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Evaluation of the Progressivity Parameters of Chronic Kidney Disease after Branched-Chain Amino Acid Supplementation in Children

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ABSTRACT

Chronic Kidney Disease (CKD) is not an uncommon issuein children. Chronic kidney disease is the abnormality of structure or function of the kidney that occurs for more than three months. The presence of a longitudinal decline in Glomerulus Filtration Rate (GFR), proteinuria, and hypertension Are the characteristics of CKD. One of the recommendations of nutritional supplementation as the prevention of CKD is by the administration of oral Branched-Chain Amino Acid (BCAA). To date, there has been no research to analyze the effects of the BCAA on children with stage 2-4CKD. This study aimed to analyze the effect of BCAA in inhibiting the progressivity of stage 2-4 CKD in children and improving nutritional status.A study with randomized pre-post test controlled trial design was performed in the Outpatient Clinic of Pediatric Nephrology in Dr. Soetomo Hospital with stage 2-4CKD. The subjects were divided into two groups, such as the BCAA and placebo, and were monitored for eight weeks to be evaluated the GFR, albumin, proteinuria, blood pressure, and nutritional status. Sixteen children with stage 2-4 CKD dominated by 71.4% of male patients were enrolled in this study. The mean age was 12.5 (SD 2.90) years. Approximately 50% (p=0.767) stage 2 chronic kidney, 50% (p=1.000) moderate malnutrition, and 64.28% (p=1.000) short stature were found, with nephrotic syndrome as the most common underlying cause of CKD (p=0.149). In BCAA group, decrease of GFR -5.08±7.13 (p=0.055), increase of serum albumin 0.20±0.23 (p=0.062), decrease of delta systole -11.57±15.08 (p=0.565) and diastole -4.85±16.25 (p=0.708), weight loss -0.07±1.01 (p=0.828), an increase of height 0.14±0.24 (p=0.771), and a decrease in BMI -0.03±0.74 (p=0.389) were reported. It was concluded that branched-chain amino acid (leucine, isoleucine, and valine) supplementation did not provide a significant effect to inhibit progressivity of stage 2-4CKD in children and improvement of nutritional status.

Keywords: Chronic kidney disease, branched-chain amino acid, progressivity of chronic kidney disease, nutritional status

INTRODUCTION

Chronic Kidney Disease (CKD) is not an uncommon issue in children. Chronic kidney disease is the abnormality of structure or function of the kidney that occurs for more than three months. According to the National Kidney Foundation's Kidney Disease Outcomes Quality Initiative (K/DOQI) guidelines, CKD is defined as kidney damage or Glomerular Filtration Rate (GFR) <60 mL/min/1.73m^2 for three months or more, irrespective of the cause.[1] Progressivity of CKD is a decrease in the level of renal function in patients who has been followed in a longitudinal and/or incidence of kidney failure and requires renal replacement therapy both for symptoms or complications of decreased kidney function. Progressivity of CKD is characterized by decrease of GFR, proteinuria and hypertension. Increased excretion of protein (proteinuria) is generally a persistent marker for kidney damage. Increased excretion of albumin (albuminuria) is a sensitive marker of CKD caused by diabetes, glomerular disease, and hypertension.[2]

Chronic kidney disease is a public health problem worldwide. Children with CKD have a risk of malnutrition, growth retardation, and disorders of nutrition due to abnormal metabolism. This situation increases the morbidity and mortality of children with CKD. One of the recommendations of nutritional supplementation as prevention of CKD is the administration of oral Branched-Chain Amino Acid (BCAA).[3]

Branched-chain amino acid is composed of leucine, isoleucine, and valine. Branched-chain amino acid may take the role in stimulating protein synthesis, inhibiting the degradation of proteins, improving nutritional status, reducing repair anorexia, maintaining the balance of acid-base in the

kidneys, preventing and delaying the progressivity of impaired renal function in CKD and toxic ammonia levels.[4-8] This study aimed to analyze the effect of BCAA in inhibiting the progressivity of stage 2-4 CKD in children and improving nutritional status.

