Evaluation of Effect of Ascorbic Acid on Ferritin and Erythropoietin Resistance in Patients of Chronic Kidney Disease

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Abstract
This study was planned to evaluate the effect of short term intravenous ascorbic acid on reducing ferritin and erythropoietin resistance in patients of chronic kidney disease (CKD) on maintenance haemodialysis (MHD).

Methods: Forty adult patients [20 patients in group A with increased serum ferritin level (>500 ng/ml), transferrin saturation (TSAT) ≤20% and 20 patients in group B with normal serum ferritin level (<200 ng/ml), TSAT ≤20%) of end stage renal disease (ESRD) with erythropoietin hyporesponsiveness undergoing maintenance hemodialysis were included in the study. Group A was given intravenous (i.v.) ascorbic acid in a dose of 500 mg once a week after each 4 hours session of dialysis for 3 weeks in a month (total 1500 mg/month), for a period of 3 months along with erythropoietin 6000 IU subcutaneous (S/C) twice weekly without iron therapy. Group B was given erythropoietin (6000 IU S/C twice weekly after each hemodialysis) and intravenous (IV) iron 100 mg/week for 3 months. Hematological and renal investigations, erythrocyte sedimentation rate (ESR), high sensitivity C-reactive protein (HsCRP), serum ferritin and TSAT were done at baseline and then one monthly intervals for three months whereas intact parathyroid hormone (iPTH) was measured at the start and end of the study.

Results: At the end of 3 months of study, in group A, Hemoglobin (Hb) and TSAT significantly increased while ferritin, HsCRP and erythropoietin resistance index (ERI) decreased significantly. In group B, the increase in Hb and TSAT were not significant statistically while ferritin increased significantly and fall in HsCRP and ERI were not significant statistically. The mean rise in Hb between subsequent months was higher in group A as compared to group B.

Conclusion: Short term i.v ascorbic acid could be a new successful adjuvant in reducing ferritin and erythropoietin resistance and enhancing Hb and TSAT in CKD patients on MHD.

Editorial Viewpoint
- Ascorbic acid, as an adjuvant therapy with epoetin, reduces epoetin requirements in CKD patients.
- This study has done to evaluate effect of short-term I.V. ascorbic acid on reducing ferritin and erythropoietin resistance in CKD.
- The study finds ascorbic acid successfully in this with enhancing Hb and TSAT in CKD patients on MHD.

Introduction
The prevalence of CKD as per a recent Indian population based study¹ was reported to be 17.2% and anemia is a major co-morbidity of CKD patients. Replacement therapy with recombinant human erythropoietin (EPO) is a key treatment of anemia. The potential role of adjuvant therapies in enhancing the efficacy of EPO in patients receiving maintenance hemodialysis has received increasing attention in recent years.²⁻³ The important reason for adjuvant therapies is that they may help to reduce epoetin requirements or allow dialysis patients to achieve increased hemoglobin concentrations, and derive more cost-effectiveness and greater clinical benefits from epoetin treatment. Recent research highlights how the use of such epoetin adjuvant like ascorbic acid has the potential to improve the efficiency of anemia therapy in patients with kidney diseases.⁴ Ascorbic acid improves

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sensitivity to erythropoietin, either by increasing iron mobilization from tissue storage or by way of antioxidant effects. A few published studies during the past decade have addressed this issue. The commonest causes of ESA resistance are non-compliance, absolute or functional iron deficiency and inflammation. Administration of intravenous ascorbic acid to hemodialysis (HD) patients with functional iron deficiency may promote better anemia control and iron utilization. But all these previous studies were small and some were uncontrolled. The results are also varied and till now no study has been done in India. Therefore the present study was designed to know whether short term intravenous ascorbic acid could be a new successful adjuvant in reducing ferritin and erythropoietin resistance in CKD patients on maintenance hemodialysis.

