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DOI: 10.1093/clinchem/34.9.1781 · Source: PubMed

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Plasma Amino Acid Pattern of Patients with HIV Infection

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We measured the free amino acids in plasma of 58 patients with HIV infection and in six persons in the risk group. The HIV+ patients had significantly increased concentrations of arginine, phenylalanine, and glutamate in comparison with both age- and sex-matched controls and the members of the risk group. Glutamate concentrations increased only in an advanced stage of the disease (WR 5 and 6 of the Walter Reed staging classification), whereas arginine and phenylalanine increased independently of the stage. There was no correlation between the amino acid concentrations and the number of T4 and T8 lymphocytes, the sedimentation rate, and the existence or absence of Kaposi's sarcoma. The amino acid pattern of HIV-infected persons is similar to that of cancer patients or those with other immune deficiencies.

Additional Keyphrases: *Kaposi's sarcoma · HIV risk group · analysis for amino acids · phenylalanine · arginine · glutamic acid · acquired immunodeficiency syndrome*

Laboratory diagnosis of the human immunodeficiency virus (HIV) infection is based on the serological evidence of HIV antibodies. So far, there are only few indicators for these patients' prognosis, such as the number of T4-positive helper-lymphocytes and the cutaneous reaction towards recall-antigens (1).

In several diseases with immunodeficiency syndromes, changes in the concentrations of specific amino acids in plasma have been used as prognostic indicators. Several groups managed to differentiate between favorable and unfavorable outcome with respect to survival or response to surgery of patients with sepsis or tumor burden (2-4). Recently, Dröge et al. (5) postulated a connection between increased concentrations of glutamate in plasma and the immune response of patients with consuming diseases.

The aim of our study was to see whether the pattern of free amino acids in plasma of patients with HIV infection differed from that of a control group. We were especially interested in correlations between amino acid concentrations and the stages of the disease, as well as the presence of Kaposi's sarcoma.

Subjects and Methods

Subjects

We examined 64 consecutive outpatients at HIV risk, all either homosexual men or intravenous drug users. Of these, 58 showed antibodies against HIV as tested by enzyme-labeled immunosorbent assay (HTLV III-EIA, Abbott, Wiesbaden). These results were confirmed by Western blot analysis at the Institute for Virology, University of Cologne (Prof. Th. Mertens).

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Received March 21, 1988; accepted May 11, 1988.

All patients (mean age of the whole group: 31.4, SD 8.2 y; women: 34.7, SD 9.5 y) were classified according to the Walter Reed staging classification (6). We preferred this classification to that of the Centers of Disease Control (7), because it more strictly discriminates during the early stages of the disease (Table 1). Seven patients had Kaposi's sarcoma: one grade WR 3, one grade WR 4, three grade WR 5, and two grade WR 6.

Table 2 lists pertinent clinical data on these patients. All persons were in a normal nutritional status (8), none had lost more than 10% of his or her original body weight during the last six months, and serum albumin concentrations were within the normal reference interval.

Because there were only nine women in the investigation group (four WR 0, three WR 2, one WR 3, one WR 4), we only used the data on the men for statistical analyses. Twenty-two male healthy volunteers (ages 34.5, SD 10.9 y) and 25 women (ages 31.8, SD 8.6 y) served as controls. All were within the reference intervals with respect to body weight and laboratory test results; all had been free of acute and chronic viral and bacterial infections during the last two months.

Laboratory Investigations

Plasma amino acids: Blood for amino acid analysis was drawn after a fast of at least 12 h between 0800 and 0900 hours as ammonium heparinated plasma. Within 2 h of venepuncture plasma was deproteinized with an equal volume of a 50 g/L solution of sulfosalicylic acid in 0.1 mol/L lithium citrate buffer (adjusted with HCl to a final pH of 2.2) containing norvaline as internal standard. The specimens were stored at -70 °C until analysis. Amino acid analyses were done by ion-exchange chromatography in a Biotronic LC 5000 analyzer (Biotronic, München, F.R.G.). The glass column (315 × 3.2 mm i.d.) was packed with BTC 2710 resin (Biotronic) to a height of 137 mm. The buffer flow rate was 0.25 mL/min. As buffers, lithium citrate buffers of different ion concentrations and pHs were used. The separation conditions were as follows: temp. 1: 35 °C, 23 min; temp. 2: 56 °C, 67 min; temp. 3: 58 °C, 27 min. The time intervals were for buffer A (pH 2.96) 8 min, for buffer B (pH 3.01) 27 min, for buffer C (pH 3.51) 26 min, for buffer D (pH 4.02) 6 min, and for buffer E (pH 3.53) 50 min. Separation time was 117 min; the regeneration period was 7 min, at 70 °C with 0.25 mol/L lithium hydroxide, and the equilibration time

