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Plasma Changes of Branched-Chain Amino Acid in Patients with Esophageal Cancer

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ABSTRACT

BACKGROUND

Studies have indicated that branched amino acids play a crucial role in gene expression, protein metabolism, apoptosis, and restoration of hepatocytes and insulin resistance. This study aimed to compare the plasma levels of branched-chain amino acids in patients with esophageal cancer and normal individuals.

METHODS

Plasma levels of leucine and isoleucine of 37 patients with esophageal cancer and 37 healthy adults were investigated by high-pressure liquid chromatography. Data analysis was performed using SPSS (version 16) software, and t test was used to compare the plasma levels of branched-chain amino acids in the two groups.

RESULTS

In the patients group, the mean age ± SD was 63 ± 13.64 years, and 21 (56.8%) individuals were male. In the control group, the mean age ± SD was 64.24 ± 13.08 years, and 21 (54.1%) individuals were male. Plasma levels of leucine (37.68 ± 105) and isoleucine (22.43 ± 59.1) in patients with esophageal cancer were significantly reduced (*p* value of isoleucine:0.007, and leucine: 0.0001).

CONCLUSION

In the present study, the plasma levels of branched-chain amino acids in patients with esophageal cancer had changed. Evidence suggests that branched-chain amino acids are essential nutrients for cancer growth and are used by tumors in various biosynthetic pathways as energy sources. Thus, studies in this field can be useful in providing appropriate therapeutic approaches.

KEYWORDS:
Esophageal Cancer, Leucine, Isoleucine

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INTRODUCTION

Esophageal cancer (EC) is the eighth most common cancer.[1] In a preliminary survey conducted by the Institute for Iranian Cancer, EC was accounted for 9% of all cancers.[2,3] Some studies have demonstrated a link between papillomavirus and

some cancers, and in Europe, high frequencies of papilloma have been seen in individuals with EC.[4]

Branched-chain amino acids (BCAAs) containing valine (Val), isoleucine (Ile), and leucine (Leu) are among essential amino acids with hydrophobic chains, constituting 20-40% of proteins in the food diet.[5,6] Amino acids (AAs) are consumed as regulators of synthesis path and protein breakdown as well as the precursors for the synthesis of alanine and glutamine amino acids. In addition, oxidation of BCAAs is regarded as one of the main energy sources for muscle. Oxidation of BCAA is controlled by the products resulting from Leu transamination in the short term and by physiological and pathological factors such as hunger, diabetes, cancer, uremia, and infection in the long term.[7]

Recent studies have investigated the diverse roles of AAs in tumors.[8] Analysis of negative nitrogen balance and skeletal muscle shows that overall protein catabolism is much more than anabolism in cancers.[9] Activation of skeletal muscle protein degradation occurs through the host GTP-ubiquitin path and mediators released from the tumor. Meanwhile, skeletal muscle protein synthesis is either unchanged or declined, while this is the consequence of losing proteins of skeletal muscle.[10] Studies have indicated that BCAAs play roles in the signaling paths of growing cells having a regulatory function in the synthesis of proteins and lipids. BCAAs also play regulatory and non-regulatory roles in cell growth and autophagy.[11,12] Furthermore, AAs play a role in energy production, the synthesis of nucleosides, and the maintenance of redox balance in cancer cells.[13]

Many types of cancer are accompanied by significant changes in the metabolism [14] of AAs according to the tumor demands and its interaction with the host cell. In this context, BCAAs supplements have been studied as a way to improve protein synthesis.[15,16] A number of studies have revealed significant changes in the use of BCAAs in various cancers and the association of levels of BCAAs with the progression of cancer.[15,17]

