Original Article

25 hydroxy vitamin D is higher when a renal multivitamin is given with cholecalciferol at hemodialysis

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Background and Objectives: Seventy six hemodialysis (HD) patients were used in a prospective randomized and clinical trial to determine if a multivitamin with vitamin D (cholecalciferol 12,000 IU/week) given during dialysis would improve the vitamin D status of hemodialysis subjects. Methods and Study Design: Subjects were randomly assigned to two groups: 37 subjects were in the renal multivitamin without vitamin D (MV) group and 39 subjects were in a multivitamin route with vitamin D (MVD) group (12,000 IU of cholecalciferol per week). All subjects were given 2 multivitamin tablets at their 3 HD sessions each week for 20 weeks. Serum 25(OH)D, calcium (Ca), and phosphorus (P) levels were evaluated. Results: At baseline, mean serum 25(OH)D were below adequate (<30 ng/mL) in the MV group (23.5±12.2 ng/mL) and in the MVD group (20.8±10.3 ng/mL). A significant increase was seen in serum 25(OH)D levels (37.7±11.4 ng/mL; p<0.001) in the MVD group after vitamin D supplementation with no rise in the MV group value (21.7±11.4 ng/mL; p=0.06). Prior to supplementation, 17.9% of patients in the MVD group had adequate serum 25(OH)D level and post supplementation 76.9% in the MVD group had adequate serum 25(OH)D. In the MV group, 18.9% subjects had adequate serum 25(OH)D levels at baseline with 18.9% having 25(OH)D >30 ng/mL at the end of the study. There were no significant differences in group values for serum Ca and P. Conclusion: The majority of HD subjects given a multivitamin with cholecalciferol at dialysis had improvement in their vitamin D status.

Key Words: cholecalciferol, hemodialysis, vitamin D, calcium, supplementation

INTRODUCTION
While vitamin D has traditionally been a nutrient associated with bone health and mineral metabolism, it is now being reported to have additional benefits in regard to immune functioning and cardiovascular health. Cardiovascular disease is still a leading cause of mortality in the hemodialysis (HD) population and HD subjects with serum 25 hydroxy vitamin D (25(OH)D) levels less than 20 ng/mL have been reported to have increased overall and cardiovascular mortality.1 Hemodialysis subjects have diminished capacity for systemic production of the active form of vitamin D, calcitriol (1,25 dihydroxy vitamin D) as the kidney is the primary location of the 1-α hydroxylase enzyme, but at least 17 tissues synthesize 1,25 dihydroxy vitamin D and 35 have vitamin D receptors (VDR) leading to significant extra-skeletal activity of vitamin D.2-5

Serum 25(OH)D levels are often low in HD subjects with 79 to 91% of subjects reported to have serum 25(OH)D levels less than 30 ng/mL.5-7 The National Kidney Foundation-Kidney Disease Outcomes Quality Initiative (NKF-K/DOQI) guidelines indicate that vitamin D supplementation is recommended in patients with stage 3 to 4 Chronic Kidney Disease (CKD) with 25(OH)D levels <30 ng/mL.8 Similarly, another study recommended that all individuals with CKD should maintain a serum 25(OH)D level of greater than 30 ng/mL.9

Due to the numerous medications that are prescribed to HD patients, their pill burden is one of the highest report-

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ed for any disease.\textsuperscript{10} Compliance with taking medications is therefore problematic with 55.4% to 62.0% of patients being noncompliant.\textsuperscript{10-11} Based on the NKF-K/DOQI guideline, vitamin D insufficiency is widespread in the HD subjects.\textsuperscript{5,6,12} and low serum 25(OH)D levels are associated with mortality risk in this population.\textsuperscript{1} Thus, the purpose of this study was to evaluate the efficacy of providing HD subjects a multivitamin with vitamin D (cholecalciferol) three times per week at dialysis improve serum 25(OH)D levels.

