Efficacy of vitamins C, E, and their combination for treatment of restless legs syndrome in hemodialysis patients: A randomized, double-blind, placebo-controlled trial

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\textbf{A B S T R A C T}

\textit{Background:} Restless legs syndrome (RLS) is a common disorder in hemodialysis patients that leads to insomnia and impaired quality of life. Because high oxidative stress has been implicated in the pathogenesis of RLS, we sought to evaluate the efficacy of vitamins C and E and their combination in reducing the severity of RLS symptoms in hemodialysis patients in this randomized, double-blind, placebo-controlled, four-arm parallel trial.

\textit{Methods:} Sixty stable hemodialysis patients who had all four diagnostic criteria for RLS developed by the International Restless Legs Syndrome Group with no acute illness or history of renal stone were randomly allocated to four fifteen-patient parallel groups to receive vitamin C (200 mg) and vitamin E (400 mg), vitamin C (200 mg) and placebo, vitamin E (400 mg) and placebo, and double placebo daily for eight weeks. International Restless Legs Scale (IRLS) scores were measured for all patients at baseline and at the end of treatment phase. The primary outcome was absolute change in IRLS sum score from baseline to the end of treatment phase.

\textit{Results:} Means of IRLS sum score decreased significantly in the vitamins C and E (10.3 ± 5.3, 95% CI: 7.4–13.3), vitamin C and placebo (10 ± 3.5, 95% CI: 8.1–11.9), and vitamin E and placebo groups (10.1 ± 6, 95% CI: 6.8–13.5) compared with the double placebo group (3.1 ± 3, 95% CI: 1.5–4.8), \((P < 0.001)\); however, no differences were observed between these treatment groups.

\textit{Conclusions:} Vitamins C and E and their combination are safe and effective treatments for reducing the severity of RLS in hemodialysis patients over the short-term.

\section*{1. Introduction}

Restless legs syndrome (RLS) is a common and well-recognized cause of insomnia and impaired quality of life in hemodialysis (HD) patients \cite{1}. Several therapeutic options are proposed for the treatment of RLS in HD patients; however, none of these treatments are conclusively effective and some have serious side effects \cite{2–4}. The complex and not fully elucidated pathogenesis of RLS in HD patients is one of the main obstacles in the way of finding more effective and safer treatments for this conundrum.

Reduced antioxidant defense mechanisms and increased production of oxidative compounds results in a high oxidative stress status in HD patients \cite{5}. High oxidative stress has been implicated in the pathogenesis of the several complications of end-stage renal disease (ESRD) including atherosclerosis, anemia, amyloidosis, and malnutrition \cite{5}. Interestingly, a recent case-control study has demonstrated that total oxidant status is significantly increased in patients with idiopathic RLS \cite{6}. Deficiency of vitamin C and reduced intracellular levels of vitamin E are among the contributing factors to high oxidative stress status in HD patients \cite{5}. Vitamins C and E are potent antioxidant agents that have been used for the prevention and treatment of several health conditions. Interestingly, there are some reports of the efficacy of vitamin E in the treatment of idiopathic RLS \cite{7,8}. Moreover, these vitamins have also been shown to be effective in the treatment of other sensorimotor and sleep disorders in HD patients, like leg cramp \cite{9}.

Considering the above mentioned facts we performed this trial to evaluate the efficacy of vitamins C and E and their combination for the treatment of RLS in HD patients.
2. Subjects and methods

This double-blind, randomized, placebo-controlled, four-arm parallel-group, phase 2 trial was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines and reviewed and approved by the Ethical Committee of Shiraz University of Medical Sciences.

