Inflammation and Erythropoietin Hyporesponsiveness – Role of Pentoxifylline - An Anti TNF-α agent.

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***Abstract** : **Introduction:** Resistance to erythropoiesis-stimulating agents (ESAs) is often associated with chronic inflammation present in patients of chronic kidney disease (CKD) on maintenance hemodialysis (MHD). This study was planned to investigate how anemia, ESA resistance and the plasma levels of biological markers of inflammation are interlinked to each other and whether Pentoxifylline (PTF) has any role in improving anemia in these subset of patients. **Methods:** 20 adult patients of end stage renal disease (ESRD) with erythropoietin hyporesponsiveness undergoing twice weekly maintenance hemodialysis were administered subcutaneously 6,000 IU of recombinant human erythropoietin (rHuEPo) twice weekly following dialysis for two months followed by addition of Pentoxifylline to erythropoietin for next four months to complete the study period of six months. The effect of erythropoietin and pentoxifylline on hemoglobin, hematocrit and inflammatory markers that included high-sensitivity C-reactive protein (hsCRP), tumor necrosis factor alpha (TNF-α), erythrocyte sedimentation rate (ESR), serum albumin and serum ferritin were studied. **Results:** There was a insignificant response of erythropoietin alone on hemoglobin and hematocrit after 2 months of rHuePo therapy ('p'>0.05), associated with insignificant decrease in hsCRP, TNF-á and ESR, respectively over 2 months but when Pentoxifylline was added to erythropoietin, the rise in hemoglobin and hematocrit was highly significant ('p'<0.01) which was associated with highly significant decrease in hsCRP, TNF-αand ESR, respectively after completion of the study ('p'<0.01). **Conclusion:** Pentoxifylline has significant effect in reducing the inflammatory parameters in CKD patients on MHD and therefore is a promising agent in improving anemia in patients of ESRD showing erythropoietin hyporesponsiveness, attributable to inflammation.*

***Key words:** Erythropoietin, Pentoxifylline, Highly sensitive C-reactive protein, Tumor necrosis factor alpha, Chronic kidney disease.*

INTRODUCTION

The incidence and prevalence of chronic kidney disease is increasing worldwide. The development of erythropoiesis-stimulating agents has resulted in substantial health benefits for patients with end-stage renal failure (ESRF), including improved quality of life, reduced blood transfusion requirements, decreased left ventricular mass, diminished sleep disturbance and enhanced exercise capacity. Most patients in CKD usually achieve desired target hemoglobin when supplemented with relatively low doses (80-120 IU/Kg/week) of Erythropoietin and parenteral iron (50-100 mg/week) with optimal rate of correction of anemia (increase in hematocrit by 4 – 6 %) or (increase in Hb by 1-2 g / dl) over four week period to achieve the goal values within 2-3 months[1]. Unfortunately, a considerable proportion of such patients exhibit a suboptimal hematologic response to ESA, as evidenced by the persistence of anemia despite adequate dosing, or the requirement of high doses to achieve recommended hemoglobin targets. The NKF-KDOQI defined the hyporesponsiveness to erythropoietin as the presence of at least one of the following three conditions: a significant decrease in Hb level at a constant ESA dose, a significant increase in the ESA dose requirement to preserve a certain Hb level, or a failure to raise the Hb level to greater than 11 g/dl despite an ESA equivalent to erythropoietin greater than 500 IU/kg/week[1]. The commonest causes of ESA resistance are non-compliance, absolute or functional iron deficiency and inflammation. Inflammation is being recognized with increasing frequency as a cause of ESA hyporesponsiveness[2]. Previous studies have clearly demonstrated an inverse association between inflammatory indices and ESA responsiveness[3]. The inflammatory cytokines are thought to directly inhibit erythropoiesis and promote apoptosis of erythroid precursors[2]. Potential treatment strategies which have been advocated include selective anticytokine therapy like anti-TNF-á antibodies, IL-1 or IL-6 receptor antagonists and statins[4]. Pentoxifylline is a competitive nonselective phosphodiesterase inhibitor, which inhibits TNF and leukotrienes synthesis, and reduces inflammation[4]. Only, few studies have shown that Pentoxifylline has a beneficial effect in reducing inflammation in CKD patients and improves anemia in patients showing hyporesponsiveness to ESA[5-7]. Hence, this study was planned to investigate how anemia, ESA resistance and the plasma levels of biological markers of inflammation are interlinked to each other and whether Pentoxifylline (PTF) has any role in improving anemia in these subset of patients.

