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Combined supplementation with α-tocopherol and vitamin C improves the blood pressure of pediatric idiopathic nephrotic syndrome patients

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SUMMARY

The purpose of the present study was to investigate the effects of combined supplementation with α-tocopherol and vitamin C on blood pressures and levels of ox-LDL and NOx in with pediatric idiopathic nephrotic syndrome patients. A total of thirty-six pediatric idiopathic nephrotic syndrome patients was involved in the study, which was assigned randomly into two groups, the control group of pediatric idiopathic nephrotic syndrome patients who received a standard therapy and placebo and the group of pediatric idiopathic nephrotic syndrome patients who received a standard therapy plus α-tocopherol and vitamin C. α-tocopherol and vitamin C were administered at a dose of 10–15 mg/kg/day for 12 weeks. An analysis of lipid profile and NOx was performed by means of a spectrophotometer. An analysis of ox-LDL was performed by using the enzyme-linked immunosorbent assay technique. There was a significant decrease in the number of prehypertensive and hypertensive patients in the supplementation group relative to the control group ($p < 0.05$). There was a decrease in the value of ox-LDL and NOx in the supplementation group relative to the control group, despite no significant difference ($p > 0.50$). In conclusion, the combined supplementation with α-tocopherol and vitamin C may improve blood pressure in pediatric idiopathic nephrotic syndrome patients. Thus, combination of

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α-tocopherol and vitamin C can be used in the clinical management of pediatric idiopathic nephrotic syndrome.

1. Introduction

Under normal conditions, reactive oxygen species will be neutralized efficiently by the cellular antioxidant defense mechanisms. Under various conditions, there is an imbalance between the production of reactive oxygen species and antioxidant defense, triggering cellular destruction and dysfunction [1]. The idiopathic nephrotic syndrome is a major cause of nephrotic syndrome in children [2]. A severe hyperlipidemia is found in idiopathic nephrotic syndrome patients, characterized by elevated levels of cholesterol, triglycerides, LDL, and VLDL. Meanwhile, HDL is normal or decreased [3,4]. Oxidative stress and oxidative modification of LDL are found in idiopathic nephrotic syndrome [5], during the acute and remission periods versus control [6]. Various studies suggested that an abnormality of nephrotic syndrome is hyperlipidemia capable of accelerating the progression of renal disease [7]. Hyperlipidemia will trigger an increase in LDL levels to be converted to ox-LDL through activation of lipoxygenase and reactive oxygen species [8]. Previous studies have shown an increase in ox-LDL in pediatric nephrotic syndrome patients relative to control [9,10].

In nephrotic syndrome an increased lipid peroxidation is also found relative to control. This increase in the markers of oxidative stress is accompanied by a decrease in antioxidants, including vitamin C and vitamin E [11–13]. To date, to the best knowledge of the author, there have been no studies that have provided supplementation with vitamin E and vitamin C to idiopathic nephrotic syndrome patients. Vitamin E acts as a lipid-soluble antioxidant, scavenging hydroperoxyl radicals in lipid environments [14]. At the molecular level, vitamin E and its various metabolites demonstrate the ability to regulate cellular signaling and gene transcription modulation [15]. Vitamin C is an essential nutrient needed for various biological processes in the body. Vitamin C is antioxidant and acts as a cofactor of biosynthesis of collagen, catecholamines, amino acids and various peptide hormones [16]. Despite the apparent benefits, there remains much controversy with regard to the pharmacological effects of both vitamins. In modulating blood pressure, there is a negative correlation between serum levels of vitamin C and systolic and diastolic blood pressures [17–20]. Administration of multivitamins is incapable of improving blood pressure in the elderly [21]. With regard to combined administration, the loss of vitamin E in plasma will be decreased by vitamin C so as to act synergistically [22]. Thus, the purpose of the present study was to evaluate the effects of combined supplementation with α-tocopherol and vitamin C on blood pressures and levels of ox-LDL and NOx in with idiopathic nephrotic syndrome patients.

2. Material and methods

2.1. Subjects

A total of 42 pediatric idiopathic nephrotic syndrome patients were involved in the study, which was randomly assigned into two groups. Inclusion criteria included an age of 1–15 years, an early/relapsed/resistant pediatric idiopathic nephrotic syndrome attack, a glomerular filtration rate of >90 ml/min/1.73 m^2, a CRP of <10 mg/dl, and not suffering from tuberculosis or other acute infections. The control group consisted of pediatric idiopathic nephrotic syndrome patients receiving a standard therapy and placebo. The treatment group consisted of pediatric idiopathic nephrotic syndrome patients receiving a standard therapy and placebo plus α-tocopherol and vitamin C. Both groups were monitored every two weeks to twelve weeks.

