

Evaluation of the Effect of Erythropoietin and Vitamin E on Highly Sensitive C Reactive Protein in Patients of Chronic Kidney Disease with Erythropoietin Hyporesponsiveness.

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Abstract : Chronic inflammation leads to higher oxidative burden in uremic patients, before and after renal replacement therapies. Higher concentrations of biomarkers of oxidative stress have been identified as a major factor associated with erythropoietin hyporesponsiveness and anemia in patients of end stage renal disease (ESRD). Highly sensitive C reactive protein (Hs-CRP) is a powerful marker of inflammation, and has been found to be associated with endothelial dysfunction. This study was planned to assess whether increased dose of erythropoietin therapy and supplementation of Vitamin E in hemodialysis patients has any favorable effect in decreasing inflammation in erythropoietin hyporesponsiveness patients. A prospective study was done on 25 adults patients of ESRD undergoing twice weekly maintenance hemodialysis having erythropoietin hyporesponsiveness. Initially all patients on maintenance hemodialysis were enrolled in the study. They were administered 4000 IU recombinant erythropoietin after each dialysis session and 100 mg of injectable iron once a week, hemoglobin and Hs-CRP levels were measured at baseline and at end of 2 months to see erythropoietin responsiveness. 25 patients having erythropoietin hyporesponsiveness were included in the study and administered recombinant erythropoietin 6000 IU subcutaneously after each dialysis, injectable iron once a week and Vitamin E 400 IU daily. The effect of increased dose of erythropoietin and Vitamin E on Hs-CRP and hemoglobin was studied at 6 months. Patients of ESRD have chronic inflammatory state as evidenced by elevated levels of Hs-CRP at baseline. Further high levels of Hs-CRP were associated with plateau effect in the response to erythropoietin on the hemoglobin concentration. Increasing the dose of erythropoietin and by administering Vitamin E simultaneously, the inflammation level attenuated which in turn improved the erythropoietin hyporesponsiveness and hemoglobin level increased significantly ($p < 0.05$). However there was no significant effect of erythropoietin and Vitamin E on ESR. There was no significant correlation between the hemoglobin levels and Hs-CRP either at the start of study, 2 months and 6 months. **Conclusion-** Increasing the dose of weekly erythropoietin and administering Vitamin E simultaneously, the level of inflammation attenuates and erythropoietin hyporesponsiveness decreases leading to increase in hemoglobin level significantly.

INTRODUCTION

Chronic kidney disease (CKD) has emerged as a major public health problem globally associated with poor outcomes and high cost of living. Anemia is directly correlated with renal function impairment, and is associated with a variety of adverse pathophysiologically consequences, poor quality of life and increased morbidity and mortality associated with ESRD¹. Etiology of anemia in CKD is multifactorial and erythropoietin deficiency is the major cause. In the recent era chronic inflammation and proinflammatory cytokines leading to oxidative stress are gaining increased recognition as an important pathological process leading to erythropoietin hyporesponsiveness and anemia as well as leading to endothelial dysfunction and cardiovascular diseases in CKD patients². Hs-CRP is a very useful non specific biological marker of inflammation the “secret killer” of ESRD, and is also a strong predictor of outcome.³ Hs-CRP, IL-6 and TNF- α had a positive correlation with the required erythropoietin dose and with an index of erythropoietin resistance.⁴ However, there are a few studies which demonstrates the effect of erythropoietin therapy in CKD patients on Hs-CRP. Hence, this study was planned to assess whether increased dose of twice weekly erythropoietin therapy and supplementation with Vitamin have any favorable effect in decreasing inflammation, in patients showing poor

MATERIAL AND METHODS

A prospective study was done on 25 adults patients of CKD undergoing twice weekly maintenance hemodialysis having

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erythropoietin hyporesponsiveness. A preformed written consent was undertaken in every case. Adult patients of CKD with hemoglobin less than 8.00 g/dl undergoing twice weekly dialysis irrespective of the cause of CKD were enrolled. Patients having chronic inflammatory conditions like rheumatoid arthritis, ankylosing spondylitis psoriasis and inflammatory bowel disease, had undergone recent surgery and malignancy were excluded from the study.

