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Original Article / Özgün Makale

Positive effect of glutamine-enforced parenteral nutrition on immune function and stress response of patients after esophageal cancer operation

Özofagus kanseri ameliyatından sonra glutamin içeren parenteral besinin hastaların immün fonksiyonu ve stres yanıtı üzerindeki olumlu etkisi

Xu Bin-Dong, Huang Guo-Zhong, Chen Hao

Department of Cardiothoracic Surgery, the Affiliated Hospital of Putian University, Putian, Fujian, China

ABSTRACT

Background: This study aims to determine the effect of glutamine-enforced parenteral nutrition on immune function and stress response of patients after esophageal operation.

Methods: A total of 143 esophageal cancer patients (71 males, 72 females; mean age 70.7±4.3 years; range 35 to 80 years) undergoing the same elective operation were randomized into two groups as the experimental group which was given additional alanyl-glutamine (0.35 g/kg/day glutamine) and the control group which was performed simple vein injection. All patients were infused with equal calorie and nitrogen by central vein during the postoperative seven days. Patients' immunological and inflammatory parameters were analyzed.

Results: Preoperatively, the levels of immunoglobulin A, G, M levels, CD3+ and CD4+ T lymphocytes, and CD4+/CD8+ T lymphocytes ratio of both groups were lower than normal values. The levels of complement 3 (C3), C4 and C reactive protein of both groups were higher than normal values. On the first postoperative day, all indicators of experimental group were superior to the control group except for levels of C reactive protein, immunoglobulin M, and CD3+ and CD8+ cells ($p>0.05$). On the seventh postoperative day, the C reactive protein level of experimental group was lower than that of control group ($p<0.05$), while the CD4+ cells, immunoglobulin A and G levels of experimental group were higher than the control group ($p<0.05$).

Conclusion: Compared with conventional parenteral nutrition, glutamine-enforced parenteral nutrition support may significantly improve the immune function and reduce stress response in patients after esophageal operation.

Keywords: Esophageal cancer; glutamine; parenteral nutrition.

ÖZ

Amaç: Bu çalışmada özofagus ameliyatından sonra glutamin uygulamalı parenteral besinin hastaların immün fonksiyonu ve stres yanıtı üzerindeki etkisi belirlendi.

Çalışma planı: Aynı elektif ameliyatı geçiren 143 özofagus kanseri hastası (71 erkek, 72 kadın; ort. yaş 70.7±4.3 yıl; dağılım 35-80 yıl) ek olarak alanil-glutamin (0.35 g/kg/gün glutamin) verilen deney grubu ve basit damar enjeksiyonu uygulanan kontrol grubu olmak üzere iki gruba ayrıldı. Ameliyat sonrası yedi gün boyunca tüm hastalara santral damardan eşit kalori ve nitrojen aşılandı. Hastaların immünolojik ve enflamatuvar parametreleri incelendi.

Bulgular: Ameliyat öncesinde, her iki grubun immünoglobulin A, G, M düzeyleri, CD3+, CD4+ hücreler ile CD4+/CD8+ hücre oranları normal değerlerden daha düşük idi. Her iki grubun komplement 3 (C3), C4 ve C reaktif protein düzeyleri normal değerlerden daha yüksek idi. Ameliyat sonrası birinci gün, deney grubunun tüm belirteçleri C reaktif protein, immünoglobulin M düzeyleri, ve CD3+ ve CD8+ hücreler hariç kontrol grubundan daha yüksek idi ($p>0.05$). Ameliyat sonrası yedinci gün, deney grubunun C reaktif protein düzeyi kontrol grubundan daha düşük iken ($p<0.05$) CD4+ hücreler ile immünoglobulin A ve G düzeyleri daha yüksek idi ($p<0.05$).

Sonuç: Konvansiyonel parenteral besin ile karşılaştırıldığında, glutamin uygulamalı parenteral besin desteği özofagus ameliyatından sonra hastalarda immün fonksiyonu anlamlı şekilde iyileştirip stres yanıtını azaltabilir.

Anahtar sözcükler: Özofagus kanseri; glutamin; parenteral besin.

