Serum Erythropoietin Concentrations and Iron Status in Patients on Chronic Hemodialysis

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We studied the relationship between serum erythropoietin (EPO) concentration and iron status in 67 patients undergoing chronic hemodialysis. Serum concentrations of EPO were measured by RIA with recombinant human EPO. The geometric mean of the serum EPO concentration was 10.9 int. units/L (mean ± SD range = 7.8 – 15.3 int. units/L) in hemodialysis patients, considerably lower than that in normal subjects (12.9 int. units/L). We found no significant correlation between concentrations of serum EPO and hemoglobin in hemodialysis patients, but found a significant negative correlation between serum concentrations of EPO and iron in hemodialysis patients. Moreover, we also found a significant positive correlation between the EPO concentration and the unsaturated iron-binding capacity (UIBC) in serum, and a significant negative correlation between the serum concentrations of EPO and ferritin in hemodialysis patients. Several patients who had relatively high EPO concentrations for hemodialysis patients also had low iron concentrations, high UIBC values, and low ferritin concentrations. These findings suggest that iron was utilized even at these EPO concentrations, which were very low for the degree of anemia observed in the hemodialysis patients.

Additional Keyphrases: iron-binding capacity - ferritin - anemia - radioimmunoassay - chronic renal failure

The major mechanisms that contribute to anemia in patients with chronic renal failure who are on regular hemodialysis are decreased production of erythropoietin (EPO) for the degree of anemia; shortened erythrocyte survival; retention of inhibitors or toxic metabolites inhibiting erythropoiesis; and blood loss to the dialyzer unit and various other locations (1–4).

EPO, a glycoprotein hormone secreted mainly by the kidney, regulates the differentiation and proliferation of erythroid precursors in bone marrow. The usual stimulus for EPO production is hypoxia, caused by anemia or other forms of impaired oxygen delivery (5).

The plasma EPO concentration has been measured by in vivo and in vitro bioassays and by RIA involving highly purified urinary human EPO. The bioassays of EPO are relatively insensitive and nonspecific. However, widespread use of EPO RIAs has been limited by the availability of both highly purified urinary human EPO for the preparation of labeled anti-urinary human EPO antisera (6–10). The cloning and expression of the human EPO gene have provided large amounts of highly purified recombinant human EPO (rhEPO) for use in the development of sensitive and specific RIAs (11–13).

In the present study, we measured serum concentrations of EPO by RIA, with rhEPO in patients with chronic renal failure maintained by regular hemodialysis, and compared the results with the values obtained in normal subjects and patients with iron-deficiency anemia. We also measured iron, unsaturated iron-binding capacity (UIBC), and ferritin concentrations in sera from hemodialysis patients and examined the relationships between these measurements and serum EPO concentrations.

Materials and Methods

Patients and Controls

We studied normal, apparently healthy subjects and two groups of patients after obtaining their informed consent (Table 1). The 80 normal subjects (35 women and 45 men), ages 27 to 65 (mean 45.7, SD 10.0) years, were controls for serum EPO concentrations. All men had hemoglobin (Hb) concentrations >140 g/L and all women had Hb concentrations >120 g/L. None had intercurrent disease, e.g., proteinuria, renal disease, or liver disease.

The 20 patients (17 women and three men) we studied with various degrees of iron-deficiency anemia ranged in age from 29 to 69 (50.0 ± 11.5) years. All had normal renal function, as determined by urinalysis and measurement of serum creatinine. Hb concentrations ranged from 40 to 113 (86 ± 21) g/L. Serum iron concentrations ranged from 200 to 510 (319 ± 84) μg/L, and serum UIBC ranged from 3690 to 5610 (4424 ± 510) μg/L.