In the screening phase of the trial, all patients aged 18–80 years who were under regular HD in the Nemazee Hospital Hemodialysis Center were asked the questions of the essential diagnostic criteria for RLS developed by International Restless Legs Syndrome Group [10]. Only the stable HD patients with no acute illness or admission who had all four diagnostic criteria were selected for the treatment phase of the study. Those who were receiving certain medications with known RLS aggravating properties, including tricyclic antidepressants, selective serotonin reuptake inhibitors, dopamine antagonists, dopamine blocking antiepileptics, lithium, and sedative antihistamines, were excluded [2,3]. Patients with a history of renal stone were also excluded due to concerns over increased risk of oxalosis and its joint and vascular complications in HD patients who consume vitamin C [11].

Of 280 screened patients, 60 fulfilled the selection criteria and gave their informed consent to participate in the treatment phase of study. These patients were dialedyzed three times a week for four hours using low flux dialyzer with polysulfone/polyamide membranes and reverse osmosis purified water and bicarbonate containing dialysate. After a washout phase of four weeks for previous treatments for RLS, including levodopa and other dopamine agonists, gabapentin, clonazepam, benzodiazepines, opioids, and multivitamins, these 60 patients were randomly assigned in a 1:1:1:1 ration to four fifteen-patient parallel groups to receive and multivitamins, these 60 patients were randomly assigned in a 1:1:1:1 ratio to four fifteen-patient parallel groups to receive one tablet of vitamin C (200 mg) and one capsule of vitamin E (α-tocopherol) (400 mg), one tablet of vitamin C (200 mg) and one placebo capsule, one capsule of vitamin E (α-tocopherol) (400 mg) and one placebo tablet, and one placebo capsule and one placebo tablet daily for eight weeks. These dosages were chosen because they were found to be safe and effective in the treatment of uremic leg cramps in a study by Khajehdehi et al. [9].

Blocked randomization with a fixed block size of four was done by an investigator who had no clinical involvement in the trial according to a randomization sequence generated by Random Allocation Software [12]. All drug and placebo tablets and capsules were made and supplied by the Shiraz School of Pharmacy in prepacked bottles numbered for each patient according to the randomization sequence. Each patient was assigned an order number and received the medications in the corresponding prepacked bottle. The placebo tablets and capsules were similar in size, color, weight, and taste to tablets of vitamin C and capsules of vitamin E, respectively. Participants and clinical investigators and other health care staff were all masked to the treatment assignment.

In order to determine the severity of RLS, prior to the onset of the treatment, 10 questions of the International RLS Study Group severity rating scale (International Restless Legs Scale or IRLS) [13] were read to the patients and completed in person by the principal investigator of the study who is an experienced nephrologist and regularly diagnoses and treats uremic RLS patients. These 10 questions each had 0–4 points; therefore, the sum score could range between 0 and 40 points. After eight weeks of therapy, IRLS scores were obtained in the same way by the same person. Furthermore, baseline levels of several laboratory parameters were measured before the commencement of treatment. The patients were also asked by clinical staff about the adverse events during each dialysis session throughout the treatment phase of the trial.

Statistical analyses were performed using the SPSS base 15 (SPSS Inc., Chicago, IL) statistical software package. The primary efficacy parameter for this trial was absolute change in IRLS sum score from baseline to the end of treatment phase in an intention-to-treat population. A one-way-between-groups ANOVA was conducted to compare the average changes in IRLS sum scores and, also, the average values of other parametric data between the four groups; when needed, post hoc comparisons were done using Scheffe test. Moreover, Kruskal–Wallis and Chi-square tests were conducted to compare the average values of nonparametric data and the gender of the patients between each of the four groups, respectively. A P value of less than 0.05 was considered to be statistically significant.