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Correspondence: Xu Bin-Dong, MD. the Affiliated Hospital of Putian University, No: 999, Dongzhen Road, Licheng District, 351100 Putian, Fujian, China.
Tel: +86 135 59 39 14 38 e-mail: xubd2002@163.com

Esophageal cancer is not an infrequent malignant tumors in China. Worldwide, almost 400,000 new cases of esophageal cancer are diagnosed annually- it is the eighth most common cancer and the sixth most common cause of cancer-related mortality.[1] China carries a big burden of esophageal cancer with an incidence of 19.3 per 100,000 in 2008,[2] and accounts for more than half of the new cases diagnosed in the world.[3] Due to tumor and intestinal obstruction, these patients are affected by negative nitrogen balance, stress, and the suppression of the immune function to some degree. Operation is the main treatment. However, operation trauma and stress aggravate the condition. Studies have shown that a weakened immune system is the main cause of infection and even death after operation.[4] Furthermore, conventional nutritional support may not improve the immune function of patients and reduce stress reaction. Until today, the commonly studied immune nutrients are glutamine (Gln), arginine, and omega-3 fatty acids.[5] According to a clinical study, adequate supplementation of Gln may improve immune response under stress, shorten recovery time, and reduce mortality rate.[6] Therefore, in this study, we aimed to determine the effect of Gln-enforced parenteral nutrition on immune function and stress response of patients after esophageal operation.

PATIENTS AND METHODS

The study included a total of 143 esophageal cancer patients (71 males, 72 females; mean age 70.7±4.3 years; range 35 to 80 years) who admitted to the Affiliated Hospital of Putian University between August 2009 and October 2013. Distribution of esophageal cancer types was as follows: upper thoracic esophageal cancer in 22 patients, middle thoracic esophageal cancer in 83 patients, and lower thoracic esophageal cancer in 38 patients. All patients were randomized into two groups as the experimental group and control group by means of New Drug Statistical Treatment. Patients in both groups had no significant differences in terms of sex, age, tumor location, operation methods of anastomosis, pathological stages, time of the beginning of preoperative symptoms, preoperative nourishment status, preoperative immune status, and stress reaction (Table 1).

Inclusion criteria: (i) operation and pathology reports for esophageal squamous cell carcinoma, negative margin; *(ii)* no indication of metabolic diseases such as diabetes or hyperthyroidism; *(iii)* no hematopoietic system disease; *(iv)* no spleen and thymus resection; *(v)* no autoimmune disease with negative effect on immune function; *(vi)* no exposure to chemotherapy, and hormone or immune stimulators.

Excluded patients: (i) patients undergoing operation difficulty; *(ii)* patients receiving more than 800 mL of blood transfusions; *(iii)* patients receiving infusion of albumin during operation.

Experimental and control groups had isonitrogenous and isocaloric intake of nutrition from the first postoperative day for seven days. The nutrition given continuously for seven days included 30 kcal/kg/day; glucose, 6:4 ratio; nitrogen, 0.3 g/kg/day; heat, 100 kcal:1g nitrogen. The experimental group was given additional alanyl-Gln, according to 0.5 g/kg/day (Gln 0.35 g/kg/day) supply.

On first preoperative day, and on first and seventh postoperative days, the following parameters were measured: concentrations of Gln in serum; stress response indicators: serum C-reactive protein (CRP), complement 3 (C3), C4; humoral immunity indexes: serum immunoglobulin (Ig)M, IgA, IgG; and lymphocyte-mediated immune parameters: CD3+, CD4+ and CD8+ lymphocytes.

The experimental protocol of the study was approved by Department of Thoracic Surgery in the Department of Thoracic Surgery in the Affiliated Hospital of Putian University. A written informed consent was obtained from each patient. The study was conducted in accordance with the principles of the Declaration of Helsinki.

Statistical analysis

All data were analyzed using SPSS for Windows version 16.0 software (SPSS Inc., Chicago, IL, USA). Student's t test was used for the measurement data such as age, stress response indicators, and immunity indexes, expressed as mean ± standard deviation. Chi square test was used for the enumeration data such as tumor location and mode of operation. P value <0.05 was considered statistically significant.

