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IMPROVING ERYTHROPOIESIS BY HEMODIALYSIS: RELATIONSHIP WITH ADEQUACY, FREQUENCY AND DURATION OF DIALYSIS

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ABSTRACT

Serum erythropoietin (EPO) concentration was measured by an enzyme-linked immunosorbent assay (ELISA) in 31 cases of chronic renal failure (CRF), including 20 hemodialysis (HD) patients, and compared with that of 31 healthy normal controls. Each patient served as a self-control comparing his or her pretreatment level with post-treatment values. There was a significant negative correlation between serum EPO and serum iron (SI) concentration in HD patients ($p<0.05$); the correlation was more prominent after three months of hemodialysis ($p<0.001$). The mean hemoglobin (Hb) concentration was significantly higher in patients who underwent HD more than once per week as compared with those who received HD once a week ($p<0.001$). In addition, patients on three months of HD had a higher Hb level as compared to those who had received HD for one month ($p<0.001$). Moreover, there was also a significant negative correlation between serum EPO concentration and dialysis adequacy ($p<0.05$), indicating that the erythropoietin-hematocrit feedback inhibition mechanism had improved due to general improvement of the patients' metabolism and possible removal of dialyzable toxins.

Keywords: Erythropoietin, anemia, chronic renal failure, hemodialysis.

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INTRODUCTION

Anemia in chronic renal failure is a multifactorial derangement,[1] although it is generally believed that impaired EPO production is the major cause of anemia in such patients.[2] However, the serum EPO level has been variously reported as elevated[3,4] or low in patients with CRF.[5,6]

In addition, in one report, hemodialysis did not affect serum EPO as previously reported,[9] but instead caused a reduction in its level in a similar study. Until now a few systematic studies have been carried out in order to evaluate the effect of adequacy, frequency and duration of dialysis on the contributory factors to anemia in CRF patients.

In the present study therefore in an attempt to resolve this controversy, we have determined serum EPO concentrations in 31 patients with terminal renal failure, before and following one or three months of hemodialysis, using a convenient randomized self-controlled program. Our main objective was to further investigate the relationship between hematocrit (Hct) levels and serum levels of EPO, iron and creatinine.

PATIENTS AND METHODS

Patients

All new patients with end stage renal disease referring to hospitals affiliated to the Shiraz University of Medical Sciences were selected for this study. Patients with polycystic

Table I. Mean values of Hct, serum EPO, Hb, iron and creatinine concentrations and TIBC, UIBC, TS, and BUN levels in patients with end stage renal disease (n=20) and controls.

Variables	Controls (n=20)	Group 1	Group 2	Group 3
EPO mIU/mL	19.5 ± 5.4	14.9 ± 5.2	12.8 ± 4.8 ($p \leq 0.001$)	12.8 ± 5.8 ($p \leq 0.001$)
Hb gm/dL	15.0 ± 1.56*	7.56 ± 1.40* ($p \leq 0.0001$)	8.21 ± 1.23* ($p \leq 0.0001$)	9.67 ± 2.46* ($p \leq 0.0001$)
Hct %	44.0 ± 4.0	23.3 ± 6.0 ($p \leq 0.0001$)	24.5 ± 3.8 ($p \leq 0.0001$)	32.0 ± 7.5** ($p \leq 0.001$)
Serum iron µg /dL	111.9 ± 49.0	131 ± 64.5	91.4 ± 31.3	87.4 ± 57.5
TIBC µg/dL	432 ± 90	384 ± 72	340 ± 59 ($p \leq 0.02$)	341 ± 88 ($p \leq 0.05$)
UIBC µg/dL	339 ± 97	268 ± 90	252 ± 83 ($p \leq 0.05$)	210 ± 112 ($p \leq 0.02$)
TS µg /dL	26.8 ± 13.5	29 ± 18.0	26.8 ± 18.0	41.7 ± 24.2
BUN Pre mg/dL	13.7 ± 4.0	56.4 ± 17.2 *** ($p \leq 0.0001$)	79.1 ± 23.2 ($p \leq 0.001$)	82.2 ± 18.6 ($p \leq 0.0001$)
BUN Post mg/dL	----------	----------	51.3 ± 17.1 ($p \leq 0.01$)‡	54.7 ± 14.0 ($p \leq 0.01$)‡
Serum creatinine mg/dL	0.85 ± 0.24	7.782 ±	11.55 ± 3.4 ($p \leq 0.0001$)	11.8 ± 2.97 ($p \leq 0.0001$)

