Evaluation of Effect of Vitamin D Deficiency on Anemia and Erythropoietin Hyporesponsiveness in Patients of Chronic Kidney Disease

N Nand¹, R Mittal²

Abstract

Background: The role of vitamin D deficiency and inflammation levels in renal anemia has been documented. However, no study is available in India where the role of vitamin D supplementation in patients with hyporesponsiveness to increased doses of erythropoietin is available. Hence this study.

Material and Methods: This study was conducted on 50 adult patients of CKD, on regular, twice weekly hemodialysis. It included 38 cases in group A with deficient serum vitamin D levels (<30 ng/ml) and 12 cases in group B with sufficient vitamin D levels (>30 ng/ml). Both groups of cases were receiving erythropoietin in a dose of 4000 I.U. subcutaneously twice weekly following dialysis and had failed to show rise in hemoglobin (Hb) >1gm/dl after one month, hence erythropoietin was increased to 6000 I.U. orally, once a week for the next three months along with oral vitamin D supplement. Group A was given additional vitamin D in a dose of 6000 I.U. subcutaneously twice weekly following dialysis and had failed to show rise in hemoglobin (Hb) >1gm/dl after one month, hence erythropoietin was increased to 6000 I.U. Group A was given additional vitamin D in a dose of 60000 I.U. orally, once a week for the next three months along with vitamin D supplement. Both groups of cases were receiving erythropoietin in a dose of 4000 I.U. subcutaneously twice weekly following dialysis and had failed to show rise in hemoglobin (Hb) >1gm/dl after one month, hence erythropoietin was increased to 6000 I.U. Group A was given additional vitamin D in a dose of 60000 I.U. orally, once a week for the next three months along with vitamin D supplement. Both groups of cases were receiving erythropoietin in a dose of 4000 I.U. subcutaneously twice weekly following dialysis and had failed to show rise in hemoglobin (Hb) >1gm/dl after one month, hence erythropoietin was increased to 6000 I.U. Group A was given additional vitamin D in a dose of 60000 I.U. orally, once a week for the next three months along with vitamin D supplement.

Results: Basal ERI, HsCRP and ESR and serum ferritin were raised in both the groups. At the end of four months, there was a significant increase in the Hb and hematocrit (Hct) (p<.001) and a significant fall in ERI, ESR, HsCRP and serum ferritin which was not significant. Basal vitamin D and ERI had a positive and insignificant correlation (r=0.05; p=0.756) in group A where as a negative and significant correlation was observed between them at the end of four months (r= -0.195; p >0.05).

Conclusion: vitamin D play an important role in reducing inflammation and thereby in the cure of anemia in EPO hyporesponsive CKD patients and needs to be supplemented, if deficiency is found.

Editorial Viewpoint

- Vitamin D deficiency place a role in CKD associated anemia.
- This study establishes role of vitamin D in reducing inflammation and thereby correction of anemia in EPO hyporesponsive CKD patients.

Introduction

Induction of erythropoiesis stimulating agents (ESAs) has been the most important advancement in treatment of anemia in CKD. Benefits of ESA therapy include reduced blood transfusions, improved survival, improvement in quality of life, reduction in cardiovascular complications including myocardial infarction, heart failure and LVH. Resistance to ESAs is an increasing problem influencing the successful management of anemia in patients of CKD. Minimising ESA resistance is a major beneficial factor in realization of full potential of ESA therapy. Identification of causes that enhance EPO responsiveness can optimize the management of anemia improving both the financial costs and safety of ESA therapy.¹

Most important causes of...
ESA resistance include absolute or functional iron deficiency, inflammation, infection, ineffective dialysis, nutritional factor deficiencies, and non compliance. Given the role of inflammation in the CKD anemia, recently the focus has shifted to explore the association of agents with anti-inflammatory properties, such as vitamin D with low hemoglobin levels and ESA resistance. More recent analysis of the Third National Health and Nutrition Examination Survey (NHANES III) has provided evidence of a linear association between 25(OH) vitamin D deficiency in renal anemia. However, studies were conducted on western population and Indian data is scarce in this regard. Hence, this study was planned to assess role of vitamin D in the cure of anemia and EPO hyporesponsiveness associated with CKD.

