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ORIGINAL ARTICLE

Serum amino acid abnormalities in pediatric patients with chronic renal failure with and without history of thromboembolic manifestations

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Abstract *Background:* Plasma amino acid concentrations have been reported to be abnormal in patients with chronic renal failure. L-Arginine has been used to improve endothelial function by increasing nitric oxide (NO) bioavailability.

The present study aim at investigating the status of plasma amino acids in pediatric patients with chronic renal failure (CRF) on regular hemodialysis (HD) with and without history of thromboembolic manifestations.

Methods: The study included 21 hemodialysis patients subdivided into two groups (those with no history of thromboembolic manifestations and those with positive history of thromboembolic manifestations) The control group included 13 age and sex matched apparently healthy subjects, After careful history taking, clinical examination, the following laboratory investigations were performed: serum calcium, phosphate, albumin, and creatinine (for controls only), complete blood count (CBC) and serum amino acid analysis.

Results: HD patients had a significantly lower concentration of threonine, valine, methionine, leucine, tyrosine, phenylalanine and tryptophane than the control group ($p = 0.032$, 0.020, 0.046, 0.011, 0.000, 0.022, and 0.004 respectively). There was no significant difference between HD

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patients and the control group as regard aspartic acid, serine, asparagine, glutamic acid, proline, glycine, alanine, cystine, isoleucine, lysine, histidine, and arginine. The mean serum L-arginine level was lower in 61.9% of HD patients than the mean of the controls with no significant difference. L-Arginine concentration was not significantly different between HD patients with and without history of thromboembolic manifestations.

Conclusion: Several abnormalities in amino acids were present in HD patients compared to controls. The mean serum L-arginine level was lower in 61.9% of HD patients than the mean of the controls with no significant difference. L-Arginine concentration was not significantly different between HD patients with and without history of thromboembolic manifestations. HD patients without history of thromboembolic manifestations had significantly lower glutamic acid concentrations and significantly higher phenylalanine concentrations than HD patients with history of thromboembolic manifestations.

1. Introduction

The uremic syndrome is a complex condition that results from an accumulation of multiple waste compounds, combined with failure of the endocrine and homeostatic functions of the kidney in end-stage chronic renal failure (CRF) patients [1].

Malnutrition is a common pathological condition which exacerbates cardiovascular morbidity and mortality in chronic renal failure patients.

The underlying mechanisms of malnutrition in CRF have not been completely clarified. Inadequate diet and a state of persistent catabolism play major roles [1]. Also protein metabolism changes with loss of renal function resulting in deterioration of nutritional status [2].

Levels of plasma and intracellular amino acids are significant early indicators of protein metabolism and nutritional status assessment [1,3]. Many of the characteristic alterations in the plasma amino acid profile that are observed in chronic end-stage renal disease are already present in mild renal insufficiency. Progressive loss of renal function generally results in increasing abnormalities; these changes in plasma amino acid concentrations were usually linear with reduction in glomerular filtration rate (GFR) [4].

Plasma protein and amino acid concentrations have been reported to be abnormal in patients with CRF, whether on conservative or regular dialysis treatment [5–8]. These abnormalities may be related to impaired protein and amino acid metabolism, to dietary deficiencies of calories and proteins, or to amino acid and protein losses due to peritoneal dialysis or hemodialysis [9]. Moreover, branched-chain amino acids are moderately decreased only in the advanced stage of renal failure and this may be, at least in part, nutritional in origin [6]. Also increased protein degradation may be the cause of increased plasma concentration of nonessential amino acids in malnourished chronic renal patients [2].

Increased cardiovascular mortality and morbidity is well recognized in adults with CRF. The adverse impact of CRF on cardiovascular mortality and morbidity in the young is however even greater with a 500 times higher rate of cardiovascular deaths than a control population. The initiation of vascular damage begins very early during the course of CRF, and involves the vascular endothelium. L-Arginine is the substrate for nitric oxide (NO) synthase and has been shown to increase endothelial function in animal models and in clinical studies of subjects with hypercholesterolemia and coronary artery disease. So L-arginine supplementation in children with CRF might increase NO bioavailability [1].

2. Aim of the work

The aim of this work is to investigate the status of plasma amino acids in pediatric patients with CRF on regular HD with and without history of thromboembolic manifestations.

