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Effect of enteral immunonutrition after radical surgery for esophageal carcinoma on anti−tumor immune response and intestinal mucosal barrier function

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ABSTRACT

Objective: To study the effect of enteral immunonutrition after radical surgery for esophageal carcinoma on anti-tumor immune response and intestinal mucosal barrier function. **Methods:** A total of 102 patients who received radical surgery for esophageal carcinoma in our hospital between May 2013 and December 2016 were selected and randomly divided into observation group and control group who received postoperative enteral immunonutrition and routine enteral nutrition respectively. 1 d before operation as well as 1 d and 7 d after operation, peripheral blood immune cell marker expression and serum intestinal mucosal barrier injury marker levels were detected. **Results:** 1 d after operation, peripheral blood T-bet, NKG2D, NKp30, NKp44 and NKp46 fluorescence intensity of both groups of patients were significantly lower than those 1d before operation while peripheral blood GATA-3 and Foxp3 fluorescence intensity as well as serum DAO, Occludin, ZO-1 and claudin-1 levels were significantly higher than those 1d before operation; peripheral blood T-bet, NKG2D, NKp30, NKp44 and NKp46 fluorescence intensity of observation group 7 d after operation were significantly higher than those 1 d after operation while peripheral blood GATA-3 and Foxp3 fluorescence intensity as well as serum DAO, Occludin, ZO-1 and claudin-1 levels were significantly lower than those 1 d after operation; peripheral blood T-bet, GATA-3, Foxp3, NKG2D, NKp30, NKp44 and NKp46 fluorescence intensity of control group 7 d after operation were not significant different from those 1 d after operation, and serum DAO, Occludin, ZO-1 and claudin-1 levels were significantly lower than those 1d after operation. **Conclusion:** Enteral immunonutrition after radical surgery for esophageal carcinoma can enhance the anti-tumor immune response and improve the intestinal mucosal barrier function.

1. Introduction

Esophageal cancer is a common malignant tumor in the digestive system and the incidence is increasing year by year. Radical resection is the preferred method for treatment of esophageal cancer, but it is quite traumatic. Esophageal cancer patients are mostly with different degrees of eating difficulties and nutrition loss before operation, and the operation trauma can make the body at a high metabolic status, increase the consumption of nutrients and increase the nutrition loss. Therefore, effective nutritional intervention is needed after radical operation for esophageal cancer. Total parenteral nutrition can directly provide the nutrients necessary for the body's metabolism, and avoid improper absorption function-induced nutritional status declining, but the long-term use of parenteral nutrition can cause metabolic disorders and intestinal mucosal barrier function injury, and in recent years, the total parenteral nutrition after radical operation for esophageal cancer has been gradually replaced by enteral nutrition[1,2]. Enteral immunonutrition is the nutritional preparation that adds arginine, glutamine, polyunsaturated fatty acids and other ingredients on the basis of traditional enteral nutrition, which helps regulate immune function and enhance antioxidant capacity[3]. In the following study, the effect of enteral immunonutrition after radical surgery for esophageal carcinoma on anti-tumor immune response and intestinal mucosal barrier function was analyzed.

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2. Subjects and methods

2.1 General information of research subjects

A total of 102 patients who received radical surgery for esophageal carcinoma in our hospital between May 2013 and December 2016 were selected, all patients were diagnosed with esophageal cancer by preoperative gastrointestinal endoscopy and pathology biopsy, and in accordance with radical resection indications, and patients associated with immune system diseases and endocrine system diseases were ruled out. Random number table was used to divide the 102 patients with esophageal cancer into two groups, each with 51 cases. Observation group included 29 men and 22 women that were 48-65 years old; control group included 30 men and 21 women that were 49-63 years old. There was no significant difference in general information between the two groups of patients.

2.2 Nutritional support methods

Both groups of patients started to received enteral nutrition from the first day after operation, total heat was 30 kcal/(kg • d), total nitrogen was 0.2 g/(kg • d), and they received 1/3 of the target total heat on the first day after operation, received 1/2 of the target total heat on the second day and received full amount of the target total heat on the third day. Control group received routine enteral nutrition preparation Nutrison Fibre that contained calories 420 kJ, protein 4 g, sugar 12.38 g and fat 3.89 g per 100 mL as well as dietary fiber, vitamins and trace elements. Observation group of patients received enteral immunonutrition preparation Supportan that contained calories 546 kJ, protein 5.85 g, sugar 10.4 g, fat 7.2 g, arginine 0.23 g, ω3 polyunsaturated fatty acids 0.3 and nucleotides 0.3 per 100 mL as well as dietary fiber, vitamins and trace elements. The therapies lasted for 7 d.

