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Influence of G308A polymorphism of tumor necrosis factor-α gene on inflammatory markers in postsurgical head and neck cancer patients with early enteral nutrition

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Abstract

Objective: Although immune dysfunction in patients with cancer could be multifactorial, the immune system may be modulated by nutritional substrates and genetic background. Our study evaluated the effect of G308A polymorphism of the tumor necrosis factor-α (TNF-α) gene on inflammatory markers in patients after surgery for head and neck cancer who received early enteral nutrition.

Methods: A population of 60 patients with oral and laryngeal cancer was enrolled. At surgery patients were treated with a hyperproteic enteral diet. Perioperatively and on postoperative day 6 the following parameters were evaluated: serum values of prealbumin, transferrin, total number of lymphocytes, interleukin-6, TNF-α, and C-reactive protein. In addition, genotyping of G308A gene polymorphism was assessed.

Results: Patients' mean age was 61.1 $\pm$ 14.6 y (four women, 56 men) with a body mass index of 25.4 $\pm$ 5.2 kg/m^2 and a previous weight loss of 0.35 $\pm$ 0.2 kg. Forty patients (37 men, 3 women; 66.6%) had the genotype *G308/G308* (wild group) and 20 patients (19 men, 1 woman; 23.4%) had the genotype *G308/A308* (mutant group). A significant increase in prealbumin and transferrin levels was detected in both groups. C-reactive protein decreased in both groups (wild group: 105.1 $\pm$ 60 versus 53.8 $\pm$ 62.3 mg/dL, $P < 0.05$; mutant group: 99.5 $\pm$ 46 versus 43.9 $\pm$ 51.9 mg/dL, $P < 0.05$). Interleukin-6 decreased in both groups (wild group: 20.1 $\pm$ 22 versus 6.2 $\pm$ 4.1 pg/mL, $P < 0.05$; mutant group: 22.3 $\pm$ 38 versus 9.2 $\pm$ 7.4 pg/mL, P = NS). Lymphocytes increased in both groups (wild group: 1102 $\pm$ 468 versus 1600 $\pm$ 537 10^3/mL, P = NS; mutant group: 1441 $\pm$ 739 10^3/mL versus 1669 $\pm$ 614 10^6/mL, P = NS). TNF-α showed no changes.

Conclusion: The G308A polymorphism of the TNF-α gene did not affect levels of inflammatory markers in patients after surgery for head and neck cancer who were treated with early enteral nutrition.

Keywords: G308A polymorphism; Head and neck cancer; Inflammatory markers

Introduction

Surgery and malnutrition have been found to depress the immune system in patients with head and neck cancer. Although immune dysfunction could be multifactorial, the immune system may be modulated by enteral nutrition [1]. For instance, some studies have shown that arginine increases lymphocytic interleukin-2 production by macrophages [2]. There is evidence suggesting that enteral nutrition, supplemented with different agents including arginine and dietary fiber, improve immune function and reduce postoperative complications in patients with head and neck cancer [3–5]. However, our group [5] found no influence on

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tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) blood concentrations with an arginine-enhanced enteral formula in a randomized clinical trial with two intervention groups. Perhaps these heterogenous results in the literature could be due to a different inflammatory genotype of these patients. Genetic background could influence stress-induced inflammatory reactions. There is little evidence on this topic in patients after surgery for cancer. However, in other patients with cardiovascular surgery, genetic background has demonstrated modulated inflammatory markers.

Mutation analysis has identified a G→A transition in the promoter region of the TNF-α gene at position −308. This polymorphic variant has been shown to affect the promoter region of the TNF-α gene leading to a higher rate of transcription compared with the wild allele [7]. In some studies, the TNF-α G308A polymorphism has been associated with enhanced cytokines and acute-phase protein production [8].

Our study evaluated the relation of the G308A polymorphism of the TNF-α gene with inflammatory markers in patients after surgery for head and neck cancer who received early enteral nutrition.

Material and methods

Patients

A population of 60 patients with oral or laryngeal cancer was enrolled. Exclusion criteria included severely impaired hepatic function (total bilirubin concentration >3.5 mg/dL) and renal function (serum creatinine concentration >2.5 mg/dL), ongoing infections, steroid treatment, and good nutritional status (weight loss <10% of body weight, 0.35 ± 0.2 kg). The study was a prospective interventional trial. Baseline studies on all patients consisted of complete history taking and physical examination. Informed consent was provided by all patients.