3. Subjects and methods

This case control prospective study was conducted at the Pediatric Nephrology Unit, Children's Hospital, Ain Shams University, Cairo, Egypt, during the period from April 2008 to February 2009.

It included 21 patients (12 males and nine females), their age ranged from 9 to 18 years with a mean of 13.99 ± 2.794. Regardinghe etiology of chronic renal failure in our studied patients, it was glumerulonephrits in 11 patients (52%), Chronic parenchemtous Renal disease in six patients (29%), bilateral renal stones in one patient (5%), Posterior uretheral valve and bilateral 4th degree vesicoureteric reflex in one patient (5%), nephrocalsinosis in one patient and the etiology was unknown in one patient. Family history of chronic renal failure was negative in our all our studied patients except for one patient who has Focal Segmental glumerulonephrits. Parent consanguinity was present among 10 (48%) of our studied patients.

Our patients were regularly dialyzed for 3–4 h/session, thrice weekly sessions per week at a blood flow rate of 160–250 ml/min through arteriovenous fistula or grafts. All patients used bicarbonate dialysate (33 mmol/L; Fresenius Medical Care, Germany) at a dialysate flow rate of 500 ml/min with volumetric ultra filtration control. All HD treatments were performed on either Gambro (AK-95, AK-200; Gambro Corp., Sweden) or Fresenius (4008 B, or 4008 S; Fresenius Medical Care, Germany) dialysis machines using low-flux polysulfone synthetic hollow fiber (F3; F4 or F5; Fresenius Medical Care, Germany). None of the patients reused the

dialyser. No bacteria or pyrogens were detected in the dialysate prepared from water obtained by reverse osmosis. Some of the used dialysis machines were equipped with an endotoxin removal filter, but the endotoxin concentration in dialysate fluids was not measured in this study. Dialysis adequacy was estimated using the kt/V method calculated by the machine (it ranged between 0.9 and 1.4).

We excluded patients on hemodialysis with diabetes mellitus or nephrotic syndrome as an etiology of ESRD.

In addition, 13 age and sex matched apparently healthy subjects were selected from relatives of patients as a control group They were six males and seven females, their age ranged from 5.5 to 18 years with a mean of 12.15 ± 3.72 years.

An informed oral consent was taken for the history taking, physical examination and sample collection from the patients, or their parents.

3.1. All children were subjected to

Full history taking with special emphasis on duration of dialysis, thromboembolic manifestations (arteiovenous fistula thrombosis, graft thrombosis, central vein thrombosis or thrombosis in any other site), protein restriction diet and medication history. Details of HD session and drugs were gathered thorough history taking and review of patients' files. They were carefully examined with special emphasis on body weight, height, edema, measurement of blood pressure ''before dialysis for the hemodialysis group'' while subjects were quietly seated, before withdrawing blood samples, cardiac and chest examination.

Ten milliliters of venous blood were withdrawn without stasis from the venous site of the arteriovenous fistula before the start of the dialysis session (before heparin administration). In the control group, blood samples were withdrawn from peripheral veins. The sample was divided to three parts one on EDTA for CBC and two clotted samples. One for albumin, Ca, P, the other one were centrifuged after clotting to obtain the serum, aliquoted, and stored frozen at −20 °C until used for amino acid analysis.

- Serum calcium, phosphate, albumin, creatinine (for controls only) assay were done immediately after sample collection using the auto analyzer 917 HITACHI, Boehringer Mannheim, Germany.
- Complete blood count (CBC) using Coulter Corporation model Gen's Ana, program level 1.99195, S/WARE Kit n/a, manufacture date, May 2001, Miami, FL 33196-2500.
- Amino acid analysis was done using The Amino Acid Analyzer SYKAM GmbH, Analytischer Messtechnik (Gewerbering 15, D_86922 Erosing, Germany in the Genetic Research Center about 4 months later.

3.2. Method of amino acid analysis

Acid precipitation of proteins

- Plasma (800 μL) were pipetted in a centrifuge tube.
- Sulphosalicylic acid (200 μL) (10%) were added to the plasma and the tube was shaken well.
- The mixture was stored in a refrigerator at 4 °C for 30 min and was then centrifuged for 10 min.
- The upper clear solution was dissolved with the sample diluting buffer (Li citrate) in the ratio of one to one. (The sample should show a pH-value of 1.80–2.00; if necessary, the pH-value was corrected by using concentrated Lithium hydroxide.
- The samples were analyzed shortly. Otherwise, it was frozen till the assay time.
- Three standards were placed with patients samples in the auto sampler. The similarity of the standards shows accuracy of amino acid analyzer.

