Trakya Univ Tip Fak Derg 2010;27(4):395-399 • doi: 10.5174/tutfd.2009.02378.2

Original Article / *Klinik Çalışma - Araştırma*

Breast Cyst Fluid Free Amino Acid Profile

Meme Kist Sıvısı Serbest Amino Asit Profili

Aynur DAĞLAR, Hakan ERBAŞ, Şendoğan GÜLEN

Trakya Üniversitesi Tıp Fakültesi, Biyokimya Anabilim Dalı, Edirne

Submitted / Başvuru tarihi: *11.03.2009* ***Accepted / Kabul tarihi:*** *20.04.2009*

Objective: Gross cystic breast disease (GCBD) is the most common benign breast disease. There are two types of breast cyst; lined by apocrine epithelium (Na/K<3) or flattened epithelium (Na/K>3). Several studies have shown that women with palpable breast cysts may have 1.7-7.5 times higher risk of developing breast cancer. Patients with malignant disease usually show abnormal amino acid profiles in the peripheral circulation. Changes in amino acid profile diagnostically correlate with organ sites of malignancy. The aim of this study was to investigate the levels of amino acids in two cyst groups and possible mechanisms involved in the development of breast cancer.

Material and Methods: The breast cyst fluid aspirated from women with GCBD were analysed. Breast cyst fluid amino acid levels were determined with HPLC.

Results: Aspartic acid, glutamic acid, hydroxyproline, serine, glycine, threonine, alanine, proline, tyrosine, methionine, isoleucine, leucine, phenylalanine and tryptophan levels were significantly higher, and lysine levels were lower in the apocrine epithelial cysts.

Conclusion: In this study, higher concentrations of amino acids in apocrine cysts, which are also known to have a higher risk of developing breast cancer, may indicate the possible role(s) of amino acids in the mechanism of breast cancer development.

Key words: Breast cyst fluid; gross cystic breast disease (GCBD); breast cancer; free amino acids; HPLC.

Amaç: Kistik meme hastalıkları kadınlarda en fazla görülen meme hastalığıdır. Apokrin epitelli (Na/K<3) ve düz epitelli (Na/K>3) olmak üzere iki tip meme kisti bulunmaktadır. Yapılan çeşitli çalışmalar memesinde kistik bir oluşum bulunan kadınların 1.7-7.5 kat daha fazla meme kanserine yakalanma riski taşıdıklarını ortaya koymuştur. Kanserli hastaların periferal dolaşımına bakıldığında ise genellikle anormal bir amino asit profili saptanmıştır. Ayrıca, amino asit profilindeki değişiklikler organ düzeyindeki kanserler ile ilişkili bulunmuştur. Bu çalışmanın amacı meme kanseri gelişimi yönünden yüksek ve düşük risk grubu kistlerdeki amino asit düzeylerini incelemek ve bu kistlerden meme kanseri gelişimi yönündeki olası mekanizmaları araştırmaktır.

Gereç ve Yöntemler: Çalışmada kistik meme hastalığı olan kadınlardan alınan meme kist sıvısı kullanıldı. Meme kist sıvısı amino asit düzeyleri HPLC metodu ile ölçüldü.

Bulgular: Apokrin epitelli kist grubunda aspartik asit, glutamik asit, hidroksiprolin, serin, glisin, treonin, alanin, prolin, tirozin, metiyonin, izolösin, fenilalanin ve triptofan düzeyleri anlamlı olarak yüksek, lizin düzeyi ise düşük bulundu.

Sonuç: Kanser gelişimi yönünden yüksek riske sahip olan apokrin epitelli kistlerde bulunan daha yüksek amino asit düzeyleri, amino asitlerin meme kanseri gelişim sürecinde potansiyel bir role sahip olabileceğini göstermektedir.

Anahtar sözcükler: Meme kist sıvısı; kistik meme hastalıkları; meme kanseri; serbest amino asitler; HPLC.