METHODS

The present study was conducted from September to November 2018 in patients of stage 2-4 CKD in the Outpatient Clinic of Nephrology Pediatric in Dr. Soetomo Hospital. This study used a randomized pre-post test controlled trial design, and the approval for the study was obtained from the Ethics Committee of Dr. Soetomo Hospital with registration number 0549/KEPK/VIII/2018 (Registered on 28/08/2018). Written and informed consent was taken from all patients before participating in the study. The diagnosis of CKD was made based on detailed clinical history, physical examination, and renal function tests. Based on the GFR, chronic kidney disease was classified into five stages, such as: stage 1 with GFR > 90 mL/min/1.73m^2, stage 2 with GFR 60-89 mL/min/1.73m^2, stage 3a with GFR 45-59 mL/min/1.73m^2, stage 3b with 30-44 mL/min/1.73m^2, stage 4 with 15-29 mL/min/1.73m^2, and stage 5 with GFR < 15 mL/min/1.73m^2.[9] The inclusion criteria were all patients with stage 2-4 CKD (GFR <89 and GFR > 15), age 6-18 years and no sign of infection. The excluded criteria were patients on dialysis (hemodialysis or peritoneal dialysis), diabetes mellitus, patient with behavioral disorders and mania, acute renal failure, and parents withdrawn from this study. The randomization process was carried out by pharmacists. Group I received BCAA with the dosage 100mg/kg BW three times in a day. Group II received a placebo or glucose. Both groups received treatment for eight weeks. All the enrolled patients were regularly followed with renal function tests, albumin, proteinuria, blood pressure and anthropometry at 0, 4 and 8 weeks of treatment. The primary data this study were collected using data collection sheet and measuring instruments such as scales and meters. Laboratory tests (bun, creatinine, albumin, and proteinuria) were carried out in the Diagnostic Center Clinical Pathology Laboratory. Bun, creatinine, and albumin levels were measured using Siemens Dimension EXL, while urine analysis, especially proteinuria, was detected using Clinical Novus Pro 12 Urinalysis Cassette. Five mL of venous blood was taken, and the blood sample was put into a yellow-top vacutainer tube. Blood pressure was measured using a cuff that fit the length of the arm of the child. The cuff bladder length must cover at least 80% of the upper arm circumference, while the cuff bladder width must be more than 40% of the upper arm circumference (or at least 2/3 the distance between the acromion and olecranon). Weight and height were measured using the CDC 2000 curve.

All adverse events (nausea, vomiting, diarrhea, constipation, anorexia, abdominal pain, headache, and allergy) experienced by a patient or observed by the investigator were recorded on standard adverse drug reaction reporting forms at each visit. The values were stated as mean±SD. Shapiro-Wilk test was used to determine the normality. Statistical significance between pre and post-treatment values in each group was determined using the Student's Paired T-test or Wilcoxon Rank test. Statistical significance between groups was analyzed using Independent T-test, and Mann-Whitney U test with $p<0.05$ was considered significant. Statistical analysis was carried out using SPSS-21 software. Samples were taken from the patient who came to the Nephrology Pediatric Outpatient Clinic of Dr. Soetomo Hospital. The minimum sample size was calculated using the formula n = (Z½α + Zβ)2δ2/ (μ1-μ2)2 = (1.96 + 0.842)2 490/187.96 = 20.43~20 patients.

RESULTS AND DISCUSSION

Out of 20 assessed patients, two patients were excluded because they were diagnosed with DM and mental retardation. Because two patients were not willing to take part in this study, sixteen patients were enrolled in the study, consisting of eight patients in the BCAA group and eight patients in the placebo group. One subject in the BCAA group got diarrhea, and parents withdrew from the study; thus, they could not continue the study until the end of the treatment. One subject in the placebo group passed away. In the second month of treatment, there was one subject who dropped out of the study due to hemodialysis. Sixteen children with stage 2-4 CKD were enrolled in this study, dominated by 71.4% of males patients (p=0.070) (Table 1). This finding was similar to the previous research. A study by Soares *et al*. showed that males (53.3%) were predominantly higher than female patients (46.7%).[10] The subjects in this study had a mean age of 12.50±2.90 (p=0.931). The prevalence of the CKD is 1.5-3 per one million children under the age of 16. A report from the ItaKid Project in Italy mentioned the prevalence of CKD as

many as 74.7 in 1 million with the incidence of approximately 12.1 per 1 million population depending on age (age range 8.8 – 13.9 years).[11] Age distribution can be explained by the influence of the main causes of CKD in children, such as congenital anomalies of the Kidney and the Urinary Tract (CAKUT) and hereditary kidney disorder, which is more common are in male than female. The disorder was observed earlier than the frequency of glomerulal abnormality.[12]