3.3. Statistical methodology

Analysis of data was performed using standard computer program Statistical Package for Social Sciences (SPSS)13.0 for windows (SPSS Incorporation, USA). Normality of variable distribution was tested by the Kolmogrov–Smirnov test. For normally distributed data description of quantitative variables in the form of mean ± standard deviation, and for non parametric data median, imterquartile ranges. Description of qualitative variables in the form of frequency and percentages. Unpaired student *t*-test, and Mann–Whitney test were used to analyze the differences between two groups for normal and non-parametric variables respectively. Chi square test was used to compare qualitative parameters.

Pearson's and Spearman's correlation coefficient tests were used to correlate normal and non parametric variables, respectively. Paired *t*-test was used to compare two readings of the quantitative variable within the same group. Multiple regression analysis was used to find out the effect of different independent factors on certain dependent variable using stepwise technique. For all tests a probability (p) value of less than 0.05 was considered statistically significant, results were tabulated and graphically represented using appropriate graphs [10].

4. Results

HD patients had a significantly lower weight, OFC, calcium and hemoglobin concentrations than the control group (p = 0.029, 0.006, 0.009, and 0.001, respectively), and they had a significantly higher phosphate concentration than the control group (p 0.000). There was no significant difference between HD patients and the control group (p = 0.267, 0.082, 0.807, and 0.266) as regard age, height, alkaline phosphatase and albumin (Table 1).

There was no significant difference between weight, height, occipitofrontal circumference, mid arm circumference, systolic blood pressure, diastolic blood pressure, age, age of onset of dialysis, duration of dialysis, calcium, phosphate, albumin and hemoglobin concentration in HD patients with and without history of thromboembolic manifestations.

HD patients had a significantly lower concentration of threonine, valine, methionine, leucine, tyrosine, phenylalanine and tryptophane than the control group (p = 0.032, 0.020, 0.046, 0.011, 0.000, 0.022, and 0.004, respectively). There was no significant difference between HD patients and the control group as regard aspartic acid, serine, asparagine, glutamic acid, proline, glycine, alanine, cystine, isoleucine, lysine, histidine, and arginine. The mean serum L-arginine level was lower in 61.9% of HD patients than the mean of the controls with no

Table 1 Descriptive and comparative statistics of some demographic data and laboratory investigations done to HD patients and controls.

	HD ($n = 21$)		Controls ($n = 13$)		T/z*	P	Sig
	Mean ± SD	Median (IQR)	Mean ± SD	Median (IQR)			
Age (years)	13.99 ± 2.794	14 (4)	12.15 ± 3.72	11 (6)	1.129	0.267	NS
Weight (kg)	28.071 ± 8.859	26.5 (8.3)	38.692 ± 16.549	35 (28.5)	2.163*	0.029	S
Height (cm)	130.14 ± 19.597	137 (27)	142.46 ± 18.59	141 (24)	1.761*	0.082	NS
OFC (cm)	53.29 ± 1.765	53 (2)	55 ± 1.871	55 (2)	2.737*	0.006	S
Ca (mg/dL)	8.495 ± 1.256	9 (2.1)	9.523 ± 0.5449	9.6 (1)	2.557*	0.009	S
P (mg/dL)	7.338 ± 1.795	7.4 (3.4)	3.938 ± 0.588	3.8 (1)	6.579	0.000	S
ALP (IU/L)	410.95 ± 401.07	212 (562)	258.77 ± 63.704	271 (112)	0.266*	0.807	NS
Alb (g/dL)	4.495 ± 0.376	4.5 (0.4)	4.654 ± 0.429	4.8 (0.9)	1.132	0.266	NS
Hb (g/dL)	8.91 ± 1.527	8.90 (2)	10.66 ± 1.243	10.7 (2)	3.478	0.001	S

HD: hemodialysis patients; SD: standard deviation; IQR: interquartile range; OFC: occipitofrontal circumference; Ca: calcium; P: phosphate; ALP: alkaline phosphatase; Alb: Albumin; Hb: hemoglobin.
* Z value of Mann–Whitney test.

significant difference. HD patients had a significantly lower concentration of threonine, valine, methionine, leucine, tyrosine, phenylalanine, and tryptophane than the control group ($p = 0.032$, 0.020, 0.046, 0.011, 0.000, 0.022, and 0.004, respectively). There was no significant difference between HD patients and the control group as regard aspartic acid, serine, asparagine, glutamic acid, proline, glycine, alanine, cystine, isoleucine, lysine, histidine, and arginine. The mean serum L-arginine level was lower in 61.9% of HD patients than the mean of the controls with no significant difference (Table 2).