2.3 Peripheral blood immune cell marker expression detection

1 d before operation as well as 1 d and 7 d after operation, 1ml of peripheral venous blood was collected from two groups of patients, anti-coagulated with EDTA, used to directly incubate the T-bet, GATA-3, Foxp3, NKG2D, NKp30, NKp44 and NKp46 fluorescence antibody, then joined by permeabilisation and continued to be incubated, and finally flow cytometer was used to detect the T-bet, GATA-3, Foxp3, NKG2D, NKp30, NKp44 and NKp46 fluorescence antibody.

2.4 Serum intestinal mucosal barrier injury marker detection

1 d before operation as well as 1 d and 7 d after operation, 5 mL of peripheral venous blood was collected from two groups of patients and centrifuged, and the upper serum was collected to detect the levels of DAO, Occludin, ZO-1 and claudin-1 by enzyme-linked immunosorbent assay kit.

2.5 Statistical methods

SPSS 20.0 software was used for variance analysis of peripheral blood and serum data, and $P<0.05$ indicated statistical significance in differences.

3. Results

3.1 Peripheral blood T cell subset marker expression

Analysis of perioperative peripheral blood T cell subset markers T-bet, GATA-3 and Foxp3 expression between two groups of patients was as follows: peripheral blood T-bet, GATA-3 and Foxp3 fluorescence intensity were not significantly different between two groups of patients 1 d before operation ($P>0.05$); 1 d after operation, peripheral blood T-bet fluorescence intensity of both groups of patients were significantly lower than those 1 d before operation while GATA-3 and Foxp3 fluorescence intensity were significantly higher than those 1 d before operation ($P<0.05$), and peripheral blood T-bet, GATA-3 and Foxp3 fluorescence intensity were not significantly different between two groups of patients ($P>0.05$); 7 d after operation, peripheral blood T-bet fluorescence intensity of observation group was significantly higher than that 1 d after operation while peripheral blood GATA-3 and Foxp3 fluorescence intensity were significantly lower than those 1 d after operation ($P<0.05$), and peripheral blood T-bet, GATA-3 and Foxp3 fluorescence intensity of control group were not significant different from those 1 d after operation ($P>0.05$).

Table 1.

Perioperative peripheral blood T cell subset marker expression.

Groups	*n*	Time	T-bet	GATA-3	Foxp3
Observation group	51	1 d before operation	13.82±1.85	9.24±1.03	3.28±0.46
		1 d after operation	6.48±0.89[a]	15.42±1.88[a]	7.68±0.93[a]
		7 d after operation	9.41±1.05[*b]	12.28±1.57[*b]	5.03±0.77[*b]
Control group	51	1 d before operation	14.02±1.77	9.42±1.08	3.31±0.49
		1 d after operation	6.61±0.84[a]	15.51±1.92[a]	7.71±0.89[a]
		7 d after operation	6.94±0.79	14.98±1.67	7.35±0.93

[*]: comparison between observation group and control group, $P<0.05$; [a]: comparison between 1 d after operation and 1 d before operation, $P<0.05$; [b]: comparison between 7 d after operation and 1 d after operation, $P<0.05$.

3.2 Peripheral blood NK cell marker expression

Analysis of perioperative peripheral blood NK cell markers NKG2D, NKp30, NKp44 and NKp46 expression between two groups of patients was as follows: peripheral blood NKG2D, NKp30, NKp44 and NKp46 fluorescence intensity were not significantly different between two groups of patients 1 d before operation (P>0.05); 1 d after operation, peripheral blood NKG2D, NKp30, NKp44 and NKp46 fluorescence intensity of both groups of patients were significantly lower than those 1 d before operation (P<0.05), and peripheral blood NKG2D, NKp30, NKp44 and NKp46 fluorescence intensity were not significantly different between two groups of patients (P>0.05); 7 d after operation, peripheral blood NKG2D, NKp30, NKp44 and NKp46 fluorescence intensity of observation group were significantly higher than those 1 d after operation (P<0.05), and peripheral blood NKG2D, NKp30, NKp44 and NKp46 fluorescence intensity of control group were not significant different from those 1 d after operation (P>0.05).