**MATERIAL AND METHODS**

**Study design**

This was a prospective randomized trial of a multivitamin containing cholecalciferol or a multivitamin without cholecalciferol for 20 weeks on a cohort of HD subjects at one dialysis center in Lubbock, Texas. Subjects received either a multivitamin without cholecalciferol (MV) or a multivitamin with supplemental cholecalciferol (MVD) three times per week at the last hour of dialysis from the nursing staff. All subjects had serum collected in the fall before supplementation and after 20 weeks of vitamin supplementation in the winter and serum 25(OH)D, serum calcium, and serum phosphorous levels were evaluated.

**Subjects**

All procedures were approved by the Texas Tech University Institutional Review Board for Human Subjects (#: 503001). The registered dietitians at the Redbud Dialysis Center in Lubbock, Texas recruited 114 subjects using a recruitment script. All subjects signed consent forms prior to participation in the study. Exclusion criteria included known bone marrow disorders, malabsorption diseases, bone-related diseases, hypercalcemia, hypervitaminosis D, or the inability to understand and sign the consent and 27 subjects were excluded based on these criteria. Based on a power analysis test, the number of patients needed to be recruited in the current study was 27, however, more patients were recruited to increase the power of the study. Based on the number of subjects in this study, the power analysis statistical test yield was 100%. Subjects that were either lost to follow-up or were unable to continue with the study were excluded as well (5 died, 1 was transferred, 2 changed to peritoneal dialysis, 2 experienced lengthy hospitalizations, and 1 was changed to a routine of HD twice a week). Data were collected from subjects of any racial group, of either sex, and of any social economic status.

Subjects were randomly assigned to one of two groups and 76 hemodialysis subjects at the Redbud Dialysis Center in Lubbock, Texas completed the study. Thirty-seven subjects received MV (RenaPlex\textsuperscript{®}, Nephro-Tech, Inc), while 39 subjects received MVD (RenaPlex-D\textsuperscript{®}, Nephro-Tech, Inc). Pre-supplementation baseline demographic characteristics and mean serum values of subjects in the MV and MVD groups are presented in Table 1.

Both de-identified medical and laboratory data of the study subjects were retrieved from the Redbud Dialysis Center database and were provided to the researchers for the study. This study compared the collected data results from pre- and post-treatment lab values taken as per the standard treatment protocols required by the dialysis center.

**Study protocol involving vitamin supplementation**

All consenting subjects who participated in the study had a baseline serum 25(OH)D level <60 ng/mL. Subjects were given 6 multivitamin tablets weekly, two at each of their three dialysis sessions. All subjects received a renal multivitamin so both the control group (MV) and the vitamin D treatment group (MVD) received adequate water-soluble vitamin intake for the duration of the study. The vitamin and mineral content for each of the renal multivitamins that were provided during the study as well as the Tolerable Upper Intake (TUI) levels for each component are presented in Table 2.

The control multivitamin tablet contained zero IU of cholecalciferol. The vitamin D treatment multivitamin tablet contained 2,000 IU of cholecalciferol. Subjects in the MVD group received a total of 12,000 IU of cholecalciferol each week (1,714 IU/day) for the 20-week duration of the study. The treatment amounts of vitamin D did not exceed the TUI levels. A nurse administered the two renal multivitamins to each subject during the last hour of each dialysis session and this administration method ensured that vitamin caplets were taken as scheduled. Thus, compliance with treatment was not a potential limitation.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Treatments</th>
<th>p-value*</th>
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<tbody>
<tr>
<td></td>
<td>MV (n=37)</td>
<td>MVD (n=39)</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>59.6±10.6</td>
<td>60.8±12.3</td>
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<tr>
<td>Gender (% women)</td>
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<td>36</td>
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<tr>
<td>Race (%)</td>
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<td></td>
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<tr>
<td>Black</td>
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<td>Hispanic</td>
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<tr>
<td>Diabetes (%)</td>
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<td>64</td>
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<tr>
<td>BMI (kg/m^2)</td>
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<td>25(OH)D Vitamin D (ng/mL)</td>
<td>23.5±12.2</td>
<td>20.8±10.3</td>
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<td>Calcium (mg/dL)</td>
<td>8.61±0.95</td>
<td>8.57±0.96</td>
</tr>
<tr>
<td>Phosphorus (mg/dL)</td>
<td>5.61±1.24</td>
<td>5.51±1.48</td>
</tr>
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</table>

BMI: body mass index.
*Group mean values for laboratory tests were not significant; independent sample’s t-test, 2 tailed significance.
of the study.