HD patients without history of thromboembolic manifestations had significantly lower glutamic acid concentrations ($p = 0.03$), and significantly higher phenylalanine concentrations ($p = 0.04$) than HD patients with history of thromboembolic manifestations (Table 3), on the other hand there was no significant difference between HD patients with and without history of thromboembolic manifestations as regard arginine concentration (Table 3).

There was no significant difference between age, age of onset of symptoms, age of onset of dialysis, duration of dialysis, weight, height, occipitofrontal circumference, mid arm circumference, systolic, diastolic blood pressure, calcium, phosphate, albumin, hemoglobin concentrations, between HD patients with low or normal arginine concentration.

HD patients with low arginine concentration had significantly lower concentrations of tyrosine and phenylalanine amino acids than those with normal arginine concentration (Patients who had L-arginine levels compared to the lowest normal level for L-arginine (normal L-arginine level 44–120 μmol/L [11] were 13 patients. Those 13 patients identified as patients with low arginine) (Table 4).

5. Discussion

Cardiovascular disease is a major cause of mortality among patients with chronic renal failure (CRF) [12]. The vascular damage in dialyzed patients is frequently observed and it is probable that disturbances in fibrinolytic activity and endothelial dysfunction may play a role in vascular complications such as stroke or ischemic heart disease [13].

Chronic renal failure patients display endothelial dysfunction, a critical element in the pathogenesis of atherosclerosis. Upon activation or apoptosis, the endothelium sheds microparticles are considered as markers of endothelial dysfunction,

Table 2 Comparison between HD patients and controls as regard serum amino acids.

		HD ($n = 21$)		Control ($n = 13$)		T/z*	P	Sig
		Mean ± SD	Median(IQR)	Mean ± SD	Median(IQR)			
Aliphatic AA	Glycine	115.24 ± 56.5638	107.7 (105.91)	113.61 ± 74.707	91.72 (65.95)	0.066	0.948	NS
	Alanine	97.90 ± 61.2268	99.53 (86.19)	605.16 ± 926.45	224.4 (622.0)	1.811*	0.074	NS
	Valine	47.9 ± 17.391	52.44 (26)	87.15 ± 45.983	75.56 (81)	2.324*	0.020	S
	Isoleucine	21.04 ± 12.1885	18.33 (18.64)	26.09 ± 10.788	29.49 (15.57)	1.153	0.259	NS
	Leucine	42.58 ± 15.545	36.88 (17.83)	64.38 ± 26.1536	64.08 (35.92)	2.725	0.011	S
Hydroxyl AA	Threonine	22.17 ± 14.10259	18.75 (23.46)	41.36 ± 27.286	34.39 (38.36)	2.280	0.032	S
	Serine	353.29 ± 922.236	44.97 (38.14)	207.62 ± 423.85	69.1 (94.55)	1.300	0.205*	NS
Sulfur AA	Cystine	12.01 ± 6.0586	13.035 (11)	24.38 ± 23.19	17.79 (9.5)	1.483	0.146*	NS
	Methionine	11.54 ± 6.775	9.97 (7.03)	16.65 ± 7.66	17.38 (13.94)	2.002	0.046*	S
Acidic AA, amides	Aspartic acid	23.87 ± 48.578	1.24 (10.7)	13.01 ± 34.403	1.725 (7.69)	0.062	0.976*	NS
	Asparagine	754.88 ± 1030.939	118.16 (1854.19)	218.98 ± 199.817	145.5 (401.5)	0.169	0.902*	NS
	Glutamic acid	10.14 ± 13.806	3.22 (11.4)	62.25 ± 44.11	56.13 (84.12)	3.001	0.002*	NS
Basic AA	Arginine	35.03 ± 24.884	33.32 (47.28)	46.66 ± 88.8127	17.5 (39.03)	1.143	0.267*	NS
	Lysine	56.59 ± 21.4578	50.36 (29.45)	139.35 ± 263.35	67.38 (35.09)	1.012	0.330*	NS
	Histidine	17.77 ± 11.08	15.67 (20.35)	35.32 ± 25.385	37.97 (51.68)	1.804	0.091	NS
Aromatic AA	Tyrosine	16.85 ± 10.479	14.115 (15.72)	40.27 ± 21.379	33.72 (33.81)	4.042	0.000	S
	Phenylalanine	30.29 ± 13.0493	27.82 (21.43)	43.48 ± 16.738	44.51 (19.55)	2.428	0.022	S
	Tryptophane	13.46 ± 9.643	11.53 (19.35)	.49 ± 5.3606	1.54 (5.29)	2.831	0.004*	S
Imino acids	Proline	128.35 ± 104.793	115.93 (203.7)	73.81 ± 38.565	64.17 (40.3)	1.281	0.229	NS

