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# *Concentrations of branched amino acids: isoleucine, valine, leucine in serum of hemodialysis patients during one-year observation*

Malnutrition, commonly reported in haemodialysis patients (HD pts), is multifactorial in origin (1, 6). This problem in HD pts is usually diagnosed by evaluating anthropometric measurements, serum protein concentrations, serum cholesterol, plasma and intracellular amino acid levels (11, 12).

It is generally accepted that nutritional status depends on the dialysis dose delivery and low dialysis efficacy is associated with higher rates of mortality and morbidity (6). The plasma amino acid profile in renal failure patients exhibits abnormal patterns, such as reduced essential and non-essential amino acids (7).

Previous studies indicated a possible relationship between plasma levels of branched-chain amino acids (BCAA): isoleucine, leucine, valine and nutritional status of HD pts (9). The BCAA have an important influence on metabolism of energy in skeletal muscles, in addition the leucine makes easy the synthesis of protein. The HD pts exhibit abnormal amino acid profile and there is lower concentration of BCAA than in healthy persons (5, 10, 14). During haemodialysis these amino acids are loosened into dialysate and this process may contribute to protein malnutrition, particularly in patients with low nutrient intake.

In the present investigation we evaluated some parameters of nutritional state and concentration of branched amino acids in haemodialysis patients during one-year observation.

## MATERIAL AND METHODS

Sixty-seven patients of mean 49 ± 11.8 years (range 23–76) undergoing haemodialysis were assessed in the first study (study I). After 12 months this assessment was repeated in 70 patients of mean 52.2 ± 12.1 years (range 24–76); (study II). Nine patients from study I were dead, 6 patients were transplanted, 15 patients were assessed at the first time.

Demographic characteristics of patients are reported in Table 1.

Table 1. Demographic characteristics of patients

| Patients | Study I | Study II | Healthy subjects |
|---|---|---|---|
| Number | 67 | 70 | 20 |
| Age (years) | 49 ± 11.8 | 52.2 ± 12.1 | 34.0 ± 9.8 |
| Sex (male : female) | 38/27 | 37/33 | 11/9 |
| Dialysis duration (years) | 105 ± 85.1 | 105.7 ± 84.7 | |
| Underlying disease | | | |
| GNC | 37 | 36 | |
| NI | 10 | 11 | |
| ACR | 7 | 7 | |
| ND | 3 | 5 | |
| NH | 1 | 1 | |
| Collag. | 4 | 7 | |
| Tbc renum | 1 | 1 | |
| DPR | 3 | 2 | |
| Amyloidosis | 1 | – | |

GNC – *glomerulonephritis chronica*, NI – *nephritis interstitialis*, ND – *nephropathia diabetica*, ACR – *anomalia congenita renum*, Tbc renum – *tuberculosis renum*, DPR – *degeneratio polycistica renum*, NH – *nephropathia hypertonica*

The patients underwent haemodialysis three times weekly for 4–5 h, reused polysulfone dialyzers and bicarbonate buffered dialysate were employed. 91% of patient received erythropoietin replacement therapy for at least 3 months prior to the study. Dialysis modality, treatment time and filters used were not changed during the study period.

Blood was drawn just before the midweek dialysis session. Morphology, plasma albumin concentration and urea for KT/V calculation were determined by routine methods. KT/V was estimated using the Daugirdas formula.

Plasma was deproteinized quickly for amino acid analysis by adding 45 mg sulphosalicylic acid in buffer pH 2.8 and precipitate was removed by centrifugation. Supernatant was quickly frozen to – 30° C until analysed. Branched amino acids: leucine, isoleucine, valine were measured by chromatography method with amino acid analyser, using a lithium-citric acid buffer system. The plasma amino acid pattern measured in the study group and compared with 20 healthy persons (14 male and 7 female) between 23–62 years of age served as the control group.

Statistical analysis. Data were expressed as means ± standard deviation ( SD). The distribution of data was tested by Chi squared test. In statistical analysis, when comparing both studied groups, data with normal distribution were analysed using t-Student test, but these without normal distribution – using Kolmogorov-Smirnov test. The comparison between the study group and control group was performed using t-Student test for normal distribution and Kruskal-Wallis test for data without normal distribution. The relationships were analysed using linear regression analysis. Statistical significance was established at level $p<0.05$. Informed consent was obtained from all the patients.