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MATERIAL AND METHODS

20 adult patients of end stage renal disease with erythropoietin hyporesponsiveness undergoing twice weekly maintenance hemodialysis were selected. All the patients included in the study were already taking 4,000 IU of erythropoietin dose s.c. twice weekly with parenteral iron supplementation for two months with inadequate response. Patients were labeled erythropoietin resistant on the basis that these patients showed inadequate rise in hemoglobin (<1g/dl rise in Hb in one month) despite giving erythropoietin in increased doses of 6,000 IU s.c. twice weekly for two months. A preinformed written consent was obtained in every case and ethical clearance for the study was taken from board of studies in medicine and allied sciences of the institution. Every patient's baseline hemoglobin, hematocrit, hsCRP, TNF-α, ESR, serum albumin, serum ferritin and renal parameters were estimated. Hs-CRP was measured using latex-enhanced immunonephalometric assay, whereas TNF-α levels were

measured using TNF-α ELISA kit which is a solid phase sandwich ELISA for the in-vitro qualitative and quantitative determination of TNF-α in supernatants, buffered solutions or serum and plasma samples. The erythropoietin resistance index (ERI) was calculated as the weekly weight-adjusted dose of EPO divided by the hemoglobin level to evaluate the dose-response effect of EPO therapy[8]. For comparison ERI was divided into two group's d" or > 34 IU/kg/g Hb in 100 ml and association with different baseline parameters was studied. ERI was calculated for each patient at baseline, at 2 months and after 6 months to assess the change in ERI reflecting change in erythropoietin resistance. The patients were continued on EPO therapy in a dosage of 6000 IU s.c. twice weekly for two months followed by addition of Pentoxifylline in a dose of 400 mg orally daily for the period of next four months and response was seen. Hematological parameters were measured at baseline and repeated every 2 monthly to look for rise in hemoglobin and hematocrit whereas inflammatory parameters that included hsCRP, TNF-α, ESR, serum albumin and serum ferritin were measured at baseline, 2 and 6 months to see whether increased dose of erythropoietin alone or after addition of Pentoxifylline has any effect on decreasing these parameters.

Statistical analysis was performed using IBM SPSS software version - 20. When comparing groups according to ERI being above or below the median, Fisher's Exact test was used (as sample size was small) and Odds ratio with 95% confidence interval was calculated. Spearman's rank correlation coefficient was used to study correlations between ERI and different variables. Variables with an effect on ERI were analyzed using forward stepwise binary logistic Regression analysis, with ERI as dependent variable between two variables. For comparison of means of different parameters at 0, 2, 4, and 6 months Repeated measures Analysis Of Variance (ANOVA) test was used and in those parameters for which p value was found to be significant, Post Hoc test i.e.(Bonferroni Multiple Comparisons Test) was used to find the actual difference in parameters at 0, 2 and 6 months.

RESULTS

Study included 15 male and 5 female patients. Mean age of the study participants was 44.75 ± 16.52 years. Hypertension (12) was the most common cause of CKD followed by diabetic nephropathy (3), chronic glomerulonephritis (2), chronic pyelonephritis (2), and autosomal dominant polycystic kidney disease (1). The various renal and hematological parameters at baseline, 2, 4 and 6 months are shown in Table I. Mean baseline ERI for the entire group was 33.15 ± 9.13 IU/kg/week/g Hb per 100 ml. ERI was found to be significantly associated with presence of fluid overload with positive correlation ('p'= 0.019, RR= 4.5) although no significant association was found with age, gender, body mass index and diabetic status (Table 2). Further, ERI was inversely correlated with hemoglobin (r = - 0.717) and hematocrit (r = - 0.663) (Table 3), however, showed positive correlation with hsCRP (r = 0.726) (Figure 1) and TNF-á (r = 0.537) (Figure 2). On performing binary logistic regression analysis in the final model (Table 4), ERI was strongly correlated with hsCRP (crude OR=8.8, 95% C.I 1.25- 26.40, 'p'= 0.029) showing that hsCRP is a major contributor for erythropoietin resistance. ESR, ferritin and albumin were found to have no significant impact on ERI ('p'>0.05).