HD: hemodialysis patients; SD: standard deviation; IQR: interquartile range. Amino acids concentrations measured by μMol/L.
* Z value of Mann–Whitney test (for non parametric data).

Table 3 Comparison between HD patients with and without history of thromboembolic manifestations.

		No history of thromboembolic manifestations (n = 12)		Positive history of thromboembolic manifestations (n = 9)		T/z*	P	Sig
		Mean ± SD	Median (IQR)	Mean ± SD	Median (IQR)			
Aliphatic AA	Glycine	125.75 ± 67.38	115.19 (121.14)	103.41 ± 42.69	95.33 (64.95)	0.804	0.434	NS
	Alanine	98.5 ± 63.43	103.76 (117.4)	97.13 ± 63.29	95.3 (82.98)	0.265*	0.837	NS
	Valine	53.64 ± 19.44	55.43 (23)	42.17 ± 13.96	42.97 (29)	1.26*	0.234	NS
	Isoleucine	26.54 ± 14.89	24.27 (22.86)	16.23 ± 7.09	16.46 (8.91)	1.751	0.103	NS
	Leucine	47.97 ± 13.36	50.26 (22.21)	37.86 ± 16.60	34.67 (10.39)	1.285	0.221	NS
Hydroxyl AA	Threonine	26.94 ± 16.77	25.35 (32.95)	15.82 ± 6.20	17.24 (10.94)	1.535	0.151	NS
	Serine	384.96 ± 1072.8	49.51 (18.24)	300.49 ± 688.89	23.43 (443.63)	1.73*	0.093	NS
Acidic AA, amides	Aspartic acid	43.23 ± 61.08	9.995 (105.24)	0.65 ± 0.53	0.06 (0.96)	1.826*	0.082	NS
	Asparagine	773.27 ± 1069.98	142.68 (1789.8)	699.72 ± 1122.5	93.65	0.277*	0.864	NS
	Glutamic acid	17.25 ± 15.68	10.49 (29.63)	1.6 ± 1.44	2.16 (2.76)	2.191*	0.03	S
Sulfur AA	Cystine	10.99 ± 5.73	12.77 (10.66)	13.60 ± 6.66	17.13 (13.05)	1.132*	0.285	NS
	Methionine	12.27 ± 7.52	10.165 (9.01)	10.57 ± 5.92	9.97 (3.3)	0.284*	0.808	NS
Aromatic AA	Tryptophane	16.32 ± 9.02	16.64 (12.78)	8.44 ± 9.73	4.42 (16.08)	1.323*	0.230	NS
	Tyrosine	21.29 ± 11.69	19.16 (20.17)	12.41 ± 7.23	13.36 (7.33)	1.937	0.071	NS
	Phenylalanine	37.03 ± 14.09	40.715 (25.26)	24.31 ± 9.01	23.86 (11.16)	2.247	0.040	S
Basic AA	Lysine	57.74 ± 19.52	50.36 (25.48)	55.58 ± 24.32	52.83 (33.71)	0.347*	0.779	NS
	Histidine	16.61 ± 13.52	14.06 (25.54)	18.92 ± 10.01	16.86 (18.69)	0.275	0.792	NS
	Arginine	39.27 ± 26.56	47.815 (50.99)	29.73 ± 23.22	24.37 (42.19)	0.622*	0.573	NS
Imino acids	Proline	141.77 ± 99.96	115.93	108.21 ± 50.30	108.21	0.309	0.778	NS

HD: hemodialysis patients; SD: standard deviation; IQR: interquartile range. Amino acids concentrations measured by μMol/L.
* Z value of Mann–Whitney test (for non parametric data).