Stage 2 CKD was found in 3 subjects (42.9%) in BCAA group and 4 subjects (57.1%) in the placebo group (p=0.767). The prevalence of stage 2-4 CKD is 2.4 times greater than the prevalence of stage 5CKD.[13] Chronic kidney disease is mostly asymptomatic in the early stages, less diagnosed, and reported. Chronic kidney disease is now considered as a remaining health problem due to the rapid increase in prevalence trends.[12] There are limited reliable data about the epidemiology of the early stages of CKD. According to the report of NHANES III, the prevalence of adult patients with early stages of CKD (CKD stage 1–4 10.8%) was ~ 50 times bigger than the prevalence of stage 5CKD (0.2%).[14] However, there was no comparison data for children. In Turkey and Belgium researchers reported that the ratio of stage 2-4 CKD to stage 5 CKD was 2.06 to 1.71.[13]

Nephrotic syndrome,as one of the basic causes of most CKD, was found in five subjects (35.7%) (p=0.149). Based on the GFR (mL/min per 1.73 m^2), patients were classified into stage 2 (stage 3 and 4 were classified into group I and II, respectively) and stage 4 (each group consisted of 2 stages of CKD) CKD in both groups. The causes of CKD in group I and II were nephrotic syndrome (14.3% and 57.1%), lupus nephritis (28.6% and 14.3%), hydronephrosis (3% and 0%), urinary tract infection, vesicolithiasis, glomerulonephritis, each of 1% (Table 2). It was similar to a research by Gheissari *et al.*, which found that the nephrotic syndrome (19.4%) was the most etiology in his research among CKD populations, followed by glomerulonephritis (15.8%), and reflux nephropathy (16.7%).[15] Such differences might occur because Dr. Soetomo Hospital is a referral hospital to which CKD patients with apparent clinical symptoms such as edema and decreased function of the

Table 1. Baseline characteristics

Variables		Placebo	P-value
Age, mean (SD), years		12.5(2.57)	0.931[1]
Age group, n(%)		3(42.9)	1.000[1]
Child (7-12 years)		4(57.1)	
Teenager (13-18 years)			
Sex, n(%)			0.070[1]
Male	3(42.9)	7(100)	
Female	4(57.1)	0(0)	
Education, n(%)			0.943[2]
Primary school	4(57.1)	4(57.1)	
Junior high school	2(28.6)	2(28.6)	
Senior high school	1(14.3)	0(0)	
No education	0(0)	1(14.3)	
Weight, mean (SD), kg	28.14(5.86)	34.5(15.84)	
Height, mean (SD), cm	132.57(12.20)	133.28(17.96)	
Arm circumference, mean, (SD), cm	19.357(2.96)	21.85(4.05)	
Head circumference, mean, (SD), cm	51.57(1.61)	54.28(2.75)	
BMI, mean (SD), kg/m^2	15.97(2.16)	18.70(4.65)	
Nutritional status, n(%)			
Normal	3(42.9)	2(28.6)	
Moderate malnutrition	4(57.1)	3(42.9)	1.000
Obesity	0(0)	2(28.6)	0.429
Short stature, n(%)			1.000
Yes	5 (71.4)	4(57.1)	
No	2(28.6)	3(42.9)	

1) Fisher's Exact test; $p < 0.05$ 2) Mann-Whitney test; $p < 0.05$

kidneys are referred. Besides, failure of the early diagnosis of congenital abnormalities in children without obvius symptoms.

In this research, approximately 50% moderate malnutrition and 64.28% short stature were found. According to a study by Wong *et al.* in 366 children with CKD, the prevalence of short stature at stage 3 CKD was 10.53%, and stage 4-5 CKD was 13.33%.[16] Damaged kidney function may cause delayed growth in children with CKD. The kidney damage causes the body to lose fluids, acid-base balance in the blood and mineral salts which are important for growth. Kidney damage can lead to metabolic acidosis and impaired secretion of growth hormones, appetite, anemia, and decreased vitamin D production, resulting in delayed growth. The cause of delayed growth is multifactorial, such as anorexia, metabolic acidosis, chronic steroid therapy, inadequate nutritional status, the reduced Insulin-Like Growth Factor-I (IGF-I), inadequate testosterone and estrogen during puberty, and bone disease.

