Amino Acid Profile

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Submission date: 26-Oct-2022 09:04AM (UTC+0700)
Submission ID: 1935522630
File name: Amino_Acid_Profile.pdf (732.3K)
Word count: 191
Character count: 27090

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Cite as: AIP Conference Proceedings **2353**, 030014 (2021); https://doi.org/10.1063/5.0052847
Published Online: 25 May 2021

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Amino Acid Profile in Patients of Chronic Kidney Disease on Hemodialysis in Indonesia

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Abstract. Protein energy wasting (PEW) is a nutritional disorder syndrome that occurs 28 - 80% in chronic kidney disease (CKD) patients on hemodialysis. Hemodialysis cause the nutrients loss including amino acids, increase protein catabolism induced by inflammation, and inhibit protein synthesis. The objective of this study was to acquire the amino acid profile in CKD patients on hemodialysis. This study used cross sectional design and involving 60 subjects of CKD patients aged >18 years on routine hemodialysis at Dr. Cipto Mangunkusumo National Referral Hospital. Amino acids examination was using dried blood spots (DBSs) sample and Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS) method. We examined 10 non-essential (alanine, arginine, aspartic acid, glutamic acid, asparagine, glycine, glutamine, proline, serine, tyrosine), 9 essentials (histidine, phenylalanine, isoleucine, leucine, lysine, methionine, threonine, tryptophan, valine), and 2 special (ornithine, citrulline) amino acids. The results showed that almost all amino acids were lower (6 non-essential, 8 essentials, and citrulline), whereas others were higher (aspartate acid, serine) or normal (glutamic acid, glycine, methionine, and ornithine) than normal reference value from Mayo. CKD patients on hemodialysis have decreased amino acid especially essential amino acids. These results can be used in modification of amino acid supplementation CKD patient on hemodialysis in Indonesia.

INTRODUCTION

Chronic kidney disease (CKD) is one of health problem with high prevalence worldwide. Its prevalence in Indonesia is estimated at 12.5% and the incidence of patients undergoing hemodialysis is increasing every year [1]. Nutritional disorder syndrome, which called as protein energy wasting (PEW), is often occurred in CKD and characterised by inadequate nutritional intake and reduced body stores of protein and energy [2]. Incidence of PEW occurs especially in CKD on hemodialysis, which is about 28 – 54% [3]. The causes of PEW in CKD on hemodialysis are multifactorial, including inadequate nutrient intake due to an anorexia, chronic inflammation, hypercatabolic status, metabolic acidosis, and hemodialysis procedure [4]. Hemodialysis procedures can cause nutrient-loss such as amino acids around 6 – 12 grams through the dialysis membrane [4]. Hemodialysis can escalate inflammatory process in CKD which increase protein catabolism, and supress amino acids utilization in protein synthesis [5]. Moreover, the loss of amino acids through dialysis membrane also lowering protein synthesis, such as albumin and eventually cause hypoalbuminemia [5]. Hypoalbuminemia is associated with higher mortality and morbidity in CKD [6].

Protein consists of amino acids as the building blocks. There are 20 types of amino acids the body needs in protein synthesis. Amino acids consist of 11 non-essential amino acids that can be formed in the body and 9 essential amino acids that should be acquired from external source or food [7]. It is known that a decrease in amino acids has occurred in CKD since the early stages of the CKD and worsening in the advance stage of CKD [8]. A study by Duranton *et al.,* found that CKD patients on hemodialysis had lower levels of total amino acids and essential amino acids than CKD patients without hemodialysis even though the patients were not on a restricted diet [8]. Study from Bolasco *et*

International Conference on Life Sciences and Technology (ICoLiST 2020)
AIP Conf. Proc. 2353, 030014-1–030014-7; https://doi.org/10.1063/5.0052847
Published by AIP Publishing. 978-0-7354-4096-8/$30.00

al., had shown that supplementation of amino acid improved nutritional status (albumin level, hemoglobin level, and body weight) and inflammatory status (C-reactive protein level) [9]. In addition, branched-chain amino acids (BCAA) supplementation also improved anorexia and nutritional status in CKD patients on hemodialysis [10].