## RESULTS

The mean values of several biochemical parameters of the studied patients are shown in Table 2.

Table 2. Baseline nutritional and biochemical parameters

| Parameter | Study I | Study II | Significance ST |
|---|---|---|---|
| Body mass index | 22.4 ± 5.5 | 21.9 ± 5.2 | N S |
| Hb (g/dl) | 10.75 ± 1.45 | 11.21 ± 1.2 | P <0.05 |
| KT/V | 0.99 ± 0.18 | 1.04 ± 0.21 | N S |
| Albumin (g/dl) | 3.96 ± 0.3 | 3.97 ± 0.3 | N S |

N S – not statistically significant

Measured data show that dialysis efficacy calculated as KT/V did not change significantly during 12 months' period. Body mass index did not change significantly, either. The haemoglobin concentration was greater during study II than during study I. In the studied group of HD pts albumin level did not differ significantly comparing both studies.

Table 3 shows the values of amino acid concentrations as total value and values of particular branched amino acids.

Table 3. Concentrations of total amino acids and baseline branched-chain amino acids

| Amino acid | Study I | Study II | Healthy subjects |
|---|---|---|---|
| Valine ummol/L | 186.04 ± 58.02 | 197.82 ± 54.23 | 286.4 ± 70.19 $^{p.m}$ |
| Leucine ummol/L | 115.94 ± 29.7 | 105.94 ± 38.55* | 187.05 ± 47.24 $^{p.m}$ |
| Isoleucine ummol/L | 72.83 ± 24.78 | 62.66 ± 24.61* | 91.45 ± 26.76 $^{p.m}$ |
| Total amino acids ummol/L | 3624.48 ± 140.32 | 3965.32 ± 125.351* | 4454.45 ± 774.91 $^{p.m}$ |

* Statistically significant in comparison Study I : Study II ( $p< 0.01$)

p – statistically significant ($p <0.05$) Study I : Healthy subject

m – statistically significant ($p <0.05$) Study II : Healthy subject ($p < 0.01$)

Mean amino acid concentration observed during study I was significantly lower than in study II. Mean amino acid concentration observed in both measurements was significantly lower than in control group. Mean concentration of particular branched amino acids in both groups were also lower than in the control group. In study I the mean concentration of leucine and isoleucine was significantly greater than in study II. There were no significant changes observed in valine concentration during the observed period.

In the studied group of patients there was observed a small negative correlation between albumin concentration and the length of dialysis therapy ($r=-0.27$ $p<0.05$). No significant correlation between: BMI, haemoglobin, length of dialysis therapy, KT/V were observed. There was a significant positive correlation between the total amino acid concentration and particular branched amino acids, as well as albumin.

These relationships were shown in Table 4 (study I) and in Table 5 (study II).

In study II the observed values of r were greater than in study I.

Table. 4 Linear regression analysis of studied parameters in study I

| | Albumin | Total amino acids | LEU | ILE | VAL |
|---|---|---|---|---|---|
| VAL | NS | r=0.49, p<0.01 | r=0.44, p<0.01 | r=0.36, p<0.36 | |
| ILE | NS | r=0.47, p<0.01 | r=0.82, p<0.01 | | |
| LEU | NS | r=0.48, p<0.01 | | | |
| Total amino acids | NS | | | | |
| Albumin | | | | | |

N S – not statistically significant, VAL – valine, ILE – isoleucine, LEU – leucine

Table 5. Linear regression analysis of studied parameters in study II

| | Albumin | Total amino acids | LEU | ILE | VAL |
|---|---|---|---|---|---|
| VAL | NS | r=0.58, p<0.01 | r=0.86, p<0.01 | r=0.81, p<0.01 | |
| ILE | NS | r=0.58, p<0.01 | r=0.92, p<0.01 | | |
| LEU | NS | r=0.60, p<0.01 | | | |
| Total amino acids | NS | | | | |
| Albumin | | | | | |

N S – not statistically significant, VAL – valine, ILE – isoleucine, LEU – leucine

## DISCUSSION

This prospective study was performed in haemodialysis patients in order to evaluate several clinically relevant issues related to nutrition. The main attention was focused on the pattern of amino acids. It should be emphasised that for each free amino acid the

plasma pool represents only a small fraction of total body content. For most amino acids the intracellular concentration in skeletal muscle (the largest pool of free amino acids immediately available for protein synthesis) is higher than in plasma (2).