Table 4 Comparison between HD patients with low and with normal arginine.

		Low arginine (n = 13)		Normal arginine (n = 8)		T/z*	P	Sig
		Mean ± SD	Median(IQR)	Mean ± SD	Median(IQR)			
Aliphatic AA	Glycine	110.04 ± 65.25	81.47 (115.45)	122.67 ± 45.12	115.19 (75.31)	0.442	0.665	NS
	Alanine	73.298 ± 57.56	74.56 (102.12)	138.91 ± 45.31	136.64 (87.17)	1.952*	0.056	NS
	Valine	45.96 ± 20.63	45.64 (31)	51.14 ± 10.99	55.45 (17)	1.085*	0.313	NS
	Isoleucine	18.31 ± 13.70	14.6 (11.69)	25.14 ± 9.05	24.89 (15.92)	1.068	0.305	NS
	Leucine	36.88 ± 13.64	33.93 (18.96)	51.13 ± 15.25	49.51 (26.91)	1.894	0.081	NS
Hydroxyl AA	Threonine	15.31 ± 5.98	16.65 (10.4)	29.04 ± 16.88	31.94 (33.21)	2.028	0.065	NS
	Serine	536.39 ± 1148.04	37.59 (455.6)	48.12 ± 18.61	50.35 (24.85)	1.085*	0.313	NS
Acidic AA,amides	Aspartic acid	21.24 ± 54.46	0.85 (1034)	28.48 ± 43.46	9.99 (70.04)	1.323*	0.23	NS
	Asparagine	569.42 ± 911.11	118.16 (1529.13)	1125.81 ± 1298.05	840.04 (2386.82)	0.679*	0.57	NS
	Glutamic acid	7.27 ± 13.13	2.31 (9.58)	13.57 ± 15.28	7.81 (22.78)	1.278*	0.247	NS
Sulfur AA	Cystine	12.71 ± 5.18	13.3 (8.49)	10.89 ± 7.54	11.24 (16.94)	0.498*	0.659	NS
	Methionine	11.06 ± 7.40	8.83 (6.4)	12.32 ± 6.01	11.84 (6.39)	1.05*	0.301	NS
Aromatic AA	Tryptophane	10.431 ± 7.52	11.08 (13.09)	18.75 ± 11.74	20.93 (22.08)	1.32*	0.230	NS
	Tyrosine	12.81 ± 10.40	11.46 (8.43)	23.20 ± 7.33	21.39 (14.28)	2.29	0.036	S
	Phenylalanine	25.74 ± 12.34	23.86 (14.69)	38.64 ± 10.51	40.82 (15.03)	2.16	0.047	S
Basic AA	Lysine	61.28 ± 21.37	62.2 (25.78)	49.55 ± 21.42	45.35 (27.18)	1.17*	0.272	NS
	Histidine	13.25 ± 10.74	10.23 (15.5)	25.29 ± 7.92	21.96	1.66	0.146	NS
	Arginine	15.26 ± 10.22	12.89 (11.68)	59.74 ± 10.64	60.18 (22.81)	3.55*	0.000	S
Imino acids	Proline	29.60 ± 39.13	29.61	194.18 ± 70.32	214.49	2.92	0.061	NS

HD: hemodialysis patients; SD: standard deviation; IQR: interquartile range. Amino acids concentrations measured by μMol/L.
* Z value of Mann–Whitney test (for non parametric data).

and it believed to behave as bioactive vectors [14]. Endothelial dysfunction is regarded as the initial reversible step in the development of atherosclerosis and has been demonstrated in all stages of renal failure, it may reflect increased atherogenic and thrombogenic properties of the endothelium, contributing to subsequent adverse cardiovascular outcome [11].

Reduced activity of the nitric oxide (NO) pathway has been implicated in the endothelial dysfunction that occurs in patients with renal failure. NO is generated from L-arginine by NO synthase, and certain uremic toxins including asymmetrical dimethyl-L-arginine (ADMA), inhibit NO synthase and might contribute to endothelial dysfunction [15].