Research on the amino acid profile in CKD on hemodialysis is still scarce. Until now, there has never been any research on the amino acid profile of CKD patients on hemodialysis in Indonesia, which encourages researchers to conduct this research. This research is very important to be carried out to become the basis for the type of amino acid supplementation in accordance with the population of CKD patients on hemodialysis in Indonesia.

EXPERIMENTAL DETAILS

Cross-sectional design with a descriptive approach had been used in this study. The research was conducted at the Laboratory of Clinical Pathology, Dr. Cipto Mangunkusumo National Referral Hospital and the Indonesian Medical Education and Research Institute (IMERI) Laboratory from October 2019 to June 2020. This research has received permission from the Ethics Committee of the Faculty of Medicine, Universitas Indonesia/Dr. Cipto Mangunkusumo National Referral Hospital with document number: 0057/UN2.F1/ETIK/2019.

The subjects of this study were CKD patients who underwent routine hemodialysis two times per week, aged ≥ 18 years, and were willing to join in this study and signed an informed consent. The sample was a dried blood sample (DBS) which was stored at -20 °C. The criteria of good quality of DBS samples are blood filled the entire filter paper circle (estimated volume 100 µL), no scratches/scrapes on the filter paper, no color change, no serum rings (separation of plasma and blood cells), and do not moldy on filter paper [11].

The method of examining amino acids used liquid chromatography-tandem mass spectrometry (LC-MS/MS) with the principle of separating amino acids from other molecules using the LC technique followed by tandem mass spectrometry to identify each amino acid. The tool used was the MS Xevo TQ tandem ultraperformance LC (UPLC) from Waters (Milford, Massachusetts, USA). The samples used were 3 punchers of 3 mm DBS. The amino acids examined were 10 types of non-essential amino acids (alanine, arginine, aspartic acid, glutamic acid, asparagine, glycine, glutamine, proline, serine, and tyrosine), 9 types of essential amino acids (histidine, phenylalanine, isoleucine, leucine, lysine, methionine, threonine, tryptophan, and valine), and 2 special amino acids (ornithine and citrulline). BCAA is a group of essential amino acids consisting of isoleucine, leucine, and valine [7].

Prior to the examination of the sample, we conducted precision test or repeatability test, which is called "within run", by running the same sample five times consecutively in the same run on the same day. Within run test used EDTA blood samples from other randomly selected adult patients. 100 µL of EDTA blood was dropped into each circle of Whatman 903 filter paper consisting of 2 circles with a diameter of 1.5 cm. The blood allowed to dry at room temperature for 4 h. These within run samples were examined as the same procedure as research sample.

Data were analyzed using Microsoft Excel Office 365. The results of within run accuracy were presented in the form of mean, standard deviation (SD), and coefficient of variation (CV). The results of amino acid levels are shown as mean, SD and median (2.5 to 97.5 percentile). The amino acid levels in this study were compared descriptively with the reference value for normal adults from Mayo (Mayo reference) [12], and result similar study from Duranton *et al.,* [8]. Mayo reference [12] is for ages > 18 years, using plasma samples and the LC-MS/MS method, while the study of Duranton *et al.,* used plasma samples of CKD patients on hemodialysis and the LC-MS/MS method [8].

RESULTS

The within run test used DBS sample material, which was carried out five times in a row on the same day (Table 1). All CV amino acid examinations were below 10%. From a total of 60 subjects, females were in a greater proportion than males. The mean age of the subjects was 50 years, ranging from 18 to 77 years (Table 2). Table 3 showed an amino acid profile of this research and compared with Mayo reference [12] and Duranton *et al.* [8]. Compared to Mayo reference [12], amino acid levels in this study were lower for most non-essential amino acids (alanine, arginine, asparagine, glutamine, proline, and tyrosine), almost all essential amino acids except methionine, and citrulline. The amino acids glutamic acid, glycine, methionine and ornithine were normal limits, whereas aspartic acid and serine had higher levels. When compared with the study of Duranton *et al.,* [8] the mean levels of non-essential, essential amino acids and total BCAAs obtained from this study were much lower, with the exception of aspartic acid, glycine, serine, and ornithine which had higher yields.