All the patients on twice weekly hemodialysis were given 1000 mg intravenous iron before the start of study to replenish the iron stores. Patients were administered twice weekly recombinant human erythropoietin 4,000 IU s/c along with injectable iron 100 mg in 100 ml of normal saline once a week. Patient's hematological profile, renal parameters and Hs-CRP were measured at baseline and after 2 months. At the end of 2 months Hs-CRP levels were reassessed using latex enhanced immunophelometric assays along with hemoglobin and packed cell volume. Patients showing a rise in hemoglobin concentration of less than 1g/dl/month were classified as poor responder and 25 such patients were included in the study. Patients not having the appropriate response of erythropoietin on hemoglobin and hematocrit levels were then administered 6000 I.U. recombinant human erythropoietin subcutaneously twice weekly and 100 mg of injectable iron after each dialysis session. All the patients undergoing the study also received Vit E 400 i.u.daily. Hs-CRP was measured at 6 months to evaluate the response of increased dose of erythropoietin and vitamin E supplementation. Paired student 't' test was used to analyse the data for change in Hs-CRP levels and hemoglobin levels.

RESULTS

Study included 16 males and 9 females. Mean age of the participants was 40.72 ± 13.70 years with mean weight 56.32 ± 11.11 Kgs.

Hypertension (10) was the most common cause of CKD followed by chronic glomerulonephritis (6), diabetic nephropathy (4), autosomal dominant polycystic kidney disease (3), obstructive uropathy and renal amyloidosis was present in one patient each.

All the patients were severely anemic with a mean baseline hemoglobin of 6.096 ± 1.191 g/dl. The various renal parameters at baseline, 2 months, 4 months and 6 months are shown in table 1. Hemoglobin levels of participants at two, four and six months were 6.248 ± 0.820 , 7.748 ± 0.672 and 8.461 ± 0.496 g/dL, respectively. The rise in hemoglobin at 2 months was not significant ($p > 0.05$) indicating erythropoietin hyporesponsiveness. However after increasing dose of erythropoietin therapy and supplementation of 400 mg of Vitamin E there was significant rise in hemoglobin levels at end of 4 and 6 months ($p < 0.05$) as compared to levels at the baseline (Table 1 and Fig 1).

Baseline mean Hs-CRP level was 3.057 ± 3.331 mg/L. the level increased significantly at two months and was 6.122 ± 6.210 mg/L ($p < 0.05$). Hs-CRP decreased at end of six months was 4.882 ± 4.778 mg/L ($p < 0.05$) indicating that increased dose of twice weekly erythropoietin and supplementation of Vitamin E had a significant effect on lowering the inflammation in CKD patients as depicted with fall of Hs-CRP levels. Patients of ESRD had chronic inflammatory state as shown with elevated levels of Hs-CRP. Hs-CRP levels were further assessed separately into three age groups of

Table-1: Showing blood parameters at baseline, 2 months and 6 months

Parameters (Blood)	Baseline	2 months	P (paired)	4 months	P (paired)	6 months	P (paired)
Hb (g/dl)	6.09 ± 1.19	6.25 ± 0.82	>0.05	7.75 ± 0.67	<0.05	8.42 ± 0.49	<0.05
PCV%	20.13 ± 3.97	21.28 ± 3.28	>0.05	26.85 ± 2.88	<0.05	30.9 ± 2.6	<0.05
B.Urea (mg%)	248.1 ± 43.5	154.6 ± 34.3	<0.0001	133.4 ± 19.4	<0.0001	115.7 ± 25.9	<0.0001
Creatinine (mg%)	9.5 ± 2.6	7.2 ± 1.7	<0.0001	6.5 ± 0.9	<0.0001	6.3 ± 0.3	<0.0001
Uric acid (mg%)	7.6 ± 2.4	6.8 ± 1.9	<0.0003	6.4 ± 1.4	<0.0004	5.6 ± 1.4	<0.0001
Calcium (mg%)	7.5 ± 1.0	8.0 ± 0.6	<0.006	8.1 ± 0.5	<0.004	8.6 ± 0.5	<0.0001
Phosphorus (mg%)	6.3 ± 1.6	5.5 ± 1	<0.0001	5.1 ± 0.7	<0.0001	4.6 ± 0.7	<0.0001