Group 1 = Patients who did not require dialysis . Group 2 = patients on hemodialysis once a week for one month. Group 3= The same patients in group 2 who were further dialyzed for two months twice a week. P values = Significant difference in comparison to controls. * Significant difference as compared to other groups. ** Significant difference as compared to group 1.*** Significant difference as compared to groups 2 and 3. ‡ Significant difference as compared to pre-dialysis values.
Hb: hemoglobin . Hct: hematocrit. EPO: erythropoietin. TS: transferrin saturation. TIBC: total iron binding capacity. UIBC: unsaturated iron binding capacity. BUN: blood urea nitrogen.

kidney disease, cardiopulmonary problems, malignancies or active infections as well as those who had recently received blood transfusion were excluded. Thirty-one patients (18 males and 13 females) with an age range of 18 to 65 years (mean=40±12.83) were studied. No patient had received any form of dialysis before the study.

Procedures

Blood samples (5mL) were drawn, their sera collected and stored at -20°C until use. Transferrin saturation (TS), total iron binding capacity (TIBC), unsaturated iron binding capacity (UIBC), EPO and creatinine (Cr) levels were measured using standard procedures.

Blood urea nitrogen (BUN), Hb concentrations and Hct levels were also measured in fresh blood samples. Healthy normal controls (n=31) were also subjected to the same measurements side-by-side and the data obtained were compared with those of CRF patients. From 31 cases with CRF, 20 needed hemodialysis. Patients who did not need dialysis (n=11) were assigned as group 1. Those who required dialysis were divided in two groups: group 2 (n=10) who were hemodialyzed once per week for a duration of one month, followed by two times a week for a duration of two months (group 3). The remaining 10 patients were dialyzed three times a week for three months (group 4). Measurements of EPO and other parameters were performed on all patients with CRF prior to and following one month and three months of dialysis. The results of each group after dialysis were compared with those of the same group prior to dialysis as well as with controls. In the present study, an enzyme-linked immunosorbent assay (ELISA) was used for the quantitative determination of serum EPO, using a kit procured from Amgen Diagnostics Co., Thousand Oaks, California, USA. The assay is highly sensitive and reliable based on the method of Noe et al.[11] in which monoclonal antibodies are employed. EPO concentration was measured in duplicates and the quantities were extrapolated from a calibration curve according to the instructions.[9] All the data obtained were analyzed by the Special Package for Social Sciences (SPSS) computer software program, ANOVA and LSD tests, and the pre-treatment data were compared with those of post-treatment. The effects of the duration and adequacy of dialysis on the level of factors believed to be responsible for anemia were determined. Adequacy of dialysis was calculated according to the following equation:

$$\text{Dialysis adequacy (\%)} = \frac{\text{BUN (initial)} - \text{BUN (final)}}{\text{BUN (initial)}} \times 100$$

RESULTS

Serum EPO levels were significantly lower in patients with end stage renal disease, as compared with those of normal controls ($p < 0.05$); no sex preference was observed. As expected, Hb and Hct levels were significantly lower in all patients with CRF as compared to controls ($p < 0.0001$). There was a reverse correlation between serum creatinine and Hb (r= -044, $p < 0.05$) on one hand, and creatinine and SI (r= -0.66, $p < 0.01$) on the other. The mean values of serum EPO, creatinine, iron, Hb, Hct, TIBC, UIBC, transferrin saturation and BUN of patients and controls are compared in Table I. Serum EPO concentration dropped after hemodialysis, but it was not of statistical significance. There was a reverse correlation between serum EPO and serum iron concentrations in groups 3 and 4 (r = -0.45, $p < 0.05$). Although there was no significant difference in serum iron,

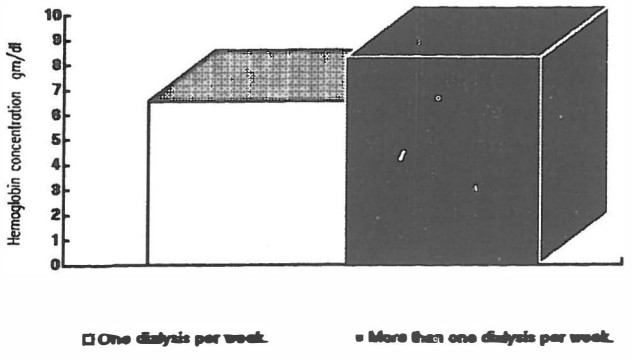

Fig. 1. Relationship between hemoglobin values and frequency of dialysis in hemodialysis patients ($p<0.001$).