3.3 Serum intestinal mucosal barrier injury marker levels

Analysis of perioperative serum intestinal mucosal barrier injury markers DAO (U/L), Occludin (μg/L), ZO-1 (μg/L) and claudin-1 levels between two groups of patients was as follows: serum DAO, Occludin, ZO-1 and claudin-1 levels were not significantly different between two groups of patients 1 d before operation (P>0.05); 1 d after operation, serum DAO, Occludin, ZO-1 and claudin-1 levels of both groups of patients were significantly higher than those 1 d before operation (P<0.05), and serum DAO, Occludin, ZO-1 and claudin-1 levels were not significantly different between two groups of patients (P>0.05); 7 d after operation, serum DAO, Occludin, ZO-1 and claudin-1 levels of both groups were significantly lower than those 1 d after operation (P<0.05), and serum DAO, Occludin, ZO-1 and claudin-1 levels of observation group were significantly lower than those of control group (P<0.05).

4. Discussion

Proper nutritional intervention after radical operation for esophageal cancer can help the gastrointestinal function to recover quickly and enhance the body's ability to endure surgical trauma. Total parenteral nutrition can directly supplement the body's nutritional requirements, but it can affect the intestinal mucosa barrier function and cause the intestinal flora translocation, so it is gradually replaced by the enteral nutrition[4,5]. Conventional enteral nutrition in radical operation for esophageal cancer can on the one hand, provide the nutrients needed for the body's metabolism, and on the other hand, help to protect the intestinal mucosal barrier function, but it has no obvious regulation on the inflammatory response, stress response or immune response[6,7]. Enteral immunonutrition contains arginine, glutamine, polyunsaturated fatty acids and other components with immunoregulatory activity on the basis of conventional enteral nutrition, and it can exactly enhance and improve the immune response. The Supportan in the study contains arginine, ω3 polyunsaturated fatty acids, nucleotides and so on, arginine can promote the differentiation of maturation of a variety of T cells, NK cells and other immune cells, ω3 polyunsaturated fatty acids can inhibit the synthesis of inflammatory mediators and

Table 2.

Perioperative peripheral blood NK cell marker expression.

Groups	n	Time	NKG2D	NKp30	NKp44	NKp46
Observation group	51	1 d before operation	4.28±0.75	2.98±0.36	1.57±0.24	3.26±0.57
		1 d after operation	2.45±0.35[a]	1.02±0.15[a]	0.67±0.09[a]	1.47±0.17[a]
		7 d after operation	3.38±0.47[*b]	1.78±0.22[*b]	1.06±0.15[*b]	2.37±0.33[*b]
Control group	51	1 d before operation	4.34±0.69	3.02±0.41	1.63±0.28	3.31±0.59
		1 d after operation	2.49±0.37[a]	1.08±0.14[a]	0.70±0.08[a]	1.50±0.20[a]
		7 d after operation	2.62±0.33	1.16±0.18	0.76±0.09	1.61±0.21

[*]: comparison between observation group and control group, $P<0.05$; [a]: comparison between 1 d after operation and 1 d before operation, $P<0.05$; [b]: comparison between 7 d after operation and 1 d after operation, $P<0.05$.

Table 3.

Perioperative serum intestinal mucosal barrier injury marker levels.

Groups	n	Time	DAO	Occludin	ZO-1	Claudin-1
Observation group	51	1 d before operation	2.37±0.36	10.37±1.38	17.68±2.25	7.58±0.93
		1 d after operation	5.86±0.62[a]	22.31±3.47[a]	33.16±4.59[a]	21.25±3.26[a]
		7 d after operation	2.98±0.38[*b]	14.52±1.93[*b]	23.31±3.59[*b]	13.52±1.88[*b]
Control group	51	1 d before operation	2.41±0.38	10.51±1.55	17.91±2.44	7.71±0.98
		1 d after operation	5.91±0.67[a]	22.19±3.31[a]	33.89±5.21[a]	22.05±3.16[a]
		7 d after operation	3.77±0.48[b]	19.84±2.26[b]	28.58±3.57[b]	17.86±2.21[b]

[*]: comparison between observation group and control group, $P<0.05$; [a]: comparison between 1 d after operation and 1 d before operation, $P<0.05$; [b]: comparison between 7 d after operation and 1 d after operation, $P<0.05$.

oxidative stress products and reduce the immunosuppressive action of above materials, and nucleotides can participate in biological processes such as DNA replication and cell division[8,9]. It has been reported that enteral immunonutrition has the effects of improving the nutritional status of radical operation for esophageal cancer and preventing the occurrence of complications, and it is the ideal enteral nutrition preparation[10].

