AMINO ACID PROFILES IN PEDIATRIC SOLID TUMORS

PEDİYATRİK SOLİD TOMÖRLERDE AMİNO ASİD PROFİLLERİ

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ABSTRACT

Purpose: *In the current study, plasma amino acid levels were determined in children with solitary tumors in order to assess whether malignant disease produces characteristic changes and whether changes are attributable to the nutritional status of patients.* ***Methods:*** *Thirty four patients (24 boys-10 girls) aged between eight months and 13 years whose tumors had recently been diagnosed and who had not previously been treated were included in the study. The diagnoses were as follows: 19 Non-Hodgkin's lymphomas , 10 Hodgkin's disease (HD), 5 Wilms' tumors. Fifteen healthy children served as controls.* ***Results:*** *Aspartate and glutamate levels were found to be increased in patients with Wilms' tumor (p<0.05), likewise arginine levels in HD were higher than that of controls (p<0.05). Branched chain AA serum levels decreased in patients with malnutrition when compared with those of without malnutrition.* ***Conclusion:*** *In our view, these alterations could be a result of specific effects of various tumors on protein and AA metabolism.*

Key Words: *Amino Acids, Pediatric Solid Tumors, Malnutrition.*

ÖZET

Amaç: *Bu çalışmada solid tümörlerde plazma amino asit düzeyleri saptanmış ve malign hastalıkların yol açabileceği karakteristik değişiklikler ve beslenme durumu ile olan ilişkileri araştırılmıştır.* ***Metod:*** *Yaşları 8 ay ile 13 yaş arasında değişen (ortalama 6.78 ± 3.76) 34 yeni tanı almış kanser hastası (19 Non Hodgkin Lenfoma, 10 Hodgkin hastalığı, 5 Wilms tümörü) ve yaşları 3 ay ile 14 yaş arasında değişen (ortalama 4.62 ± 4.71) 15 sağlıklı çocukta plazma amino asit düzey ölçümü yapılmıştır.* ***Sonuçlar:*** *Aspartat ve glutamat düzeyleri Wilms tümörlü hastalarda yüksek bulunmuştur (p<0.05). Hodgkin Hastalığı'nda arjinin kontrol grubuna göre anlamlı olarak yüksektir (p<0.05). Dallı zincirli amino asit serum düzeyleri malnütrisyonu olan hastalarda olmayanlara göre düşük bulunmuştur (p<0.05).* ***Tartışma:*** *Bu bozukluklar değişik tümörlerin protein ve amino asit metabolizması üzerine olan özel etkilerinin sonucu olabilir.*

Anahtar Kelimeler: *Amino Asid, Pediyatrik Solid Tümörler, Malnutrisyon.*

INTRODUCTION

Cancer patients have been shown to have various metabolic derangements (1). These disturbances include decreased muscle protein synthesis, increased protein catabolism, and increased gluconeogenesis etc. All these alterations lead to changes in circulating amino acid (AA) concentrations (2-7).

Animal experiments (8) and clinical studies in adult patients with various cancer types have revealed certain plasma AA patterns (9). As a result, the determination of plasma AA concentrations have been thought to be helpful in establishing the diagnosis of certain types of cancer, as well as identifing the origin of metastatic tumors with unknown origin (9). In the current study, plasma aminoacid levels were determined in children with solid tumors in order to assess whether malignant disease produces characteristic changes and whether changes are attributable to the nutritional status of the

patients.

MATERIALS AND METHODS

Fifteen healthy well-nourished control subjects; 9 males and 6 females aged between three months and 14 years (mean 4.62 ± 4.71) and 34 newly diagnosed cancer patients; 24 males and 10 females aged between 8 months and 13 years (mean 6.78 ± 3.76) were included in this study. Their diagnoses were as follows: 19 non-Hodgkin's lymphoma (NHL), 10 Hodgkin's disease (HD), 5 Wilms' tumor.

Since cancer treatment as well as malnutrition are known to markedly affect amino acid metabolism, these factors should be taken into consideration when assessing tumor-related alterations in plasma AA levels. To provide evidence in support this hypothesis; none of the patients had undergone any kind of treatment, transfusion or parenteral nutrition. Those with cardiac, hepatic, metabolic, or endocrine disease were excluded. In both groups liver and kidney function tests were within normal limits. After an overnight fasting, heparinised venous blood samples were obtained. All the samples were centrifuged in 3000 rpm for 10 minutes and kept at -70°C until the time of analysis. Plasma AA levels were then analysed by using high-performance liquid chromatography (Waters: Milipore Corporation). For data analysis AA were divided into groups as total AAs(TAA),essential AAs (EAA), branched-chain AAs (BCAA; e.g.,valine,leucine and isoleucine), and acidic AAs (AAA;e.g.,aspartic and glutamic acid).

