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Original Paper

The effect of recombinant human erythropoietin on serum selenium levels in hemodialysis patients

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Abstract

Succesful results in the treatment of anemia, one of the main complications of chronic renal failure, can be achieved by the use of recombinant human erythropoietin (RhEPO), which is available almost fifteen years in clinics. On the other hand, as both chronic renal failure and maintenance hemodialysis reduce the levels of trace elements, this study was designed to evaluate the interaction potential of RhEPO with serum concentrations of selenium (Se) during four months. Thirty one adult hemodialysis outpatients participated in the study. Ten of them, not on any drug therapy to interact with RhEPO, recruited as "Control Group", and the remainder, on RhEPO therapy, as "RhEPO Group". Blood was drawn from the Control Group at the beginning of the study, and from the RhEPO Group at every month for four months. Serum erythropoietin levels were measured by a radioimmunoassay method and Se status by a spectrofluorometric method. It was found that Se levels were not affected by RhEPO treatment during 3 months of therapy, while an increase was seen on the fourth month. The observation indicates that the increase in serum Se levels would be significant in longer than three-month RhEPO treatment.

Key words: recombinant human erythropoietin, RhEPO, selenium, hemodialysis, chronic renal failure

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Introduction

One of the main causes of anemia in chronic renal failure is the decrease in erythropoietin (EPO), a natural hormone, production in kidneys (1, 2). Formerly, the treatment choices for this situation were androgens and blood transfusions despite the adverse effects (1, 3, 4). After the isolation of RhEPO in 1977 by Miyake et al. (5), the preparation was produced by DNA technology in 1985 (6). RhEPO is a glycoprotein containing 165 amino acids (7). The treatment with RhEPO was found more convenient in the population with renal anemia (8-10). The main indications of RhEPO except renal anemia include other anemias due to cancer or zidovudin therapy, prematurity, chronic diseases, and autologous blood donation preoperationally (9, 11). Since the level of EPO is sensitive to intrarenal hypoxia, if hematocrit decreases below 30–35%, the level of EPO increases from 10 mU/ml to 100 mU/ml (3). Although it is controversial between countries and/or clinicians, the target levels for hemoglobin is 9.95 g and 30.95% for hematocrit in Turkey (9, 12–14).

The effects of the treatment with RhEPO on the levels of trace elements were investigated in several studies (15–17). Se, a nutritient having antioxidant properties, is found in all tissues mostly kidneys, liver and skeletal muscle (18, 19). It forms at least 13 selenoproteins (20–22). Se, is an integral component of glutathione peroxidase (GSHPx), which protects cells from oxidative stress catalysing the reduction of reactive peroxides (22, 23). It was recently demonstrated that iodothyronine 5'-deiodinase enzyme, which took place in thyroid metabolism, was a selenoprotein. (24). Se has been also found to have antiinflammato-

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ry, antiviral and immun stimulant effects and the correlation between decrease in blood Se levels and cancer or myocard infarction has been investigated (25–28).

The aim of this study was to assess the serum levels of Se in hemodialysis patients on RhEPO therapy and to evaluate if there was an interaction between the serum concentrations of RhEPO and Se during four months.

Materials and methods

Subjects

The study was performed in the Hemodialysis Units of Nephrology Departments of two hospitals of Ankara. Prior to the study "Approval of Ethics Committee" was received from each one of them.

Se and EPO levels were prospectively assessed in adult outpatients for a total period of 4 months. Although the study was started with 45 patients, 14 of them had to be excluded from the study due to transportation to another hospital or dialysis center, infectious diseases and excitus. All of the subjects were undergoing maintenance hemodialysis three times a week for at least 6 months. Causal nephropaties were polycystic kidney, chronic glomerulonephritis, chronic pyelonephritis, urolithiasis, mesenchial proliferative glomerulonephritis, diabetic nephropaties and amyloidosis. Ten of the patients were enrolled in the "Control Group", in which no one had any other chronic disease but renal failure and received any drug interacting with serum EPO levels and trace element supplements. The "RhEPO Group" consisted of 21 patients to whom RhEPO treatment were started depending on their hematocrit levels below 30% and/or hemoglobin levels below 9 g % in accordance with the decisions of nephrologists. No patient in this group also had any other chronic disease but renal failure, and received any drug interacting with serum EPO levels and were on trace element supplements. The demographic data of the groups were summarized in Tables 1 and 2.