Blood draw and lab protocol
Blood samples were taken in a non-fasting state pre-dialysis. Spectra East Laboratories Inc. (Rockleigh, New Jersey) completed the analyses on the pre- and post-treatment blood samples as per the dialysis center protocol and the laboratory test results were obtained from the subjects’ medical records. The 25(OH)D was analyzed using a chemiluminescent assay.

Statistical analysis
Descriptive statistics comprised of mean and standard deviation values were determined for all study variables. A p-value of p<0.05 was considered statistically significant when comparing the differences of mean values. A paired sample t-test was implemented to define any statistically significant difference between pre- and post-supplementation means for all laboratory test results for each group. An independent sample student’s t-test was used to assess for any significant differences between mean laboratory test values at baseline or after vitamin D supplementation in the MV and MVD groups.

RESULTS
In the baseline data pre-supplementation, mean serum 25(OH)D levels were below adequate (<30 ng/mL) in the MV group and the MVD group (23.5±12.2 and 20.8±10.3 ng/mL for the MV and MVD groups, respectively; Table 2) and no significant differences (p=0.32) were observed between mean values in the two groups. Pre-supplementation, the range of serum 25 (OH)D was 8.0 to 55.9 ng/mL in the MV group and 4.5 to 49.6 ng/mL in the MVD group. In the MV group, 7 subjects (18.9%) and in the MVD group, 7 subjects (17.9%) had serum 25(OH)D values above 30 ng/mL. As obesity is associated with lower serum 25(OH)D levels,1 body mass index (BMI) was determined (kg/m²). There were no significant differences in the mean BMI values of the MV and MVD groups or in pre- and post-supplementation values in either the MV or the MVD groups (Table 3).

Post-supplementation, the range of serum 25(OH)D was 9.8 to 50.9 ng/mL in the MV group and 12.8 to 64 ng/mL in the MVD group. In the MVD group, thirty subjects (76.9%) had serum 25(OH)D levels greater than 30 ng/mL post-supplementation as compared to only seven (17.9%) subjects with levels >30 ng/mL pre-supplementation. In the MV group, pre- and post-supplementation serum 25(OH)D levels were not significantly different (p=0.06) (23.5±12.2 versus 21.7±11.4 ng/mL). Only 7 (18.9%) subjects in the MV group had serum 25(OH)D levels pre-supplementation >30 ng/mL, which did not change during the study period. No subjects had a serum 25(OH)D level >100 ng/mL at any time during the study.

There were no significant differences between MV and MVD group mean serum calcium or phosphorus pre- or post-supplementation, and pre-supplementation and post-

<table>
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<tr>
<th>Vitamin/mineral</th>
<th>Multivitamin Renaplex® 1 tablet</th>
<th>Multivitamin with D Renaplex-D® 1 tablet</th>
<th>Intake per day: Multivitamin Renaplex®</th>
<th>Intake per day: Multivitamin with D Renaplex-D®</th>
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<td>E, IU</td>
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<td>17</td>
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<td>1714</td>
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TUL: Tolerable upper limit per day (Institute of Medicine 2000); ND: Not determined.

Table 3. Pre- and post- vitamin D supplementation mean values of multivitamin group (MV) and multivitamin with vitamin D (MVD) group of hemodialysis subjects

<table>
<thead>
<tr>
<th>Vitamin/mineral</th>
<th>MV (n=37)</th>
<th>Pre-trial</th>
<th>Post-trial</th>
<th>p-value</th>
<th>MVD (n=39)</th>
<th>Pre-trial</th>
<th>Post-trial</th>
<th>p-value</th>
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<tbody>
<tr>
<td>25 (OH)D (ng/mL)</td>
<td>23.5±12.2</td>
<td>21.7±11.4</td>
<td>0.063</td>
<td>20.8±10.3</td>
<td>37.7±11.4</td>
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<tr>
<td>Calcium (mg/dL)</td>
<td>8.61±0.95</td>
<td>8.67±1.02</td>
<td>0.616</td>
<td>8.50±0.95</td>
<td>8.57±0.96</td>
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<tr>
<td>Phosphorus (mg/dL)</td>
<td>5.61±1.24</td>
<td>5.64±1.70</td>
<td>0.913</td>
<td>5.51±1.48</td>
<td>5.37±1.29</td>
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<td>BMI (kg/m²)</td>
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<td>30.1±8.21</td>
<td>30.0±8.37</td>
<td>0.822</td>
<td></td>
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</table>

BMI: Body mass index.