At present, there is no clear report on the effect of enteral immunonutrition on the immune response after radical operation for esophageal cancer. T lymphocytes are important cells that mediate anti-tumor immune response, and $CD4^+$T cell subgroups Th1, Th2 and Treg can interact with each other and participate in anti-tumor immune response process[11]. Th1 has significant anti-tumor activity, which is activated under the action of transcription factor T-bet, and then secrete a large number of cytokines to kill cancer cells; Th2 and Treg are inhibitory cell subgroups that are regulated by transcription factor GATA-3 and Foxp3 respectively[12,13]. In order to define the effect of intestinal immunonutrition on T cell-mediated immune response after radical surgery for esophageal cancer, perioperative different T cell subgroup surface marker expression was analyzed in the study. Peripheral blood T-bet fluorescence intensity of both groups of patients 1 d after operation were significantly lower than those 1 d before operation while GATA-3 and Foxp3 fluorescence intensity were significantly higher than those 1 d before operation. This suggests that radical operation for esophageal cancer can cause different levels of immune disorders, inhibit the function of Th1 cells and increase the function of Th2 and Th17 cells. Peripheral blood T-bet fluorescence intensity of observation group 7d after operation was significantly higher than that 1 d after operation while GATA-3 and Foxp3 fluorescence intensity were significantly lower than those 1 d after operation, and peripheral blood T-bet, GATA-3 and Foxp3 fluorescence intensity of control group 7 d after operation were not significant different from those 1 d after operation. This indicates that the conventional enteral nutrition cannot regulate the immune response after radical operation for esophageal cancer; and enteral immunonutrition can correct the immune disorders after radical operation for esophageal cancer, enhance the function of Th1 cells and inhibit the function of Th2 and Th17 cells.

The anti-tumor immune response in vivo is not only dependent on the specific immune response mediated by T lymphocytes, but also closely related to the non-specific immune response mediated by NK cells. On the one hand, NK cells can identify TCR/CD3 complex to activate ADCC and then kill tumor cells; on the other hand, they can secrete multiple cytokines to kill tumor markers. The activating receptors NKG2D, NKp30, NKp44 and NKp46 on cell surface play a crucial role in the activation of NK cells[14,15]. In order to clarify the effect of enteral immunonutrition on the immune response mediated by NK cells after radical operation for esophageal cancer, the perioperative surface marker expression on different NK cells were analyzed in the study. Peripheral blood NKG2D, NKp30, NKp44 and NKp46 fluorescence intensity of both groups of patients 1 d after operation was significantly lower than that 1 d before operation. This indicates that the radical operation for esophageal cancer can inhibit the activation of NK cells to different extent. Peripheral blood NKG2D, NKp30, NKp44 and NKp46 fluorescence intensity of observation group 7 d after operation were significantly higher than those 1d after operation, and peripheral blood NKG2D, NKp30, NKp44 and NKp46 fluorescence intensity of control group 7 d after operation were not significant different from those 1 d after operation. This means that conventional enteral nutrition cannot regulate the activity of NK cells after radical operation for esophageal cancer, and enteral immunonutrition can correct the immune disorder after radical operation for esophageal cancer, and promote the activation of NK cells.

The prominent value of enteral nutrition after radical operation for esophageal cancer is to maintain the integrity of the intestinal mucosal barrier function, prevent the systemic inflammatory response caused by intestinal flora translocation and endotoxin release into the blood, reduce the body damage and promote the functional recovery. In the process of intestinal mucosal barrier injury, the DAO that is involved in nucleic acid and protein metabolism in intestinal mucosal epithelial cells will be released into the blood circulation, and the Occludin, Claudin-1 and ZO-1 that participate in the regulation of intestinal mucosal epithelial intercellular junction will also be released into the blood with the destruction of the mucosal barrier[16,17]. In order to clarify the effect of enteral immunonutrition on the intestinal mucosal barrier function after radical operation for esophageal cancer, the contents of the intestinal mucosal barrier injury markers in perioperative period were analyzed in the study. Serum DAO, Occludin, ZO-1 and claudin-1 levels of both groups of patients 1 d after operation were significantly higher than those 1 d before operation. This indicates that radical operation for esophageal cancer can cause different levels of intestinal mucosal barrier injury. Serum DAO, Occludin, ZO-1 and claudin-1 levels of both groups 7 d after operation were significantly lower than those 1 d after operation, and serum DAO, Occludin, ZO-1 and claudin-1 levels of observation group 7 d after operation were significantly lower than those of control group. This means that both conventional enteral nutrition and enteral immunonutrition can protect the intestinal mucosa barrier function, and enteral immunonutrition is better than the conventional enteral nutrition in improving the intestinal mucosal barrier function.

Enteral immunonutrition after radical operation for esophageal cancer can enhance the anti-tumor immune response, enhance the function of Th1 cells and NK cells, and inhibit the function of Th2 and Th17 cells; it can also improve the intestinal mucosa barrier function.

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