The declined GFR in the BCAA group was 5.08±7.13, and increased GFR in the placebo group was 9.98±5.14 (p=0.055) (Table 3). The declined GFR in the BCAA group was due to the condition of the subjects who received BCAA treatment already suffered damage, decreased levels of kidney function, and severe progressivity of primary diseases. According to Wilson, it is caused by the damage starting from the glomerulus. Another area of undamaged glomerulus will undergo hypertrophy and eventually became sclerotic. Hyperfiltration and hypertrophy from remaining nephron, although it appears to be beneficial. It is suspected as the cause of progressivity of primary renal dysfunction.[17] This result is contradictory with the previous study by Cano *et al*. suggesting that branched-chain amino acids were able to cause correction on CKD and were found to improve the status of protein, avoid toxicity and delay the progressivity of kidney disease.[18]

In patients with severe kidney disease, BCAA supplementation is always associated with a low-protein diet. Nutritional BCAA interventions

Table 2. CKD stages and comorbidities

CKD	BCAA	Placebo	p-value
Stages of CKD, n(%)			0.767[1]
Stage 2	3(42.9)	4(57.1)	
Stage 3a	1(14.3)	1(14.3)	
Stage 3b	1(14.3)	0(0)	
Stage 4	2(28.6)	2(28.6)	
Comorbidities, n(%)			0.149[1]
Lupus nephritis	2(28.6)	1(14.3)	
Nephrotic syndrome	1(14.3)	4(57.1)	
Hydronephrosis	3(42.9)	0(0)	
UTI	0(0)	1(14.3)	
Vesicolithiasis	0(0)	1(14.3)	
Glomerulonephritis	1(14.3)	0(0)	

1) Chi-Square tests; p < 0.05

Table 3. Comparison between BCAA and placebo in stage 2-4 CKD after eight weeks treatment

Delta	BCAA	Placebo	P-value
GFR, mean (SD),mL/min/1.73 m^2	-5.08(7.13)	5.14(9.98)	0.055[1]
Albumin, mean (SD), g/dL	0.20(0.27)	5.14(9.98)	0.560[1]
Proteinuria	+1 (0 - +4)	+1 (0 - +4)	0.629[2]
Blood pressure, mean (SD), mmHg			
Systole	-11.57(15.08)	-7.00(12.19)	0.565[1]
Diastole	-4.85(16.25)	-7.50(4.23)	0.708[1]
Weight, mean (SD), kg	0.07(1.33)	0.58(1.20)	0.828[2]
Height, mean (SD), cm	0.14(0.24)	0.16(0.40)	0.771[2]
Upper arm circumference, mean (SD), cm	0.18(0.24)	0.08(0.20)	0.360[2]
Head circumference, mean (SD), cm	0.21(0.39)	0.35(0.39)	0.561[2]
BMI, mean (SD), kg/m^2	-0.03(0.74)	0.28(0.50)	0.389[1]

1) Independent samples test; p < 0.05 2) Mann-Whitney test; p < 0.05

Table 4. Side effect after BCAA and placebo treatment in eight weeks

Side Effect	BCAA n (%)	Placebo n (%)	P-value
Yes	2(25)	0(0)	0.467[1]
Nausea	1(8.3)	0(0)	
Vomiting	0(0)	0(0)	
Diarrhea	1(8.3)	0(0)	
Constipation	0(0)	0(0)	
Anorexia	0(0)	0(0)	
Abdominal pain	0(0)	0(0)	
Headache	0(0)	0(0)	
Allergy	0(0)	0(0)	
No	6(75)	8(100)	

1)Fisher's Exact test; p < 0.05

have been shown to improve insulin sensitivity, hyperparathyroidism, and may reduce proteinuria in CKD patients.[18] It was contrast to a finding suggesting that BCAA on renal can activate mammalian Target of Rapamycin (mTOR) where BCAA can play a central role in the regulation of cell growth (size and cell proliferation), stress response (apoptosis, autophagy, and necroptosis) and cell metabolism of nutrients and energy balance. It can be activated in response to nutrients, growth factors, stress, and cellular energy.[19,20] Eventhough there was a decrease in kidney function during BCAA supplementation, the decline was not significant. There was no rapid but merely slow decline in the glomerular filtration rate every month. In the placebo group, the increase in glomerular filtration rate was usually found in children with Acute on Chronic Kidney Disease (ACKD). Patients with chronic kidney disease, as evidenced by low GFR or the presence of proteinuria, are at a higher risk of developing acute kidney injury, a condition known as acute kidney injury.

