Effects of combined supplementation of vitamins C and E on the oxidative modification of low-density lipoprotein, soluble form of CD36, soluble vascular cell adhesion molecule-1, and nitrite/nitrate oxide levels in idiopathic nephrotic syndrome

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Abstract This study aimed to investigate whether a combined supplementation of vitamins C and E was able to modify the oxidized low-density lipoprotein (ox-LDL), soluble form of CD36 (sCD36), soluble vascular cell adhesion molecule-1 (sVCAM-1), and nitrite/nitrate oxide (NOx) levels in pediatric nephrotic syndrome (NS) cases. The study included 36 children with NS. The patients were randomly allocated to either the treatment group or the placebo group (18 children each). The treatment group received a combined supplementation of vitamins C and E. The serum levels of ox-LDL, sCD36, and sVCAM-1 were assayed by enzyme-linked immunosorbent assay. The serum levels of NOx were assayed by colorimetric assay. Results showed that...
the levels of ox-LDL, sVCAM-1, and NOx were decreased after treatment with a combined supplementation of vitamins C and E, but there was no statistical difference (p > 0.05). After treatment, there was an increase in the level of sCD36 in both groups, although this was not significantly different (p > 0.05). The level of ox-LDL was significantly lower in the remission-idiopathic NS (remission-INS) group compared with the nonremission-INS group (p < 0.05). The levels of ox-LDL and sVCAM-1 were significantly lower in the remission-treated group than in the nonremission-treated group (p < 0.05). In conclusion, the combined supplementation of vitamins C and E cannot modify the ox-LDL, sCD36, sVCAM-1, and NOx levels in children with INS. In remission cases, the combined supplementation of vitamins C and E reduces the ox-LDL and sVCAM-1 levels.

Materials and methods

Study patients

The study included 42 children with NS, who were randomly allocated to either the treatment group or the placebo group, with each group consisting of 21 children. Patients were enrolled in this study after informed and written consent had been obtained. Patients were seen on an outpatient basis at 2-week intervals. Criteria for eligibility were as follows: age 1–15 years, persistent proteinuria (≥1 g/m²/d), total cholesterol (T-Chol) more than 200 mg/dL or LDL-cholesterol more than 160 mg/dL, and glomerular filtration rate more than 80 mL/min/1.73 m² (estimated by the Schwartz formula).

Definitions

Primary (idiopathic) NS was defined by heavy proteinuria (urinary protein > 50 mg/kg/d or dipstick ≥ 2+), hypoalbuminemia (serum albumin < 2.5 g/dL), and hypercholesterolemia (serum cholesterol > 5.72 mmol/L or > 200 mg/dL), with or without edema. The initial treatment includes oral administration of prednisone (2 mg/kg/d;
maximum 60 mg/d) in three doses for 4 weeks followed by therapy (three times per week, at Monday-Wednesday-Saturday) on alternate days for another 4 weeks. The daily dose was then tapered down for 4–7 months and finally stopped. The active stage of NS was defined as increased urinary protein excretion (Albustix >2+ for at least 3 consecutive days or >50 mg/kg/d) and serum albumin concentration less than 2.5 g/dL. Remission was defined as normal protein excretion (Albustix trace or negative for at least 3 consecutive days). Patients were considered steroid resistant if their proteinuria disappeared (negative or trace for 3 consecutive days) following 4 weeks of prednisone treatment. Patients who still had proteinuria were considered steroid sensitive.

Treatment with vitamins C and E

Vitamin C (Sigma-Aldrich, USA) at a dose of 10–15 mg/kg body weight (maximum dose 400 mg) and vitamin E (D1-α-tocopherol acetate) at a dose of 10–15 mg/kg body weight (maximum dose 400 mg) were orally administered for 12 weeks.

Blood samples

Blood sample was drawn from an antecubital vein into 10-mL serum Vacutainer tubes. After approximately 45 minutes, the tubes were centrifuged at 3000 rpm (5000g) for 10 minutes at room temperature. Serum was separated from blood cells and stored at −20°C until further analysis.

Lipid profile analysis

Fasting blood samples (10 mL) were collected in 1 mmol disodium EDTA and 0.5 mg sodium azide. Level of Total Cholesterol(T-Chol), LDL-cholesterol (LDL-C), HDL-C and TGs were measured directly by routine laboratory method on Dimension RxL (Siemens Dimension, Clinical Chemistry System, Camberley, UK).

