

Effect of Erythropoietin on Highly Sensitive C - Reactive Protein Levels in Cases of Chronic Kidney Disease undergoing Maintenance Hemodialysis

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Abstract: The role of chronic inflammation in the development of anemia and erythropoiesis stimulating agents (ESA) hypo responsiveness is now gaining increasing attention as potential factor that might adversely affect patient outcome. In cases of chronic kidney disease (CKD) C-reactive protein (CRP) is probably the most notorious inflammatory marker. Recent research is destined to focus on future therapy of inflammation and its related complications. Anti-inflammatory properties of erythropoietin have been recently recognized. Hence, this study was undertaken to assess the effect of erythropoietin therapy on highly sensitive C-reactive protein (Hs-CRP) levels in CKD patients. 25 adult patients of end stage renal disease (ESRD) who were undergoing twice weekly maintenance hemodialysis were administered subcutaneously 4000 I.U. of recombinant human erythropoietin (rHuePo) twice weekly following dialysis. The effect of erythropoietin on Hs-CRP and hemoglobin over six months was studied. There was significant response ($p < 0.05$) of erythropoietin on hemoglobin after 2 months of rHuePo therapy, but a plateau effect was observed thereafter. Hs-CRP levels were within normal range in the study participants and rHuePo showed no significant effect ($p > 0.05$) in decreasing Hs-CRP levels. Erythropoietin improves anemia of CKD, though target levels can only be achieved by increasing the dose of erythropoietin. Hs-CRP levels didn't decrease with anti-inflammatory (rHuePo) therapy as envisaged with twice weekly erythropoietin therapy.

INTRODUCTION

CKD is emerging as a major public health problem globally. Anemia is a well known and is a major complication of CKD. Its presence is considered one of the hallmarks of chronicity of renal disease and the degree of anemia correlates well with loss of renal function.¹ The role of chronic inflammation in the development of anemia and erythropoiesis stimulating agents hypo responsiveness is now gaining increasing attention as potential factors that might adversely affect patient outcomes.^{2,3} Hyperparathyroidism, chronic inflammation, aluminium toxicity and iron deficiency are major causes of erythropoietin resistance in CKD with anemia.

Inflammation in CKD could be due to bacterial or viral infections, surgical trauma including vascular access surgery, heart failure and renal or systemic inflammatory diseases.⁴ It leads to worsening anemia, resistance to hormones such as erythropoietin and insulin, catabolism, and oxidative stress. The inflammatory and reactive oxygen species systems, besides enhancing each other, could lead to endothelial dysfunction, an important predictor of long term prognosis.⁴ Therefore; the focus of ongoing research is directed to look for various molecules which can reduce the inflammation in CKD patients thereby decreasing excess morbidity arising out of anemia and cardiovascular disease mortality. Potential treatment strategies which have been advocated include selective anticytokine therapy like anti-TNF- α antibodies, soluble TNF receptors and IL-1, IL-6 receptor antagonists.³ Statins are also reported having some role in reducing inflammation.⁵

Recognizing Hs-CRP as a cause of poor response to erythropoietin in ESRD patients, there arises a need to decrease its levels with various treatment options available. Further, as erythropoietin has been shown to have anti-inflammatory effect, this study was planned to assess whether there is chronic inflammation in cases of CKD undergoing maintenance hemodialysis and erythropoietin has (if any) Hs-CRP lowering effect or not.

MATERIAL AND METHODS

A total of 25 adult patients of CKD who were undergoing twice weekly maintenance hemodialysis were included. A pre-informed consent was obtained in every case. Patient's baseline hemoglobin, total leucocytes

count, differential leucocytes count, Hs-CRP, and other baseline renal parameters including blood urea, serum creatinine, serum uric acid, serum calcium, serum phosphate, serum sodium, serum potassium and Creatinine clearance were estimated. Patients were administered twice weekly recombinant human erythropoietin 4000 I.U. s/c after 4 hours of hemodialysis session along with injectable iron 100 mg in 100ml of normal saline weekly. Patients were followed for 6 months and hematological and renal parameters were assessed every 2 months. Hs-CRP levels were reassessed after 6 months of rHuepo therapy. Data was analyzed for change in Hs-CRP and hemoglobin concentration using Paired student 't' test. Their correlation analysis was done using Pearson correlation test.