Nutrition

At surgery patients received a high protein enteral diet (20.4% proteins, 46.8% carbohydrates, and 33.8% fats). Table 1 presents the composition of the enteral diet. Enteral feeding was started within 24 h of surgery at a rate of 20 mL/h through an intraoperatively placed nasogastric tube. The infusion rate was progressively increased every 24 h until the daily nutritional goal (32 total kcal/kg, 1.7 g protein/kg) was reached, on postoperative day 4. All patients reached 100% of calculated requirements. Prophylactic antibiotic treatment was given for 3 d postoperatively (500 mg of ceftazidime intravenously three times daily and 300 mg of clyndamicine intravenously three times daily).

Table 1
Composition of enteral diet (per 100 mL)

Total energy (kcal)	125
Protein (g) (casein)	6.3
Total lipid (g)	4.9
ω-6/ω-3	5/1
Linoleic acid	1.3
α-Linolenic acid	0.3
Carbohydrate (g)	14.1
Dietary fiber (g)*	0.9

* Oligofructose, inulin, soy polysaccharide, resistant starch, arabic gum, and cellulose.

Patient monitoring

Perioperatively and on postoperative day 6 the following parameters were evaluated: serum values of prealbumin (milligrams per deciliter), transferrin (milligrams per deciliter), albumin (grams per deciliter), total number of lymphocytes (10^6/mL), IL-6 (picograms per milliliter), TNF-α (picograms per milliliter), and C-reactive protein (milligrams per deciliter; CRP).

Assays

Fasting blood samples were drawn for measurement of prealbumin (18–28 mg/dL) and transferrin (250–350 mg/dL) with an autoanalyzer (Hitachi, ATM, Manheim, Germany).

Interleukins were measured by an Immulite analyzer (DPC, Los Angeles, CA, USA). One hundred microliters of serum (heparinized plasma) was required to determine IL-6 and TNF-α; the analytical sensitivities of IL-6 and TNF-α were 5 and 1.7 pg/mL, respectively. CRP was measured by immunoturbimetry (Roche Diagnostics GmbH, Mannheim, Germany); the analytical sensitivity was 0.5 mg/dL. Samples were assayed in duplicate in 1 d by the same investigator to avoid interinvestigator variability.

Genotyping of G308A gene polymorphism

Oligonucleotide primers and probes were designed with Beacon Designer 4.0 (Premier Biosoft International, Los Angeles, CA, USA). The polymerase chain reaction was carried out with 50 ng of genomic DNA, 0.5 μL of each oligonucleotide primer (primer forward: 5′-CTG TCT GGA AGT TAG AAG GAA AC-3′; primer reverse: 5′-TGT GTG TAG GAC CCT GGA G-3′), and 0.25 μL of each probe (wild probe: 5′-Fam-AAC CCC GTC CTC ATG CCC-Tamra-3′; mutant probe: 5′-Hex-ACC CCG TCT TCA TGC CCC-Tamra-3′) in a 25-μL final volume (Termociclador iCycler IQ, Bio-Rad, Hercules, CA, USA). DNA was denatured at 95°C for 3 min; this was followed by 50 cycles of denaturation at 95°C for 15 s and annealing at 59.3°C for 45 s. The polymerase chain reaction was run in a 25-μL final volume containing 12.5 μL of IQTM Supermix (Bio-Rad) with hot start Taq DNA polymerase.

Statistical analysis

The results were expressed as mean ± SD. The distribution of variables was analyzed with the Kolmogorov-Smirnov test. Quantitative variables with normal distribution were analyzed with two-tailed paired or unpaired Student's *t* test, as needed. Non-parametric variables were analyzed with Friedman's and Wilcoxon's tests. $P < 0.05$ was considered statistically significant.

Results

Sixty patients were enrolled in the study. Their mean age was 61.1 ± 14.6 y (4 women, 56 men). Five patients underwent resection of a tumor located in the oral cavity with unilateral or bilateral neck dissection; 55 patients underwent laryngectomy (total or partial) or pharyngolaryngectomy.

Forty patients (37 men, 3 women; 66.6%) had the genotype *G308/G308* (wild group) with an average age of 63.3 ± 11 y, and 20 patients (19 men, 1 woman; 23.4%) had the genotype *G308/A308* (mutant group) with an average age of 60.7 ± 11.9 y, without statistical differences in other parameters (Table 2).