Inflammatory markers that included hsCRP, TNF-α, ESR, serum ferritin and serum albumin were measured before and after giving the drug for four months. HsCRP, TNF-α and ESR decreased significantly ('p'<0.01) from 2.96 ± 1.15 mg/dl, 61.10 ± 27.50 pg/ml and 46.50 ± 11.37 mm in Ist hour, respectively at 2 months (before giving the drug) to 1.11 ± 0.66 mg/dl, 27.99 ± 16.28 pg/ml and 32.45 ± 7.66 mm in Ist hour, respectively at 6 months (after giving the drug), but the change in serum ferritin and serum albumin levels from 631.15 ± 314.32 ng/ml and 3.0 ± 0.12 mg/dl at 2 months to 542.05 ± 159.5 ng/ml and 3.06 ± 0.12 mg/dl, respectively was not found to be statistically significant ('p'>0.05). All these parameters were also measured at the start of the study with baseline hsCRP, TNF-α, ESR values of 3.07 ± 1.56 mg/dl, 68.43 ± 32.24 pg/ml and 49.70 ±15.78 mm in Ist hour, respectively, which served as controls and the decrease in these parameters between 0 and 2 months was found to be statistically of no significance ('p'>0.05) suggesting that there is negligible effect of erythropoietin alone in suppressing inflammation. Similarly, baseline serum ferritin and serum albumin levels were 639.16 ± 446.90 ng/ml and 3.07 ± 0.29 g/dl, which did not showed significant change after 2 months ('p'>0.05). The average decrease in ERI between baseline and 2 months was 1.71 IU/kg/g Hb in100 ml which was statistitically not significant ('p'>0.05), but the average decrease in ERI between 2 and 6 months was 8.30 IU/kg/g Hb in100 ml found to be statistically significant (p <0.01) as shown in Figure 3.

Table 1: *Basic parameters during the study*

Parameter	Baseline (Mean ±S.D)	At 2Months (Mean±S.D)	At 4 Months (Mean ±S.D)	At 6Months (Mean±S.D)	ANOVA (P value)
Hemoglobin (g/dl)	6.82 ± 1.24	6.97 ± 1.07	7.99 ±0.89	9.02 ±0.77	**<0.0001***
Hematocrit (%)	20.45 ± 3.37	21.35 ± 2.97	24 ± 2.86	26.90 ±2.88	**<0.0001***
B.Urea (mg%)	207.25±52.26	145.20 ±19.01	123.00±23.45	125.75 ±23.86	**< 0.001***
S.Creatinine(mg%)	8.81 ± 3.09	6.99 ± 1.55	6.27 ±0.83	5.99 ± 1.33	**< 0.001***
S.Calcium(mg%)	8.47 ± 0.67	8.58 ± 0.69	8.54 ± 0.50	8.87 ± 0.37	0.1181
S.Phosphate(mg%)	7.02 ± 2.29	6.76 ± 1.28	6.13 ± 0.88	5.95 ± 0.80	**< 0.001***
S.Uric Acid(mg%)	8.03 ± 2.26	7.15 ± 1.15	6.55 ± 0.76	6.62 ± 0.95	**< 0.001***