RESULTS

On preoperative first day, levels of CRP, C3, and C4 of both groups were higher than normal, but the comparison between two groups showed no significant difference. On postoperative first day, levels of C3 and C4 of the experimental group were lower than the control group (p>0.05), while CRP level of the experimental group was higher than the control group (p>0.05). On seventh postoperative day, CRP level of the experimental group was

Table 1. Clinical parameters of patients in two groups

Parameters	Experimental group (n=73)		Control group (n=70)			
	n	Mean±SD	n	Mean±SD	χ^2/t value	*p*
Gender					0.154	0.950
Male	35		36			
Female	38		34			
Age (years)		70.2±9.7		71.4±10.2	0.469	0.812
Tumor location						
Upper	10		12		0.838	0.626
Middle	45		38		1.553	0.403
Lower	18		20		0.544	0.706
Mode of operation						
Cervical anastomosis	52		48		1.670	0.336
Aortic arch anastomosis	10		8		1.832	0.177
Anastomosis below aortic arch	11		14		1.701	0.235
The time of the beginning of preoperative symptoms						
Less than two weeks	23		27		0.662	
More than two weeks	50		43		0.177	
Preoperative nourishment status (Ideal body weight %)						
>90	12		10		0.521	
80-90	21		20		1.826	
60-79	30		28		0.906	
<60	10		12		1.533	
Pathological stages						
Stage I	14		12		0.523	
Stage II	20		21		0.819	
Stage III	33		30		1.330	
Stage IV	6		7		0.144	

SD: Standard deviation.

significantly lower than that of the control group ($p<0.05$), (Table 2).

On preoperative first day, levels of IgA, IgG, IgM, CD3+ and CD4+ cells, and CD4/CD8 ratio of both groups were lower than normal, but the comparison between two groups revealed no significant difference. On postoperative first day, levels of IgA and IgG of the experimental group were higher than the control group ($p>0.05$), while IgM of the experimental group was lower than the control group ($p>0.05$). Levels of CD4+ and CD8+ cells, and CD4/CD8 ratio of the experimental group were higher than those of the control group ($p>0.05$), while CD3+ cells of the experimental group was lower than the control group ($p>0.05$). On postoperative seventh day, levels of CD3+ cells, CD4/CD8 ratio, and IgM level of the experimental group were higher than those of the control group with no statistical significance ($p>0.05$), level of CD8 of the experimental group was lower than the control group with no statistical significance ($p>0.05$), while levels of CD4+ cells, IgA, and IgG of the experimental group were significantly higher than those of the control group ($p<0.05$), (Table 3, 4).

On preoperative first day, the concentrations of Gln in serum of both groups were normal. On postoperative first day, the concentrations of Gln of both groups were lower than those of first preoperative day in each group ($p<0.05$). The concentrations of Gln of the experimental group were higher than the control group ($p>0.05$). On seventh postoperative day, the concentrations of Gln of the experimental group were higher than that of the control group ($p<0.05$). The concentrations of Gln of the control group were lower than those of first preoperative day ($p<0.05$), (Table 5).

The incidence of pulmonary infection and anastomotic leakage of the experimental group was lower than that of the control group ($p<0.05$). The incidences of incision infection, blood infection, urinary infection, and abdominal infection of the experimental group were lower than that of the control group ($p>0.05$), (Table 6).

Table 2. Comparison of inflammatory parameters before and after operation

Parameters	D1 (preoperative)	D1	D7
	Mean±SD	Mean±SD	Mean±SD
C-reactive protein (mg/L)			
Experimental group	63.32±81.71	169.31±43.07*	55.39±3.16*‡
Control group	54.60±2.91	134.76±50.12*	84.40±2.70
C3 (mg/L)			
Experimental group	1.97±0.18	2.66±0.17*	0.54±0.06*
Control group	2.07±0.53	2.86±0.30*	2.17±0.11
C4 (mg/L)			
Experimental group	1.14±0.16	1.59±0.33*	0.35±0.24*
Control group	0.95±0.14	1.66±0.18*	0.87±0.11

SD: Standard deviation; * p values <0.05 when comparing inflammatory parameters between first (D1) and seventh (D7) postoperative day and preoperative values (D1); ‡ p value <0.05 when comparing inflammatory parameters between experimental group and control group. C3: Complement 3