A part this study was presented as a poster at the 18th National Biochemistry Congress, Trabzon, 2004.

Correspondence (İletişim adresi): Dr. Hakan Erbaş. Trakya Üniversitesi Tıp Fakültesi, Biyokimya Anabilim Dalı, Edirne, Turkey.
Tel: 0284 235 78 57 e-mail (e-posta): herbas@trakya.edu.tr

INTRODUCTION

Gross cystic breast disease (GCBD) is the most common benign disease of the breast, occurring in 7-10% of adult women. There are two types of breast cysts[1] Type I cysts are lined by apocrine epithelium and contain fluid with high concentrations of potassium and low concentrations of sodium (Na/K ratio <3). This group of cysts also has high concentrations of steroid hormones, including androsterone, epiandrosterone and dehydroepiandrosterone and their conjugates. Type II cysts are lined by flattened attenuated epithelium and contain fluid with an electrolyte composition similar to that of plasma (Na/K ratio>3) and have lower concentrations of steroid hormones and their conjugates than type I cysts. Patients with type I cysts have shown to be more likely to develop further cysts than women with type II cysts, and patients who develop large numbers of cysts almost always have type I cysts.[2] Several large studies have shown that the risk of breast cancer in women with GCBD to be 1.7 to 7.5 times higher, and women with an apocrine breast cyst may have a higher risk of developing breast cancer than women with breast cyst lined by flattened epithelium.[3-5] Histological risk factors for breast cancer have been also reported to be more common with type I cysts.[3]

A variety of cancer bearing patients have been shown to have disturbances in carbohydrate, lipid and protein metabolisms.[6] The presence of a malignant tumour is usually associated with negative nitrogen balance,[7] increased gluconeogenesis from amino acids, decreased muscle protein synthesis and increased muscle breakdown. It has been suggested that the complex of metabolic derangements of protein in cancer patients may be reflected by alterations in the plasma free amino acid (PFAA) profile.[8] Levels of plasma amino acids may represent the net effect of all the factors influencing the total flux of amino acids in the body.[9] There have been reports focused on the role of the plasma amino acid profile as a marker of cancer-linked protein metabolism alterations.[10-14] The description of different plasma amino acid profiles for specific types of cancer, such as the small cell lung cancer[11] and the hepatocellular carcinoma in patients with cirrhosis,[12] suggest that the metabolic alterations of each type of tumour determine their own, distinctive profile of plasma amino acids. It has been proposed that the possibility of some cancers can induce characteristic plasma free amino acid profiles may suggest the roles of amino acids in diagnosing and finding the site of the cancer.[13,15]

On the other hand, in the present literature, there is no report on the breast cyst fluid free amino acid profile, except one which reports the cystic concentrations of homocysteine, cysteine, cysteinylglycine and glutathione.[16] The importance of amino acids in cancer metabolism, and a relatively higher risk of developing breast carcinoma especially from the low electrolyte ratio breast cyst group together, led us to perform this study. In this study, we aimed to determine the breast cyst fluid free amino acid composition in cyst groups which have low and high risks of developing breast carcinoma. In the breast cyst fluid, several different substances were found to be present. However, the exact mechanism underlying this risk for breast cancer development is still unclear. Determination of free amino acid profile of breast cyst fluid may help to understand at least some part of this complex mechanism.

PATIENTS AND METHODS

Patient Samples

Breast cyst fluids (BCF) were obtained by fine needle-aspiration from 17 women (9 apocrine and 8 flattened cyst group) attending the Breast Clinic at the General Surgery Department. Samples were centrifuged for 20 minutes at 1000G and supernatants were stored at-80°C until assayed. The study was approved by the Local Ethics Committee.

Measurement of Na and K

Intracystic Na and K concentrations were measured by an auto analyser (Synchron LX20, Beckman Coulter, USA).