Uremic patients and in particular HD pts, show significant deviations in their plasma amino acids concentrations, compared to healthy patients (7, 8). These abnormalities are partly the result of metabolic disturbances and partly the result of haemodialysis losses or damaged kidney function. The results of our investigation demonstrated that mean concentration of amino acids was significantly lower than in the control group. This finding is in agreement with other reports (3, 15).

The main supplier of amino acids (other than food intake) is the muscle protein pool. All tissues metabolise amino acids, but the liver and kidney are important sources for the synthesis of nonessential amino acids. When the kidney is damaged, reduced plasma concentrations for many essential amino acids and particularly for valine, leucine and isoleucine are found.

We estimated that mean concentration of these important BCAA were lower than in the control healthy group. Qureshi et al. (15) also showed that mean plasma concentration of branched-chain amino acids were reduced below the control levels. These amino acids are primarily metabolised in brain and muscles. Therefore some investigators attempted to define the association between some parameters of nutritional status and BCAA (4).

Hiroshige et al. (10) indicated a possible relationship between plasma levels of branched-chain amino acids and appetite. In our study body mass index (BMI) and that of albumin level as a marker of nutritional status were assessed. We did not find any relationship between BMI and albumin as well as total concentration of amino acids, but we observed the negative correlation between the long duration of dialysis therapy and albumin. The reason for this observation is multifactorial. Several factors such as: leptin, insulin, nitric oxide synthetase inhibitors and proinflammatory cytokines are responsible for decreased albumin concentration after a long time of therapy.

Among factors causing protein catabolism and malnutrition in HD pts metabolic acidosis has attached much interest in recent years. It has been evident that acidosis, rather than uremia may be an important stimulus for net protein catabolism, by stimulation of BCAA catabolism and proteolysis in skeletal muscle (12, 15).

Boratyńska et al. (5) showed that administration of recombinant human erythropoietin (rhEPO) not only affects erythropoesis, but also influences the plasma amino acid pattern. Our patients were treated rhEPO before and during the whole study. We recorded that the mean haemoglobin concentration was higher in study II than during study I. We estimated, that the mean concentration of leucine and isoleucine were higher in study I. We did not find correlation between haemoglobin and BCAA.

Hiroshige et al. (10) recorded that branched-chain amino acids and especially leucine were correlated with BMI. The investigations of Chazot et al. (6) confirmed these observations and indicated that especially leucine has correlated with BMI in HD pts

treated for over 304 months. But our patients were treated not so long, so we did not find such correlation.

We observed a significant positive correlation between the total amino acids concentration and particular BCAA as well as albumin. In study II r values were greater than in study I. Recent reports showed a close relationship between the augmentation of appetite and BCAA supplementation. Gill et al. (9) suggested that the use of BCAA-enriched parenteral nutrition might minimise the reduction in food intake. There is a tight correlation of plasma BCAA level with appetite or voluntary food intake.

The BCAA play a central role in metabolism as a precursor for the synthesis of proteins, fatty acids, regulation of protein turnover and insulin release (1). Among BCAA leucine stimulates protein synthesis and its keto analogue inhibits proteolysis, due to inhibition of glucocorticoid synthesis. The normal concentrations of all BCAA are necessary for nutritional study (1, 2). Especially, normalisation of plasma leucine concentration improves nutritional status. Qureshi et al. (15) suggested that leucine may be at least as good a predictor as valine. The results of this study confirm the significant deviations in branched-chain amino acids concentrations in haemodialysis patients.

