other basic data between the two groups ( $P > 0.05$ ), thus indicating comparability (Table 1).

### Methods

#### Treatment

All patients underwent computed tomography (CT). Continuous spiral CT scanning was performed with the upper boundary at the upper edge of the fourth cervical vertebra and the lower boundary at the lower edge of the second lumbar vertebra. The scanning images were transmitted to the three-dimensional (3D) treatment planning system; the target area was determined according to the examination results, and 3–5 coplanar fields were selected for irradiation. The radiotherapy techniques used were Varian linear accelerator 3D conformal radiotherapy, image-guided intensity-modulated radiotherapy, or volume rotation intensity-modulated radiotherapy. A total radiotherapy dose of 60

**Table 1** Characteristics of patients in observation group

Factor	n	%
Age (years)		
≤ 60	21	24.1
> 60	66	75.9
Sex		5
Male	55	63.2
Female	32	36.8
Smoking		
No	29	33.3
Yes	58	66.7
Pathological types		
Squamous	73	83.9
Adenosquamous cell carcinoma	14	16.1
Tumour site		
Upper 1/3	30	34.5
Middle 1/3	41	47.1
Lower 1/3	16	18.4
T stage		
II	2	2.3
III	35	40.2
IV	50	57.5
N stage (I)		
0	55	63.2
1	32	36.8
Clinic stage		
II	4	4.6
III	33	37.9
IV	50	57.5

Gy (2 Gy/fraction) was delivered, and the radiotherapy was performed for 30–32 cycles.

#### Fasting venous blood

Fasting venous blood was collected before and 1 month after radiotherapy in the observation group. Fasting venous blood was collected from the control group in the morning of the physical examination day and centrifuged at 3,000 r/min for 10 min; the serum was separated and stored at 2 °C to 6 °C for detection. Levels of the following serum tumor markers were measured using an Abbott I2000 chemiluminescence analyzer and the associated reagents: carcinoembryonic antigen (CEA), carbohydrate antigen 19-9 (CA19-9), carbohydrate antigen 72-4 (CA72-4), cytokeratin 19 fragment (CYFRA21-1), and SCC antigen. The levels of T cell subsets, including CD3+, CD4+, CD8+, and CD4+/CD8+ cells, were measured using a FACS Canto II flow cytometer.

### Statistical analysis

The SPSS22.0 software was used to perform all data analyses. The measurement data were expressed as mean  $\pm$  standard. Independent-sample *t* test was used to perform a between-group comparison, while paired *t*-test

was used to perform a within-group comparison.

## Results

### Comparison of serum tumor markers

The CEA, CA19-9, CA72-4, CYFRA21-1, and SCC antigen levels before radiotherapy in the observation group were higher than those in the control group, and the differences were significant ( $P < 0.05$ ). The CEA, CA19-9, CA72-4, CYFRA21-1, and SCC antigen levels in the observation group after radiotherapy were lower than those before radiotherapy, but were still higher than those of the control group; the differences were significant ( $P < 0.05$ ; Table 2).

### Comparison of T cell subsets

The levels of CD3+, CD4+, and CD4+/CD8+ cells before and after radiotherapy in the observation group were lower than those in the control group, while the levels of CD8+ were higher than those in the control group; the differences were significant ( $P < 0.05$ ). The levels of CD3+, CD4+, and CD4+/CD8+ cells in the observation group after radiotherapy were lower than those before radiotherapy, while the levels of CD8+ cells were higher than those before radiotherapy; the differences were significant ( $P < 0.05$ ; Table 3).

## Discussion

Esophageal cancer is a common malignant tumor of the digestive system and poses a serious threat to the life and health of Chinese residents. At present, surgery is the primary treatment method for early-stage esophageal cancer; however, for patients with middle and advanced stage esophageal cancer, surgical resection is no longer

effective, and thus, radiotherapy is often preferred for the clinical treatment of middle and advanced stage esophageal cancer [4]. In recent years, continual development in 3D conformal radiotherapy technology has allowed focus on the target area of tumor cells for irradiation, while reducing unnecessary damage to the surrounding normal tissues. However, radiotherapy may inhibit the immune function of the body, thus affecting its therapeutic effects. Therefore, it is of great clinical significance to explore effective detection indicators to evaluate the outcomes of radiotherapy and detect changes in immune function.

Comprehensive treatment is recommended for patients with inoperable advanced esophageal cancer. In this group, patients with medical diseases, of older age, or those unwilling to synchronize radiotherapy and chemotherapy prior to radiotherapy were selected. This study aimed to understand the effects of radiotherapy alone on tumor markers and immune cells and to provide a preliminary understanding of the mode of radiotherapy combined with other treatments. For patients with long-term follow-up, comprehensive treatment was preferred; for patients with advanced stage esophageal cancer, chemotherapy or immunotherapy combined with traditional Chinese medicine was provided.