Table 1

<table>
<thead>
<tr>
<th>Demographic and clinical characteristics and laboratory parameters in patients.</th>
<th>Vitamins C &amp; E</th>
<th>Vitamin C &amp; placebo</th>
<th>Vitamin E &amp; placebo</th>
<th>Double placebo</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>n = 15</td>
<td>n = 15</td>
<td>n = 15</td>
<td>n = 15</td>
<td>–</td>
</tr>
<tr>
<td>Causes of ESRD</td>
<td>6</td>
<td>8</td>
<td>6</td>
<td>7</td>
<td>–</td>
</tr>
<tr>
<td>Diabetic nephropathy</td>
<td>4</td>
<td>3</td>
<td>2</td>
<td>3</td>
<td>–</td>
</tr>
<tr>
<td>Hypertensive nephropathy</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>–</td>
</tr>
<tr>
<td>Glomerulonephritis</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>–</td>
</tr>
<tr>
<td>Polycystic kidney disease</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>–</td>
</tr>
<tr>
<td>Obstructive nephropathy</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>–</td>
</tr>
<tr>
<td>Reflux nephropathy</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>–</td>
</tr>
<tr>
<td>Amyloidosis</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>–</td>
</tr>
<tr>
<td>Unknown</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>–</td>
</tr>
<tr>
<td>Age (years)</td>
<td>47.4 ± 14.2 (22–73)a</td>
<td>54.6 ± 15.2 (25–76)</td>
<td>49.3 ± 12.9 (25–68)</td>
<td>59.5 ± 17.9 (24–77)</td>
<td>NSb</td>
</tr>
<tr>
<td>Female/male</td>
<td>9/6</td>
<td>9/6</td>
<td>11/4</td>
<td>6/9</td>
<td>NS</td>
</tr>
<tr>
<td>Duration on HD (months)</td>
<td>14.7 ± 14.8</td>
<td>14.9 ± 8.1</td>
<td>18.4 ± 10.7</td>
<td>15.7 ± 10.3</td>
<td>NS</td>
</tr>
<tr>
<td>IRLS sum score</td>
<td>20.5 ± 4.6</td>
<td>18.8 ± 5.5</td>
<td>21.3 ± 6.4</td>
<td>16.9 ± 5.5</td>
<td>NS</td>
</tr>
<tr>
<td>Weekly Kt/V</td>
<td>2.8 ± 1</td>
<td>3.5 ± 1</td>
<td>2.8 ± 0.8</td>
<td>2.6 ± 0.9</td>
<td>NS</td>
</tr>
<tr>
<td>Calcium (mg/dL)</td>
<td>8.8 ± 1</td>
<td>8.7 ± 1</td>
<td>8.8 ± 0.7</td>
<td>8.7 ± 0.7</td>
<td>NS</td>
</tr>
<tr>
<td>Phosphate (mg/dL)</td>
<td>5.3 ± 1.5</td>
<td>5.6 ± 1.3</td>
<td>5.4 ± 1.6</td>
<td>4.9 ± 1.1</td>
<td>NS</td>
</tr>
<tr>
<td>PTH (pg/dL)</td>
<td>329.2 ± 125.4</td>
<td>266.4 ± 113.3</td>
<td>334.7 ± 174.6</td>
<td>258.7 ± 117.9</td>
<td>NS</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>3.5 ± 0.6</td>
<td>3.8 ± 0.6</td>
<td>3.9 ± 0.5</td>
<td>3.8 ± 0.4</td>
<td>NS</td>
</tr>
<tr>
<td>Iron (µg/dL)</td>
<td>65.5 ± 20.4</td>
<td>95.2 ± 55.9</td>
<td>56.5 ± 12.7</td>
<td>76.8 ± 45.3</td>
<td>NS</td>
</tr>
<tr>
<td>Ferritin (µg/L)</td>
<td>295 ± 180.2</td>
<td>429.5 ± 360.8</td>
<td>316 ± 225.4</td>
<td>373.1 ± 302.8</td>
<td>NS</td>
</tr>
<tr>
<td>Total iron binding capacity (µg/dL)</td>
<td>657.7 ± 714.7</td>
<td>472.8 ± 408.9</td>
<td>501 ± 593.2</td>
<td>285.3 ± 108.3</td>
<td>NS</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>33 ± 5.3</td>
<td>32.9 ± 5.6</td>
<td>35.4 ± 5.1</td>
<td>34.1 ± 6.2</td>
<td>NS</td>
</tr>
</tbody>
</table>

a Values are expressed as means ± standard deviations.
b No significant difference between the four groups.
This trial is registered with ClinicalTrials.gov, number NCT01125033.