TABLE 1. The results of the accuracy test within the run for amino acid examination with DBS sample material

Amino Acid (µmol/L)	Mean	Standard deviation	Coefficient of Variation (%)
Non-essential			
Alanine	75.23	1.95	2.59
Arginine	15.53	1.06	6.8
Aspartic acid	94.36	5.46	5.78
Glutamic acid	41.06	2.43	5.93
Asparagine	15.2	1.26	8.27
Glycine	334.19	7.77	2.33
Glutamine	11.34	0.46	4.03
Proline	37.82	2.63	6.95
Serine	426.38	8.24	1.93
Tyrosine	20.35	1.59	7.8
Essential			
Histidine	21.96	2.13	9.7
Phenylalanine	15.25	0.35	2.3
Isoleucine	15.95	1.05	6.57
Leucine	22.7	0.81	3.55
Lysine	43.37	3.7	8.53
Methionine	7.21	0.38	5.33
Threonine	15.26	0.69	4.55
Tryptophan	10,6	0.29	2.77
Valine	17.87	1.4	7.86
Special			
Ornithine	79.53	7.08	8.9
Citrulline	24.28	2.02	8.3

TABLE 2. Characteristic of subject

Parameter	Frequency (%) Mean ± SD Median (min - max)
Gender	
Male	26 (43.3)
Female	34 (56.7)
Age (year)	50 ± 14
Duration of hemodialysis (year)	4 (1 - 20)

TABLE 3. The comparison of several studies of amino acid profile

Amino Acid (µmol/L)	This Study[a]		Mayo Reference[b] (nmol/mL) [12]	Duranton *et al.*[c] (µmol/L) [8]
	Mean ± SD	Median (2.5 − 97.5 percentile)		
Total				
Amino acid non-essential*	769.12 ± 266.30	852.49 (174.70−1242.22)	-	1400.8 ± 379.8
Amino acid essential	162.69 ± 31.44	161.73 (93.07−243.10)	-	762.1 ± 284.0
BCAA*	54.78 ± 14.65	52.34 (34.40−95.71)	-	314.7 ± 127.2
Non-essential				
Alanine	78.49 ± 16.16	81.41 (43.76−112.01)	200−579	232.7 ± 90.4
Arginine	20.04 ± 9.27	21.13 (2.52−38.66)	32−120	68.0 ± 29.6
Aspartic acid*	101.39 ± 65.65	112.00 (0.15−252.42)	< 7	15.7 ± 23.1
Glutamic acid*	25.03 ± 8.29	24.87 (3.75−39.09)	13−113	58.3 ± 49.0
Asparagine	11.68 ± 4.69	12.59 (2.25−21.09)	37−92	40.5 ± 14.6
Glycine	179.71 ± 62.40	190.48 (1971−269.35)	126−490	166.8 ± 54.0
Glutamine*	10.07 ± 1.75	10.94 (6.98−12.22)	371−957	473.2 ± 117.8
Proline*	56.18 ± 21.14	51.70 (29.24−128.75)	97−368	279.7 ± 108.7
Serine*	278.62 ± 127.79	315.54 (1831−515.92)	63−187	65.9 ± 27.6
Tyrosine*	7.9 ± 9.01	5.70 (0−34.91)	31−90	43.5 ± 17.8

TABLE 3. Cont.

Amino Acid (µmol/L)	This Study[a]		Mayo Reference[b] (nmol/mL) [12]	Duranton *et al.*[c] (µmol/L) [8]
	Mean ± SD	Median (2.5 − 97.5 percentile)		
Essential				
Histidine*	5.79 ± 8.52	0 (0−24.46)	39−123	67.8 ± 25.6
Phenylalanine	19.31 ± 3.96	19.32 (11.69−28.56)	35−80	59.2 ± 18.0
Isoleucine*	17.74 ± 4.75	16.60 (11.40−33.32)	36−107	61.5 ± 25.4
Leucine*	21.41 ± 4.78	20.62 (13.78−35.18)	68−183	87.3 ± 47.6
Lysine*	46.82 ± 24.53	51.87 (0−84.97)	103−255	150.3 ± 62.9
Methionine	6.64 ± 1.09	6.66 (4.55−9.93)	4−44	26.8 ± 12.0
Threonine	19.03 ± 4.02	18.85 (8.80−28.38)	85−231	75.8 ± 36.5
Tryptophan	10.32 ± 1.29	10.27 (7.73−13.44)	29−77	24.0 ± 14.1
Valine	15.63 ± 7.59	15.66 (1.68−33.72)	136−309	165.9 ± 62.1
Special				
Ornithine	71.39 ± 32.79	74.86 (6.98−129.67)	38−130	56.1 ± 19.3
Citrulline*	12.50 ± 12.99	9.95 (0−43.89)	17−46	67.5 ± 26.1