**Material and Methods**

Forty adult patients [20 patients in group A with increased serum ferritin level (>500 ng/ml), transferrin saturation (TSAT) ≤20% and 20 patients in group B with normal serum ferritin level (<200 ng/ml), transferrin saturation ≤20%] of ESRD with erythropoietin hyporesponsiveness undergoing maintenance hemodialysis were included in the study. All the patients were subjected to detailed history, clinical examination and investigations with special reference to renal, hematological and special investigations included in the study protocol. A pre-informed written consent was obtained in every case. Group A was given intravenous ascorbic acid in a dose of 500 mg once a week after each dialysis for 3 weeks in a month (total 1500 mg/month), for a period of 3 months along with erythropoietin 6000 IU S/C twice weekly after each hemodialysis and iron therapy was not given to this group due to hyperferritinemia. Group B was given erythropoietin (6000 IU S/C twice weekly after each hemodialysis) and IV iron 100 mg/week for 3 months. Hematological and renal investigations, ESR, HsCRP, serum ferritin and transferrin saturation were done at baseline and then one monthly intervals for three months whereas iPTH was measured at the start and end of the study. Serum ferritin was measured using a two-site sandwich immunoassay based on direct chemiluminometric technology, which uses constant amounts of two anti-ferritin antibodies. Serum transferrin was measured using calorimetric method with precipitation. Hs-CRP was measured using latex – enhanced immunonephelometric assay. Intact Parathyroid hormone (iPTH) was measured on the Elecsys 1010 using a sandwich principle. Statistical analysis was performed using SPSS software version – 17.0. For comparison of means of same parameter in two groups unpaired students t test was used and p-values obtained to determine the statistical significance. For comparison of means of same parameter in a single group at two point of time during follow up paired students t test was used and p-values obtained to determine the statistical significance. For comparison of means of different parameters at 0, 1, 2, and 3 months repeated measures analysis of variance (ANOVA) test was used and p-values obtained to determine the statistical significance. The p values were two tailed and probability level of significant difference was set at <0.05 for both paired and unpaired students t test and ANOVA test.

**Results**

113 Patients of ESRD undergoing regular maintenance hemodialysis were screened. 62 patients were found to be erythropoietin resistant. The serum ferritin and transferrin saturation levels of these 62 patients were repeated and 52 patients with transferrin saturation of 20% or less were selected for the study. Depending on the serum ferritin levels, these 52 patients were divided into two groups A and B. Group A included 25 patients with increased serum ferritin level (>500 ng/ml) and Group B included 27 patients with normal serum ferritin level (<200 ng/ml). Out of these 52 patients, 12 patients couldn’t complete the study. 5 patients (2 in group A and 3 in group B) left the study in between due to some unknown causes and 7 patients (3 in group A and 4 in group B) expired during the study. So finally 40 patients (20 in each group) completed the study. Study included 12 males and 8 females patients in group A and 15 male and 5 female patients in group B. Mean age of the study participants was 45.5±14.54 years in group A and 49.8±12.79 years in group B. Hypertension was the most common cause of CKD (7 patients in each group) followed by diabetic nephropathy (6 in group A and 5 in group B), chronic glomerulonephritis (3 in each group) and obstructive uropathy (3 in each group). 1 patient in each group had chronic pyelonephritis and autosomal dominant polycystic kidney disease was present in 1 patient of group B. The various hematological and renal parameters in group A and group B were alike at baseline. The parameters at 1, 2 and 3 months are shown in Tables 1 and 2.

At the end of 3 months of study, in group A, Hb significantly increased from 6.78±0.75 to 10.01±0.54 g/dl (P <0.001) and TSAT increased from 18.55±1.41 to 34.21±3.25% (P <0.001), while ferritin, ESR, HsCRP and ERI decreased significantly from 1286±217.31 to 814.10±104.46 ng/ml (P <0.001), 53.05±8.09 to 30.08±7.03 mm 1st hr (P <0.001), 3.40±1.73 to 1.87±1.27 mg/dl (P <0.05) and 32.17±9.12 to 22.18±5.24 IU/kg/g Hb in 100 ml (P <0.001). In group B, the increase in Hb and TSAT were not significant statistically (P=0.359,
Table 1: Basic parameters in two groups