**p value <0.05*

Table 2: *Association of ERI with physical parameters*

VARIABLE		ERI (17- ≤34) n=12	ERI (>34-51) n=8	P value
Age (years)	20-49	8	7	0.60
	50-80	4	1	
Sex	Male	10	5	0.34
	Female	2	3	
BMI (kg/m²)	15-22	6	6	0.37
	22-25	6	2	
Diabetes	Yes	2	1	1.00
	No	10	7	
Fluid Overload	Yes	2	6	**0.019***
	No	10	2	

**p value <0.05*

Table 3: *Correlation of ERI with baseline hematological and inflammatory parameters*

VARIABLE	MEAN ±S.D	SPEARMAN COEFFICIENT (r)	P value (<0.05)*
Hemoglobin (g/dl)	6.82±1.24	- 0.717	**0.0004***
Hematocrit %(PCV)	20.45±3.35	- 0.663	**0.0014***
S.Ferritin (ng/ml)	639.16±446.9	- 0.181	0.44
S.Albumin (mg/dl)	3.032±0.35	0.285	0.22
ESR(mm in Ist hour)	49.7±15.7	0.183	0.43
hsCRP (mg/dl)	3.07±1.55	0.726	**0.0003***
TNF-α (pg/dl)	68.43±32.24	0.537	**0.014***

**p value <0.05*

Table 4: *Binary logistic regression analysis*

Variables in the Equation	Sig.	Exp(B)	95% C.I. for EXP(B)	
			Lower	Upper
hsCRP (mg/dl)	.029*	8.842	1.253	26.40
TNF alpha (pg/ml)	.518	-	-	-
Hb (g/dl)	.386	-	-	-
PCV(%)	.758	-	-	-