DISCUSSION

Esophageal cancer is one of the least studied and most lethal cancers worldwide,[7-9] with a five-year survival rate of less than 10%.[10] It is one of the most common malignant tumor in China. Its incidence has risen in recent years.[11] Patients usually have various degrees of malnutrition, even the emergence of cachexia due to patients' difficulty in eating and the metabolic disorder of the organism because of increased production of cytokines and catabolic hormone.[5] At the same time, preoperatively, esophageal cancer often causes low immune function and stress response in different degrees. These also may increase the risk of infection and tumor recurrence, and affect prognosis. Therefore, improving the nutritional status and recovering the immune function of patients postoperatively are important to improve their stress response.

Glutamine is a nonessential amino acid, which is an important energy metabolism of immune cells and intestinal mucosal cells. It can also promote the division and differentiation of lymphocytes and macrophages. It plays a significant role in human nutrition metabolism, immune protection, and regulation of inflammation.[12] When the body is under stressful or pathological conditions, when there is a relative lack of endogenous synthesis of Gln, or in case of Gln depletion, exogenous Gln must be supplied. Under these conditions, Gln becomes an essential amino acid. Therefore, Gln is considered to be a conditionally essential amino acid for the body in case of stress, which is important for cell metabolism and immune cell function. In our study, the concentrations of Gln in serum of both groups were normal preoperatively. Postoperatively,

Table 3. Comparison of humoral immune parameters before and after operation

Parameters	D1 (preoperative)	D1	D7
	Mean±SD	Mean±SD	Mean±SD
IgG (g/L)			
Experimental group	13.91±2.72	10.93±2.32*	14.70±3.36*‡
Control group	12.77±3.23	9.35±2.71*	10.61±2.35
IgA (g/L)			
Experimental group	2.39±0.74	1.76±0.57*	2.84±0.57*‡
Control group	2.21±0.58	1.66±0.65*	2.00±0.92
IgM (g/L)			
Experimental group	1.88±0.41	1.53±0.92*	1.83±0.64*
Control group	1.90±0.72	1.57±0.58*	1.68±0.29

SD: Standard deviation; * p values >0.05 when comparing humoral immune parameters between first (D1) and seventh (D7) postoperative day and preoperative values (D1); ‡ p values <0.05 when comparing humoral immune parameters between experimental group and control group.

Table 4. Comparison of cellular immune parameters before and after operation

Parameters	D1 (preoperative)	D1	D7
	Mean±SD	Mean±SD	Mean±SD
CD3+ cells (%)			
Experimental group	0.61±0.03	0.57±0.02	0.66±0.05*
Control group	0.65±0.05	0.58±0.03	0.64±0.04
CD4+ cells (%)			
Experimental group	0.43±0.01	0.41±0.02*	0.48±0.03*‡
Control group	0.42±0.02	0.40±0.02	0.40±0.04
CD8+ cells (%)			
Experimental group	0.26±0.02	0.28±0.03	0.25±0.02*
Control group	0.25±0.02	0.27±0.02	0.26±0.01
CD4/CD8 ratio			
Experimental group	1.68±0.14	1.62±0.09*	1.72±0.13*
Control group	1.66±0.16	1.54±0.21*	1.67±0.14

SD: Standard deviation; * p values <0.05 when comparing cellular immune parameters between first (D1) and seventh (D7) postoperative day and preoperative values (D1); ‡ p value <0.05 when comparing cellular immune parameters between experimental group and control group.

esophageal cancer patients have continuous high rates of metabolism decomposition, while Gln consumption and blood Gln utilization rate increase. Similarly, on first postoperative day in our study, the concentrations of GLN of both groups were lower than those of the first preoperative day in each group. Insufficient nutrition supply may induce intestinal mucosal atrophy, which aggravates stress reaction of metabolism. In their study, Zhou et al.[13] demonstrated Gln as an additional nutrition for some severely burned rats and that it can reduce intestinal mucosal reaction of metabolism. Therefore, in traumatic stress, percutaneous enteral or parenteral Gln nutrition support might maintain the structure and function of intestinal mucosa and reduce skeletal muscle protein degradation, improve nitrogen balance, and reduce the stress reaction of metabolism. In our study, when the body's immunological function gradually restored, the concentrations of Gln in serum of experimental group became normal. However, on the seventh postoperative day, the concentrations of Gln in serum of control group were lower than the experimental group because of the relative lack of endogenous synthesis of Gln, or even body Gln depletion. In addition, CRP is a sensitive index, which reflects stress status, while C3 and C4 synthesis increases