The 67 patients (29 women and 38 men) we studied with chronic renal failure, who had been on intermittent hemodialysis for one to 189 (65.1 ± 55.4) months, ranged in age from 27 to 69 (51.7 ± 11.0) years. Bicarbonate dialysis was performed two or three times a
week for 4 h on each patient. The etiology of end-stage renal disease in these patients included chronic glomerulonephritis (51 patients), diabetes mellitus (eight patients), nephrotic syndrome (three patients), gouty nephropathy (two patients), chronic pyelonephritis (one patient), polycystic kidney disease (one patient), and toxemia of pregnancy (one patient). Their Hb concentrations ranged from 49 to 112 (77 ± 15) g/L. Serum iron concentrations ranged from 390 to 2140 (958 ± 351) μg/L, and serum UIBC ranged from 310 to 4090 (1898 ± 786) μg/L. Serum ferritin concentrations ranged from 6.2 to 3000 μg/L, with a geometric mean of 139.4 (mean ± SD range = 23.9–794.9) μg/L.

Venous blood was drawn from normal subjects and patients with iron-deficiency anemia after an overnight fast, and immediately before hemodialysis in the hemodialysis patients. The serum was kept at −70 °C until we measured serum EPO concentrations.

RIA of Serum EPO

We determined the serum concentrations of EPO with an RIA kit (Nippon DPC Corp., Tokyo, Japan) that included 125I-labeled rhEPO as the tracer, rabbit antiserum against rhEPO as the primary antibody, goat anti-rabbit IgG antiseraum as the secondary antibody, and rhEPO standards at 0, 5, 10, 20, 40, 80, and 160 int. units/L. The rhEPO (Kirin Brewery Corp., Tokyo, Japan) in this kit was manufactured from Chinese hamster ovary cells transformed with the human EPO gene. The 125I-labeled rhEPO was prepared by lactoperoxidase–glucose oxidase methods, and was separated from unreacted iodine on a Sephadex G-25 column.

The assay was performed as follows: add 200 μL of primary antibody to 200 μL of serum sample of rhEPO standard, and incubate for 20 h at 25 °C. Add 100 μL of rhEPO tracer and react for 20 h at 25 °C. Then add 1.0 mL of the secondary antibody solution with polyethylene glycol as sediment retainer. Centrifuge at 2000 × g and 4 °C for 15 min. Aspirate the supernate and count the radioactivity of the pellet. Draw a standard curve and interpolate the concentrations of EPO in the serum samples from this curve. The intra-assay coefficients of variance (CVs) for the samples (mean concentrations: 17.2, 38.7, 56.8, and 64.7 int. units/L) were 4.3%, 4.5%, 4.7%, and 4.4%, respectively (n = 10). Interassay CVs were 6.0%, 4.6%, 2.4%, and 3.5% (n = 5).

Other Tests

Hb serum creatinine concentrations were measured and reticulocytes counted by routine laboratory procedures. Concentrations of iron and UIBC in serum were measured by the bathophenanthroline method. Serum ferritin concentrations were measured by RIA (Baxter Corp., Tokyo, Japan).

Statistical Analysis

We performed the statistical evaluation by using Student's unpaired t-test, Pearson's correlation coefficient, and linear-regression analysis. Because serum EPO concentrations were log-normally distributed in the healthy subjects (Figure 1), we used geometric mean values and log-transformed data from serum EPO concentrations for the statistical evaluation. We also used log-transformed data for serum ferritin concentrations in hemodialysis patients. When mean values were arithmetic, the ranges were given as mean ± SD. P values <0.05 were considered significant.

Results

Normal subjects. The serum EPO concentrations ranged from 7.7 to 25.8 int. units/L, with a geometric

![Graph A](image)

![Graph B](image)

Fig. 1. Distribution of serum EPO concentrations, int. units/L (A), and of log concentrations (B) in normal subjects (n = 80)

Minimum and maximum EPO concentrations were 7.7 and 25.8 int. units/L, respectively.
mean of 12.9 (mean ± SD range = 10.2–16.4) int. units/L in normal subjects. The 95% confidence limits of the normal range values for the 80 normal subjects were 8.1 and 20.8 int. units/L. We found no significant difference in serum EPO concentrations between men and women (13.1 vs 12.8 int. units/L) (Table 1). We observed no significant relationships between serum EPO concentration and age, or between Hb concentration and age. In addition, we found no significant relationship between serum EPO and Hb concentrations (Figure 2).