## CONCLUSIONS

1. Mean amino acids concentration observed during both study groups was significantly lower than in control group.
2. Mean concentration of branched amino acids: valine, leucine, isoleucine were lower than in control group.
3. There was a small negative correlation between albumin concentration and the duration of dialysis therapy.
4. There were significant positive correlations between the total amino acids, branched amino acids as well as albumin, especially in study II.

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## SUMMARY

Malnutrition is commonly reported in haemodialysis patients (HD pts) and is multifactorial in origin. Previous studies indicated a possible relationship between plasma levels of branched-chain amino acids (BCAA): isoleucine, leucine, valine and nutritional status of HD pts. The BCAA have important influence on metabolism of energy in skeletal muscles, in addition the leucine makes easy the synthesis of protein. In the present investigation we evaluated some parameters of nutritional state and the concentration of branched-amino acids in haemodialysis patients during one-year observation. Sixty-seven patients undergoing haemodialysis were assessed in the first study (study I), after 12

months this assessment was repeated in 70 patients (study II). Blood was drawn before the midweek dialysis session. Morphology, plasma albumin concentration and urea for KT/V calculation were determined by routine methods. Branched amino acids were measured by chromatography method with amino acid analyser AAA 400, using a lithium-citric buffer system. The plasma amino acid pattern was measured in the study group and compared with 20 healthy persons. Mean amino acids concentration observed in both study groups was significantly lower than in the control group. Mean concentration of BCAA was also lower than in control group. There was a small negative correlation between albumin concentrations and the length of dialysis therapy. There were significant positive correlations between: total amino acids, branched amino acids as well as albumin, especially in study II.

Zachowanie się stężeń aminokwasów rozgałęzionych: izoleucyny, waliny, leucyny w surowicy krwi chorych przewlekle hemodializowanych podczas rocznej obserwacji

Skład aminokwasów w przestrzeni wewnątrzkomórkowej oraz zewnątrzkomórkowej ulega zmianie w przewlekłej niewydolności nerek i koreluje ze stopniem ubytku filtracji kłębkowej oraz objawami mocznicy. Utrata aminokwasów przez błonę dializacyjną oraz katabolizm białek indukowanych hemodializą nasila zaburzenia w obrębie puli przede wszystkim egzogennych aminokwasów. Celem podjętych badań u chorych przewlekle hemodializowanych było określenie w surowicy stężenia puli wszystkich wolnych aminokwasów oraz aminokwasów rozgałęzionych: waliny, izoleucyny, leucyny. Te egzogenne aminokwasy mają istotny wpływ na stan odżywienia, syntezę białek w obrębie mięśni oraz na apetyt. Badania wykonano u 67 chorych przewlekle hemodializowanych (badanie I) i powtórzono po rocznej obserwacji w grupie 70 chorych (badanie II). W oznaczeniu stężenia i rozdziału aminokwasów zastosowano metodę chromatografii jednowymiennej jednokolumnowej. Ponadto w surowicy krwi chorych zbadano stężenia: hemoglobiny, albuminy oraz wskaźnik masy ciała, a także wydajność dializy (KT/V). Uzyskane wyniki porównano z wartościami uzyskanymi w grupie osób zdrowych. Na podstawie przeprowadzonych badań stwierdzono, że średnie stężenie wszystkich aminokwasów było istotnie niższe w porównaniu z grupą kontrolną i nie uległo istotnym zmianom po rocznej obserwacji. Średnie stężenie wszystkich aminokwasów rozgałęzionych było w obu etapach badania również istotnie niższe w porównaniu z grupą kontrolną. Ponadto średnie stężenie izoleucyny oraz leucyny było znamiennie wyższe w badaniu I w porównaniu z wartościami uzyskanymi w badaniu II. Wyższe średnie wartości morfologii uzyskane po 12 miesiącach obserwacji nie miały istotnego wpływu na stężenia badanych rozgałęzionych aminokwasów egzogennych. Wykazano obecność istotnej ujemnej korelacji między stężeniem albuminy oraz wyrażonym w miesiącach czasem prowadzonej dializoterapii. W rocznej obserwacji stwierdzono obecność wzajemnych dodatnich zależności między wszystkimi aminokwasami rozgałęzionymi oraz albuminą.