The nutritional status of patients were assessed by the criteria of weight for height (WFH), mid upper arm circumference (MUAC) and triceps skinfold thickness (TSFT). When two of these three anthropometric measurements were found to be below the normal limits, the patients were then considered as having malnutrition. Mann- Whitney U test was used for the comparison of plasma AA levels between the controls and patients groups with or without malnutrition. Chi-square and Spearman correlation analysis were also used for evaluating the data.

RESULTS

Table 1. summarizes the demographic data of control and patient groups. We measured 25 types of AAs in both groups. Among of them only the dicarboxylic aminoacids aspartic, glutamic acid and arginine were significantly increased in three types of tumor patients (Table 2). In patients with NHL, total and grouped AA levels were not significantly different from that of control group ($p>0.05$) (Table 2, 3). Arginine in HD was significantly higher than that of controls ($p<0.05$) (Table 2). Acidic AA levels were found to be increased in patients with Wilms' tumor ($p<0.05$) (Table 2, 3). 18% of patients with cancer had malnutrition (Table 1). BCAA and ornithine

Table - 1: Demographic Data.

Diagnosis	Male			Female			Nutritional status
	No	Age range (yrs)	Stage	No	Age range (yrs)	Stage	
Control (n: 15)	9	3*-12		6	11*-14		Well nourished
NHL (n:19)	13	1.5-13	II-IV	6	2.5-12	II-IV	5 malnourished
HD (n: 10)	8	3.5-12	I-IV	2	2-8	I-III	1 malnourished
Wilms' (n: 5)	3	2-4.5	II	2	8*-2	III	Well nourished

* months

NHL: Non-Hodgkin's Lymphoma, HD: Hodgkin's Disease

Table - 2: Serum Levels of Three Amino acids*.

Aminoacids	Control (n=15)	NHL (n=19)	HD (n=10)	Wilm's Tumor (n=5)
Arginine	0.50±1.94	2.47±4.60	4.84±5.15**	1.43±3.20
Aspartate	6.36±3.41	9.61±6.58	5.88±4.54	12.42±7.97**
Glutamate	8.47±5.86	8.80±5.54	7.34±3.65	10.17±3.90**

NHL: Non-Hodgkin's Lymphoma, HD: Hodgkin's Disease,

*mmo l/ L; Mean ± Standard Deviation

**p - 0.05 Compared with control subjects

Table - 3: Serum Amino acids Groups*.

	TAA	EAA	BCAA	AAA
Control (n=15)	237.03±40.88	52.17±19.11	39.04±14.78	14.84±4.59
NHL (n=19)	253.73±49.0	50.94±23.09	34.94±10.92	18.43±6.67
HD(n=10)	255.59±54.12	50.06±21.59	39.32±10.53	13.22±3.99
Wilm's Tumor (n=5)	229.59±29.22	55.38±32.41	46.04±32.59	22.59±5.52**
All Patients (n=34)	250.73±47.90	51.33±23.42	37.86±15.49	17.52±6.50

NHL: Non-Hodgkin's Lymphoma, HD: Hodgkin's Disease,
TAA: Total amino acids, EAA: Essential amino acids, BCAA: Branched-chain amino acids, AAA: Acidic amino acids
*mmol /L; Mean ± Standart Deviation
**p< 0.05 Compared with control subjects

levels were found to be lower in patients with malnutrition when compared with patients without malnutrition ($p< 0.05$).