The present study showed that serum leucine and valine were significantly lower in HD patients than in the control group. This is in accordance with [16] who reported that plasma leucine and valine levels were significantly decreased in uremic children from those in the controls. [17] reported that the low plasma and cellular valine together with low plasma leucine are secondary to abnormal muscle and hepatosplanchnic AA metabolism. In muscle, metabolic acidosis induces protein breakdown via an activation of both cytosolic ATP-ubiquitin–dependent proteolytic pathway and BCKA dehydrogenase, responsible for an irreversible BCAA breakdown. The decrease in hepatosplanchnic retention of nonessential AAs con-

tributes to the abnormalities of arterial AAs associated with renal insufficiency. Moreover, a significant decrease of branched chain AA concentration is related to calorie-protein malnutrition, inflammation, and a long period of hemodialysis [10].

In the current study there was a significantly lower serum tryptophane in HD patients compared to the control group. Laurence et al. (2007) [18] confirmed the reduction in plasma tryptophan in patients on dialysis. They reported that plasma tryptophan concentration is reduced in CRF patients that is in turn associated with elevated precursors of tryptophan metabolism, including l-kynurenine and quinolinic acid, both of which have been implicated in the neurotoxic manifestations of CRF. Druml et al. (1994) [19] suggested that binding of tryptophan to albumin is reduced because of the accumulation of competing solutes in the plasma leading to acceleration of tryptophan metabolism by the fall in the protein bound fraction associated with elevated tryptophan clearance. Our result is in contrary with Schefold et al. (2009) [9] who investigated tryptophane catabolism, indoleamine 2,3-dioxygenase activity (the enzyme that catabolizes tryptophane) and the role of inflammation in moderate to very severe CKD and hemodialysis in 40 adult CKD patients aged 57 ± 14 years with different stages of renal disease. They reported that tryptophane levels were unchanged in CKD, indoleamine 2,3-dioxygenase activity and serum levels of tryptophan catabolites of the kynurenine pathway increase with CKD severity. In CKD, induction of indoleamine 2,3-dioxygenase may primarily be a consequence of chronic inflammation. The difference between our study and their study may be related to the difference in age distribution (adult vs. pediatric), and severity of renal disease (patients at different stages of renal disease vs. hemodialysis patients).

The present study showed a significantly lower serum tyrosine and phenylalanine in HD patients compared to the control group. This is in accordance with Canepa et al. (1992) [16] who reported that plasma tyrosine was significantly decreased in uremic children compared to controls, which may be explained according to Kopple (2007) [20] by the impairment in the conversion of phenylalanine to tyrosine in CRF patients as the conversion of phenylalanine to tyrosine to a large extent takes place in the kidneys. Similarly, Yokoyama et al. (2002) [21] found that the reduction of quinoid type BH2 to BH4 is modified in patients with CRF which may play a role in the impaired phenylalanine hydroxylase activity.

In the current study there was significantly lower serum methionine in HD patients compared to the control group. This may be due to the high clearance of methionine in HD patients [18]. This contradicts with Suliman et al. (2002) [8] who examined the relationship of plasma sulfur amino acids to cardiovascular disease and nutritional status in 151 patients with CRF close to the start of regular dialysis treatment. They reported that plasma methionine level was normal. The difference between this study and that of Suliman et al. (2002) [8] may be related to the difference in dialysis duration (patients on dialysis for >6 months vs. patients close to the start of regular dialysis treatment) and the different number of patients.

In the current study serum threonine was significantly reduced in HD patients compared to the controls. This is in accordance with Bergström et al. (1990) [22] who stated that in the hemodialysis patients threonine was significantly reduced in plasma compared to the controls.

The present study showed that 61.9% of HD patients had lower L-arginine levels compared to the normal level for L-arginine (normal L-arginine level [23]. However, lower L-arginine in HD patients was not significant. This is in accordance with Kielstein et al. (1999) [24] who found that plasma L-arginine concentrations were not significantly decreased in patients with ESRD. Moreover, Schmidt et al. (1999) [25] stated that plasma arginine level was normal and plasma levels of citrulline and the endogenous NOS Inhibitor, ADMA, were markedly elevated in patients with ESRD compared to controls. The hemodialysis patients had, as a whole, less abnormal free amino acid concentrations in plasma and muscles, compared to patients with untreated chronic uremia, although the general pattern tended to be similar. Muscle arginine was low in the untreated patients. However, it was not significantly decreased in the hemodialysis patients. This may presumably be a consequence of higher protein intake and better nutritional status in the hemodialysis patients than in the non-dialyzed patients in whom anorexia might have adversely affected the intake of nutrients [21].