**Material and Methods**

Patients of CKD, undergoing regular maintenance hemodialysis in this institute were screened. All these patients were receiving injection Recombinant Human Erythropoietin (rHuEPO) 4000 I.U. subcutaneously twice weekly and injectable iron in a dose of 100 mg/ week following dialysis session. The patients were observed for one month and rise in hemoglobin values were seen for each patient. 65 patients were found to show EPO hyporesponsiveness as they had inadequate rise in Hb (<1 g/dl rise in Hb in one month). Depending on serum vitamin D levels, these patients were divided into two groups A and B. Group A included 49 patients with deficient serum vitamin D levels (<30 ng/ml) and group B included 16 patients with sufficient vitamin D levels (>30 ng/ml). For both, group A and B patients, dose of erythropoietin was increased to 6000 I.U. and response was seen after one month. Both the groups were given daily doses of calcium and vitamin D (500 mg and 250 I.U. respectively). Group A was given additional vitamin D (cholecalciferol) in a dose of 60000 I.U. orally, once a week for three months where as Group B served as control. 15 patients couldn’t complete the study, therefore 50 patients (38 in group A and 12 in group B) completed the study. Hematological and renal parameters including ESR and HsCRP, serum ferritin were repeated every month for four months. Serum vitamin D [25(OH) D3], serum iPTH, serum vitamin B12 and folic acid were measured at the start and end of the study.

25(OH)D3 was measured on Elecsys 2010 by electrochemiluminescence method. iPTH was measured on the Elecsys 1010 using a sandwich principle. Serum ferritin was measured using a two-site sandwich immunoassay based on direct chemiluminometric technology. Hs-CRP was measured using latex enhanced immunonephelometric assay. ERI was used as an index to evaluate dose response effect by calculating weekly EPO dose per kg of body weight, divided by the Hb concentration (weekly EPO dose/ kg weight/g/dl Hb).

For comparison of parameters in two groups, Student’s t test was used and p-values were obtained to determine the statistical significance. For comparison of a parameter within the group at two point of time during follow up, paired students t test was used. For comparison of means of different parameters at 0, 1, 2, and 3 months Baseline, Group A and Group B were compared using paired student’s t test. Table 1: Baseline parameters and treatment response 0 to 4 months