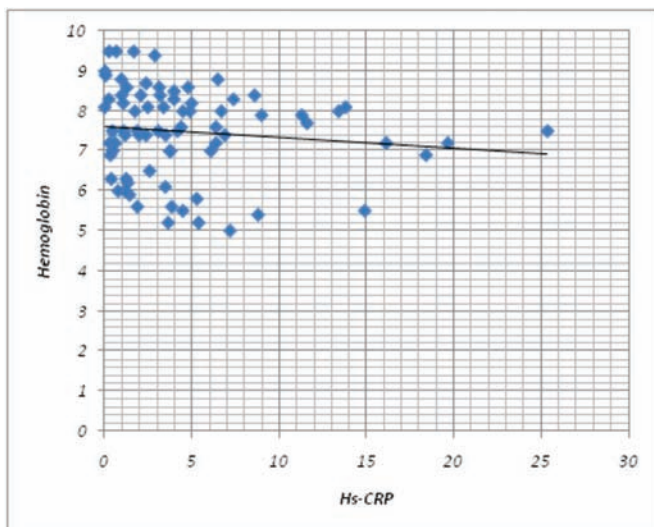


Fig. 1: Correlation between Hs-CRP and Hemoglobin

Table-2: Showing Hs-CRP levels at baseline, 2 and 6 months

parameters	0 month	2 month	P (paired)	6 month	P (paired)
Hs-CRP (mg/l)	3.057 ± 3.331	6.122 ± 6.210	<0.05	4.882 ± 4.778	<0.05
S.Ferritin(μ g/L)	310.796 ± 243.86	600.972 ± 407.92	<0.05	490.08 ± 325.42	<0.05
ESR (mm 1 st hr)	78.64 ± 38.93	93.28 ± 28.80	<0.05	86.96 ± 15.92	>0.05

20-40, 41-60 and > 60 years separately to assess if there was significant effect in some subgroup. Results were statistically non significant in any subgroups ($p > 0.05$).

Correlation analysis was done to assess the effect of baseline Hs-CRP levels and hemoglobin levels at baseline, at 2 month and at six months and it was found that there was negative correlation between Hs-CRP and Hemoglobin over the course of study ($r = 0.149$, -0.048 and -0.264 , respectively). It was observed that there was no statistically significant effect of baseline Hs-CRP levels on serial hemoglobin levels over six which was probably due to small sample size. Further when in order to find any relationship between hemoglobin and Hs-CRP all the data irrespective of time were plotted and it was found that there was a negative correlation between the two but it was not statistically significant ($r = -0.122$, " p " > 0.05).

It was observed that three out of 25 patients had deterioration in their hypertension control. Their, antihypertensive medications had to be increased appropriately. One patient had flu like episodes three times after the administration of subcutaneous erythropoietin which was controlled with paracetamol. In two patients there was clot formation in the A-V fistula, None of the patients developed seizures with the erythropoietin therapy during the course of study period.

DISCUSSION

CKD is associated with significantly higher rates of morbidity and mortality including a substantial loss of quality of life despite improvement in renal replacement technology over the last five decades. Cardiovascular disease is the leading cause of morbidity and mortality in CKD⁵. The multifold increase in cardiovascular risk in these patients may be related to traditional risk factors and those specific to CKD comprising of anemia, hyperphosphatemia, hyperparathyroidism, inflammation, fluid imbalance and endothelial dysfunction. Evidence suggests that inflammation is an important factor associated with hemoglobin variability and it has emerged as a major confounding factor associated with worsening of anemia, resistance to hormones such as erythropoietin and insulin, catabolism, cardiovascular diseases and oxidative stress leading to increased mortality as well as morbidity⁶. Gunnell et al in a study concluded that inflammation was a major determinant of erythropoietin dose requirement⁷. Inflammation may be due to bacterial or viral infections, surgical trauma including vascular access surgery, heart failure and renal or systemic inflammatory diseases and type of dialyzer etc⁸.

Renal failure is associated with increase in oxidative agents like reactive oxygen species, as well as loss of antioxidants including superoxide dismutase, catalase, Vitamin C, vitamin E, zinc, selenium etc leading to increased oxidative stress⁹. Vitamin E is a potent antioxidant that terminates the lipid peroxidation and hence inhibiting the production of malondialdehyde, reduces the oxidative stress which in turn is associated with decreased atherogenesis, cardiovascular risk and erythropoietin resistance¹⁰. Treatment with Vitamin E may reduce the requirement for recombinant erythropoietin by decreasing the erythropoietin resistance, in addition it also increases the

erythroid colony formation.

Exposure of blood to bio-incompatible dialysis membranes causes activation of circulating mononuclear cells and has been implicated as a potential cause of inflammation induction within the patients of ESRD¹¹. The inflammatory and reactive oxygen species systems could lead to endothelial dysfunction which is an important predictor of long term prognosis. Inflammatory response begins with the release of interleukin - 1 (IL-1) and tumor necrosis factor- α (TNF- α) by monocytes and macrophages that subsequently activates a complex cascade of other inflammatory mediators including IL-2, IL-6 and IL-8. IL-1 and TNF- α stimulate the production of acute phase proteins which include High sensitivity C- reactive protein (HsCRP), serum amyloid A (SAA), ferritin, haptoglobin, complement 3 and β_1 -acid glycoprotein. IL-6 augments the synthesis of fibrinogen, α -1 antitrypsin and β_2 macroglulin¹². Levels of IL-6, IL-1 and TNF- α have been found to be significantly raised in renal failure patients and negative acute phase reactants including Serum albumin, S. transferrin, S. Iron, S. Fetuin etc are decreased.