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group A (Mean±SD)</th>
<th>Group B (Mean±SD)</th>
<th>P value (paired)</th>
<th>P value (paired)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin (g/dl)</td>
<td>6.78±0.75</td>
<td>10.01±0.54</td>
<td>&lt;0.001</td>
<td>7.25±0.88</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>20.35±2.25</td>
<td>30.04±1.62</td>
<td>&lt;0.001</td>
<td>21.13±2.08</td>
</tr>
<tr>
<td>B.Urea (mg%)</td>
<td>114.10±16.63</td>
<td>100.15±13.09</td>
<td>0.03</td>
<td>118.4±13.12</td>
</tr>
<tr>
<td>S.Creatinine (mg%)</td>
<td>5.90±1.28</td>
<td>5.11±0.98</td>
<td>0.04</td>
<td>6.90±0.85</td>
</tr>
<tr>
<td>S.Phosphate (mg%)</td>
<td>6.45±1.02</td>
<td>5.65±0.69</td>
<td>0.04</td>
<td>6.49±1.08</td>
</tr>
<tr>
<td>Ferritin (ng/ml)</td>
<td>7.27±0.88</td>
<td>6.55±0.73</td>
<td>0.02</td>
<td>8.1±1.77</td>
</tr>
<tr>
<td>S.Uric Acid (mg%)</td>
<td>1286±217.31</td>
<td>814.10±104.46</td>
<td>&lt;0.001</td>
<td>178±13.90</td>
</tr>
<tr>
<td>S.Phosphate (mg%)</td>
<td>6.45±1.02</td>
<td>6.45±1.08</td>
<td>0.04</td>
<td>6.49±1.08</td>
</tr>
<tr>
<td>Ferritin (ng/ml)</td>
<td>349.05±44.53</td>
<td>238.2±49.60</td>
<td>&gt;0.05</td>
<td>252.6±31.47</td>
</tr>
<tr>
<td>TSAT (%)</td>
<td>18.55±1.41</td>
<td>34.21±3.25</td>
<td>&lt;0.001</td>
<td>18.69±1.40</td>
</tr>
<tr>
<td>iPTH (pg/ml)</td>
<td>34.0±1.73</td>
<td>18.71±1.27</td>
<td>&lt;0.05</td>
<td>4.03±1.10</td>
</tr>
<tr>
<td>S.albumin (mg/dl)</td>
<td>3.8±0.80</td>
<td>3.89±0.69</td>
<td>0.886</td>
<td>3.59±0.44</td>
</tr>
</tbody>
</table>

Table 2: Change in ERI in two groups

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group A Mean±SD</th>
<th>Group B Mean±SD</th>
<th>P value (at 3 months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ERI (IU/kg/g) Hb in 100 ml</td>
<td>0 Month</td>
<td>1 Month</td>
<td>2 Months</td>
</tr>
<tr>
<td>Group A</td>
<td>32.17±9.12</td>
<td>32.57±9.11</td>
<td>30.44±8.41</td>
</tr>
<tr>
<td>Group B</td>
<td>29.25±9.03</td>
<td>27.27±9.14</td>
<td>30.07±8.62</td>
</tr>
</tbody>
</table>

P=0.810 respectively) while ferritin increased significantly from 178±13.90 to 269.75±21.04 ng/ml (P <0.001) and fall in ESR, HsCRP and ERI were not significant statistically (P=0.282, P=0.628, P=0.769 respectively). The mean rise in hemoglobin between subsequent months was higher in group A as compared to group B. ERI in group A was correlated with baseline hemoglobin and other inflammatory parameters by using Spearman coefficient of correlation and it was observed that ERI was negatively correlated to hemoglobin, hematocrit, serum ferritin and serum albumin (p >0.05) and positively correlated to ESR and HsCRP (p >0.05) but this correlation was statistically not significant. The changes in serum sodium, serum potassium, serum calcium, serum proteins, iPTH, proteinuria and GFR, blood pressure in both the groups were not found to be statistically significant (p>0.05).

**Discussion**

Management of anemia in patients with ESRD with EPO has been a major advance. The use of EPO has decreased the amount of blood transfusions and enhanced the quality of life in the ESRD patients. EPO hyporesponsiveness is reported in HD patients. To resolve EPO hypo responsiveness, it has been recommended that the dosage of EPO be gradually increased. However, the probable undesirable side effects related to the use of high erythropoietin doses in theory has led physicians to reduce the dosage of erythropoietin.