Serum tumor markers are expressed in malignant tumor cells or are generated after the tumor tissue is stimulated; they play a predictive role in the occurrence and development of tumors [5]. CEA is a broad-spectrum tumor marker, and its high level of expression in the serum has suggestive effects on esophageal cancer, breast cancer, gastric cancer, among others [6]. CA19-9 is a glycoprotein tumor marker, and its serum level is increased in various types of cancer [7]. CA72-4 dynamic monitoring can be performed to assist in the clinical

**Table 2** Comparison of serum tumor markers

Groups	<i>n</i>	CEA (ng/mL)	CA19-9 (U/mL)	CA72-4 (U/L)	CYFRA21-1 (ng/mL)	SCC (ng/mL)
Observation group	87					
Before radiotherapy		4.25 ± 0.98 <sup>a</sup>	15.25 ± 3.98 <sup>a</sup>	5.64 ± 1.08 <sup>a</sup>	4.45 ± 0.98 <sup>a</sup>	2.45 ± 0.78 <sup>a</sup>
After radiotherapy		2.37 ± 0.86 <sup>ab</sup>	11.67 ± 2.86 <sup>ab</sup>	3.67 ± 0.89 <sup>ab</sup>	2.27 ± 0.56 <sup>ab</sup>	0.87 ± 0.32 <sup>ab</sup>
Control group	87	1.02 ± 0.73	7.52 ± 2.53	1.11 ± 0.33	1.02 ± 0.73	0.18 ± 0.05

a: Compared with the control group,  $P < 0.05$ ; b: compared with that before radiotherapy,  $P < 0.05$

**Table 3** Comparison of T cell subsets

Groups	<i>n</i>	CD3 <sup>+</sup>	CD4 <sup>+</sup>	CD8 <sup>+</sup>	CD4 <sup>+</sup> /CD8 <sup>+</sup>	NK cells	Treg cells
Observation group	87						
Before radiotherapy		62.25 ± 7.58 <sup>a</sup>	35.95 ± 8.13 <sup>a</sup>	27.45 ± 6.42 <sup>a</sup>	1.31 ± 0.29 <sup>a</sup>	15.64 ± 2.23 <sup>a</sup>	17.31 ± 2.29 <sup>a</sup>
After radiotherapy		55.32 ± 8.75 <sup>ab</sup>	32.21 ± 6.17 <sup>ab</sup>	32.07 ± 7.69 <sup>ab</sup>	0.81 ± 0.16 <sup>ab</sup>	20.01 ± 0.53 <sup>ab</sup>	10.48 ± 1.76 <sup>ab</sup>
Control group	87	66.42 ± 6.01	40.32 ± 5.53	23.89 ± 2.57	1.69 ± 0.73	26.19 ± 2.93	3.37 ± 0.43

a: Compared with the control group,  $P < 0.05$ ; b: compared with that before radiotherapy,  $P < 0.05$

diagnosis of esophageal cancer and observation of the curative effects of treatments<sup>[8]</sup>. CYFR21-1 can be used as a tumor marker to predict the tumor changes in lung and breast cancers, and to effectively distinguish patients with cancer from those without cancer<sup>[9]</sup>. SCC antigen is a tumor-related glycoprotein fragment that can be detected in the tissues of patients with esophageal, lung, and cervical cancer. Although CA19-9, CA125, and CA72-4 have been used as tumor markers in the clinical detection of digestive tract adenocarcinoma, some studies have reported that esophageal SCC antigen can also be used as a tumor marker to help clinically judge the prognosis of esophageal SCC; therefore, this marker was also included in the current study<sup>[8]</sup>. In the observation group, the levels of CEA, CA19-9, CA72-4, CYFRA21-1, and SCC antigen after radiotherapy were lower than those before radiotherapy, which was evidently related to the response of the tumor to radiotherapy. The results from this study showed that the levels of CEA, CA19-9, CA72-4, CYFRA21-1, and SCC antigen in the observation group before and after radiotherapy were higher than those in the control group; moreover, radiotherapy could inhibit the proliferation of cancer cells. However, whether the effect on the level of tumor markers is different from that after chemotherapy has not been reported.

T cell subsets are the main markers that reflect the immune function of the body. The co-receptors of CD3<sup>+</sup> T cells are common markers on the surface of T lymphocytes. CD4<sup>+</sup> cells can induce the differentiation of lymphocytes and production of antibodies, to induce an immune response. CD8<sup>+</sup> cells can act as inhibitory T cells, and often show cytotoxic activity, while inhibiting the secretion of antibodies. Tumor cells can directly activate or induce the increase in the expression of CD8<sup>+</sup> T cells to inhibit cellular immune responses, resulting in a decrease in the ratio of CD3<sup>+</sup>, CD4<sup>+</sup>, and CD4<sup>+</sup>/CD8<sup>+</sup> cells, and a decrease in the immune function of the body, thus allowing the tumor cells to evade immune surveillance and grow progressively<sup>[10]</sup>. The results of this study showed that the levels of CD3<sup>+</sup>, CD4<sup>+</sup>, and CD4<sup>+</sup>/CD8<sup>+</sup> cells in the observation group before and after radiotherapy were lower, while the levels of CD8<sup>+</sup> cells were higher than those in the control group; this indicated that the immune function of esophageal cancer patients was significantly reduced, which was consistent with the above results. In addition, the levels of CD3<sup>+</sup>, CD4<sup>+</sup>, CD4<sup>+</sup>/CD8<sup>+</sup>, and NK cells in the observation group after radiotherapy were lower, and the levels of CD8<sup>+</sup> and Treg cells were higher

than those before radiotherapy; this suggested that the immune function of patients with esophageal cancer was suppressed after undergoing radiotherapy, which may be caused by the significant non-selective killing effect of radiotherapy on normal tissues.

In conclusion, radiotherapy can effectively reduce the level of serum tumor markers in patients with esophageal cancer, but has adverse effects on the immune function of the body; hence, further clinical studies are needed to obtain a better clinical efficacy.

### Conflicts of interest

The authors indicated no potential conflicts of interest.

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