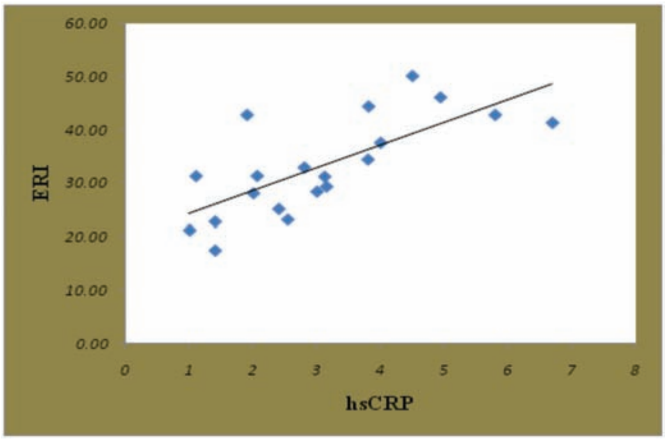

Figure 1. *Correlation of baseline ERI with baseline hsCRP Levels*

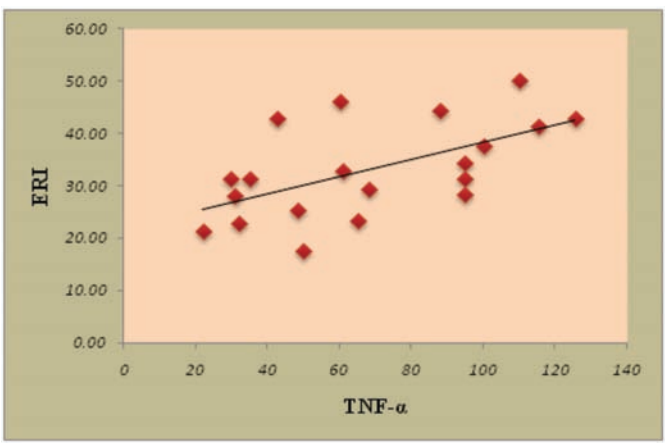

Figure 2. *Correlation of baseline ERI with baseline TNF-á Levels*

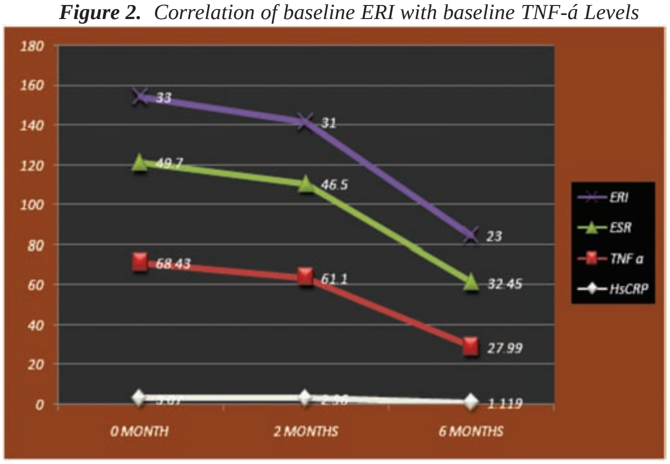

Figure 3. *Showing progressive decline in inflammatory markers (hsCRP, TNF-α , ESR and ERI over the 6 months of study with major effect seen between 2 to 6 months.*

The baseline hemoglobin and hematocrit values of our study participants were 6.82 ± 1.24 g/dl and 20.45 ± 3.37 %, respectively. After 2 months there was a marginal increase in hemoglobin and hematocrit values to 6.97 ± 1.07 g/dl and 21.35 ± 2.97 %, respectively which was statistically not significant ('p'>0.05), suggesting that these patients were erythropoietin resistant, but when Pentoxifylline was added to these patients for next four months, there was highly significant increase in hemoglobin and hematocrit values to 9.02 ± 0.77 g/dl and 26.90 ± 2.88% respectively ('p'<0.01), with a mean difference of -2.20 g/dl and -6.45% from baseline hemoglobin and hematocrit values, suggestive of increased response to erythropoietin.

DISCUSSION

Erythropoiesis-stimulating agent's hyporesponsiveness occurs in approximately 5-10% of patients receiving ESA and represents an important diagnostic and management challenge. Resistance to ESA clearly has the potential to increase mortality in patients on hemodialysis. To assess the response to treatment, we used the relationship between body weight-adjusted dose of EPO and hemoglobin concentration (ERI), as described in earlier studies[8] and observed that ERI was significantly associated with presence of fluid overload. Several inflammatory biomarkers[8] namely CRP, IL-6, adiponectin, S. ferritin, 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 have been defined for consideration as predictors of outcome in ESRD. Inflammatory response begins with the release of interleukin-1 (IL-1) and TNF-α by monocytes and macrophages that subsequently activate a complex cascade of other inflammatory mediators including IL-2, IL-6 and IL-8. IL-1 and TNF-á stimulates the production of these acute phase proteins. Among these hsCRP, which is a pentameric protein synthesized in liver, opsonises infection and activates complement has attracted the most interest. 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 an inexpensive test. It is also a long-term predictor of cardiovascular risk and mortality in the general population and in CKD patients. Furthermore, high plasma concentrations of C-reactive protein (CRP) have been shown to be associated with anemia and ESA hypo-responsiveness in chronic haemodialysis patients[3]. Our study showed that ERI was inversely correlated with hemoglobin and hematocrit, however showed positive correlation with inflammatory markers hsCRP and TNF-α. On performing logistic regression analysis in the final model, ERI was strongly correlated with hsCRP, showing that hsCRP is the best parameter showing correlation with erythropoietin resistance.