*Data distribution is not normal

DISCUSSION

In general, all the CV amino acids in the study were in accordance with a recommendation from Bowron, namely < 10% [13], but there were 8 amino acid parameters with a higher CV when compared to the study of Kosen *et al.,* [14]. The tools used in this study were the same as those used by Kosen *et al.,* but they used a kit as a reagent, and this study used a column [14]. The operation using the kit can only identify a limited number of amino acids (13 types), however the reagent and working procedure have been standardized according to the kit instructions from manufacture. Column processing could identify more amino acids (21 types in this study), however the processing method and reagents had to be developed independently by inhouse Laboratory. The use of a kit can produces a better CV because the work process and kit reagents have been standardized. Prinsen *et al.,* stated that higher CV can be caused by sample preparation techniques, tool calibration, tool optimization process, and sample homogeneity on filter paper. DBS sample preparation is more complex than plasma sample with the possibility that sample homogeneity is not as good as plasma sample [15].

The research subjects were slightly more female, namely 56.7%. This result is in line with the research of Farida *et al.,* in Surabaya-Indonesia, namely the distribution of female CKD patients (51.6%) more than male (48.4%) [16]. Slightly different from what was found by Bawazier's study in Jakarta-Indonesia, namely the distribution of CKD patients on hemodialysis was slightly more in male (51%) than in female (49%) [17]. The mean age in this study was 50 years which is consistent with that found by Bawazier [17]. The median duration of hemodialysis was 4 years. Bawazier also found that most patients at Dr. Cipto Mangunkusumo National Referral Hospital underwent hemodialysis for about 1 − 4 years [17].

Most of the amino acids in this study were lower than the Mayo reference [12] and study of Duranton *et al.,* [8], especially glutamine, except aspartic acid and serine which were found to be higher. Mayo reference [12] and Duranton *et al.,* [8] used plasma samples. The study by van Vliet *et al.,* found that the amino acid levels (phenylalanine and tyrosine) of DBS were lower than that of plasma EDTA [18]. Research by Bloom *et al.,* also reported that DBS samples had a high correlation, but amino acid levels from DBS tended to be lower than plasma [19]. DBS amino acid levels are lower than plasma due to the lower plasma content of DBS due to the presence of erythrocytes. Haematocrit variations can also affect the plasma content in DBS contributing to the difference in amino acid levels in DBS and plasma samples. Therefore, the American college of Medical Genetics and Genomics (ACMG) recommends that each laboratory should have its own reference value. This is based on differences in research methods and the types of samples used by each laboratory so that they can be a source of variation [20].

Variations in amino acid levels can also be caused by food. Amino acids are precursors of gluconeogenesis, so they will experience a decrease in plasma levels after 12 hours of fasting because amino acids are being used to maintain glucose homeostasis. In starvation conditions, amino acids consumption will increase for gluconeogenesis [21]. Conversely, the peak of amino acid levels occurs 2 hours after eating. A study conducted by Worthley *et al.,* found that amino acid levels were higher in non-fasting patients than in fasting patients, even though statistical test was not performed [22]. Furthermore, Milsom *et al.,* found no difference in the levels of amino acids (phenylalanine and tyrosine) that were checked every two hours for total 16 hours which indicated that the effect of food was almost similar for the two types of amino acids [21]. ACMG still recommends fasting plasma samples to avoid errors in the assessment of amino acid test results [20].

Normally, glutamine is the most abundant amino acid in the body, especially intracellularly. The function of glutamine in the body is mainly used in cells with a high level of division such as immune system cells, gastrointestinal mucosa, as well as for the formation of glutathione and to maintain acid and alkaline levels in the body [7]. Food sources that contain natural glutamine levels are relatively low and glutamine in our food source is easily damaged by heating [23]. Therefore, glutamine source is mostly dependent on protein degradation (30%) and de novo synthesis of glutamic acid in muscle and other tissues (70%) [24]. Another possibility of low glutamine level is that the efficiency of glutamine extraction in the DBS sample is not good. Uyen *et al.,* reported that glutamine in DBS samples could result in deamination or dehydration to pyroglutamic acid, which did not occur in plasma samples [25].