2.2. α-Tocopherol and vitamin C

Vitamin E administered was α-tocopherol at a dose of 10–15 mg/kg/day. This dose was divided into two doses (a maximum of 400 mg/day). Vitamin C was administered at a dose of 10–15 mg/kg/day and was divided into two doses (a maximum of 400 mg/day). Both preparations were administered after meals for 12 weeks.

2.3. Serum isolation

After 12 weeks, 7 ml of blood was collected from the antecubital vein using a vacutainer tube. After 45 min, the tube was centrifuged at 3000 rpm (5000 g) for 10 min at room temperature. Furthermore, blood serum was separated and stored at −20 °C until analysis of biomarkers.

2.4. Analysis of ox-LDL levels

Serum levels of ox-LDL were analyzed using the enzyme-linked immunosorbent assay (Mercodia Oxidized LDL ELISA kit, catalog no 10-1143-01, Uppsala, Sweden). The analysis was performed according to the kit's detailed instructions.

2.5. Analysis of NOx levels

Serum levels of NOx were analyzed using a spectrophotometer with the Cayman nitrate/nitrite colorimetric assay kit, catalog no. 780001, Cayman Chemical, Ann Arbor, MI, USA. The analysis was performed according to the kit's detailed instructions.

2.6. Analysis of lipid profile

The analysis of lipid profile included total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), and triglycerides. The analysis was performed using Dimension RxL (Siemens Dimension, clinical chemistry system, Camberley, UK).

2.7. Ethics

This study has passed the study of human research ethics from the institutional ethics committee of Diponegoro University, Dr. Kariadi Hospital, Semarang, Central Java, Indonesia.

2.8. Statistical analysis

Data were presented in mean ± standard deviation and analyzed using the Chi-square and Mann Whitney test. The statistical analysis was performed using IBM SPSS for Windows version 20.0 statistical package. The p-value of <0.05 was statistically significant.

3. Results

Until the end of the study, 36 patients completed the study and six dropped out. During the observation period, no side effects of vitamins or placebo was found. Age, gender, diet, serum lipid profile, blood pressure, remission status of the two groups showed no significant difference ($p > 0.05$), as shown in Table 1.

Table 2 shows the values of blood pressure in both study groups. There was a significant decrease in the number of prehypertensive and hypertensive patients in the supplementation group relative to the control group ($p < 0.05$).

The values of lipid profile, ox-LDL, and NOx are presented in Table 3. There was a decrease in the values of ox-LDL, and NOx in the supplementation group relative to the control group, despite no significant difference ($p > 0.50$).

Table 1
Baseline clinical characteristics and food record among subjects.

Level	Placebo (n = 18)	Treatment (n = 18)	*p* Value
Age (month)	4.8 ± 3.1	7.3 ± 3.1	0.304
Sex (male/female)	11/7	11/7	
Onset (year)	4.8 ± 3.1	5.6 ± 3.7	0.601
Duration (year)	1.9 ± 2.3	2.0 ± 2.2	0.924
Blood pressure			
Normotension	6	8	0.733
Prehypertension	12	10	
Food record and recall			
Energy (kcal/day)	1353.8 ± 411.7	1354.6 ± 329.6	0.509
Carbohydrate (gram)	200.2 ± 56.7	187.9 ± 38.5	0.355
Carbohydrate (%)	56.3 ± 8.70	56.5 ± 8.1	0.798
Lipid (gram)	47.7 ± 14.7	45.1 ± 17.5	0.649
Lipid (%)	29.5 ± 7.4	29.5 ± 7.4	0.480
Protein (gram)	49.3 ± 12.1	52.0 ± 20.8	0.636
Protein (%)	14.1 ± 2.7	15.1 ± 3.6	0.351
PUFA (gram)	5.0 ± 3.5	6.7 ± 3.7	0.181
MUFA (gram)	8.3 ± 4.6	9.2 ± 4.5	0.632
SFA (gram)	21.5 ± 6.8	21.8 ± 8.5	0.884
Vitamin E	3.7 ± 2.1	3.5 ± 1.5	0.750
Vitamin C	40.8 ± 34.0	65.5 ± 1.8	0.082

Note: PUFA: polyunsaturated fatty acid; MUFA: monounsaturated fatty acid; SFA: saturated fatty acid; %: percentage; mg: milligram.