Table 1. Demographic Data of the Control Group

No	Gender	Age (years)	Etiology	Duration of Hemodialysis (months)
1	M	22	PRD	109
2	F	37	CGN	156
3	F	38	CPN	129
4	F	60	CGN	161
5	F	56	?	75
6	F	33	Urolithiasis	108
7	M	47	?	131
8	M	54	CGN	85
9	F	59	?	32
10	M	49	?	11
Mean		46		100
SD		12		50
Median		48		109
Minimum		22		11
Maximum		60		161

Note: M: Male; F: Female; PRD: Polycystic renal disease; CGN: Chronic glomerulonephritis; CPN: Chronic pyelonephritis

Procedure

RhEPO (Eprex® or Recormon®) doses were given subcutaneously three times a week after hemodialysis session. Blood flow rate was maintained at 200 ml/min and dialysate flow rate was at 500 ml/min. Biocompatible membranes were used in hemodialyzers. The water supply was analyzed monthly for sodium, potassium, aluminum, calcium, magnesium, zinc, sulfate, chloride, nitrate and heavy metals. All of them were within acceptable limits during the study period.

To assess the levels of endogenous EPO, selenium, and zinc, 5 ml of venous blood was drawn only once before hemodialysis session in the Control Group. The RhEPO Group included the patients both the ones who were started before the study but given a break as "wash out period" for one week, and were decided to start with RhEPO treatment prospectively to correct their renal anemia. Five ml blood was drawn before the treatment re/started and monthly for 3 month-study-period before hemodialysis sessions, four times in total. Blood samples were centrifuged (at 3000 rpm for 15 minutes) for the measurement of serum RhEPO and Se levels. The serum samples

Table 2. Demographic Data of the RhEPO Group

No	Gender	Age (years)	Etiology	Duration of Hemodialysis (months)
1	M	38	MPGN	11
2	F	68	CRF	113
3	M	43	MPGN	72
4	M	33	CGN	27
5	F	71	CRF	13
6	F	64	Amiloidosis	144
7	F	34	CGN	32
8	F	59	PRD	74
9	F	41	CRF	94
10	F	37	CPN	60
11	M	34	Amiloidosis	24
12	F	60	DN	75
13	M	48	CRF	120
14	F	39	CRF	93
15	M	28	CPN	74
16	F	38	CPN	145
17	F	53	CRF	109
18	F	29	CPN	101
19	F	28	CGN	36
20	M	46	CGN	54
21	F	37	CRF	82
Mean		44		74
SD		13		39
Median		39		74
Minimum		28		11
Maximum		71		145

Note: M: Male; F: Female; MPGN: Mezenchial proliferative glomerulonephritis; CRF: Chronic renal failure; CGN:Chronic glomerulonephritis; PRD: Polycystic renal disease; CPN: Chronic pyelonephritis; DN: Diabetic nephropathy

were stored at −20 °C until analyzed. Se samples were kept in trace metal-free polypropylene tubes.

Standard routine biochemical and special tests interfering with the results of EPO tests, such as ferritin, and parathormone, were performed in patients during the study period. The subjects were examined for pulse, blood pressure and weight before their bloods were drawn. A special diet with restricted in phosphorous and potassium for chronic renal failure was given to all of the patients.