*Pre- and post-supplementation values significantly different (p<0.0001); paired sample t-test.
**MV and MVD group mean values significantly different post-supplementation (p<0.0001); independent sample student’s t-test, 2 tailed significance.
supplementation values were not significantly different for either group (Table 3). The K/DOQI (2003) guidelines define hypercalcemia as a corrected total calcium value greater than 10.2 mg/dL. Only one subject in the MV group was found to have high corrected serum calcium of 10.8 mg/dL after supplementation.

DISCUSSION
Consistent with previous studies, the percentage (81.4%) of HD subjects in the current study with below optimal serum 25(OH)D levels was high and the mean pre-supplementation level of 25(OH)D was lower than the KDOQI recommended 30 ng/mL level. The current study further confirms the fact that vitamin D inadequacy is common in the HD population as both groups had inadequate vitamin D (<30 ng/mL) levels prior to the initiation of the study with mean±SD serum 25(OH)D values of 23.5±12.2 ng/mL in the MV group and 20.8±10.3 ng/mL in the MVD group. Jean et al12 found that 89% of HD subjects (mean serum 25(OH)D 12.8 ng/dL) in their cholecalciferol supplementation study had low serum vitamin D prior to the initiation of vitamin D supplementation. Similarly, Blair et al13 indicated that 80% of HD subjects involved in their ergocalciferol supplementation study were found to have vitamin D levels less than 31 ng/mL. Saab et al14 had a similar finding with 91% of hemodialysis subjects having 25(OH)D levels below the recommended amount of 30 ng/mL. These and the current study confirm that suboptimal vitamin D status is very common in the HD population.

Cardiovascular disease is still a leading cause of death in HD subjects and HD subjects with serum 25(OH)D levels less than 20 ng/mL were found to have increased overall and cardiovascular mortality.1 In subjects in this study, 54.3% of subjects had serum 25(OH)D levels less than 20 ng/mL. Vitamin D deficiency (serum 25(OH)D <10 ng/mL) in HD subjects with diabetes was found by Drechsler et al14 to be associated with a three-fold increase in the risk of sudden cardiac death as well as a 30% increase in stroke risk. For every unit decrease in 25(OH)D levels there was a two-fold increase in deaths related to infections in the subjects with a severe vitamin D deficiency (<20 ng/mL). Based on the possible negative health consequences and prevalence of low serum vitamin D levels, research on the efficacy of different administration methods of vitamin D is warranted.

Both ergocalciferol and cholecalciferol have been used in research trials either in a high dose monthly or daily dosing schedules but not in the form of cholecalciferol in a multivitamin given at dialysis as in the current study. Vitamin D2 (ergocalciferol) and vitamin D3 (cholecalciferol) are similar in structure, metabolism and function.16,17 Ergocalciferol has a unique side chain distinguishable from cholecalciferol, but both undergo the same two hydroxylation mechanisms to initially transform into 25(OH)D then eventually become hydroxylated to form activated vitamin D.16,18 While there is still some data to the contrary, cholecalciferol has generally been found to be more effective in correcting low serum vitamin D levels than equivalent doses of ergocalciferol in non-dialysis subjects.19