Patients with iron-deficiency anemia. The geometric mean of the serum EPO concentration was 189.8 (mean ± SD range = 45.0–800.3) int. units/L in patients with iron-deficiency anemia. We observed no significant relationship between serum EPO concentration and age. Serum EPO concentrations were inversely related to Hb concentrations (r = −0.88, P < 0.001) (Figure 2). We found a negative correlation between serum concentrations of EPO and iron (r = −0.64, P < 0.01), whereas serum EPO concentrations were not significantly correlated with serum UIBC.

Hemodialysis patients. Serum EPO concentrations in hemodialysis patients ranged from 6.4 to 28.4 int. units/L, with a geometric mean of 10.9 (mean ± SD range = 7.8–15.3) int. units/L. The mean serum EPO concentration was significantly less in these patients than in normal subjects (10.9 vs 12.9 int. units/L, P < 0.01); however, the hemodialysis patients had markedly lower Hb concentrations (77 ± 15 g/L). We found no significant relationship between serum EPO concentra-
tion and age, and no significant difference in serum EPO concentrations between men and women (11.5 vs 10.2 int. units/L) (Table 1). We observed no significant relationship between serum EPO concentration and Hb concentration in hemodialysis patients (Figure 2). However, serum EPO concentrations were significantly inversely correlated with serum concentrations of iron and ferritin (r = −0.25, P < 0.05, and r = −0.24, P < 0.05, respectively), and positively correlated with serum concentrations of UIBC in these patients (r = 0.32, P < 0.01) (Figure 3).

Serum EPO concentrations were also significantly inversely correlated with serum creatinine concentrations (r = −0.33, P < 0.01). However, when we divided the hemodialysis patients into two groups according to the frequency of hemodialysis, the mean serum EPO concentration was not greater in the patients who received hemodialysis twice a week (n = 34) than in those who received hemodialysis three times a week (n = 33). Concentrations of serum EPO were not correlated with the concentrations of serum creatinine in the patients who received hemodialysis three times a week.

Serum EPO concentrations demonstrated no correlation with reticulocyte counts or duration of hemodialysis. We found no significant difference in serum EPO concentrations between those patients whose renal failure was due to chronic glomerulonephritis and those with diabetic nephropathy (11.2 vs 11.1 int. units/L, respectively).

![Fig. 2. Relationship between serum EPO (int. units/L) and hemoglobin concentrations](image)

![Fig. 3. Correlation between serum EPO concentration (int. units/L) and iron status in hemodialysis patients: (A) serum iron, (B) serum UIBC, and (C) serum ferritin](image)
No significant difference was seen in the mean serum EPO concentrations between hemodialysis patients with a blood transfusion within one month (n = 8) and those without a blood transfusion within one month (n = 59), or between patients receiving androgen therapy (n = 16) and those not taking androgen (n = 51). However, the mean serum EPO concentration was significantly greater in hemodialysis patients (n = 8) receiving iron treatment than in those not receiving it (n = 59): 15.1 vs 10.4 int. units/L (P < 0.01).

Hb concentrations were significantly inversely correlated with concentrations of iron and ferritin (r = -0.53, P < 0.01, and r = -0.36, P < 0.01, respectively) and were significantly positively correlated with UIBC concentrations (r = 0.53, P < 0.001) (Figure 4). We also found a significant correlation between Hb concentration and the duration of hemodialysis (r = 0.47, P < 0.001) (Figure 5). Furthermore, in hemodialysis patients, serum iron concentrations were inversely correlated with UIBC concentrations (r = -0.68, P < 0.001), and were positively correlated with serum ferritin concentrations (r = 0.54, P < 0.001).

Discussion

A decrease in the concentration of Hb is well known to produce a marked increase in EPO concentration in patients with simple anemia not complicated by renal disease (9, 14). In the present study, the negative correlation between concentrations of Hb and serum EPO in patients with iron-deficiency anemia was observed as expected. However, we found no significant correlation between Hb and EPO concentrations in hemodialysis patients. This result is consistent with reports by Ohi-gashi et al. (12), Umezu et al. (13), and Rhyner et al. (14), indicating that serum EPO concentrations in hemodialysis patients were markedly low for the degree of anemia.