DISCUSSION

Protein, carbohydrate and fat metabolism can be altered in patients with cancer. Tumors arising in a particular organ site may impose a characteristic plasma AA pattern (9). Kluthe (10), identified high arginine levels in adult patients with HD. Arginine levels were also found to be increased in localized squamous cell carcinoma of the esophagus, and cervix and breast carcinomas (9, 11, 12). In our study, only serum arginine levels were found to be increased when all the patients were considered. Arginine levels were found to be significantly higher in HD. Arginine is a semi-essential AA (13). The physiological role of arginine is as substrate for protein, creatinine and polyamine synthesis.It is also a substrate for production of nitric oxide via the deaminase pathway (14, 15). Arginine has been shown to have potent effects on immune defence, this effect may be mediated primarily by enhancing T cell mediated responses (16). Recent in vitro experiments have shown that the key role of arginine on macrophage and lymphocyte-mediated toxicity and/or infected cells is largely via production and release of nitric oxide (NO) (17). NO is the intermediate metabolite generated during biochemical transformation of arginine to citrulline (18). The increased levels of arginine in our HD patients, in correlation with the literature, may presumably be either due to the overstimulation of the T lymphocytes leading to over production of Arginine or to some other unknown mechanism in the production and release of NO.

Aspartate and glutamate were found to be increased in patients with Wilms' tumor. Glutamic acid levels were observed to be increased in adult patients with pancreas (19), breast, colon and gynecologic cancers, especially in metastatic cases (20, 21). Zhang et al. (22), have identified high aspartic and glutamic acid levels in liver, lung, colon and breast cancer. The reason for high aspartic acid levels in cancer-bearing patients has not yet been fully understood (21), however, the utilization of this AA in peripheral tissues and increased release in malignant tissues have been postulated. Like arginine, glutamic and aspartic acids have a role in the urea cycle. The increased level of glutamic acid could be a sign of impaired nitrogen utilization in cancer-bearing patients (22). It was deduced that both glutamate release from tumor tissue to the plasma pool and reduced glutamate utilization by peripheral tissues at the tumor bearing host occurred. The nutrient intake and the nutritional status of the patients have no influence on the glutamate concentrations (20). On the other hand, the tumor induced stress metabolism of the patient is characterized by catabolism of muscle proteins. The released free AAs are shifted to the visceral organs, where they are used as precursors of the stimulated visceral protein synthesis. The amino acids may be used also as energy-yielding substrates, so that the glutamate pool is increased as a consequence of increased AA degradation (22).

Malnutrition in cancer-bearing children develops in a shorter time than normal children (23). When nourishment is sufficient, excess calorie expenditure and malabsorption are the main reasons for malnutrition (24-30). In cancer bearing patients the malnutrition rate has been reported as 5-37.5% (28-31). This wide range may be due to the different parameters used for the assessment of malnutrition and is closely related to the tumor stages (31). When we used

WFH, TSFT and MUAC criteria together, the malnutrition rate of our patients was identified as 18 %. Although we used the parameters of Smith et al.(30), our malnutrition rate was found to be higher when compared with that of Smith's. This difference may be due to the advanced stages of our patients (mostly stage III or IV). Serum albumin levels were lower than 3gr/dl in only four patients. It has been shown that there was no correlation between serum albumin levels and nutritional status in cancer-bearing patients. The level of albumin does not reflect the protein and calorie intake of the patients (32, 33). We compared the serum AA levels in patients with or without malnutrition, and as a result we observed that valine, leucine, ornithine levels were decreased in patients with malnutrition. When AA groups were taken into consideration, levels of BCAA were found to be decreased in cancer-bearing patients with malnutrition. EAA were found to be decreased in patients with malnutrition, while a relative increase in the levels of non EAA was identified (34, 35). A reduced protein intake would lead to a decrease of EAA, which was not observed in our patients. No change in the levels of BCAA in adult cancer-bearing patients with malnutrition has been reported in the literature (36). Our results are in accordance with that of Kurzer et al. (8), who identified a decrease in the levels of BCAA in rats with MCA sarcoma and malnutrition, and stated that this could be due to the tissue invasiveness or decreased release from tissues or both. In another study , an increase in ornithine levels was shown in patients with malnutrition (37). On the contrary, we identified a decrease in ornithine levels in cancer bearing patients with malnutrition. This could be due to the different metabolism of the cancer itself. Marquez et al. (37), showed that tumor cells needed exogenous ornithine during the proliferation phase. The authors also identified a net ornithine flux towards tumoral tissues, and this finding could explain the decreased serum levels of ornithine in patients with cancer.

In conclusion, a definite profile of AAs has not been obtained in patients with solid tumors. However we have found that arginine levels were increased in HD, AAA levels were increased in patients with Wilms' tumor and BCAA and ornithine levels were decreased in patients with malnutrition. The clinical role of these alterations in the diagnosis and nutritional management of childhood cancer needs to be confirmed by means of other studies in a large series of patients.

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