MATERIALS AND METHODS

Study population

The present study was a case-control study, for which 37 patients with EC referred to Golestan Gastroenterology and Hepatology Research Center were selected. The patients group was selected from those who were diagnosed with EC and had no treatment (chemotherapy, radiation) or surgery. Additionally, they were in good nutritional status and did not have absorbing problems. Furthermore, patients with other metastatic and metabolic diseases were excluded. The control group included individuals with perfect health and those who did not have a history of cancer and malignant diseases, and those who had no problem in absorbing. Moreover, this study was approved by the Ethics Committee of Golestan University of Medical Sciences (Code:781589304082). All the samples were taken during fasting. For this purpose, 5 ml of peripheral blood of all participants were taken in tubes containing anticoagulant ethylenediaminetetraacetic acid (EDTA), and then the plasma were separated after 10 minutes by 1000 g centrifugation, and they were frozen at -80°C. The plasma concentration of BCAAs was measured using the HPLC model KNAUER (Germany). The basis of this method was the high-performance liquid chromatography (HPLC), and the gradient method with the flow rate of 1 and pH = 7.02 [18]

We first mixed 200 µl of the sample with 50 µl of standard homoserine and 800 µl of methanol and incubated it for 5 minutes at 4°C, and the supernatant was centrifuged for 5 minutes at 4000 rpm, then 250 µl of the supernatant was separated and mixed with 100 µl of borate buffer. At this step, they were vortexed for 5 seconds. Afterward, 25 microliters of normal HCL 75% was added to the above mixture and again was vortexed for 5 seconds. Additionally, 50 microliters of the resulting solution were mixed with 200 microliters of solution A and were vortexed for 5 seconds. Then, we injected 20 microliters of the resulting solution into the device HPLC with a Hamilton syringe. Plasma levels of Leu and Ile amino acids were measured for 60 minutes.

Statistical analysis

Data analysis was conducted using SPSS software version 16 and by calculating the standard deviation and the mean concentration of amino acids in patients with EC and the control group. p values less than 0.05 were considered statistically significant. To test the normality of the data, Kolmogorov-Smirnov was used. According to the results, normality assumptions for Ile and Leu had a normal distribution. For amino acids with normal distribution, the t test was selected to measure a significant difference in the

Table 1: Mean concentration of BCAA in the case and control groups with t test

Variables				Ile(µm)	Leu (µm)
Age	Case (N = 37)	Pearson correlation		-0.08	0.061
		p-value		0.638	.719
	Control (N = 37)	Pearson correlation		-0.089	-0.019
		p-value		0.602	0.913
Sex	Case (N = 37)	Mean ± SD	Male	47.27 ± 12.72	87.36 ± 21.32
			Female	45.03 ± 14.04	82.29 ± 24.39
	Control (N = 37)	Mean ± SD	Male	64.73 ± 24.87	115.18 ± 37.26
			Female	52.48 ± 17.65	93.01 ± 35.56
A comparison of amino acids with t test		Control	Mean ± SD	59.1 ± 22.43	105 ± 37.68
		Case	Mean ± SD	46.3 ± 13.16	85.17 ± 22.52
		p value		0.007	0.0001
		Equal variances assumed		0.004	0.008
		Equal variances not assumed		0.004	0.008

plasma level of BCAA in patients with EC and the control group.

RESULTS

In the patients group, the mean age ± SD was 63 ± 13.64 years, and 21 (56.8%) individuals were male. In the control group, the mean age ± SD was 64.24 ± 13.08 years, and 21 (54.1%) individuals were male. Plasma levels of leucine and isoleucine amino acids in patients with EC were significantly reduced ($p < 0.05$). Totally, Table 1 and Figure 1 present all results.

DISCUSSION

EC is one of the fast-growing cancers. Despite the advances in early detection of the disease, the survival rate of these patients is less than 10%. Currently, endoscopic diagnosis of biopsy is the main method to detect EC. However, with significant constraints, screening for early detection of EC extremely important from the clinical point of view.