At the end of the current study, the mean serum 25(OH)D was significantly higher in subjects that received the MVD than in subjects that received the MV with a mean increase of 16.9 ng/mL and 76.9% of subjects had serum 25(OH)D levels greater than 30 ng/mL. Holick et al16 reported that each 100 IU dose of cholecalciferol per day would increase the serum 25(OH)D level by one ng/mL and the results of the current study are as would have been predicted with a 1,714 IU daily dose resulting in a 16.9 ng/mL increase in mean serum 25(OH)D levels (0.98 ng/mL rise per 100 IU). Jean et al12 documented the efficiency of a set monthly dose of 100,000 IU cholecalciferol provided to HD subjects that were vitamin D deficient and had a 29.5 ng/mL increase for a 0.89 ng/mL rise in 25(OH)D per 100 IU vitamin D. At the end of the 15-month study, 91% of the HD subjects had an improved 25(OH)D level that was above the target of 30 ng/mL with a mean value of 40 ng/mL. Matias et al19 similarly found that serum 25(OH)D improved compared to the baseline (22.3±12.0 vs 42.0±12.1 ng/mL for pre- and post-supplementation, respectively) in HD patients when supplemented with either 10,000 or 50,000 IU cholecalciferol weekly for a year. Saab et al15 used 50,000 IU per month of ergocalciferol in HD subjects and reported a 36.7 ng/mL rise in serum 25(OH)D in 6 months. In a retrospective study, Blaire et al13 found that in stage 5 CKD subjects, a 50,000 IU ergocalciferol supplement each week improved serum 25(OH)D by 23.6 ng/mL in 24 weeks. Due to variation in initial serum 25(OH)D levels in subjects, varying doses, forms, administration schedules, season of the year when values were measured, and length of time of the studies, it is difficult to draw conclusions about which type or administration schedule for vitamin D is best in the HD population but the vitamin D protocols all resulted in improvement in mean serum vitamin D levels.

Conclusion
Administration of vitamin D in a multivitamin at dialysis was a safe and effective means to improve serum 25(OH)D levels in most HD subjects. As some of the subjects in the MVD group did not have acceptable serum levels of 25(OH)D at the conclusion of the supplementation period, more research is needed regarding the optimal dose of cholecalciferol to give at dialysis for this population. Subjects with serum 25(OH)D levels indicative of deficiency will frequently need higher doses of vitamin D to achieve normal serum levels. In conclusion, the findings in this study provide further support for previous research on the effectiveness of cholecalciferol in improving serum 25(OH)D levels in HD subjects. This study also indicates that an oral dose of 12,000 IU cholecalciferol a week in a multivitamin is a dose that is safe and effective for the treatment of low vitamin D in most patients undergoing HD treatment.

REFERENCES
2. Norman AW. From vitamin D to hormone D: fundamentals of the vitamin D endocrine system essential for good health.
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Original Article

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患者在血液透析时给予含胆钙化醇的复合维生素其血清25-羟维生素D浓度高

背景与目的：为确定在透析时给予含维生素D（胆钙化醇12,000 IU/周）的复合维生素是否能够改善血液透析患者的维生素D状态，进行该项包括76位血液透析（HD）患者的前瞻性随机临床试验。方法与研究设计：受试者被随机分为两组：37位分在不含维生素D的复合维生素组（MV），另外39位分在含维生素D的复合维生素组（MVD，胆钙化醇12,000 IU/周）。所有受试者在HD第3阶段每周给予2片复合维生素，持续20周。评估其血清25(OH)D、钙和磷浓度。结果：两组患者基线平均血清25(OH)D均不足(<30 ng/mL)，MV组为23.5±12.2 ng/mL，MVD组为20.8±10.3 ng/mL。补充维生素D之后，MVD组血清25(OH)D浓度显著升高37.7±11.4 ng/mL；p<0.001，而MV组血清25(OH)D浓度没有升高(21.7±11.4 ng/mL；p=0.06)。补充前，MVD组有17.9%的患者血清25(OH)D充足，补充后，MVD组有76.9%的患者血清25(OH)D充足。在MV组，基线时有18.9%的患者血清25(OH)D充足，试验结束时，血清25(OH)D>30 ng/mL的患者仍然只占18.9%。两组血清钙和磷在补充前后没有显著变化。结论：大多数HD患者在透析时给予含胆钙化醇的复合维生素可以改善他们的维生素D状态。

关键词：胆钙化醇、血液透析、维生素D、钙、补充