“Trapped” iron storage or decreased availability of iron is the most common factor for the resistance to the effect of ESAs. Hemodialysis patients may suffer from anemia, despite an iron overload which we call as “functional iron deficiency anemia”. This is because in patients with kidney diseases, especially on dialysis, iron tends to be shifted out of circulation into storage tissues, making it less available for erythropoiesis. This syndrome of decreased accessibility of storage iron is referred to as functional iron deficiency anemia. This condition is characterized by low TSAT, despite normal or increased total body iron storage (TSAT ≤20% and ferritin ≥500 ng/ml). Inflammation has also been considered as one of the most important cause of erythropoietin hyporesponsiveness and in turn anemia. Inflammation also causes increased ferritin production and impaired transferrin saturation and this prevents iron delivery to erythrocytes precursors by shunting iron to reticuloendothelial storage pool leading to a state of functional iron deficiency.

In this study, we found that the use of low amount of intravenous ascorbic acid for short duration in patients who had hyperferritinemia and EPO hyporesponsiveness (Group A) improved anemia, transferrin saturation significantly and also reduced the high level of ferritin, inflammatory parameters (HsCRP, ESR) and erythropoietin resistance significantly in these patients. In group B patients with normal ferritin level and EPO hyporesponsiveness, where only erythropoietin and intravenous iron was given (no adjuvant therapy), the rise in hemoglobin, transferrin saturation was not significant and there was also a significant rise in serum ferritin levels, with no significant fall in erythropoietin resistance index, suggestive of persistent EPO hyporesponsiveness.

The results of this study in group A is consistent with the hypothesis that ascorbic acid (a reducing
Excessive vitamin C treatment may worsen uremic-related oxalosis. Hence, supplementation not exceeding 150 mg/day (1050 mg weekly) is still considered a safe dose. Therefore in this study, the dose 500 mg of IV ascorbic acid once a week for 3 weeks in a month (total 1500 mg/month), was less than the recommended regimen. The bioavailability is variable in HD patients receiving oral ascorbic acid. Furthermore, gastrointestinal upset (especially at a large dose) and noncompliance may reduce the efficacy of oral treatment. Therefore we administered ascorbic acid intravenously in this study. We did not measure the plasma oxalate level, which is another limitation of this study. The significant decrease in inflammatory parameters levels in this study in group A could be attributed to amelioration of oxidative stress and inflammation by ascorbic acid.

In this study, ERI was used as an index to evaluate the dose-response effect of EPO therapy. In group A, it was found that there was a fall in ERI at the end of 3 months, which was statistically significant, whereas in group B, the fall in ERI was not significant statistically. This suggests that ascorbic acid given to group A cases has decreased EPO resistance probably by overcoming EPO hyporesponsiveness secondary to functional iron deficiency state through improved iron utilization and anti-inflammatory property. Other workers also showed a decrease in erythropoietin resistance and dose of EPO decreased by 24% in 50% of the patients, 30% in 65% of the patients respectively, following intravenous ascorbic acid therapy.

The main differences of this study from the others are as follows: (1) fixed dose of erythropoietin was used so that the adjuvant effect of ascorbic acid could be evaluated (2) at the time of hyperferritinemia, IV iron was not given to the patients (3) we used low dosage of intravenous ascorbic acid (total 1500 mg/month). The decision to use the lower dosage was based on limiting the probable collection of oxalate in patients, because we were not measuring oxalate levels.

We tried to exclude all other factors responsible for erythropoietin resistance. In this study rain canal water was used which did not contain aluminum. Every bout of infection was treated aggressively as early as possible. Therefore, probable factor which was operative for the less rise in hemoglobin and the marked rise in ferritin level in group B was most probably due to chronic inflammatory state which is essentially a basic feature of CKD.

Therefore short term intravenous ascorbic acid could be a new successful adjuvant in reducing ferritin and erythropoietin resistance in CKD patients on maintenance hemodialysis. However, further studies are needed to determine at what ferritin levels maximum response from intravenous ascorbic acid treatment could be attained, and to ascertain the best dosage interval for optimal effect and minimal possible toxicity.

References