### Table 1: Baseline parameters and treatment response 0 to 4 months

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group A (mean±S.D.)</th>
<th>P value (paired)</th>
<th>Group B (mean±S.D.)</th>
<th>P value (paired)</th>
<th>P value (un-paired)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin (g/dl)</td>
<td>6.94±0.42</td>
<td>11.01±0.46</td>
<td>&lt;0.001</td>
<td>7.15±0.42</td>
<td>11.05±0.64</td>
</tr>
<tr>
<td>Hematocrit % (PCV)</td>
<td>20.82±1.28</td>
<td>33.04±1.40</td>
<td>&lt;0.001</td>
<td>21.47±1.26</td>
<td>33.15±1.93</td>
</tr>
<tr>
<td>Blood Urea (mg%)</td>
<td>107.15±13.68</td>
<td>64.2±7.85</td>
<td>&lt;0.001</td>
<td>113.83±10.39</td>
<td>61.33±6.32</td>
</tr>
<tr>
<td>Serum Creatinine (mg%)</td>
<td>7.30±1.01</td>
<td>4.91±0.74</td>
<td>&lt;0.001</td>
<td>6.93±1.42</td>
<td>4.57±0.58</td>
</tr>
<tr>
<td>Serum Calcium (mg%)</td>
<td>7.25±0.55</td>
<td>10.0±0.32</td>
<td>&lt;0.001</td>
<td>9.04±0.60</td>
<td>8.9±0.2</td>
</tr>
<tr>
<td>Serum Phosphate (mg%)</td>
<td>2.45±0.31</td>
<td>3.88±0.31</td>
<td>&lt;0.001</td>
<td>3.90±0.62</td>
<td>3.81±0.22</td>
</tr>
<tr>
<td>Serum Urine acid (mg%)</td>
<td>7.44±0.99</td>
<td>5.17±0.95</td>
<td>&lt;0.001</td>
<td>6.94±0.77</td>
<td>4.05±0.57</td>
</tr>
<tr>
<td>Serum protein (g/dl)</td>
<td>6.05±0.68</td>
<td>6.26±0.30</td>
<td>0.07</td>
<td>6.33±0.43</td>
<td>6.6±0.25</td>
</tr>
<tr>
<td>Proteinuria (g/day)</td>
<td>1.16±0.39</td>
<td>1.05±0.30</td>
<td>0.30</td>
<td>1.31±0.55</td>
<td>1.21±0.32</td>
</tr>
<tr>
<td>G.F.R.(ml/min/1.73m²)</td>
<td>9.90±2.84</td>
<td>8.6±2.45</td>
<td>0.07</td>
<td>9.10±0.22</td>
<td>7.98±1.01</td>
</tr>
<tr>
<td>Vit. B12 (ng/ml)</td>
<td>445.34±61.22</td>
<td>455.63±58.56</td>
<td>0.07</td>
<td>437.33±73.39</td>
<td>458.25±50.76</td>
</tr>
<tr>
<td>Folic acid (pg/ml)</td>
<td>7.36±1.29</td>
<td>7.59±1.32</td>
<td>0.122</td>
<td>6.9±1.15</td>
<td>7.15±1.24</td>
</tr>
<tr>
<td>iPTH (pg/ml)</td>
<td>300.81±44.98</td>
<td>130.56±30.12</td>
<td>&lt;0.001</td>
<td>128.83±11.72</td>
<td>125.41±11.93</td>
</tr>
<tr>
<td>Vitamin D (ng/ml)</td>
<td>21.28±4.60</td>
<td>77.47±5.49</td>
<td>&lt;0.001</td>
<td>77.08±5.33</td>
<td>79.83±8.61</td>
</tr>
<tr>
<td>ESR (mm 1st hr)</td>
<td>54.31±5.94</td>
<td>24.31±2.33</td>
<td>&lt;0.001</td>
<td>31.33±5.31</td>
<td>26.75±3.13</td>
</tr>
<tr>
<td>S. Ferritin (ng/ml)</td>
<td>537.13±77.02</td>
<td>301.69±40.17</td>
<td>&lt;0.001</td>
<td>308.5±54.35</td>
<td>256.3±51.22</td>
</tr>
<tr>
<td>HsCRP (mg/dl)</td>
<td>3.87±0.55</td>
<td>1.39±0.43</td>
<td>&lt;0.001</td>
<td>2.7±0.42</td>
<td>2.03±0.30</td>
</tr>
</tbody>
</table>

(NS: Not significant)
3 and 4 months, repeated measures analysis of variance (ANOVA) test was used. The p values were two tailed and probability level of significant difference was set at <0.05.

**Results**

There were 20 males and 18 females in group A and 9 males and 3 females in group B. No statistically significant difference (P>0.05) was observed between the baseline hematological parameters of both groups (Table 1). There was no statistically significant difference between the baseline biochemical parameters of both groups (P>0.05) except serum calcium, serum phosphate, iPTH and vitamin D which were found to be significant (P<0.001) in group A compared to group B (Table 1) as group A patients were vitamin D deficient.

The basal ERI, ESR, HsCRP and serum ferritin were raised and were 33.67±7.40, 54.31±5.94, 3.87±0.55, 437.71±33.59 and 29.16±2.94, 30.58±3.57, 2.7±0.42, 308.5±54.35 in groups A and B respectively. All the parameters were significantly higher (P<0.001) in group A as compared to B suggesting higher levels of inflammation in vitamin D deficient group. Further, these patients failed to have a rise in Hb and Hct even after increase in dose of erythropoietin for one month and Hb and Hct were 6.94±0.42 and 20.82±1.28 and 7.05±0.55 and 21.17±1.66 respectively after one month of 6000 I.U. erythropoietin (p=0.211). On addition of vitamin D 60000 I.U., there was a significant fall in all the inflammatory markers (ESR, HsCRP and serum ferritin levels) and rise in Hb after one month and this trend continued for next three months (Figures 1 and 2). On the other hand, there was rise in Hb and Hct after one month of increase in erythropoietin in group B (P>0.05) except serum calcium, serum phosphate, iPTH and vitamin D which were found to be significant (P<0.001) in group A compared to group B (Table 1) as group A patients were vitamin D deficient.