Determination of the ox-LDL level

The ox-LDL level was determined in the serum samples collected. Enzyme-linked immunosorbent assay (ELISA) for ox-LDL (Mercodia Oxidized LDL ELISA, Catalog nr-10-1143-01, Uppsala, Sweden) was performed according to the manufacturer’s instructions, with an intra-assay coefficient of variation (CV) less than 6% and interassay CV less than 7%. ELISA was performed in triplicate.

Determination of the sCD36 level

The sCD36 level was determined in the serum samples collected. ELISA for sCD36 (human sCD36 ELISA kit; Aviscera Bioscience, Catalog No. SK000196, Santa Clara, CA, USA) were performed according to the manufacturer’s instructions, with an intra-assay CV less than 5% and interassay CV less than 9%. ELISA was performed in triplicate.

Determination of the sVCAM-1 level

The sVCAM-1 level was determined in the serum samples collected. ELISA for sVCAM-1 (Quantikine human sVCAM-1 ELISA kit; R&D systems, Catalon No. DVC00, Shanghai, PRC) was performed according to the manufacturer’s instructions, with an intra-assay CV less than 5% and interassay CV less than 8%. ELISA was performed in triplicate.

Determination of the NOx level

The NOx level was determined in the serum samples collected. Colorimetric assays (ELISA) for NO (Cayman nitrate/nitrite colorimetric assay kit, Catalog No. 780001, Cayman Chemical, Ann Arbor, MI, USA) were performed according to the manufacturer’s instructions, with an intra-assay CV of 2.7% and interassay CV of 3.4%. ELISA was performed in triplicate.

Ethics

Human experimental procedures were approved by the Institutional Ethics Committee of University of Diponegoro/Dr. Kariadi Hospital, Semarang, Central Java, Indonesia.

Statistical analysis

Data are presented as mean ± standard deviation and the differences between groups were analyzed using independent sample t test for normal distribution or Mann–Whitney U test for abnormal distribution with IBM SPSS for Windows version 20.0 statistical package. A p value less than 0.05 was considered statistically significant.

Results

Patients

Six of the 42 children (14.3%) dropped out of the study. Thus, we tested our hypothesis on 36 children (18 in each group). The age, sex, frequency of hypertension, and food record at baseline were not significantly different between groups (p > 0.05; Table 1). During the observation period, there were no side effects due to the vitamin or placebo treatment. In addition, the remission status, frequency of hypertension, and lipid profile were not significantly different between the two groups (p > 0.05; Table 2).

Levels of ox-LDL, sVCAM, NO, and sCD36

The mean ox-LDL level at baseline in the treatment and placebo groups was 127.2 U/L and 125.2 U/L, respectively (Table 3). The mean sVCAM-1 level at baseline in the treatment and placebo groups was 929.4 ng/mL and 927.1 ng/mL, respectively. The mean NOx level at baseline in the treatment and placebo groups was 8.9 μM and 8.2 μM, respectively. After treatment, there was a decrease in the ox-LDL, sVCAM, and NOx levels in both groups, but this was not significantly different (p > 0.05). The mean
Table 1  Baseline clinical characteristics and food record among patients.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>INS</th>
<th>INS + vitamins C and E</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (mo)</td>
<td>80.48 ± 42.24</td>
<td>87.81 ± 45.10</td>
<td>0.458</td>
</tr>
<tr>
<td>Sex (male/female)</td>
<td>12/9</td>
<td>13/8</td>
<td>0.753</td>
</tr>
<tr>
<td>Normotension</td>
<td>8 (38.1%)</td>
<td>9 (42.9%)</td>
<td>0.753</td>
</tr>
<tr>
<td>Prehypertension stage 2</td>
<td>13 (61.9%)</td>
<td>12 (57.1%)</td>
<td>0.753</td>
</tr>
<tr>
<td>Energy (kcal/d)</td>
<td>1452.6 ± 2777.73</td>
<td>1358.8 ± 308.67</td>
<td>0.307</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>199.9 ± 56.86</td>
<td>186.7 ± 37.75</td>
<td>0.378</td>
</tr>
<tr>
<td>Carbohydrate (%)</td>
<td>54.3 ± 9.50</td>
<td>55.9 ± 8.38</td>
<td>0.575</td>
</tr>
<tr>
<td>Lipid (g)</td>
<td>50.6 ± 15.37</td>
<td>45.94 ± 17.00</td>
<td>0.359</td>
</tr>
<tr>
<td>Lipid (%)</td>
<td>30.5 ± 6.98</td>
<td>28.9 ± 6.79</td>
<td>0.456</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>53.7 ± 17.6</td>
<td>52.5 ± 19.6</td>
<td>0.840</td>
</tr>
<tr>
<td>Protein (%)</td>
<td>15.1 ± 5.8</td>
<td>15.3 ± 3.5</td>
<td>0.308</td>
</tr>
<tr>
<td>Cholesterol (mg)</td>
<td>268.7 ± 200.79</td>
<td>196.8 ± 149.34</td>
<td>0.704</td>
</tr>
<tr>
<td>PUFA (g)</td>
<td>5.3 ± 3.3</td>
<td>7.0 ± 4.14</td>
<td>0.195</td>
</tr>
<tr>
<td>MUFA (g)</td>
<td>8.65 ± 4.4</td>
<td>9.89 ± 5.39</td>
<td>0.633</td>
</tr>
<tr>
<td>SFA (g)</td>
<td>23.5 ± 8.68</td>
<td>21.7 ± 7.97</td>
<td>0.491</td>
</tr>
<tr>
<td>Fiber (g)</td>
<td>6.7 ± 3.68</td>
<td>8 ± 4</td>
<td>0.263</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>4.3 ± 2.76</td>
<td>3.7 ± 1.65</td>
<td>0.439</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>40.8 ± 37.6</td>
<td>62.5 ± 45.26</td>
<td>0.061</td>
</tr>
</tbody>
</table>

