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The impact of preoperative immunonutrition and other nutrition models on tumor infiltrative lymphocytes in colorectal cancer patients

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Abstract

AIM: The importance of the alteration of tumor infiltrative lymphocytes ($CD4^+$, $CD8^+$, $CD16^+$, and $CD56^+$) in colorectal cancer prognosis is well known. In this study, we analyzed the effect of preoperative immunonutrition and different nutritional models on the clinical condition of colorectal cancer patients.

METHODS: Twenty-eight colorectal cancer patients were grouped into 4 groups according to their nutrition regimens randomly and were given immunonutrition (IMN), standard enteral (SE), total parental nutrition (TPN), and normal nutrition (NN) regimens, all of which contained the same calorie-nitrogen content within a 7-day preoperative period. All patients had an endoscopic biopsy before and after the regimen, and the lymphocyte population infiltrating mucosal parts of the resected tumor tissue were evaluated. Immunohistochemical analysis of the tissue specimens was performed by staining with antihuman $CD4^+$, $CD8^+$, $CD16^+$, and $CD56^+$ antibodies.

RESULTS: After nutrition, there were significant increases in each of the 4 groups of $CD4^+$ and $CD8^+$ cells within the tumor. Comparing the rates of augmentation, the increased rates of the $CD8^+$ cells infiltrating the tumor after nutrition in the patients who were fed with IMN were significantly more than the ones in other groups ($P = .01$). $CD16^+$ cell infiltration was significantly higher in all groups except the SE and IMN groups. The SE group had increased $CD56^+$ cell infiltration compared with the other groups.

CONCLUSIONS: In the colorectal cancer patients who had nutrition in the 7-day preoperative period, except for the SE nutrition group, there were significant increases of infiltration of $CD56^+$ cells at the mucosal part of the tumor tissue within the $CD4^+$ and $CD8^+$ cell population. When postnutrition values were compared, there was a marked increase of $CD8^+$ cells in the IMN group.

There have been a lot of articles published concerning tumor infiltrative lymphocytes (TILs) that affect the prognosis of cancer.[1–5] Rapponen et al[6] reported that for overall and disease-free survival, the stage of disease in colorectal tumor patients is significantly related with the existence of TILs.[6] Because of the positive effects of TILs on prognosis, most studies focused on increasing the number of TILs in tumor tissue. Li et al[7] observed a considerable TIL increase in the gastrointestinal tumor tissue of the patients who used cimetidine before operation compared with those who did

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Table 1 Distribution of patients according to the groups

	Patients (n)	Nutrition model	Nutritional products
Group 1	n = 7	TPN	With subclavian catheter infusion* Freamin %8.5 Lipovenöz%10−20 Dekstroz%10,%20,%30 Soluvit N Vitalipid N adult Tracutil
Group 2	n = 7	IMN	Arginine, omega-3 fatty acid and RNA added enteral product (Impact)†
Group 3	n = 7	NN	Normal feeding planned by a dietitian
Group 4	n = 7	SE	SE product without RNA or omega-3 fatty acid (Fresubin)

SE = standard enteral; IMN = immunonutrition; NN = normal nutrition; TPN = total parental nutrition.

*TPN: glucose (%dextrose as needed; Eczacibaşi, Sakarya, Turkey), amino acid (Freeamine 8.5%; Eczacibaşi, Sakarya, Turkey), lipids (20% lipid; Fresinus-Kabi Deutschland; Hamburg, Germany), and trace element (Tracutil; Braun Deutschland, Melsungen, Germany).

†One liter of Impact (Novartis Nutrition, Bern, Switzerland) contains 780 nonprotein calories, 43 g of protein, 12.5 g of arginine,33 g of omega-3 fatty acids, and 1.2 g of RNA.

not.[7] Heys et al[8] reported that the patients supplemented with L-arginine had a significant TIL increase compared with those who were not.[8]

Protein and calorie malnutrition is observed in 30% to 90% of cancer patients, which affects immunity inversely.[9] Because they have a positive effect on immune parameters, immunonutrition products such as arginine, glutamine, omega-3 fatty acid, and ribonucleic acids that are added to nutritional treatments and their support to the immune response for malnutrition as a nutritional support have been searched. Most prospective randomized clinical studies discuss the effects of immunonutritional support, postoperative complications, the frequency of the development of infection, hospital stay, wound healing, weight gain, cost, and mortality.[10–16] The mechanisms with a positive impact have not been studied in detail yet.

The basic defense response formed by host against tumor has been directed by cellular immunity. The studies concerning alterations in cellular immune response after immunonutrition are about peripheral blood T lymphocytes and their natural killer and lymphokine-activated killer (LAK) activities.[17–19] Is there a relation with the increase in TILs ($CD4^+$ $CD8^+$, $CD16^+$, and $CD56^+$) and the antitumor immune response? If so, is immunonutrition different from the other nutrition styles? To find out answers to these questions, we compared the amount of TILs in endoscopic biopsies before nutrition with the amount of TILs in tumor tissues that were resected after nutrition in colorectal cancer patients. In this study, we aimed to search the impact of immunonutrition and other nutrition models on the amount of TILs.

Material and Methods

In this study, 28 patients who were admitted to Surgical Clinics of Haydarpasa Numune Training and Research Hospital, Istanbul, Turkey, for colorectal cancer surgery were included. Patients were informed and gave their consent. The study was approved by the local ethics committee. Exclusion criteria were patients with the following: clinical findings of vitamin and element deficiency, diabetes mellitus, a history of renal and hepatic deficiency and active infection, and immunosuppressive drug use.

The patients were divided into 4 groups randomly according to their nutrition models (Table 1). For nutritional evaluation, anthropometric, biophysical, and laboratory measurements were performed. The patients had nutrition according to the determined models during the 7-day preoperative period. TILs in the mucosal part of the endoscopic biopsy tissue before the nutrition regimen and in the surgically resected tumor tissue after nutrition were compared quantitatively. Absolute levels of lymphocytes were analyzed and compared. The microscope slides were reviewed by 1 pathologist (SO), and $CD4^+$, $CD8^+$, $CD16^+$, $CD56^+$ counts were performed by 1 researcher (PA) in a blinded fashion.

The energy needs were calculated using the Harris-Benedict formula after the anthropometric measurements. Except for standard enteral nutrition, all other regimens were isonitrogenous and isocaloric. The nonprotein calorie/nitrogen ratio was 1/142 in the standard enteral and normal groups and 1/78 in the immunonutrition group.

Four-micrometer-thick biopsy sections and tumor mucosal tissues were analyzed microscopically. Tissues were deparaffinized with incubation with a xylene and 100% alcohol solution at 37°C overnight. To suppress endogenous peroxidase activity, slides were incubated for 20 minutes in a 3% H_2O_2 solution. For antigen retrieval, tissues were processed with a high-temperature microwave application. After deparaffinization, slides were heated in a 1-mmol (pH = 8) EDTA 1/10 (Pro Taqstura; Quartett, Berlin, Germany) diluted solution for recovering CD4 antigen and in a 10-mmol (pH = 6) citrate buffer 1/10 diluted solution (Bio-Optica, Milan, Italy) for 10 minutes for recovering CD8, CD16, and CD56 antigens. Slides were cooled at