In ESRD, Hs-CRP has been proven to be a strong predictor of both cardiovascular and all cause mortality associated with oxidative stress, erythropoietin hyporesponsiveness, vascular calcification and endothelial dysfunction⁶. Hs-CRP, a pentameric protein synthesized in liver, opsonizes infection and activates complement, remains unaffected by food intake and circadian variation and remains stable over time, is inexpensive and widely available as compared to other markers, and hence emerged as an important inflammatory marker in CKD¹³. Studies have also estimated that on average, the required erythropoietin dose to maintain a certain hemoglobin level may be increased by 30-70% in dialysis patients with serum CRP > 2 mg/L as compared to those with a lower CRP concentration¹⁴.

The availability of erythropoiesis-stimulating agents (ESAs) has revolutionized the treatment of anemia in patients with chronic kidney disease. Fluctuation of hemoglobin levels or 'Hemoglobin variability' during treatment with ESAs is a well-documented phenomenon. Several factors are believed to contribute to variation in the Hemoglobin level, including patient comorbidities and intercurrent events. Inflammation is an important factor associated with hemoglobin variability and that Hs-CRP (a widely used surrogate marker of inflammatory activity) is a predictor for less stable hemoglobin control in CKD patients⁷. Recent experimental evidence suggests that EPO has biologic effects distant from its traditional site of action, via the erythroid tissue. EPO has been shown to possess antiapoptotic effects in many (nonerythroid) cell lines. Further, animal experiments demonstrate a beneficial effect of EPO after induced ischemia of various organs (heart, brain, spinal cord, kidney, and retina), and EPO was found to be protective in ischemic and toxic acute renal failure models¹⁵. In a rat study in which animals subjected to subtotal nephrectomy were administered darbepoietin alfa or saline, the former group had a reduced death rate and a lesser degree of kidney damage. A recent area of interest relates to certain circulating cells of bone marrow origin called endothelial progenitor cells (EPC), which migrate to sites of vascular injury and lead to repair. CKD is associated with a decrease in the number and a decline in the function of EPCs, and EPO boosts EPC number¹⁶.

ESR rose significantly ('p' <0.05) at 2 months probably due to inflammation, but it did not change significantly at 6 months indicating that increased dose of erythropoietin had no significant effect on ESR. Serum ferritin level increased significantly (<0.05) at

2 months as well as 6 months as compared to baseline which was due to administration of intravenous iron 1 every week. An attempt was made to find out the factors responsible for erythropoietin hyporesponsiveness. In this study, rain canal water was being used in reverse osmosis (R. O.) system which did not contain aluminium. Iron stores were replenished by administering 1000 mg of iron intravenously and citrate dialysate was used for hemodialysis procedure. Elemental calcium carbonate and activated Vitamin D was used to suppress secondary hyperparathyroidism and arteriovenous grafts of all cases had been working properly. We used biocompatible polysulphone dialysis membrane (single use) for each dialysis session. Therefore only factor which was operative in these cases was chronic inflammatory state. After administration of increased doses of recombinant erythropoietin and Vitamin E supplementation there was significant rise in hemoglobin and fall in Hs-CRP was significant, indicating that increased dose of erythropoietin and addition of vitamin E attenuated inflammation and decreased oxidative stress. Further rise in Hs-CRP at end of 2 months suggest that erythropoietin resistance was most probably due to inflammation. It was observed during the study that there was a negative correlation between hemoglobin levels and Hs-CRP levels at 2 months (r= -0.084) and 6 months (r= -0.264) however this correlation was found to be statistically insignificant (p > 0.05). Reason behind this could probably be due to small sample size.

CONCLUSIONS

High Sensitivity C-Reactive Protein - a sensitive marker of inflammation is raised in CKD patients. Moderate doses of erythropoietin though effective in treating anemia CKD. By increasing the dose of weekly erythropoietin and administering Vitamin E the level of inflammation attenuates and erythropoietin hyporesponsiveness decreases leading to increase in hemoglobin level significantly.

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