INS = idiopathic nephrotic syndrome; MUFA = monounsaturated fatty acid; PUFA = polyunsaturated fatty acid; SFA = saturated fatty acid.

sCD36 level at baseline in the treatment and placebo groups was 72.5 ng/mL and 56.9 ng/mL, respectively. After treatment, there was an increase in the sCD36 level in both groups, although this was not significantly different (p > 0.05).

Table 4 presents the ox-LDL, sVCAM, NOx, and sCD36 levels in the remission and nonremission cases. The ox-LDL level was significantly lower in the remission-INS group compared with the nonremission-INS group (p < 0.05). The levels of ox-LDL and sVCAM-1 were significantly lower in the remission-treated group compared with those in the nonremission-treated group (p < 0.05).

Discussion

Hyperlipidemia results from hypoalbuminemia due to inhibition of the reaction (conversion of cholesterol of HDLs to cholesterol esters) catalyzed by lecithin cholesterol acyltransferase and to an inhibition of HDL particle formation from very-low-density lipoproteins due to reduced activity of lipoprotein lipase.25 In this study, we found that ox-LDL showed a decreasing trend in both groups, although this was not significantly different (p > 0.05). This finding indicated that a combined supplementation of vitamins C and E cannot affect the lipid peroxidation process in patients with NS. In a previous study of 15 teenage children with familial hypercholesterolemia and familial combined hyperlipidemia, treatment with vitamins C (500 mg/d) and E (400 IU/d) for 6 weeks did not have an effect on biomarkers of oxidative stress; however, this treatment improved the brachial artery dilatation.26 In addition, another study showed that the absence of improvement in urinary isoprostane levels and atherosclerotic lesion area in vitamin-E-supplemented mice may be due to their high-fat-diet consumption and/or their extreme elevations in plasma lipid levels.27 Our data show that the level of ox-LDL was significantly lower in the remission-INS group compared with the nonremission-INS group (p < 0.05) or in the remission-treated group than in the nonremission-treated group.

Table 2  Clinical and laboratory parameters after treatment with vitamins E and C.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>INS</th>
<th>INS + vitamins C and E</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Remission:nonremission</td>
<td>9:9</td>
<td>10:8</td>
<td>0.738</td>
</tr>
<tr>
<td>Normotension</td>
<td>9 (50%)</td>
<td>15 (83.4%)</td>
<td>0.077</td>
</tr>
<tr>
<td>Prehypertension stage 2</td>
<td>9 (50%)</td>
<td>3 (16.6%)</td>
<td>0.077</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>265.33 ± 158.12</td>
<td>329.05 ± 212.796</td>
<td>0.443</td>
</tr>
<tr>
<td>LDL (mg/dL)</td>
<td>173.05 ± 132.76</td>
<td>211.28 ± 162.5</td>
<td>0.628</td>
</tr>
<tr>
<td>HDL (mg/dL)</td>
<td>45.88 ± 20.18</td>
<td>51.44 ± 16.58</td>
<td>0.373</td>
</tr>
<tr>
<td>VLDL (mg/dL)</td>
<td>42.61 ± 22.35</td>
<td>75.67 ± 83.27</td>
<td>0.563</td>
</tr>
<tr>
<td>Triglyceride (mg/dL)</td>
<td>161.89 ± 135.89</td>
<td>203.89 ± 184.24</td>
<td>0.719</td>
</tr>
</tbody>
</table>