RESULTS

The mean age of the patients was 43.28 ± 29.11 years. There were 20 men and 5 women. Hypertension was the most common cause of CKD with 8 patients followed by chronic glomerulonephritis (7), diabetic nephropathy (4), obstructive uropathy (3), autosomal dominant polycystic kidney disease (2) and anlysis from (1) renal amyloidosis.

All patients had severe anemia and mean hemoglobin was 7.168 ± 0.9114 g/dL at the baseline with total leucocyte count of 10296 ± 4276.143 /mm³. The various renal parameters at baseline, two months, four months and six months are shown in table 1. The level of hemoglobin increased significantly however it reached a plateau effect at two months as shown in fig. 1. Hemoglobin rose from 7.168 ± 0.9114 g/dL at baseline to 7.984 ± 1.673 , 7.912 ± 1.619 , 8.028 ± 1.001 g/dL at two, four and six months respectively and rise was significant ($p < 0.05$) at two, four and six months.

Mean baseline Hs-CRP values were within normal limits as defined in KDQOI guidelines. Hs-CRP changed from 1.919 ± 0.7722 mg/L at baseline to 1.702 ± 0.4444 mg/L at six months as shown in fig 2. The difference was statistically non-significant ($p > 0.05$) indicating that twice weekly erythropoietin therapy didn't influence the Hs-CRP levels. Further, correlation analysis was done between change in Hs-CRP levels over the study period and Hemoglobin levels over the same period as shown in fig 3. It shows that correlation between the variables was statistically non-significant ('p' = 0.185, r = -0.274) at start and at six months of study.

Hs-CRP levels were further assessed in three different age groups (at baseline and at 6 months) of 20-40, 40-60 and 60-80 years to determine the effect of age. However, there was no statistically significant effect ('p' > 0.05). Data was further analyzed in males and females separately.

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It was observed that though mean values decreased from 1.890 ± 0.826 mg/L to 1.663 ± 0.477 mg/L in the male participants and from 2.036 ± 0.564 mg/L to 1.696 ± 0.424 mg/L in the female participants, they were not statistically significant ($p > 0.05$).

When Hs-CRP levels were subdivided into two groups with values less than 2 mg/L (group 1) and more than 2mg/L (group 2), Group 1 had 13 patients with mean Hs-CRP levels of 1.30 ± 0.36 mg/L and Group 2 had 12 patients with Hs-CRP levels of 2.58 ± 0.48 mg/L. It was observed that Hemoglobin levels in Group 1 varied from 6.97 ± 0.94 g/dl at baseline, to 8.13 ± 2.22 at 2 months, to 8.05 ± 2.14 at 4 months to 7.87 ± 1.29 g/dL at six months. Similarly, Hemoglobin levels in group 2 varied from 7.37 ± 0.85 at baseline, to 7.82 ± 0.80 at 2 months, to 7.75 ± 0.79 at 4 months to 8.19 ± 0.55 g/dL at 6 months. However, change in hemoglobin was non-significant ($p > 0.05$) between the two groups as shown in fig 4.

Patients were evaluated for the development of complications of twice weekly erythropoietin therapy. It was observed that 4 had deterioration in their hypertension control. One patient had flu like episodes. 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.



Figure 1: Shows hemoglobin levels at baseline, two, four & six months after erythropoietin therapy.



Figure 2: Shows Hs-CRP levels at baseline & six months.

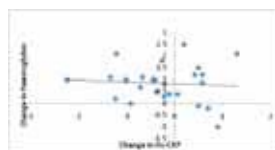


Figure 3: Shows correlation between rise in hemoglobin & fall in Hs-CRP levels after erythropoietin therapy.