Derivatisation of Amino Acids

BCF samples were mixed in a 1:1 ratio with the internal standard solution (methionine sulfone, 0.4 mM in 0.1 M HCl) and passed through 0.45 μm filter. A volume of 25 μl standard or sample was dried. The samples were then reconstituted with 20 μl drying solution [2:2:1, 1 M sodium acetate:methanol:triethylamine (TEA)], dried and dissolved in 20 μl of derivatization solution [methanol:water:TEA:phenylisothiocyanate (PITC), 7:1:1:1]. The derivatisation of both primary and secondary amino acids occurred in 20 min at 25°C and produced the corresponding phenylthiocarbamoyl derivatives. The samples were then re-dried. Finally, the dried samples were dissolved in 100 μl PicoTag sample buffer. After sonicating for a few seconds the samples were injected into HPLC column.

HPLC

The Alliance 2690 Separation Module (Waters, Milford, MA, USA) HPLC system, which consisted of two solvent delivery pumps, an auto injector, a column heater (46°C) and a Waters 487 UV detector set at 254 nm, was used in the reverse phase HPLC analysis. A Waters Millennium 32 chromatography manager system was used to control the system operation, to collect and process data. All separations were generated on a Waters PicoTag column (30 cmx3.9 mm) operating at a flow-rate of 1.0 ml/min. Samples were injected in volumes of 20 μl. The mobile phase consisted of a gradient of two eluents (PicoTag Eluent A and B). The gradient employed in the separation started with eluent B rising from 3 to 34% in 60 min. After a washing step of 10 min with 100% B, the column was re-equilibrated for 20 min with 100%

A. A constant flow-rate of 1 ml/min was maintained. HPLC-grade water was generated with a MilliQ water purification system from Millipore (Billerica, MA, USA).

Statistics

The results were expressed as mean±SEM. The Mann-Whitney U test was used for statistical analysis of two groups. $p<0.05$ was considered statistically significant.

RESULTS

Aspartic acid, glutamic acid, hydroxyproline, serine, asparagine, glycine, taurine, histidine, citrulline, threonine, alanine, arginine, proline, tyrosine, valine, methionine, cystathionine, cystine, isoleucine, leucine, hydroxylysine, phenylalanine, tryptophan, orninthine and lysine levels were determined in two groups of cyst fluid and shown in Table 1. While most of the amino acids were higher in the low electrolyte ratio cyst group, the levels of asparagine, citrulline, cystathionine and cystine were similar in the two cyst groups. The lysine levels were higher in the high electrolyte ratio cyst group, but this increase was not statistically significant (Table 1).

Amino acids were grouped according to their biochemical characteristics as aromatic amino acids (AAA; tyrosine, phenylanlanine, tryptophan), branched-chain amino acids (BCA; valine, isoleucine, leucine), glucogenic amino acids (GAA; alanine, aspartic acid, serine, asparagine, glycine, threonine), essential amino acids (EAA; threonine, valine, methionine, isoleucine, leucine, phenylanlanine, tryptophan and lysine) and nonessential amino acids (NEAA). Total amino acid (TAA) levels were also calculated and shown in Table 2.

There were statistically significant differences between the two cyst groups for concentrations of aspartic acid, glutamic acid, hidroxyproline, serine, glycine, threonine, alanine, proline, tyrosine, methionine, isoleucine, leucine, phenylanlanine and tryptophan (Table 1).

There were also significant differences in the two cyst groups for the concentrations of aromatic, branched-chain, gluconeogenic, essential, nonessential and total amino acids in the two cyst groups (Table 2, Figure 1 and 2).