Assessment of RhEPO:

EPO levels were measured using a radioimmunoassay kit (EPO-Trac™ ^{125}I RIA Kit-INCSTAR Corporation, Minnesota) at Hacettepe University, Faculty of Medicine- Department of Nuclear Medicine. The detection limit was <4.4 mU/ml.

Serum Selenium Determination:

Se levels in serum were measured by a spectrofluorometric method at Hacettepe University, Faculty of Pharmacy (29). Calibration of the spectrofluorometric instrument, quality assessment of the analytical data, verification of precision, accuracy and sensitivity were accomplished by the direct use of standard preference material (SRM): (Seronorm™ Trace Element S by Nycomed). Results were in good aggreement with certified values. The limit of detection of the method was 0.7 µg/L; within-day precision was 2.4% CV, between-day precision was 2.6% CV, and recovery was determined to be 98.10 ± 0.04%.

Statistical Analysis

As most of the variables were not in normal distribution, the results were defined in median and since the number of the patients who finished the study was not enough to use parametric tests, nonparametric tests were performed. The results were expressed as mean ± standard deviation (SD) and have been compared using Kruskal-Wallis followed by Mann-Whitney U test. Spearman test was used to evaluate the correlation between the serum EPO and Se levels. A p value less than 0.05 was considered significant. All statistical analysis was conducted using SPSS/PC software.

Results

Serum EPO levels

The results of the Control Group and the RhEPO Group were given in Table 3. Although no significant difference was found between the groups ($p = 0.670$) and genders ($p = 0.931$), the difference was significant between months ($p < 0.05$).

Serum Selenium status

The results of serum Se levels of the Control Group and the RhEPO Group were given in Table 4. The values of the Control Group were compared to the mean levels of 4 months of the RhEPO Group. The difference was found insignificant ($p = 0.1131$).

A significant monthly difference was found in between the Control Group and the RhEPO Group ($p < 0.05$). When months were evaluated separately against the mean value of the Control Group, serum Se levels were higher only in the fourth month than the values in previous months. (Control versus monthly p values of RhEPO Group were found as 0.1975, 0.5684, 0.1697 and 0.012, respectively) (Figure 1).

Selenium-EPO correlation

The correlation between mean serum EPO levels and mean Se levels was evaluated in the RhEPO Group (n = 21) and no correlation was found between them (Table 5).

Table 3. Serum EPO Levels

	Serum EPO Levels (mU/ml)					
	Control Group (n = 10)	RhEPO Group (n = 21)				
	EPO	EPO^1	EPO^2	EPO^3	EPO^4	EPO_{av}
Mean	20.3	16.7	22.7	20.4	19.0	19.7
SD	2.7	7.9	10.2	9.2	5.9	5.9
Median	19.8	15.7	22.1	20.0	17.4	19.5
Minimum	18.0	4.3	9.8	11.4	10.8	11.3
Maximum	27.5	39.6	44.1	50.5	32.3	36.2

Note: ^{1}: First month, ^{2}: Second month, ^{3}: Third month, ^{4}: Fourth month, $_{av}$: Average

Table 4. Serum Selenium Levels

	Serum Selenium Levels (µg/L)					
	Control Group (n = 10)	RhEPO Group (n = 21)				
	Se	Se^1	Se^2	Se^3	Se^4	Se_{av}
Mean	57.9	63.8	61.5	65.2	72.6	65.8
SD	11.5	13.8	16.1	14.6	13.8	10.8
Median	54.3	62.1	60.1	64.9	70.1	65.1
Minimum	44.6	35.8	36.7	40.9	38.4	47.2
Maximum	76.6	89.1	101.3	105.3	107.2	97.5

Note: ^{1}: First month, ^{2}: Second month, ^{3}: Third month, ^{4}: Fourth month, $_{av}$: Average