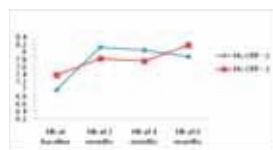


Figure 4: Shows change in hemoglobin in the two subgroups of patients having Hs-CRP > 2 and < 2 mg/L.

Table 1: Basic Parameters of Study Participants

Parameter	Baseline	At two months	At four months	At six months
Hemoglobin (g/dL)	7.168 ± 0.9114	7.984 ± 1.673* (<0.05)	7.912 ± 1.619* (<0.05)	8.028 ± 1.001* (<0.05)
PCV (%)	27.016 ± 1.781	29.20 ± 2.843* (<0.001)	29.120 ± 2.743* (<0.001)	29.52 ± 2.124* (<0.001)
TLC (per cm)	10296 ± 4276.143	8046 ± 2181.739* (0.011)	7288 ± 1239.73* (0.003)	7684 ± 1644.34* (0.002)
B. urea (mg/dL)	197.12 ± 76.94	138 ± 44.636* (<0.001)	118.96 ± 53.27* (<0.001)	108.80 ± 30.62* (<0.001)
S. creatinine (mg/dL)	8.648 ± 3.418	7.308 ± 2.198* (0.005)	6.6 ± 1.693* (<0.001)	6.560 ± 1.271* (<0.001)
S. calcium (mg/dL)	8.788 ± 1.241	9.196 ± 0.670* (0.02)	8.8 ± 0.924 (0.966)	9.020 ± 0.758 (0.423)
S. phosphate (mg/dL)	6.628 ± 2.044	6.056 ± 1.639 (0.065)	5.224 ± 1.638* (<0.001)	4.692 ± 1.335* (<0.001)
S. uric acid (mg/dL)	7.632 ± 3.033	6.556 ± 1.797* (0.032)	6.484 ± 1.415* (0.021)	6.272 ± 1.623* (0.010)
Hs-CRP (mg/L)	1.919 ± 0.7722	—	—	1.702 ± 0.4444

DISCUSSION

The main cause of mortality and morbidity in CKD patients is cardiovascular disease with an annual mortality rate of approximately 9% which is 10 to 20 fold higher than in general population, even when adjusted for age, gender, race and the presence of diabetes mellitus.⁶ A number of risk factors that provide rationale for the remarkable prevalence of vascular disease in ESRD have been recently identified and amongst these, inflammation has been the most important.⁷ Also inflammation in ESRD has been found to be associated with malnutrition, anemia and erythropoietin resistance.⁸ Renal failure contributes to inflammation as

a result of accumulation of pro-inflammatory compounds or products of metabolism. Inflammation and acute phase response interact with hematopoietic system at several levels resulting in reduced erythropoiesis, accelerated destruction of erythrocytes and blunting of the reactive increase in erythropoietin in response to reduced hemoglobin levels.⁹ Cytokines also cause anemia by inhibiting erythropoietin secretion.¹⁰

Several inflammation biomarkers¹¹ namely CRP, IL-6, Adiponectin, S. Ferritin, TLC count, Intercellular adhesion molecule-1 (ICAM-1), Vascular cell adhesion molecule (VCAM-1) and inflammatory molecules with negative acute-phase reaction namely S. Albumin, S. Transferrin, S. Iron, S. Fetuin have been defined for consideration as predictors of outcome in ESRD. Among these Hs-CRP, which is a pentameric protein synthesized in liver, opsonizes infection and activates complement has attracted the most interest.⁷ CRP is secreted by liver and inflammation causes a rapid increase in its serum concentration. It plays a role in host defense by interacting with complement. Compared to measurement of other markers of inflammation and the acute phase reaction, serum CRP has several advantages. It is a simple, reliable, readily available and inexpensive test. It is also a long term predictor of cardiovascular risk and mortality in the general population and in CKD patients.⁴ Furthermore, high plasma concentrations of C-reactive protein (CRP) have shown to be associated with anemia and ESA hypo responsiveness in chronic hemodialysis patients.¹²