Table 1. Walter Reed Staging Classification of HIV Infection (1)

WR	Characteristics
0	Member of high-risk group
1	Serological diagnosis of HIV infection
2	Additional chronic lymphadenopathy (of more than two non-inguinal lymph nodes)
3	T-helper cells <400/mm ³
4	Additional cutaneous hyperpigmentation
5	As WR 3, additional cutaneous anergy, and/or oral candidiasis
6	Opportunistic infection, additional to HIV infection

Table 2. Characteristics of HIV Patients and Risk Group (WR 0)

WR	Sex	Age, y	Body weight, % IBW	T4 cells		T8 cells		T4/T8 ratio	Total protein, g/L
				number/mm ³					
0	2m, 4f	35.7 ± 11.7	99.8 ± 8.2	1125 ± 282	639 ± 13	1.73 ± 0.4		82 ± 6	
1	4m	33.5 ± 8.3	97.9 ± 4.2	611 ± 142	882 ± 487	0.79 ± 0.31		84 ± 5	
2	20m, 3f	32.0 ± 8.5	96.0 ± 13.8	630 ± 192	1060 ± 460	0.68 ± 0.32		80 ± 7	
3	10m, 1f	35.3 ± 11.0	93.1 ± 10.6	277 ± 96	627 ± 263	0.53 ± 0.3		79 ± 5	
4	8m, 1f	33.2 ± 6.3	100.4 ± 7.1	240 ± 123	761 ± 238	0.36 ± 0.2		81 ± 7	
5	9m	38.6 ± 10.5	94.3 ± 12.1	180 ± 122	629 ± 445	0.29 ± 0.18		78 ± 7	
6	2m	44.0 ± 18.5	91.1 ± 12.2	93 ± 89	488 ± 341	0.18 ± 0.06		67 ± 5	

Values are means ± standard deviation. Abbrev.: IBW, ideal body weight; m, male; f, female; WR, Walter Reed staging classification.

with buffer A was 55 min. Amino acids were detected as reaction products with a 20 g/L ninhydrin solution at a wavelength of 570 nm. The solution was made under nitrogen atmosphere with 7.5 parts ethylene glycol monomethyl ether and 2.5 parts 4 mol/L lithium acetate buffer, containing 0.75 g of titanium chloride.

Differentiation of T-lymphocytes: T-cell subsets were enumerated by flow cytometry (FACS 2; Becton Dickinson, Mountain View, CA) with use of monoclonal antibodies OKT4 and OKT8 to identify T-helper and T-suppressor cells.

Other laboratory data: For determination of liver enzymes, total plasma proteins, urea, and creatinine we used an automated analyzer (Model 794; Hitachi Inc., Tokyo, Japan).

Statistics: For statistical evaluation we made use of the U-test (Wilcoxon, Mann, Whitney). All values are expressed as means and standard deviations. *P* values below 0.05 were considered to be significant.

Results

Table 3 lists those amino acids that differed significantly from the reference intervals in percentage of total amino acids (the absolute concentrations of all amino acids are available from the authors on request). Our reference intervals were in accordance with published data (9-11). There were no significant differences between values for the amino acids of HIV-infected patients and those of either the controls or the risk group (WR 0), with the exception of arginine, phenylalanine, and glutamate. The means for the women are within one standard deviation of that for the male group with respect to these three amino acids. The concentrations of alanine, valine, methionine, isoleucine, and leucine are known to be significantly lower in women than they are in men (12). This is also true of our female study group, without significant differences between female patients and controls.

The glutamate values are only increased in advanced

stages of the infection (WR 5, 6), whereas the arginine and phenylalanine concentrations differ in all HIV+ patients from the reference intervals. There is no correlation between the absolute or relative (to the total amino acid amount) concentrations. The data for patients with Kaposi's sarcoma do not differ from those without it of the same WR grade. Furthermore, the changes of the amino acids also do not correlate with the activity of the liver enzymes, with the sedimentation rate, or with immunoglobulin concentrations. No subject classed as WR 0 had an increased sedimentation rate, and only small increases (<10/>20) were seen in three WR 2 and one WR 5 patients. Distinct increases were observed in five WR 2, four WR 3, four WR 4, four WR 5, and all WR 6.