3. Results

Enrollment of patients started in March 2008; the trial was completed in February 2009 with no loss or exclusion. As demonstrated in Table 1, no statistically significant differences were found between the four groups in terms of age, sex ratio, baseline IRLS sum scores, and laboratory parameters. All of the enrolled patients were under regular treatment with erythropoietin and intravenous iron according to our regional HD guideline.

At the end of treatment, the IRLS sum scores decreased in all four groups; however, the decrements in means of IRLS sum scores in the vitamins C & E (10.3 ± 5.3, 95% CI: 7.4–13.3), vitamin C and placebo (10 ± 3.5, 95% CI: 8.1–11.9), and vitamin E and placebo groups (10.1 ± 6, 95% CI: 6.8–13.5) were significantly higher than in the double placebo group (3.1 ± 3, 95% CI: 1.5–4.8), (P < 0.001). Moreover, no differences in decrements of IRLS sum scores were observed between the treatment groups.

Two patients in the vitamins C & E, one in the vitamin E & placebo, and one in the double placebo group reported nausea during the first week of treatment; furthermore, one patient in the vitamins C & E, one in the vitamin C & placebo, and one in the vitamin E & placebo group reported dyspepsia during the first two weeks of the treatment. These symptoms were elicited by clinical staff during the HD sessions and none of them were severe enough to compel the patients to stop the treatment. Furthermore, no evidence of oxalois or its complications was observed in patients treated with vitamin C.

4. Discussion

Our study, for the first time, demonstrates the superior efficacy of vitamin C (200 mg daily), vitamin E (400 mg daily), and their combination in the treatment of RLS in HD patients compared with placebo. Moreover, these supplements were found to be safe and without serious side effects in the short-term.

Possible efficacy of vitamin E in the treatment of RLS was initially noticed when it alleviated symptoms of idiopathic RLS and leg cramp in some patients taking this vitamin for treatment of their dermatologic diseases [7]. Thereafter, it was found to be effective in a limited number of other patients suffering from idiopathic RLS [8]. This observed efficacy was attributed to the antioxidant properties of vitamin E. However, there are no reports on the efficacy of this vitamin or of vitamin C in the treatment of uremic RLS.

The pathophysiology of uremic RLS is complex and not well understood. Dopaminergic dysfunction is considered one of the major players in the pathophysiology of this disorder [14,15]. Dopaminergic insufficiency in RLS appears to be a functional insufficiency resulting from reduced synthesis of dopamine or its receptors in certain areas of the central nervous system [15]. Increased oxidative stress in HD patients is implicated in the pathogenesis of typical complications of ESRD [5]. Recently, patients with idiopathic RLS were found to have significantly higher total oxidant status than normal controls [6]. Furthermore, production of paraoxonase1 (PON1), a potent antioxidant enzyme, was found to be increased in these patients, as well as the serum levels of acetylcholinesterase. It is suggested that paraoxonase1 protects acetylcholinesterase from oxidative stress. In return, acetylcholinesterase protects dopamine from oxidative injury [6]. Therefore, these changes were considered as compensatory mechanisms that protect dopamine from oxidative injury. Moreover, PON1 activity in ESRD patients has been demonstrated to be lower than controls in several studies [16,17]. Therefore, oxidative stress may play an important role in the pathogenesis of uremic RLS. Levels of Vitamin C are usually low in HD patients due to its loss during HD and the dietary restriction of foods rich in vitamin C, like fresh fruits and vegetables, to avoid hyperkalemia [11]. Furthermore, HD patients have low intracellular levels of vitamin E [5]. These two antioxidants have been proposed as potential treatments for HD complications resulting from high oxidative stress like atherosclerosis and anemia [5,18]. Therefore, the perceived effect of vitamins C and E in the reduction of RLS symptoms in this study may be due to their antioxidant properties. However, this should be further evaluated in future studies by measuring the markers of oxidative injury before and after the treatment.