The results of the current study indicate that the plasma concentration of BCAA in patients with EC was reduced. In previous studies, in some cases, changes in BCAA in patients with EC were inconsistent; their plasma concentration was significantly reduced in the current study. Ananieva and colleague,[7] studied the metabolism of BCAA in cancer treatment and diagnosis, and the results of their studies revealed that these amino acids were essential nutrients for cancer growth and were used by tumor cells in different directions as energy sources. Norton and others 19 measured the plasma concentration of AAs in 15 controls and 55 patients with cancer. Additionally, the profile of AAs in 16 patients with metastatic sarcoma was measured. In four groups of cancer patients (lymphoma, sarcoma, osteosarcoma, and metastatic sarcoma) without or with minimum weight loss, most plasma AA levels were similar to those of the control group. The results of their study indicated that the plasma levels of amino acid proline in patients with lymphoma and sarcoma were significantly reduced. The total amount of AAs was reduced in patients with EC who lost 20% weight more than control group. Patients with parenteral nutrition had higher plasma levels of lysine and tyrosine than those of the control group during the chemotherapy period.[19] Hong and colleagues 20 examined the profile of plasma AAs in 51 patients with EC and 60 individuals as the control group. They showed a significant difference in the two groups and found that the plasma level of BCAAs was significantly reduced using HPLC in patients with EC than in the control group.[20] Clarke and co-workers [21] measured the plasma concentrations of blood AAs in a fasting state in four groups of patients, including 1: Healthy individuals, 2: Cancer patients with weight loss, 3: Cancer patients with more than 20% weight loss, and 4: Cancer patients with more than 20% weight loss due to reduced food intake because of cancer. Their study demonstrated that the pattern of changes in AAs varied in cancer patients and that some gluconeogenic AAs in

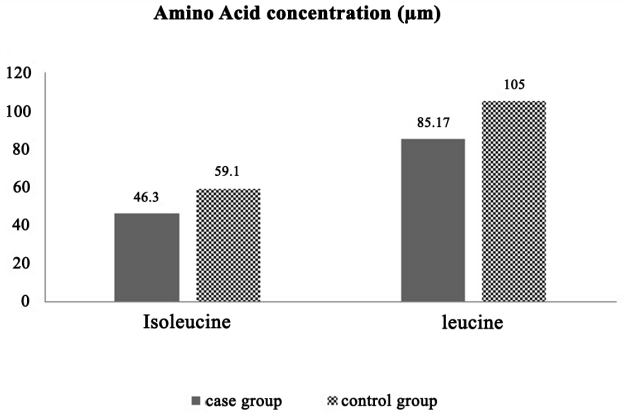

Fig.1: Changes of branched-chain amino acid levels in the patients group and the control group

patients with the disease compared with the control group were significantly decreased.

In this study, the plasma level of BCAA was also significantly reduced, which may be because amino acids (BCAAs) were essential AAs used as the main source for energy production and were as pre-fabrication for the synthesis of AAs and proteins.[5,6] It may be expected that increased proteolysis should lead to increased plasma AAs, but this increase leads to increased oxidative paths.[7] Tumors also use catabolysis of BCAAs more than other AAs do for protein synthesis. It should be noted that the balance of catabolism, oxidation, and protein synthesis varies in different tumors and different tumor levels, and thus levels of BCAAs may vary considering the metabolism amount and the phenotype profile.[22]

Limitations of this study

One of the main limitations of this study was the presence of sampling limitations because in this study, only patients with EC who were in the early stages of the disease and before any treatment and surgery entered the study, which made it difficult to collect samples. And also, because of time constraints in presenting a master's thesis, only 37 patients with these special conditions were able to enter the study in one year.

CONCLUSION

Since BCAAs play a role in different biological activities, and clinical studies of BCAA can be used for cancer management and its prognosis, there is considerable competition for entrance to the brain between BCAAs and aromatic AAs. However, further studies are required in the future.

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ETHICAL APPROVAL

There is nothing to be declared.

CONFLICT OF INTEREST

The authors declare no conflict of interest related to this work.

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