Table 1. Amino acid levels in two sub-groups of breast cyst (μmol/L)

	Na/K<3 m±SEM; n=9	Na/K>3 m±SEM; n=8	z	p
Aspartic Acid	222.1±49.1	52.9±6.6	-3.368	<0.001
Glutamic Acid	8,839.5±1,059.6	997.6±230.9	-3.464	<0.001
Hydroxyproline	44.1±8.2	14.6±2.8	-3.079	<0.001
Serine	503.9±106.8	203.7±20.6	-2.502	<0.05
Asparagine	64.3±17.8	66.7±26.7	0.000	>0.05
Glycine	2,061.1±332.5	362.2±72.2	-3.464	<0.001
Taurine	142.9±44.6	65.9±11.5	-1.251	>0.05
Histidine	112.4±36.4	48.1±13.9	-1.061	>0.05
Citrulline	31.7±14.1	31.8±10.7	-0.387	>0.05
Threonine	339.5±96.3	184.3±62.1	-1.540	<0.05
Alanine	669.4±117.2	254.3±54.5	-2.502	<0.01
Arginine	287.8±28	278.8±32.3	0.000	>0.05
Proline	13.8±6.8	0±0	-2.069	<0.05
Tyrosine	275.1±58.1	106.5±27.2	-2.117	<0.05
Valine	395.9±113.1	147.5±28.0	-1.828	>0.05
Methionine	54.5±12.8	17.4±2.4	-2.887	<0.01
Cystathionine	27.1±4.0	27.5±9.4	-0.337	>0.05
Cystine	204.7±24.5	212.4±15.7	-0.289	>0.05
Isoleucine	188.3±57.6	52.4±7.3	-2.983	<0.01
Leucine	352.0±89.6	91.4±13.6	-3.129	<0.001
Hydroxylysine	28.0±6.0	16.8±3.4	-1.107	>0.05
Phenylalanine	178.7±42.9	51.7±8.0	-2.983	<0.01
Tryptophan	63.7±14.9	10.1±2.7	-3.175	<0.001
Ornithine	51.9±8.3	43.1±8.3	-0.770	>0.05
Lysine	47.3±10.4	101.3±22.1	-1.732	>0.05

Table 2. Categorised amino acid levels in two sub-groups of breast cyst (μmol/L)

	Na/K<3 m±SEM; n=9	Na/K>3 m±SEM; n=8	z	p
Aromatic	517.5±112.8	168.3±34.5	-2.69	<0.01
Branched-chain	936.3±256.5	291.7±47.6	-2.59	<0.01
Glucogenic	3,860.7±639.5	1,124.1±172.7	-3.36	<0.001
Essential	1,620.0±413.1	655.9±109.0	-2.11	<0.05
Nonessential	13,445.3±1667.8	2,611.6±379.9	-3.46	<0.001
Total Amino Acids	15,176.3±1974.6	3.424±462.2	-3.46	<0.001

DISCUSSION

Relatively little is known about the pathophysiology and pathogenesis of gross cystic disease of the breast. In an attempt to elucidate the endocrinology of cystic breast disease, many investigators have measured different constituents in breast cyst fluid. Higher intracystic concentrations of certain mitogenic polypeptides, such as transforming growth factor α,[17] epidermal growth factor, gastrin releasing peptide[18] and sex hormones, in particular estradiol,[19] were found in the low electrolyte ratio group than in the high electrolyte ratio group. This may provide an explanation for the higher risk of breast cancer which has been observed in the low electrolyte ratio group, and indicate that these substances may have a role in mammary carcinogenesis.[20] However, this complex mechanism is still mainly unknown.

Several investigators have pointed out that there are significant changes in PFAA profiles in cancer patients.[21] The changes of protein metabolism, as reflected in the PFAA profiles, may be used as an additional