Table 5. Correlation Between Serum EPO Levels and Selenium Levels

Variables	p	r
Control Group		
EPO × Se	0.5367	−0.2175
RhEPO Group		
EPO 1 × Se 1	0.4234	−0.1845
EPO 2 × Se 2	0.4419	−0.1773
EPO 3 × Se 3	0.4730	0.1656
EPO 4 × Se 4	0.3388	0.2196
EPO av × Se av	0.6298	−0.1117

Note: r: Correlation coefficient

Discussion

Along with dietary restrictions and symptomatic intervention in the treatment of chronic renal failure, which is a very serious worldwide health problem including our country, hemodialysis is one of the most frequently and effectively used therapeutic approaches (30). For this reason, while waiting for kidney transplantation, many of patients can maintain a healthier life and continue their jobs despite short interruptions.

The leading complications of chronic renal failure are anemia and hypertension. When hematocrit and hemoglobin values decrease below normal limits, the patient feels extremely tired and quality of life deteriorates. Since kidneys, where EPO is synthetized, are sensitive to oxygene, they have vital importance regarding anemia (2). In the past, androgens (fluoxymesteron, oxymesterone, testosteron) were used to correct anemia. However, both interpersonal differences and adverse effects limited their use effectively (1, 4). Therefore, until 1985, in spite of its adverse effects, blood transfusions seemed to be the most popular choice for the treatment (3). Today successful results in the treatment of anemia can be achieved by the use of RhEPO, which came into the practice almost fifteen years ago (5, 6). Nevertheless, according to the earlier studies, 5% of the patients in various countries and 10% of Turkish patients who were given RhEPO, were found to be less responsive or "resistant" to RhEPO (14, 31). The main causes of resistance include iron deficiency, infectious diseases, chronic inflammatory conditions and hyperparathyroidism (32–35).

On the other hand, the effect of RhEPO treatment on trace elements status in serum were also studied. It was reported that serum Se levels decreased in chronic renal failure and during hemodialysis (36–38). It was also shown that while zinc, nickel, and manganese concentrations increase, aluminum, and silisium concentrations decrease with RhEPO treatment (15–17).

Since antioxidant activity decreases and lipid peroxidation increases, the survival of erythrocytes is shortened in chronic renal failure. Accumulation of uremic toxins, and loss of trace elements, such as Se and zinc during hemodialysis can be mentioned as the main causes of this situation (39). In a recent report, it was claimed that cardiovascular complications of hemodialysis could be associated with deficiency of antioxidant systems (40). However, there are some studies showing that RhEPO treatment could decrease lipid peroxidation and the levels of malondialdehyde (39, 41, 42). Because of this treatment, the levels of antioxidant enzymes, such as superoxide dismutase and GSHPx, were found to be high on the membranes of young erythrocytes. This finding was explained by erythropoiesis and cellular hemoglobin synthesis due to RhEPO, followed by increase of circulating young red cells (39). There are some studies reporting that the supplements of Se and vitamin E to hemodialysis patients could be beneficial in resistant cases of RhEPO treatment (40, 41).

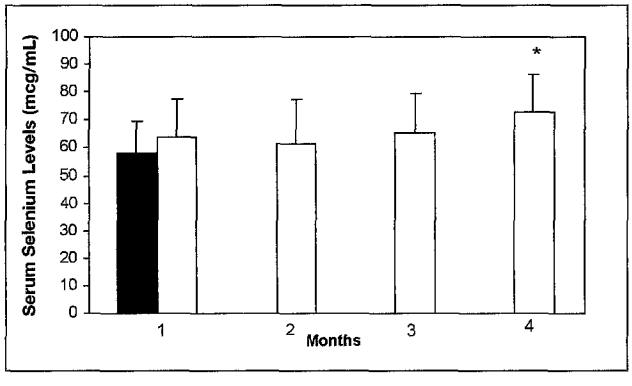

Figure 1. Serum Selenium Levels. Values are given as mean ±SD. 1: First month, 2: Second month, 3: Third month, 4: Fourth month, *: $p < 0.05$ versus Control, ■: Control Group (n = 10), □: RhEPO Group (n = 21).