INS = idiopathic nephrotic syndrome; LDL = low-density lipoprotein; HDL = high-density lipoprotein; VLDL = very-low-density lipoprotein.
group (p < 0.05). This finding shows that the level of ox-LDL will decrease with or without combined vitamin treatment.

Macrophage CD36 is surface molecule that is able to bind and internalize LDL for the formation of foam cells. Elevated plasma sCD36 levels have been proposed to be a marker of insulin resistance and atherosclerosis risk. In this study, we found that the sCD36 level increased in both groups, but this was not significantly different (p > 0.05). We hypothesize that this increase might have been due to the increased number of platelets and monocytes. A previous report showed that the circulating form of the sCD36 receptor is associated with MPs coming from platelets, leukocytes, and endothelial cells upon receiving stimuli or apoptosis signal. Besides, low-grade inflammation also induces the production of MPs. Asymmetric dimethylarginine (ADMA) is an endogenous inhibitor of NO synthase. The inhibition of NO synthesis is known to be associated with an increase in the expression of endothelial adhesion molecule (sVCAM-1). In this study, there was no significant difference in the levels of NOx and sVCAM-1 between the two studies (p > 0.05). In a previous study of 15 teenage children with familial hypercholesterolemia and familial combined hyperlipidemia, treatment with vitamins C (500 mg/d) and E (400 IU/d) for 6 weeks did not have an effect on ADMA. In other words, our study shows that the combined supplementation of vitamins C and E cannot modulate the endothelial dysfunction in patients with NS. Interestingly, the level of sVCAM-1 was significantly decreased in the remission-treated group, compared with the nonremission-treated group (p < 0.05). This finding shows that the combined supplementation of vitamins C and E modulates the endothelial dysfunction in patients with remission of NS. In conclusion, the combined supplementation of vitamins C and E cannot modify the ox-LDL, sCD36, sVCAM-1, and NOx levels in children with INS. In remission cases, the combined supplementation of vitamins C and E reduces the ox-LDL and sVCAM-1 levels.

### Table 4. Levels of ox-LDL, sVCAM-1, sCD36, and NOx in remission and nonremission cases.

<table>
<thead>
<tr>
<th>Biomarkers</th>
<th>INS</th>
<th>Before</th>
<th>After</th>
</tr>
</thead>
<tbody>
<tr>
<td>ox-LDL (U/L)</td>
<td>Nonremission (n = 9)</td>
<td>107.8 ± 66.1</td>
<td>45.5 ± 39.2 *</td>
</tr>
<tr>
<td>sVCAM-1 (ng/mL)</td>
<td>Remission (n = 9)</td>
<td>943.9 ± 342.3</td>
<td>85.8 ± 150.0</td>
</tr>
<tr>
<td>sCD36 (ng/mL)</td>
<td>Nonremission (n = 8)</td>
<td>58.5 ± 51.7</td>
<td>59.3 ± 58.3</td>
</tr>
<tr>
<td>NOx (μM)</td>
<td>Remission (n = 10)</td>
<td>7.0 ± 3.1</td>
<td>8.2 ± 5.6</td>
</tr>
</tbody>
</table>

* p < 0.05, in comparison with the nonremission-INS group.
** p < 0.05, in comparison with the nonremission-INS + vitamins C and E group.

INS = Idiopathic nephrotic syndrome; NOx = nitrite/nitrate oxide; ox-LDL = oxidized low-density lipoprotein; sVCAM = soluble vascular cells adhesion molecule.

### References

7. Rosoky RM, Wlosok N, Nasser M, et al. Oxidized low-density lipoprotein and ankle-brachial pressure index in patients with...
clinically evident peripheral arterial disease. *Clinics (Sao Paulo).* 2010;65:383–387.