Table 5. Comparison of the concentrations of glutamine in serum before and after operation (μmol/L)

Groups	D1 (preoperative)	D1	D7
	Mean±SD	Mean±SD	Mean±SD
Experimental group	618±75	496±45‡	612±62*
Control group	614±55	486±32‡	490±33*‡

SD: Standard deviation; * p value <0.05 when comparing the concentrations of glutamine in serum between experimental group and control group; ‡ p value <0.05 when comparing the concentrations of glutamine in serum between first (D1) and seventh (D7) postoperative day and preoperative values (D1).

Table 6 Comparison of the infectious complication between two groups

Characteristic	Experimental group (n=73)	Control group (n=70)	χ^2 value	*p*
Incision infection	4	6	0.157	>0.05
Pulmonary infection	5	16	7.309	<0.05
Blood infection	1	3	0.302	>0.05
Urinary infection	2	7	1.595	>0.05
Abdominal infection	2	6	1.329	>0.05
Anastomotic leak	2	14	10.713	<0.05

the condition of stress.[14] Our study confirmed these results. Preoperative CRP levels in both groups were higher than normal values, suggesting that patients were under the state of stress response before operation. On the first postoperative day, CRP levels of the two groups were further increased, indicating that operation trauma aggravates stress response even more; however, on the seventh postoperative day, CRP level of the experimental group significantly decreased, while that of the control group showed no obvious decrease. There was a significant difference between the two groups ($p<0.05$), suggesting that Gln-enforced parenteral nutrition might ameliorate stress response in patients with esophageal cancer postoperatively.

On the first preoperative day, levels of IgA, IgG, and IgM of both groups were lower than normal, suggesting that patients were under the protection of humoral immunity suppression. On the seventh postoperative day, levels of IgA and IgG of the experimental group were significantly higher than the control group ($p<0.05$), indicating that Gln-enforced parenteral nutrition might enhance humoral immunity of patients with esophageal cancer postoperatively. It may be so since Gln is the precursor to purine, pyrimidine, nucleic acid, and protein. Also, it is a carrier of nitrogen between tissues and organs. Moreover, Gln plays an important role in promoting B lymphocyte synthesis and secretion of antibodies, and this effect cannot be replaced by other kinds of amino acid.[15]

On the first preoperative day, the levels of CD3, CD4, and CD4/CD8 of both groups were lower than normal, suggesting that patients were under the protection of cell-mediated immunity suppression. On the seventh postoperative day, CD4 level of the experimental group was significantly higher than the control group ($p<0.05$), indicating that Gln-enforced parenteral nutrition might enhance cell immunity in patients with esophageal carcinoma postoperatively. It may be so since Gln is the precursor to synthesis metabolism, given the fact that the need for Gln increases in the condition of stress. Glutamine supplementation can improve the protein metabolism, thereby inhibiting muscle protein breakdown, improving nitrogen balance, and promoting gluconeogenesis. At the same time, Gln is capable of improving the physiological function of immunologically competent cells, such as intestinal mucosal cells and lymphocytes, macrophages and neutrophils.[11] Meanwhile, whether in enteral or parenteral application, Gln is equally effective.[16] Therefore, the incidence of pulmonary infection and anastomotic leakage of the experimental group was lower than the control group in our study ($p<0.05$).

This study has some limitations. The outcome is only in the form of biochemical surrogate markers. No information is given on postoperative mortality between the two groups. And this is essential information since it is a major outcome. Furthermore, in this case, although the study of parenteral glutamine could be useful enough for proof of principle, enteral administration may have more clinical relevance. These questions should be substantially evaluated in future studies.

In conclusion, this study showed that patients with esophageal cancer are affected by decreased immune function and high stress postoperatively. Compared with conventional parenteral nutrition, glutamine-enforced parenteral nutrition support may improve the immune status, reduce stress response significantly, and thereby promote recovery.

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