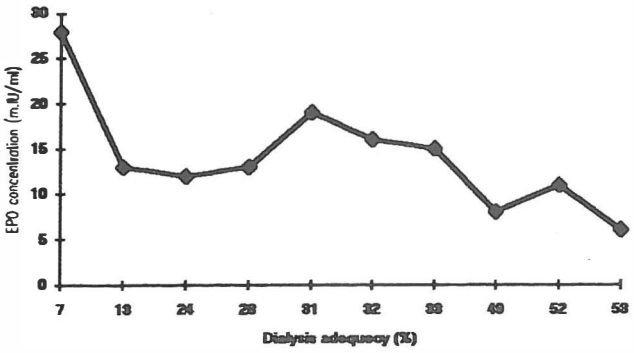

Fig. 2. Correlation between serum erythropoietin concentration (mIu/mL) and dialysis adequacy in patients on three month hemodialysis (r=-0.67, $p<0.05$).

TIBC, UIBC and transferrin saturation in group 2 as compared with the controls, TIBC and UIBC decreased significantly following hemodialysis ($p<0.05$ and $p<0.01$, respectively). A significant reverse correlation was observed between serum EPO and iron levels as well as EPO and UIBC of groups 2 and 3 (r = -0.71, $p<0.01$; r= -0.77, $p<0.001$, respectively). The mean Hb value was lower in patients who received dialysis once a week as compared with those who underwent dialysis more frequently (Figure 1, $p<0.001$). Furthermore, there was a significant reverse correlation between serum EPO concentration and dialysis adequacy (Figure 2, $p<0.05$). Our results did not show any significant difference between groups 3 and 4. With regard to other parameters, adequacy and frequency of dialysis did not result in any statistically significant changes.

DISCUSSION

The main mechanisms contributing to anemia in CRF patients are decreased production of erythropoietin, shortened erythrocyte half-life, retention of toxic metabolites inhibiting erythropoiesis and blood loss in the dialysis unit and other locations. Uremic toxins also play a role by depressing renal or extra-renal EPO production. Other mechanisms that contribute to anemia in CRF are development of osteitis fibrosa, iron and/or folate deficiency, aluminium toxicity, transfusion-induced erythroid suppression, and other factors due to hemolysis such as hypersplenism.[12,13] A decrease in the concentration of Hb is well known to produce a marked increase in EPO production in patients with anemia not complicated by renal failure.[14] Anephrics as well as patients with advanced renal failure have very low levels of EPO.[15] No significant correlation has been found between Hb and EPO concentrations in end stage renal disease and hemodialysis patients. The results presented herein are in agreement with those reported by Seguchi et al,[5] showing that serum EPO concentrations are markedly low in patients with end stage renal disease. In the present study, there was a significant negative correlation between serum iron and UIBC ($p<0.05$). These findings suggest that renal endocrine function deteriorates in parallel to excretory kidney functions, thus causing progression of anemia due to loss of renal mass. Serum iron concentration was considerably reduced in patients with CRF, a finding that has been reported by other investigators.[16] On the other hand, Finch et al.[17] have observed normal serum iron levels in dialysis cases who needed little or no transfusion, whereas dialysis patients with severe anemia requiring repeated transfusions had an increased serum iron concentration. In this study, no significant difference was found in serum iron, TIBC, UIBC and transferrin saturation of patients before dialysis as compared to normal controls. A negative correlation was observed between serum iron and serum EPO level in patients on one month hemodialysis (r=-0.45, $p<0.05$), as well as those on hemodialysis for three months (r= -0.71, $p<0.01$). In the latter there was a reverse correlation between serum EPO and UIBC (r= -0.71, $p<0.01$), serum creatinine, and Hb concentrations as well as between serum iron and hemoglobin levels (r= -0.77, $p<0.001$). These findings suggest that iron is utilized in spite of the progression of renal disease and low serum EPO concentration in patients on hemodialysis.

The effects of the adequacy and frequency of hemodialysis on the level of the factors responsible for the degree of anemia in CRF patients have not been investigated adequately so far. In the present study, we have found a significant reverse correlation between serum EPO concentration and dialysis adequacy ($p<0.05$). The mean Hb concentration in patients on hemodialysis for three months was greater than those who received hemodialysis

for one month ($p<0.001$). These results suggest that anemia of CRF patients is possibly due to inhibition of erythropoiesis by dialyzable toxins.

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