In the present study, we also examined concentrations of serum iron, UIBC, and ferritin in hemodialysis patients. EPO stimulates erythropoiesis mainly by amplifying the pool of erythroid-committed cells in the bone marrow, and then initiates iron uptake and Hb synthesis (5). Accordingly, the incorporation of 59Fe into erythrocytes or heme has been used to measure plasma EPO concentrations in bioassays (5, 9).

Serum iron concentrations vary considerably in patients with chronic renal failure. Some investigators (15, 16) have observed that serum concentrations of iron and UIBC were often decreased in patients with chronic renal failure. On the other hand, Finch et al. (17) reported that dialysis patients with severe anemia requiring repeated transfusions had an increased plasma iron concentration, a prolonged iron turnover, a reduced erythron-iron turnover, and a decreased erythrocyte utilization of radioisotopic iron, whereas dialysis patients requiring little or no transfusion had a normal plasma iron concentration, an intermediate to normal erythron-iron turnover, and a nearly normal erythrocyte utilization of radioisotopic iron.

Mann et al. (18) and Coles and Cavill (19) demonstrated improvement of ferrokinetics in patients with chronic renal failure without an attendant increase in plasma EPO concentrations after peritoneal dialysis, suggesting that renal anemia resulted from a failure of erythropoiesis related to the uremic inhibitors, as well as decreased EPO production.

In the present study, we demonstrated the inverse relationship between serum concentrations of EPO and iron in hemodialysis patients, as well as in patients with iron-deficiency anemia, though we did not find a significant relationship between serum concentrations of EPO and Hb in hemodialysis patients. Moreover, we demonstrated the positive correlation between serum

Fig. 4. Correlation between Hb concentration and iron status in hemodialysis patients: (A) serum iron, (B) serum UIBC, (C) serum ferritin

O, iron treatment (n = 8); △, blood transfusion within one month (n = 8). Hb concentrations ranged from 49 to 112 g/L.

Fig. 5. Correlation between Hb concentration and duration of hemodialysis

Dialysis was performed two or three times a week for 4 h on each patient.
concentrations of EPO and UIBC, and the negative correlation between serum concentrations of EPO and ferritin in hemodialysis patients.

These findings indicate that several hemodialysis patients who had relatively high EPO concentrations for hemodialysis patients had not only renal anemia attributable to decreased EPO production, but also iron-deficiency anemia, because these patients had low serum iron and high UIBC concentrations. Accordingly, these results suggest that iron was utilized even at these EPO concentrations, which were very low for the degree of anemia in these patients. This concept is also consistent with the findings that serum ferritin—the most useful variable for assessing iron stores (20)—was decreased in these patients.

In contrast, most hemodialysis patients who had relatively low EPO concentrations for hemodialysis patients had high serum iron concentrations, low serum UIBC concentrations, and high ferritin concentrations. These results suggest that iron was not utilized because of very low EPO concentrations in this group of patients, who had only pure renal anemia.

Therefore, correcting iron deficiency is valuable in hemodialysis patients with low serum concentrations of iron and ferritin, whose serum EPO concentrations are at least not lower than the normal range.

The significant correlation we demonstrated between Hb concentrations and the duration of hemodialysis in hemodialysis patients (Figure 5) is consistent with the reports by Charles et al. (21) and Umezu et al. (13). Ishikawa (22) reported that prolonging the dialysis period increased the incidence, number, and size of acquired renal cysts and that an improvement of hematocrit in patients on long-term dialysis had also been noted, suggesting that the increased hematocrit was related to the acquired cystic disease of the kidney. Rhyner et al. (14) reported that a puncture of a solitary renal cyst decreased the serum EPO and hematocrit concentrations, and the accumulation of cystic fluid in a renal cyst increased the serum EPO and hematocrit concentrations in a patient who had secondary polycythemia due to a solitary renal cyst. However, we found no relationship between serum EPO concentration and the duration of hemodialysis in hemodialysis patients, even though Ohigashi et al. (12) did find a significant correlation.

References