Table 2
The blood pressure in pre and post treatment.

Blood pressure	Placebo group		Treatment group		*p* Value
	Pre	Post	Pre	Post	
Systolic (mmHg)	114.8 ± 22.3	106.7 ± 13.8	109.2 ± 14.9	103.8 ± 11.0	0.460
Diastolic (mmHg)	74.7 ± 16.8	70.0 ± 10.4	67.8 ± 16. 6	64.7 ± 11.2	0.220
Normotension	6	9	8	15	0.494
Prehypertension–hypertension stage II	12	9	10	3	0.034

Note: mmHg: milimeterHg.

Table 3
The lipid profile, level ox-LDL, level NOx in pre and post treatment.

Biomarker	Placebo group		Treatment group		*p* Value
	Pre	Post	Pre	Post	
Total cholesterol (mg/dL)	394.0 ± 129.33	265.3 ± 158.13	413.4 ± 168.78	329.1 ± 212.80	0.438
LDL-cholesterol (mg/dL)	260.9 ± 115.48	173.1 ± 132.76	286.2 ± 155.34	211.3 ± 162.50	0.624
HDL-cholesterol (mg/dL)	50.5 ± 26.22	45.9 ± 20.18	55.5 ± 28.38	51.4 ± 16.59	0.373
Tryglyceride (mg/dL)	295.3 ± 132.79	161.9 ± 135.89	308.7 ± 166.53	203.9 ± 184.24	0.716
ox-LDL (mg/dL)	125.2 ± 57.2	76.7 ± 61.7	127.2 ± 51.7	89.5 ± 63.9	0.424
NOx (μm)	8.2 ± 4.7	7.9 ± 3.2	8.9 ± 6.8	8.5 ± 5.9	0.938

Note: LDL: low density lipoprotein; HDL: high density lipoprotein; ox-LDL: oxidized-low density lipoprotein; NOx: nitric oxide; mg/mL: milligram/desiliter; μM: micromolar.

4. Discussion

In the present study, the values of age, gender, diet, serum lipid profile, blood pressure, remission status of the two groups showed no significant difference ($p > 0.05$). And so did the lipid profile, ox-LDL, and NOx levels before treatment. This shows that both groups can be compared.

There was a significant decrease in the number of prehypertensive–hypertensive patients in the supplementation group relative to the control group ($p < 0.05$). This suggests that combined supplementation with α-tocopherol and vitamin C was capable of improving the blood pressure of pediatric idiopathic nephrotic syndrome patients. The declined blood pressure was caused by changing levels of ox-LDL and NOx. The present study showed a trend of changing values, despite no significant difference ($p > 0.05$).

Supplementation with α-tocopherol is capable of lowering blood pressure in spontaneously hypertensive rats and diabetes mellitus-induced spontaneous hypertension [22,23]. Tocopherol increases the activity of nitric oxide synthase in the spontaneously hypertensive rats' vasculature [24]. As an antioxidant, vitamin C provides protection against oxidative stress, the trigger of cellular damage through the scavenging of reactive oxygen species, neutralization of vitamin E-dependent hydroperoxyl radicals, and protein protection from alkylation of lipid peroxidation products. With regard to blood pressure regulation, vitamin C plays a role in modulating the function of nitric oxide synthase through the eNOS cofactor recycling, tetrahydrobiopterin [14]. Administered in combination, the hydroxyl groups of tocopherol will react with peroxyl radicals to form lipid hydroperoxide and tocopheryl radicals. Tocopheryl radicals will react with vitamin C to restore vitamin E in its reduced state. Furthermore, the availability of tocopherol will increase the activity of nitric oxide synthase [14,24]. Another study demonstrated that the combination of vitamins C and E had beneficial effects in endothelial-dependent vasodilation and arterial stiffness [25].

In conclusion, the combined supplementation with α-tocopherol and vitamin C may improve blood pressure in pediatric idiopathic nephrotic syndrome patients. Thus, combination of α-tocopherol and vitamin C can be used in the clinical management of pediatric idiopathic nephrotic syndrome.

Conflict of interest statement

All authors' state there is no conflict of interest with regard to this study or the publication of this article.

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