Pentoxifylline, a nonselective phosphodiesterase inhibitor with anti-TNF-α properties, in erythropoietin resistant anemia has been studied many years ago when a small prospective study done by Navarro JF et al.[5] showed that Pentoxifylline significantly improved hemoglobin over six months study period along with decrease in serum TNF-α concentration and concluded that Pentoxifylline can improve the hematologic status in this population. Few studies have also shown that Pentoxifylline has been found to reduce other inflammatory parameters such as hsCRP, ESR, serum fibrinogen and TNF-α in patients of CKD and is therefore helpful in reducing inflammation in CKD which is a major contributor of ESA hyporesponsivness [7]. But these studies are small and some are uncontrolled with variable results, so we planned this study. Out of the various inflammatory parameters studied, our study demonstrated that hsCRP, TNF-α and ESR decreased significantly ('p'<0.001) during the six months study period. Serum ferritin also decreased marginally but the decrease in S. ferritin was not found to be statistically significant ('p'= 0.168). Similarly, there was a slight increase in serum albumin levels but it was not found to be statistically significant ('p'= 0.445). Secondly, the important observation made was that when we compared the change in different parameters at 0, 2 and 6 months, it was found that the mean decrease in hsCRP, TNF-α and ESR between baseline and 2 months was statistically not significant ('p'>0.05) when we treated patients with erythropoietin alone in increased doses but the mean decrease in hsCRP, TNF-α and ESR between 2 and 6 months, when Pentoxifylline was added to the treatment regimen, was found to be statistically significant ('p'<0.01) which implies that although there was negligible effect of increased dose of erythropoietin itself on reducing inflammatory parameters, which can be attributed to anti-inflammatory properties of erythropoietin[9], the significant effect was seen only after addition of Pentoxifylline to erythropoietin treatment to the subjects. Also, ERI which signifies erythropoietin resistance decreased slightly between baseline and 2 months, which was statistitically not significant ('p'> 0.05), but the average decline in ERI between 2 and 6 months was statistically significant ('p'<0.01) which is an indirect evidence of potential role of pentoxifylline in overcoming erythropoietin resistance.

CONCLUSION

Patients showing erythropoietin hyporesponsiveness have high titers of inflammatory markers and remain fluid overloaded. ERI can be considered as a marker related to comorbidity and inflammatory status. It is easy to calculate and is highly useful in the bedside evaluation of the patient's clinical status. A sudden increase in ERI should be a motive for concern and should lead to a search for possible underlying inflammatory processes. Pentoxifylline, by virtue of its anti-inflammatory properties, acts as an adjuvant to erythropoietin in management of anemia in patients of CKD who show erythropoietin hyporesponsiveness. Thus future research should focus on understanding the influence of inflammatory biomarkers and cytokines in decreasing effectiveness of erythropoietin stimulating agents and possible future pharmacological interventions for management of inflammation associated hyporesponsiveness to ESA therapy.

REFERENCES

1. *National Kidney Foundation. KDOQI clinical practice guidelines for chronic kidney disease, evaluation, classification, and stratification. Am J Kidney Disease 2002; 39: 1 – 266.*
2. *Macdougall IC, Cooper AC. Hyporesponsiveness to erythropoietin therapy due to chronic inflammation. Eur J Clin Invest 2005;35 (3):32-5.*
3. *Barany P, Divino Filho JC, Bergstrom J. High C-reactive protein is a strong predictor of resistance to erythropoietin in hemodialysis patients. Am J Kidney Dis 1997;29:565-8.*
4. *Stenvinkel P. Inflammation in end-stage renal failure: Could it be treated? Nephrol Dial Transplant 2002;17(8):33-8.*
5. *Navarro JF, Mora C,Garcia J, Rivero A, Macia M, Gallego E et al. Effects of pentoxifylline on the hematologic status in anemic patients with advanced renal failure. J Urol Nephrol 1999;33(2):121-5.*
6. *Cooper A, Mikhail A, Lethbridge MW, Kemeny DM, MacDougall IC. Pentoxifylline improves Hb levels in patients with EPO-resistant anemia in renal failure. J Am Soc Nephrol 2004;15(7):1877-82.*
7. *Goicoechea M, de Vinuesa SG, Quiroga B, Verdalles U, Barraca D, Yuste C et. al. Effects of pentoxifylline on inflammatory parameters in chronic kidney disease patients: a randomized trial: J Nephro 2012;25(6):969-75.*
8. *Lopez-Gomez JM, Portolés JM, Aljama P. Factors that condition the response to erythropoietin in patients on hemodialysis and their relation to mortality. Kidney Int 2008 ;111:75-81.*
9. *Tanaka Y, Joki N, Hase H, Iwasaki M, Ikeda M, Ando R et al. Effect of erythropoietin-stimulating agent on uremic inflammation. J Inflamm 2012;9:17.*