Erythropoietin has revolutionized the treatment of anemia associated with CKD. However a proportion of patients treated with erythropoietin respond poorly or not at all, and in a subset of these, no obvious cause such as iron deficiency could be found. The interactions between different inflammatory mediators and erythropoietin response appear to be complex. Iain C. Macdougall,¹³ presented information regarding nonerythroid effects of erythropoietin. EPO receptors have been detected in many tissues, such as the brain and heart. EPO has been shown to possess antiapoptotic effects in many (nonerythroid) cell lines. In view of the increasing recognition of Hs-CRP as a cause of poor response to erythropoietin in ESRD patients, there arises a need to decrease its levels with various treatment options available. Further, as erythropoietin has been shown to have anti-inflammatory effect, this study was planned to assess whether twice weekly erythropoietin in patients undergoing maintenance hemodialysis has any Hs-CRP lowering effect or not.

The results of this study indicated that erythropoietin had no significant effect in lowering the inflammatory state. This finding may be related to the fact that Hs-CRP observed at baseline were.

REFERENCES

- McGonigle RJS, Wallin JD, Shaddock RK, Fisher JW. Erythropoietin deficiency and inhibition of erythropoiesis in renal insufficiency. *Kidney Int* 1984; 25: 437-44.
- Druke T. Hyporesponsiveness to recombinant human erythropoietin. *Nephrol Dial Transplant* 2001; 16(Suppl 7): 25-28
- Stenvinkel P. The role of inflammation in the anaemia of end-stage renal disease. *Nephrol Dial Transplant* 2001; 16(Suppl 7): 36-40
- Stenvinkel P, Heimburger O, Lindholm B, Kaysen GA, Bergstrom J. Are there two types of malnutrition in chronic renal failure? Evidence for relationships between malnutrition, inflammation and atherosclerosis (MIA syndrome). *Nephrol Dial Transplant* 2000; 15: 953-60.
- Menon V, Wang X, Greene T, Beck GJ, Kusek JW, Marcovina SM, et al. relationship between C-reactive protein, albumin and cardiovascular disease in patients of chronic kidney disease. *Am J Kidney Dis* 2003; 42: 44-52.
- Stenvinkel P, Alvestrand A. Inflammation in End-stage renal disease: sources, consequences and therapy. *Semin Dial* 2002; 15(5): 329-337.
- Stenvinkel P, Lindholm B. C-reactive protein in End stage renal disease: Are there reasons to measure it? *Blood Purification* 2005; 23: 72-8
- Barany P. Inflammation, serum C-reactive protein and erythropoietin resistance. *Nephrol Dial Transplant* 2001; 16: 224-7
- Dellanna F, Hetzel GR, Backus G et al. Hb-variation in ESRD patients- association between risk factors and ESA dose [abstract SA-PO028]. Presented at American Society of Nephrology Annual Congress, 14-19 November 2006, San Diego, USA
- Jelkmann W, Pagel H, Wolff M, Fandrey J. Monokines inhibiting erythropoietin production in human hepatoma cultures and isolated perfused rat kidneys. *Life Sci* 1992; 50: 301-8.
- Kalantar-Zadeh K. Inflammatory marker mania in chronic kidney disease: pentraxins at the crossroad of universal soldiers of inflammation. *Clin J Am Soc Nephrol* 2: 872-875, 2007.
- Barany P, Divino F, Bergstrom J. High C-reactive protein is a strong predictor of resistance to erythropoietin in hemodialysis patients. *Am J Kidney Dis* 1997; 29: 565-568
- McDougall IC. Role of erythropoietin therapy in CKD and CKD: Beyond hemoglobin. Non-conventional cardiovascular risks in chronic kidney disease. Program and abstracts of the American Society of Nephrology Renal Week 2006; November 13-19, 2006; San Diego, California
- de Francisco A. L. M., Stenvinkel P, Vaulont S. Inflammation and its impact on anaemia in chronic kidney disease: from haemoglobin variability to hyporesponsiveness. *NDT Plus* (2009) 2 [Suppl 1]: i18-i26
- Barany P. Inflammation, serum C-reactive protein and erythropoietin resistance. *Nephrol Dial Transplant* 2001; 16: 224-7