In this study, it was assessed that whether an interaction occurs between RhEPO and serum Se levels in hemodialysis patients. As a matter of fact, it was very difficult to find patients meeting the admission criteria for RhEPO Group and continue the study with them since they hesitated to give blood because of their fear of not replacing it. Therefore, the study had to be performed in a population of small size and two separate hospitals.

In the Control Group, endogen average serum EPO levels were found to be 20.30 ± 2.66 mU/ml (median 19.75 mU/ml) based on only one measure at the beginning of the study period. These results are comparable with earlier ones reporting 10–20 mU/ml as endogen EPO levels in hemodialysis patients (3). The levels were in the same range in the RhEPO Group also [average of four months were 19.69 ± 5.85 mU/ml (median 19.45 mU/ml)]. It was found that the difference was significant between months, namely, the level of EPO was higher in the second month ($p < 0.05$). Since the first 4–6 weeks are "initiation phase", this finding which might be due to changing of the erythroid cells sensitivity, was not surprising (43).

On the other hand, average serum Se levels were found as 57.93 ± 11.54 mg/l (median 54.30 mg/l) in the Control Group. It was attributed that the reason for high standart deviation is inadequacy of subjects. Kallistratos et al. (44) performed the study on healthy controls, uremic nonhemodialysis patients, and uremic hemodialysis patients, and reported that serum Se levels were lower in uremic patients than the controls, however there was not an effect of hemodialysis on them. The results for Se levels were 11% ± 1 mg for uremic hemodialysis patients, which is approximately twice of ours. Nonetheless, endogen serum Se levels show differences geographically (18). The average level of Se of healthy adult controls in Ankara, where the study was performed, is 74 ± 16 mg/l (45). Bonomini et al. (46), also, showed that uremic patients had lower blood Se levels than control. It has been planned to expand this study with nonhemodialysis and hemodialysis RhEPO patients besides healthy controls.

In the RhEPO Group, average serum Se levels after one-week-wash out period for the RhEPO were found as 63.83 ± 13.81 mg/l (median 62.09 mg/l). The average of four-month period was 65.76 ± 10.83 mg/l (median 65.10 mg/l).

The values of the Control Group were compared to the average levels of 4 months of the RhEPO Group, and the difference between groups was found insignificant ($p > 0.05$). When the values of each month compared seperately with the Control Group, the difference between the Control Group and the fourth month was found significant ($p < 0.05$). Since the difference between months significant, it was concluded that RhEPO treatment could be responsible for the increasing trend in Se levels in the fourth month.

It was observed that quality of life of anemic hemodialysis patients was improved significantly with RhEPO therapy for two years, hence, serum zinc, nickel and manganese levels, which are positively correlated with erythrocyte count, hemoglobin value and hematocrit level, were improved eventually (15). Although such an improvement could be expected in Se status in a similar way, the results of this study demonstrates that RhEPO administration for 3 months was not enough to make such an assumption for Se.

In the RhEPO Group, which contains more patients, gender differences in Se levels were evaluated, also. The mean levels of 4 months were found to be 62.09 ± 5.28 mg/l in males, and 67.60 ± 12.50 mg/l in females. However, it is difficult to make a conclusion since the number of females are twice of males.

In conclusion, four-month RhEPO use does not interact with mean serum Se levels. Although serum Se levels were not affected in the first three months, the increase observed in the fourth month implied that the increase in serum Se levels would be more significant in a longer period of RhEPO treatment. Although the main drawbacks of this study are the size of the study population and the duration of the study period, it has been suggested that the difference between groups can be significant in a longer-period and/or with more-patient study due to the increase of intake of dietary Se and other nutrients, such as thiamine, pyridoxine, and total protein that increase bioavailability of Se (47). In order to assess the effect of Se supplements to RhEPO patients on the amount and duration of decrease in oxidative stress, another study is in progress.

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