Discussion

Several diseases, especially consuming illnesses, can induce specific patterns in plasma amino acids, with increased concentrations of phenylalanine, glutamate, and arginine (9, 13). The amino acid pattern of HIV+ patients strongly resembles the pattern seen in tumor patients generally. The relative increase (i.e., relative to the total amino acid concentration) in glutamate and in the other two amino acids is much more distinct than it is when expressed in terms of absolute concentrations.

In the literature there is only one study as yet on amino acid concentrations of HIV+ patients (5), and in it only glutamate was examined. The results are quite in contrast to ours, because they show much more pronounced increases in glutamate. The reasons for these differences are unclear. Glutamate values are incorrectly increased if plasma is not deproteinized or chilled quickly after venepuncture at very low temperatures (10). We deproteinized our specimen within 2 h after venepuncture, and the storage temperature was -70 °C.

The pathogenetic mechanism inducing the changed amino acid pattern of HIV+ and tumor patients is not known. Increased concentrations of phenylalanine can be seen in all

Table 3. Plasma Amino Acids That Differed Significantly from Reference Intervals (in Proportion of Total), Grouped by Walter Reed Staging Classification

Amino acids (% total AA)	Male								KS (n = 9)
	controls (n = 22)	WR 0 (n = 6)	WR 1 (n = 4)	WR 2 (n = 20)	WR 3 (n = 10)	WR 4 (n = 8)	WR 5 (n = 9)	WR 6 (n = 9)	
Glutamate	1.86 ± 0.86	1.90 ± 0.36	2.71 ± 1.69	2.18 ± 1.2	2.14 ± 1.26	2.36 ± 1.86	2.69 ^b ± 0.87	2.97 ^b ± 0.6	3.1 ^b ± 0.66
Phenylalanine	2.24 ± 0.5	2.1 ± 1.56	2.89 ± 1.28	3.08 ^b ± 0.94	2.71 ^b ± 0.83	3.0 ^b ± 0.94	2.91 ^b ± 0.66	3.06 ^b ± 0.70	2.91 ^b ± 0.70
Arginine	2.02 ± 0.58	1.86 ± 0.67	2.83 ^a ± 0.85	2.57 ^a ± 0.89	2.87 ^a ± 0.7	2.88 ^a ± 0.8	3.12 ^b ± 1.02	3.16 ^b ± 0.75	3.16 ^b ± 1.04

^a *P* < 0.05, ^b *P* < 0.01 versus controls, and WR 0. Abbrev.: KS: patients of WR 3-6 with Kaposi's sarcoma.

kinds of stress metabolism, e.g., trauma and sepsis (14) as well as in oncological diseases (13). That is why the increase of phenylalanine seems to be a nonspecific indicator of stress metabolism, as ferritin also is known to be in HIV+ patients (15). The amino acids could possibly be released from the muscle—a consequence of the amino acid shift from the periphery to visceral organs during stress metabolism (16).

Glutamate concentrations reportedly are increased in patients with gastrointestinal (17, 18), gynecological (19, 20), hematological (21–23), and lung cancer (21). Glutamate plays a central role in the metabolism of amino acids and ammonia. It is formed in the degradation of arginine, ornithine, proline, and histidine. Its metabolite, *N*-acetylglutamate, may regulate the synthesis of carbamyl phosphate (24) and, as a consequence, the turnover rate of the urea cycle. Glutamate is used directly for protein synthesis, and a minor pathway involves the decarboxylation to gamma-aminobutyrate. That is why this amino acid is of importance both as a precursor of a neurotransmitter and as an active transmitter (25). Regarding the inhibitory influence of gamma-aminobutyrate on the lateral hypothalamic dopaminergic system, which may reduce the spontaneous food intake (26), it is of interest that most of the diseases accompanied by increased plasma glutamate concentrations are often characterized by anorexia.

There seems to be a significant correlation between increased glutamate concentrations on the one hand and diminished proliferative responses to mitogens of peripheral blood lymphocytes on the other (5). Therefore, increased glutamate concentrations possibly can contribute to the impaired immune reactivity of patients with consuming diseases. Arginine may also play a role as an immune modulator. Barbul et al. (27) were able to demonstrate that an arginine supplementation of 30 g per day stimulates the mitogenic reactivity of the peripheral lymphocytes in healthy volunteers.

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