There were an increase and decrease of serum albumin in the BCAA group (0.20±0.23) and the placebo group (-0.16±0.09)(p=0.062). Branched-chain amino acid can increase plasma albumin. Branched-chain amino acid can accelerate protein synthesis in the liver and other tissues via the mTOR pathway. Mammalian Target of Rapamycin is a serine or threonine-protein kinase which can regulate transcription, protein synthesis, and other cellular functions.[21] This finding is in accordance with the research by Iijichi, which observed hepatocytes and albumin secretion of rats by administering BCAA. As the results, rapamycin, one of the mTOR inhibitors, can reduce the production of albumin that is formed by the leucine. This observation indicates that BCAA via mTOR can synthesize albumin in rat hepatocytes.[22]

In this study, hypertension was observed in 64.28% of subjects consisting of 14.28% pre-hypertension, 35.71% stage 1, and 14.28% stage 2. In this study, there was a decrease in delta systole and diastole in the BCAA group (-11.57±15.08) compared with the placebo group (-4.85±16.25), with a p-value of 0.565 and 0.708, respectively. According to the research by Bellizzi, intake of ketoanalog BCAA supplements VLPD was associated with lower proteinuria and more controlled blood pressure compared to the group, which only received LPD intake. This research showed that BCAA caused vasodilatory effect and turned through the response of the BCAA levels resulting in decreased blood pressure Thus, it can inhibit the progressivity of CKD.[23]

Proteinuria for 8 weeks between BCAA and placebo administration BCAA was +1(0-+4) (p=0.629). Proteinuria in CKD is an important indicator of kidney damage. Soares *et al.* discovered that a child with severe proteinuria had a six times greater decrease of GFR than for children without proteinuria. In this study, proteinuria was found 72%, suggesting a higher result than previous research.[10] Wong *et al*. mention that proteinuria occurred in 62% of patients. The difference was probably because research by Wong *et al*. included all patients with GFR 30 to 90 mL/min/1.73 m^2 who already received ARBs and ACE inhibitor therapy, which has renoprotective effects.[16] Resullt the BCAA, especially leucine, is a strong activator and activate mTOR signaling of protein synthesis with the initiation of translation. BCAA will drop capillary pressure causing the glomerulus and proteinuria; thus, it may slow progressivity of CKD.

In BCAA group, weight loss -0.07±1.01 (p=0.828), an increase height 0.14±0.24 (p=0.771), and a decrease in BMI -0.03±0.74 (p=0.389) were reported. Leusin can induce neurons expressing proopiomelanocortin anorexigenic (POMC), which reduces appetite and weight gain.[24] Anorexia associated with the accumulation of unidentified anorexigenic materials, inflammatory cytokine changes settings of appetite due to interference an imbalance of amino acids, which can increase free tryptophan transport passing through the blood-brain barrier. This mechanism leads to the condition of the hiperserotoninergic resulting in decreased appetite.[25]

In this study, there were side effects of BCAA and placebo consumption (Table 4). In the BCAA group obtained diarrhea was found in one subject, and complained appeared after three days of BCAA supplementation. However, there was one subject with complaints of nausea after the consumption of BCAA. Walser *et al*. showed that BCAA supplementation at a dose of 6-14 g/day for 15-60 days in ten patients of severe uremia produced no toxicity.[26] Mitch *et al*. found no side effect or toxicity of ketoanalogues of amino acid supplementation in patients of CKD.[27] Adverse Drug Reactions (ADRs) might be the manifestations of underlying renal pathology or due to other co-administered drugs. Meng *et al*. reported two patients with abdominal pain and one patient with diarrhea; however, all these symptoms ceased spontaneously, and no patients ended the BCAA supplementation. There were no serious incidents caused by BCAA reported in these studies.[28] The limitation of this study was the condition of the patients with severe underlying illness. Also, the number of subjects in each stage of CKD was different. Therefore, further research to analyze further BCAA supplementation with a larger population, and the same stage of CKD was expected.

CONCLUSION AND SUGGESTION

Branched-chain amino acid (leucine, isoleucine, and valine) supplementation did not provide a significant effect in inhibiting the progressivity of stage 2-4 CKD in children and improving nutritional status.

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