ERI in group A was correlated with vitamin D at baseline in group A patients by using Spearman coefficient of correlation and an insignificant correlation was observed between them (r=0.05; p=0.756) (Figure 3). Where as a negative and insignificant correlation was observed between them at the end of four months. (r= -0.195; p >0.05). These findings infer that there was negligible effect of increased dose of erythropoietin itself on reducing inflammatory parameters, the significant effect was seen only after vitamin D was administered in addition to erythropoietin treatment to the subjects.

**Discussion**

Anemia is an important complication of CKD and it is usually severe in ESRD. ESAs have been among the most important advancement in the treatment of anemia in CKD, however, approximately 5–10% of patients with CKD demonstrate resistance to ESAs which is reportedly associated with adverse outcomes, such as increased cardiovascular morbidity, faster progression to ESRD and all-cause mortality. Inflammation has been considered one of the most important cause of erythropoietin hyporesponsiveness and in turn renal anemia.

Various studies show that patients with CKD have increased levels of inflammatory markers such as C-reactive protein and the cytokines IL-1, IL-6, interferon-γ (IFN-γ) and tumor necrosis factor-α (TNF-α). In CKD patients, vitamin D deficiency may stimulate immune cells within the bone marrow micro-

![Fig. 1: Change in levels of HSCRP & ESR during the study in both groups](image-url)
A number of possible mechanisms have been suggested to explain the effect of vitamin D on the required EPO dosage in ESRD patients. First, both in vitro and in vivo studies have shown the effect of Vitamin D on bone marrow precursor cells and their efficacy in the formation of red blood cells. Also, this vitamin is known to decrease inflammation in the tissues which might decrease the hyporesponsiveness to ESAs to some extent. And at last, the fact that many types of tissues express the Vitamin D receptors such as parathyroid and erythroid precursor cells, both of which are known as the tissues involved in ESA hyporesponsiveness, suggests that the modulation of these tissues with Vitamin D might increase the response to EPO treatment.

Further, the continued high levels of HsCRP in group B patients suggest inflammation as the cause of hyporesponsiveness in these cases.

In a comparable study, twelve patients received oral alfacalcidol at a dosage of 6-7 micrograms per week and calcium carbonate during the first 12 months, calcium without alfacalcidol during the next 3 months, and again alfacalcidol and calcium during the last 3 months. There was an increase in Hb during the first 3 months (P<0.005 vs before), a stabilization in Hb during the next 9 months (n.s.), and a decrease in Hb during the second phase (P<0.005 vs 12 months), and a re-increase in Hb during the three phases: third phase (P<0.005 vs 15 months). The results of the study further substantiated these findings as CKD cases with deficient vitamin D levels had raised inflammatory markers including ESR, HsCRP and serum ferritin. All these markers significantly decreased following supplementation of vitamin D with rise in Hb and hematocrit.
In this study, observed values of serum calcium, serum phosphate and serum iPTH show statistically significant difference from baseline to end of four months in group A while no such difference was observed in group B over the same period on these parameters. These findings are similar with the results of a recent study which showed that mean plasma iPTH decreased significantly in the calcifediol recipient group as compared to placebo group (p <0.005) and concluded that oral modified release calcifediol appears safe and highly effective in treating secondary hyperparathyroidism associated with vitamin D insufficiency in CKD. Reflecting on the underlying mechanism, a study has shown that oral modified release calcifediol appears safe and highly effective in treating secondary hyperparathyroidism associated with vitamin D insufficiency in CKD. Considering the potential importance of vitamin D in the treatment of secondary hyperparathyroidism, it is important to investigate the role of vitamin D in the pathogenesis and treatment of anemia in CKD patients. The findings of the present study suggest that vitamin D supplementation may have a beneficial effect on anemia management in these patients, especially in those with vitamin D deficiency. Therefore, further studies are needed to investigate the role of vitamin D in the treatment of anemia in CKD patients.

References