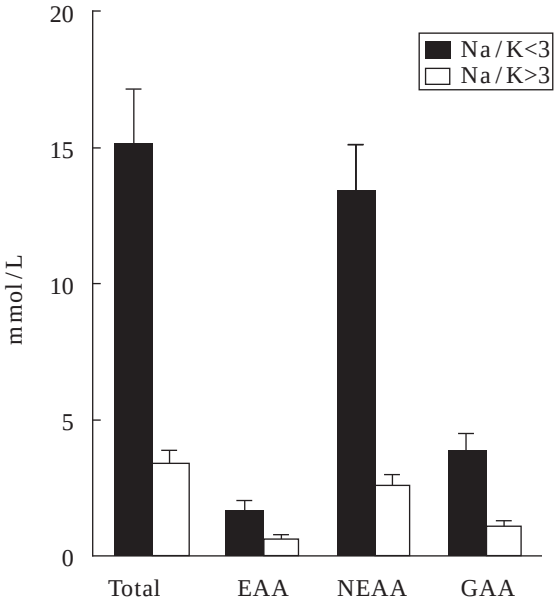

Figure 1. *Breast cyts fluid; total (TAA), essential (EAA), nonessential (NEAA) and glucogenic (GAA) amino acid levels in low and high electrolyte ratio cyst groups.*

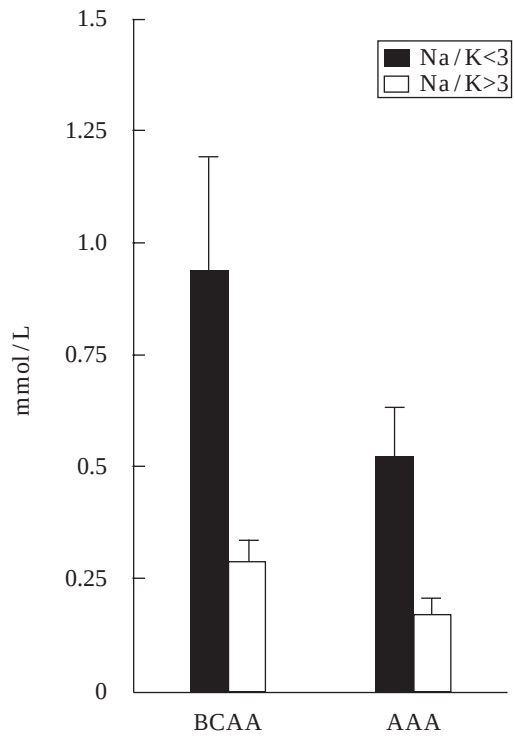

Figure 2. *Breast cyts fluid; branched-chain (BCA) and aromatic (AAA) amino acid levels in low and high electrolyte ratio cyst groups.*

tool for diagnosing cancer. The possibility of developing a cancer should be taken into consideration in a patient who shows abnormal PFAA levels. Meanwhile, the changes in either individual or group amino acids can be useful for the diagnosis of a specific cancer.[21]

Although there are a number of studies on the free amino acid pattern of different cancer, but at present, there are only three studies on the PFAA profile of breast cancer patients. Kubota et al. found decreased levels of cysteine and glutamine in breast cancer. Their study also showed increased levels of alanine, arginine and threonine in breast cancer patients. The TAA, EAA, AAA, BCAA and GAA levels all increased in breast cancer patients. They suggested that seven amino acids (glutamine, threonine, histidine, cysteine, alanine, arginine and ornithine) had a close link with specific cancers, indicating that PFAA profiles correlate with the organ-site origin among the three different malignant tumours.[13]

Another study has found significant increases of ornithine, glutamic acid and free tryptophan levels in breast cancer patients. They came to the conclusion that the effects of the tumors on the host's PFAA profile depend on cancer type.[22]

Proenza et al. also measured the levels of individual amino acids in blood, plasma and blood cell compartments in breast cancer patients.[23] Their data demonstrated a decreased level of aspartic acid, but increased levels of asparagine, glutamine and hydroxyproline in breast cancer. They also demonstrated that cancer, a situation of increased amino acid demand, is accompanied by a decrease in the amino acid availability, of which the blood cell pool can be the main contributor. Thus, they concluded that the amino acids profile characteristic of different types of cancers cannot only be found in plasma but also in blood. They have suggested that the amino acid profiles in other compartments can be informative as well.