Because vitamin C augments the antioxidant function of vitamin E by regenerating x-tocopherol [19], we expected combination supplementation of vitamins C and E to be more effective than their separate administration, as was observed in the study of efficacy of these vitamins in the treatment of uremic leg cramps [9]. Although we did not observe the superior efficacy of combination therapy in our study, it may be observed in future studies with larger sample sizes or longer durations.

Tyrosine hydroxylase (TH) is one of the essential enzymes for the synthesis of dopamine in the brain [14]. There are in vitro studies that show vitamins C and E can induce TH production and increase dopamine synthesis in the neural cell lines [20,21]. Direct stimulation of TH by vitamins C and E and the resultant increased production of dopamine may be another mechanism for the observed efficacy of these supplements in the treatment of uremic RLS.

Iron deficiency is strongly implicated in the pathogenesis of RLS. HD patients may have higher than normal serum ferritin levels, as is observed in our patients. However, because ferritin is a positive acute phase reactant, these high levels are due to the chronic inflammatory state and functional iron deficiency that many HD patients suffer from and are not indicative of iron deficiency [22]. Iron supplementation has been shown to be effective in the treatment of uremic RLS [23]. Vitamin C as a reducing agent has been shown to increase gastrointestinal absorption of iron, to increase the bioavailability of IV iron after injection, and to release iron from ferritin and mobilize iron from the reticuloendothelial system to transferrin [11,18]. Of note in this regard, vitamin C administration to HD patients has been shown to reduce serum ferritin levels and to increase transferrin saturation [18]. Therefore, it may be interesting to compare the efficacy of vitamin C plus iron or iron placebo or, alternatively, iron with vitamin C or vitamin C placebo in the treatment of uremic RLS in future randomized blinded trials.

Deficiency of the endogenous opioid system is also implicated in the pathogenesis of RLS and a variety of opioids have been reported to be effective in the treatment of this disorder [3,24]. Vitamin C has been demonstrated to relieve pain and decrease opioid consumption in cancer patients [25]. Moreover, high concentrations of antioxidants such as vitamin C have been reported to inhibit the endogenous opioid degrading metalloenzyme and increase endorphin levels in in vitro studies [25]. Interestingly, oral administration of vitamin C supplemented with vitamin E has been shown to successfully suppress withdrawal syndrome in heroin addicts [25]. Therefore, induction of the endogenous opioid system by these vitamins may be another explanation for their observed efficacy in the treatment of uremic RLS.

Compared to larger randomized trials of various therapeutic agents for idiopathic RLS, most of uremic RLS trials, including our study, show lower placebo response [4,23,26]. This may be explained by the fact that ESRD patients usually consume a greater number of medications compared to idiopathic RLS patients in order to control for several metabolic derangements that result from...
renal failure. Therefore, addition of new placebo medications may have less psychological impact in uremic RLS patients than idiopathic RLS patients. Furthermore, placebo response has been shown to increase in RLS patients with increasing duration of trial [26]; therefore, the short duration of our study may be another explanation for its small placebo response.

Although an increase over most RLS studies [4], the low sample size is the main limitation to our study and the extrapolation of its results. The other limitation of this study is its short duration.

In conclusion, our study shows that vitamin E and C and their combination are safe and effective treatments for uremic RLS in the short term. These medications may be considered as alternative or additive treatments to current therapeutic remedies for this conundrum. Further studies with larger sample sizes are needed to evaluate their long term efficacy and safety and their possible mechanisms of action.

Conflict of Interest

The ICMJE Uniform Disclosure Form for Potential Conflicts of Interest associated with this article can be viewed by clicking on the following link: doi:10.1016/j.sleep.2011.11.010.

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