As presented in Table 3, no significant intergroup differences in the trend of plasma proteins were detected, with a significant increase in prealbumin and transferrin levels.

C-reactive protein and IL-6 decreased in both groups. TNF-α showed no changes. Lymphocytes increased in both groups. No differences between groups were detected.

Discussion

Malnutrition and immunosuppression are two characteristics of patients with head and neck cancer [9]. Malnutrition is due to reduced dietary intake secondary to dysphagia and interleukins secreted by the tumor, with catabolic action playing a dominant role [10]. IL-6 concentrations correlate with the prognostic and inflammatory nutritional index [11].

There is evidence suggesting that enteral nutrition improves immune function and decreases postoperative complications in patients with head and neck cancer [1,5,12]. However, effects of enteral nutrition on cytokine concentrations of these patients are not well documented [6,13]. The genetic background might play a role in these unclear responses and a main actor could be G308A polymorphism of the TNF-α gene. This genetic variation in the TNF-α–308 gene seems to be consistently associated with adverse clinical outcomes and nutritional status in patients with end-stage renal disease [14] and those with inflammatory bowel disease [8].

Certain nutrients such as the semi-essential amino acid arginine, RNA, and ω-3 fatty acids may act pharmacologically on the immune system. It has been suggested that these nutrients improve host immune defences [1,15]. Our group [13] found no differences between two enteral formulas (enhanced with arginine and fiber versus no arginine and no fiber) [6]. In our first study, fiber may have biased the results due to the dietary fiber contained in the arginine-enhanced formula. In our second study [6], with only arginine as the different nutrient between the two enteral formulas, IL-6 and CRP concentrations decreased in both groups. Perhaps this decrease in IL-6 concentration (in the previous study [13] only decreased CRP was found) could be due to different genetic statuses of patients (TNF-α polymorphisms) [16]. In this present study without an immunoenhanced formula, we found that decreased CRP and Il-6 concentrations after surgery are not related to the G308A polymor-

Table 2
Patient characteristics in wild group (G308G) and mutant group (G308A)*

	G308G	G308A
Age (y)	63.3 ± 11	60.7 ± 11.9
Men/women	3/37	1/19
Body mass index	25.4 ± 4.9	25.5 ± 5.2
Previous weight loss	0.4 ± 0.3	0.3 ± 0.2
Disease stage		
I	0	0
II	3	1
III	4	2
IV	33	17
Diagnosis of disease		
Oral cavity	4	1
Larynx	36	19

* No statistical differences.

Table 3
Biochemical and hematologic parameters

Parameters	Basal	Day 6
Prealbumin (mg/dL)		
Group I†	12 ± 4.4	25.3 ± 3.9*
Group II‡	13.9 ± 5.4	20.2 ± 5.8*
Transferrin (mg/dL)		
Group I	142.7 ± 19.3	187.1 ± 39.5*
Group II	149.8 ± 48.1	200.7 ± 52*
C-reactive protein (mg/dL)		
Group I	105.1 ± 60	53.8 ± 62.3*
Group II	99.5 ± 46	43.9 ± 51.9*
Interleukin-6 (pg/mL)		
Group I	20.1 ± 22	6.2 ± 4.1*
Group II	22.3 ± 38	9.2 ± 7.4*
Tumor necrosis factor-α (pg/mL)		
Group I	8.3 ± 5.8	7.7 ± 4.6
Group II	9.1 ± 6.1	5.7 ± 1.8
Lymphocytes (10^3/mL)		
Group I	1102 ± 468	1600 ± 537*
Group II	1441 ± 739	1669 ± 614*

* $P < 0.05$ versus basal values. No statistical differences were detected between groups.
† Wild group (G308G).
‡ Mutant group (G308A).

phism of the TNF-α gene. It is clear that enteral nutrition is a good option to attenuate the immunosuppression status of these patients [17,18], with better results than with parenteral nutrition [19]. Further studies are needed to evaluate the interaction between artificial nutritional support after surgery and genetic background.

In conclusion, the G308A polymorphism of the TNF-α gene had no effect on levels of inflammatory markers in patients after surgery for head and neck cancer who were treated with early enteral nutrition.