In our study, as in these studies, we have found increased levels of alanine, threonine, glutamic acid, tryptophan and hydroxyprolin levels, especially, in low electrolyte ratio group breast cysts which were shown to bear higher risk of developing breast carcinoma. There was no difference between two cyst groups for arginine leves. Although, ornitine levels were higher in the high risk group of cysts, the difference was not significant. We have also shown that all TAA, EAA, AAA, BCAA and GAA levels were significantly higher in low electrolyte and high risk of breast carcino group than high electrolyte ratio group.

The metabolism of protein and amino acids in cancer patients has been shown to be closely linked to glucose metabolism and regulated by the same hormones and their metabolites.[24] The rate of glucose utilization has been found to increase in cancer patients.[25,26] Glycogen stored in the liver and skeletal muscle is expected to meet the increased demand of glucose in cancer patients, but is often insufficient for the demand due to decreased

food intake. However, blood glucose levels remain stable because of increased gluconeogenesis in hepatic cells. Heber et al. demonstrated that the rate of whole-body glucose production rises significantly in cancer patients.[6] The increase in alanine flux for gluconeogenesis is probably the major metabolic abnormality in cancer patients. The finding of higher levels of both alanine and total GAA levels in type I breast cysts may suggest the presence of a similar mechanism in BCF.

We have also observed that the major amino acids of BCF were glutamic acid, glycine, alanine, serine, valine and leucine. All these amino acids were significantly higher with a low electrolyte ratio and high risk for developing breast cancer.

On the other hand, plasma free tryptophan levels were determined in several types of cancer, including breast cancer, and found to be higher with respect to healthy controls.[27] Our finding of higher concentrations of tryptophan in type I cysts is in parallel with those of the others. It has been shown that tryptophan concentrations were decreased significantly after removal of breast tumours and it is suggested as a new useful marker for monitoring neoplastic diseases.[28]

Many reports have focused on the potential use of the amino acid profile as a tumour marker. These studies suggest that the metabolic alterations of various cancers can determine their own distinctive amino acid prolifes, as in breast cancer. Our finding of significantly higher concentrations of amino acids in type I cyst may indicate their important role(s) in this complex mechanism. In the light of the present literature, we may state that further studies are needed on breast cysts to elucidate the possible role of amino acids in breast cancer development.

Conflict of Interest

No conflict of interest declared by the authors.