Abnormalities in amino acids in chronic uremia have been attributed to low protein intake, deficiency of excretory and metabolic functions of the diseased kidneys, toxic effects of uremia on the intermediary metabolism of amino acids, altered distribution of some amino acids between the extra- and intracellular compartments and, in dialysis patients, loss of protein and amino acids by the dialytic procedure [21] .

Malnutrition may be one of the causes of AA abnormalities detected in these HD patients, as evidenced by the significant lower weight and OFC in HD patients compared to the control group. Also, serum albumin was lower in HD patients compared to the control group. However, this was not statistically significant. The insignificant difference in serum albumin may be explained by the fact that no one of our patients was on protein restriction diet. This is in accordance with Sen and Prakash (2000) [26], who found that malnutrition is a common clinical problem in dialysis patients, which is multifactorial in origin, and Magorzewicz et al. (2008) [27] who reported that despite quite good nutritional status, dialyzed patients have abnormalities in their AA profiles. This is in contrary with Sezer et al. (2007) [28], who reported that in their patients there were 34 hypoalbuminemic and 34 normoalbuminemic patients. The difference between this study and their study may be related to the difference in patients groups (CRF patients on hemodialysis vs. hemodialysis, continuous ambulatory peritoneal dialysis and predialytic patients).

There was no significant difference between HD patients with and without history of thromboembolic manifestations (arteiovenous fistula thrombosis, graft thrombosis, central vein thrombosis or thrombosis in any other site) as regard age, calcium, phosphate, alkaline phosphatase, albumin and hemoglobin concentration.

The present study showed that serum glutamic acid concentration was significantly lower in HD patients without history of thromboembolic manifestations compared to those with history of thromboembolic manifestations. Morrell et al. (2008) [13]reported that despite the presence of glutamate in platelet granules, the role of glutamate during hemostasis is under research, they showed that activated platelets release glutamate, that platelets express alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor (AMPAR) subunits, and that glutamate increases agonist-induced platelet activa-

tion. Furthermore, they demonstrate that glutamate binding to the AMPAR increases intracellular sodium concentration and depolarizes platelets, which are important steps in platelet activation. In contrast, platelets treated with the AMPAR antagonist CNQX or platelets derived from GluR1 knockout mice are resistant to AMPA effects. Importantly, mice lacking GluR1 have a prolonged time to thrombosis *in vivo* their data identify glutamate as a regulator of platelet activation, and suggested that the AMPA receptor is a novel antithrombotic target. Thus we suggest a state of chronic platelet activation in HD patients with history of thromboembolic manifestations.

The present study showed that serum phenylalanine was significantly lower in HD patients with history of thromboembolic manifestations than those without history of thromboembolic manifestations. Zhang et al. (2009) [29] used an ultra fast liquid chromatography coupled with IT-TOF mass spectrometry (UFLC/MS-IT-TOF) metabonomic approach to study the plasma and urine metabolic profiling of atherosclerosis rats, potential biomarkers were screened by using *S*-plot and were identified by the accurate mass and MS(n) fragments. They reported that concentrations of leucine, phenylalanine, tryptophan, acetylcarnitine, butyrylcarnitine, propionylcarnitine and spermine in plasma decreased in atherosclerosis rats. The alternated metabolites demonstrated abnormal metabolism of phenylalanine, tryptophan, bile acids and amino acids. There research proved that metabonomics is a promising tool for disease research.

In conclusion, several abnormalities in amino acids were present in HD patients compared to controls in the form of significantly lower serum concentration of threonine, valine, methionine, leucine, tyrosine, phenylalanine and tryptophane than the control group. There were low concentrations of aspartic acid, serine, asparagine, glutamic acid, alanine, cystine, isoleucine, lysine, histidine and arginine with no significant difference. There were high concentrations of glycine and proline with no significant difference. The mean serum L-arginine levels were lower in 61.9% of HD patients than the mean of the controls with no significant difference. L-Arginine concentration was not significantly different between HD patients with and without history of thromboembolic manifestations. HD patients without history of thromboembolic manifestations had significantly lower glutamic acid concentrations and significantly higher phenylalanine concentrations than HD patients with history of thromboembolic manifestations.

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