References

[1] Daly JM, Reynolds J, Thom A. Immune and metabolic effects of arginine in the surgical patient. Ann Surg 1988;208:521–3.
[2] Barbul A. Arginine and immune function. Nutrition 1990;6:53–8.
[3] Van Bokhorst-de van der Schueren MA, Quak JJ, Von Blomberg-van der Flier ME, Kuik DJ, Langendoen SI, Snow GB, et al. Effect of perioperative nutrition, with and without arginine supplementation, on nutritional status, immune function, postoperative morbidity, and survival in severely malnourished head and neck cancer patients. Am J Clin Nutr 2001;73:323–32.
[4] De Luis DA, Izaola O, Aller R, Cuellar L, Terroba MC. Postsurgery enteral immunonutrition in head and neck cancer patients. Eur J Clin Nutr 2002;56:1126–9.
[5] De Luis DA, Arranz M, Aller R, Izaola O, Cuellar L, Terroba MC. Immuonenhanced enteral nutrition, effect on inflammatory markers in head and neck cancer patients. Eur J Clin Nutr 2005;59:145–7.
[6] Gaudino M, Di Castelnuovo A, Zamparelli R, Andreotti F, Burzotta F, Iacoviello L, et al. Genetic control of postoperative systemic inflammatory reaction and pulmonary and renal complications after coronary artery surgery. J Thorac Cardiovasc Surg 2003;126: 1107–12.
[7] Wilson AG, Simons JA, McDowell TL, McDevitt HO. Effects of polymorphism of TNF alpha promoter on transcriptional activation. Proc Natl Acad Sci USA 1997;94:3195–9.
[8] Martinez-Borra J, González S, Lopez Larrea C, Rodrigo L, Deschamps EM, Diéguez MA. Efecto del polimorfismo del promotor de TNF alpha en la producción de TNFalpha y en la respuesta de fase aguda en pacientes con enfremedad de Croöhn. Quim Clin 2002; 21:433–6.
[9] Riboli E, Kaaks R, Esteve J. Nutrition and laryngeal cancer. Cancer Causes Control 1996;7:147–56.
[10] Myrianthefs PM, Batistaki C. Cancer cachexia and immunomodulation. J Balkan Union of Oncology 2005;10(2):181–8.
[11] Walsh D, Mahmoud F, Barna B. Assessment of nutritional status and prognosis in advanced cancer: interleukin-6, C-reactive protein, and the prognostic and inflammatory nutritional index. Support Care Cancer 2003;11:60–2.
[12] Riso S, Aluffi P, Brugnani M, Farinetti F, Pia F, Dandrea F. Postoperative enteral immunonutrition in head and neck cancer patients. Clin Nutr 2000;19:407–12.
[13] De Luis DA, Izaola O, Aller R, Cuellar L, Terroba MC. Effect of CPR and interleukin blood levels in Postsurgery enteral arginine-enhanced enteral nutrition in head and neck cancer patients. Eur J Clin Nutr 2003;57:96–9.
[14] Stenvinkel P, Pecoits R, Lindholm B. Gene polymorphism association studies in dialysis: the nutrition-inflammation axis. Semin Dial 2005;18:322–30.
[15] Senkal M, Kemen M, Homann HH, Eickhoff U, Beier J, Zumtobel V. Modulation of postoperative immune response by enteral nutrition with a diet enriched with arginine, RNA, and omega3 fatty acids in patients with upper gastrointestinal cancer. Eur J Surg 1995; 161:115–22.
[16] Jacob CO, Fronek Z, Lewis GD, Koo M, Hansen JA, Mc Devitt HO. Heritable major histocompatibility complex II–associated differences in the production of tumor necrosis-alfa: relevance to genetic predisposition of systemic lupus erythematosus. Proc Natl Acad Sci USA 1990;87:1233–7.
[17] Takagi K, Yammamori H, Toyoda Y, Nakajima N, Tashiro T. Modulating effects of the feeding route on stress response and endotoxin translocation in severely stressed patients receiving thoracic esophagectomy. Nutrition 2000;16:355–60.
[18] Windsor AC, Kanwar S, Li AG, Barnes E, Guthrie JA, Spark JI, et al. Compared with parenteral nutrition enteral feeding attenauates the acute phase response and improves disease severity in acute pancreatitis. Gut 1998;42:315–6.
[19] Mangiante G, Rossi L, Carluccio S, Marchiori L, Colucci G, Ciola M, Nicoli N. Influence of enteral nutrition on cytokine response in resective liver surgery. Chir Ital 2002;54:613–9.