REFERENCES

1. Dixon JM, Miller WR, Scott WN and Forrest APM. The morphological basis of human breast cyst populations. Br J Surg 1983;70:604-6.
2. Dixon JM, McDonald C, Elton RA, Miller WR. Risk of breast cancer in women with palpable breast cyst: a prospective study. Lancet 1999;353:1742-5.
3. Dixon JM, Lumsden AB, Miller WR. The relationship of cyst type to risk factors for breast cancer in patients with breast cystic disease. Eur J Cancer Clin Oncol 1985;21:1047-50.
4. Haagensen CD. Diseases of the breast. 3rd ed. Philadelphia: WB Saunders; 1986.
5. Mannello F, Tonti GAM, Papa S. Human gross cyst breast disease and cystic fluid: bio-molecular, morphological and clinical studies. Breast Can Res Treat 2006;97:115-29.
6. Heber D, Byerly LO, Chlebowski RT. Metabolic abnormalities in the cancer patient. Cancer 1985;55:225-9.
7. Brennan MF, Burt ME. Nitrogen metabolism in cancer patients. Cancer Treat Rep 1981;65:67-78.
8. Lee JC, Chen MJ, Chang CH, Tiai YF, Lin PW, Lai HS, et al. Plasma amino acid levels in patients with colorectal cancers and liver cirrhosis with hepatocellular carcinoma. Hepatogastroenterology 2003;50:1269-73.
9. Abumrad NN, Miller B. The physiologic and nutritional significance of plasma-free amino acid levels. JPEN 1983;7:163-70.
10. Norton JA, Gorschboth CM, Wesley RA, Burt ME, Brennan MF. Fasting plasma amino acid levels in cancer patients. Cancer 1985;56:1181-6.
11. Russell DM, Shike M, Marliss EB, Detsky AS, Shepherd FA, Feld R, et al. Effects of total parenteral nutrition and chemotherapy on the metabolic derangements in small cell lung cancer. Cancer Res 1984;44:1706-11.
12. Watanabe A, Higashi T, Sakata T, Nagashima H. Serum amino acid levels in patients with hepatocellular carcinoma. Cancer 1984;54:1875-82.
13. Kubota A, Meguid MM, Hitch DC. Amino acid profiles correlate diagnostically with organ site in three kinds of malignant tumors. Cancer 1992;69:2343-8.
14. Rossi Fanelli F, Cangiano C, Muscaritoli M, Conversano L, Torelli GF, Cascino A. Tumor-induced changes in host metabolism: a possible marker of neoplastic disease. Nutrition 1995;11:595-600.
15. Zhang PC, Pang CP. Plasma amino acid patterns in cancer [letter], Clin Chem 1992;38:1198-9.
16. Tallova J, Tomandi J, Bicikova M, Simickova M. Homocysteine in breast cyst fluid. Eur J Clin Invest 2001;31:623-7.
17. Lai LC, Siraj AK, Erbas H, Lennard TWJ. Transforming growth factor alpha, beta 1 and beta 2 in breast cyst fluid. Anticancer Res 1994;14:2805-10.
18. Lai LC, Ghatei MA, Takahashi K, Patel KV, Schrey MP, Ghilchik MW, et al. Mitogenic peptides in breast cyst fluid: relationship with intracystic electrolyte ratios. Int J Cancer 1990;46:1014-6.
19. Boccardo F, Torrisi R, Zanardi S, Valenti G, Pensa F, De Franchis V, et al. EGF in breast cyst fluid: realationships with intracystic androgens, oestradiol and progesterone. Int J Cancer 1991;47:523-6.
20. Goustin AS, Leof EB, Shipley GD, Moses HL. Growth factors and cancer. Cancer Res 1986;46:1015-29.
21. Lai HS, Lee JC, Lee PH, Wang ST, Chen WJ. Plasma free amino acid profile in cancer patients. Seminars in Cancer Biol 2005;15:267-76.
22. Cascino A, Muscaritoli M, Cangiano C, Conversano L, Laviano A, Ariemma S, et al. Plasma amino acid imbalance in patients with lung and breast cancer, Anticancer Res 1995;15:507-10.
23. Proenza AM, Oliver J, Palou A, Roca P. Breast and lung cancers are associated with a decrease in blood cell amino acid content. J Nutr Biochem 2003;14:133-8.
24. Heber D, Tchekmedyian NS. Cancer cachexia and anorexia. In: Heber D, Blackburn GL, Go VLW, editors. Nutritional oncology. California: Academic Press; 1999;537-46.
25. Pisters PW, Brennan MF. Amino acid metabolism in human cancer cachexia. Ann Rev Nutr 1990;10:107-32.
26. Lang CH, Dobrescu C, Bagby GJ. Tumor necrosis factor impairs insulin action on peripheral glucose disposal and hepatic glucose output. Endocrinology 1992;130:43-52.
27. Cascino A, Cangiano C, Ceci F, Franchi F, Mineo M, Mulieri M, et al. Increased plasma free tryptophan levels in human cancer: a tumour related effect? Anticancer Res 1991;11:1313-6.
28. Laviano A, Cascino A, Muscaritoli M, Fanfarillo F, Rossi Fanelli F. Tumor-induced changes in host metabolism: a possible role for free tryptophan as a marker of neoplastic disease. Adv Exp Med Biol 2003;527:363-6.