Aspartic acid and serine obtained in studies are consistently higher in levels than the normal Mayo reference [12] and Duranton *et al*. [8]. The increasing level of amino acids in the body can be caused by increasing de novo synthesis of amino acids, food intake, and degradation of protein into amino acids; or decreasing amino acid excretion, amino acid oxidation, and protein synthesis. Duranton *et al.,* found a significant increase of aspartic acid in the plasma among hemodialysis patients and a decrease in urinary aspartate excretion along with the worsening of CKD [8]. Aspartic acid is a non-essential amino that has a role in the energy cycle, the formation of pyrimidine purines, and the production of antibodies [26]. Aspartic acid is formed from glutamate and oxaloacetic in the Krebs cycle, in other words, the source of aspartic acid are abundant in the body. In addition, sources of aspartic acid from food can come from oysters, sausage meat, soybeans and their products, green beans, brown rice, red beans, peanuts, sugar cane, and artificial sweetener aspartame [27-32]. Approximately 40% of aspartame will be degraded in the body to aspartate acid [33]. Foods containing monosodium glutamate (MSG) can increase aspartic acid due to transamination of glutamic acid [34]. According to Basic Health Research in the year 2013, the unhealthy consumption behavior among Indonesians aged > 10 years is the consumption of MSG (77.3%) and foods/drinks that contain high sugar (53.1%) [35]. In addition, Indonesians often consume vegetable protein products, especially soybeans such as tempeh and tofu, which are high in glutamic acid and aspartic acid.

Serine is a major contributor to glycine-forming carbon, cysteine, taurine; and plays a role in the methylation process of homocysteine into methionine [36]. Serine synthesis can be derived from glucose (glycolysis) and pyruvate, protein degradation, and conversion of glycine [36,37]. The kidneys have an important role in the conversion of glycine to serine which will then be released into the circulation. In CKD, serine levels should decrease, but in CKD there is also a disturbance of serine excretion which causes an increase in serine levels in the blood [38]. The increase in serine in this study can occur due to increased serine synthesis, increased protein degradation and decreased protein synthesis. Serine synthesis from glycolysis can continue to increase in a protein-restricted diet as long as calorie adequacy is maintained [36]. In addition, food sources that contain serine can come from egg whites, soy products, green beans, cheese, meat, nuts, tuna, fish, and wheat gluten [23,31,32,39].

The strength of this study is that it is the first study in Indonesia that measures amino acid levels in adult patients, especially CKD patients with routine hemodialysis. Measurement of amino acid levels using the LC-MS / MS method which is the gold standard examination for amino acids and is still of limited use in Indonesia. The DBS sample used in this study has several advantages, such as easy to collect the sample, transport and store [11].

The use of DBS samples also has limitations in terms of standardization when dropping blood onto filter paper in terms of the volume of bloodshed and the drying process which must be carried out horizontally so that the distribution of blood is evenly distributed on the filter paper surface. Pre-analytic samples of amino acid testing requiring fasting have not been carried out in this study, so that variations in amino acid yields in this study can be influenced by food. In addition, this study has not compared the amino acid profile with the reference value of amino acids in normal adults in Indonesia.

SUMMARY

The majority of amino acids were lower in CKD patients on hemodialysis, especially essential amino acids including BCAA, compared with the normal value of amino acids from Mayo and other similar study. Non-essential amino acids, especially glutamine, were found to be very low in this study, but aspartic acid and serine were higher compared to previous studies. Based on these findings, essential amino acid supplementation (especially BCAA) and non-essential (especially glutamine) in CKD patients on hemodialysis is necessary. Further research to set the reference value of amino acids in Indonesian adults is needed.

ACKNOWLEDGMENTS

We appreciate the Grant from Ministry of Research, Technology, and Higher Education of the Republic of Indonesia [Contract No. NKB-39/UN2.RST/HKP.05.00/2020]. We also appreciate to Biologists at IMERI Laboratory, Faculty of Medicine, Universitas Indonesia for their assistance in conducting this study.

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