

Selenium Associates With Response to Erythropoiesis-Stimulating Agents in Hemodialysis Patients



Minoru Yasukawa¹, Shigeyuki Arai¹, Michito Nagura¹, Ryo Kido^{2,3}, Shinichiro Asakawa¹, Daigoro Hirohama¹, Osamu Yamazaki¹, Yoshifuru Tamura¹, Michitaka Fujimaki⁴, Sawako Kobayashi⁵, Masakazu Mimaki⁶, Hiroko Kodama⁷, Shunya Uchida^{1,8}, Yoshihide Fujigaki¹ and Shigeru Shibata^{1,9}

¹Division of Nephrology, Department of Internal Medicine, Teikyo University School of Medicine, Tokyo, Japan; ²Medical Checkup Center, Inagi Municipal Hospital, Tokyo, Japan; ³Research Center for Social Systems, Shinshu University, Nagano, Japan; ⁴Department of Nephrology, Nerima Hikarigaoka Hospital, Tokyo, Japan; ⁵Department of Nephrology, Tokyo-Kita Medical Center, Tokyo, Japan; ⁶Department of Pediatrics, Teikyo University School of Medicine, Tokyo, Japan; ⁷Graduate School of Health Sciences, Teikyo Heisei University, Tokyo, Japan; ⁸Department of Health Care, Teikyo Heisei University, Tokyo, Japan; and ⁹Advanced Comprehensive Research Organization, Teikyo University, Tokyo, Japan

Introduction: Impaired response to erythropoiesis-stimulating agents (ESAs) is associated with increased mortality in patients with end-stage kidney disease. However, the underlying mechanisms are not fully elucidated. Accumulating data reveal that selenium (Se), a trace element, plays a key role in stress erythropoiesis and erythrocyte homeostasis. We evaluated the relationship between serum Se levels and the response to ESAs in hemodialysis patients.

Methods: In this cross-sectional study, we determined serum Se levels in 173 hemodialysis patients. We analyzed the association of serum Se with ESA responsiveness, as defined by ESA resistance index.

Results: Of the study participants, 50% had lower Se levels than the population-based reference values. We found that serum Se levels were significantly and inversely correlated with erythropoiesis resistance index (ERI) but not transferrin saturation (TSAT) or ferritin levels. Multiple regression analyses confirmed the association between Se levels and ESA hyporesponsiveness, independently of other known factors, such as iron status, being female, and dialysis vintage ($\beta = -0.11$, $P < 0.001$). When patients were divided according to Se levels and iron status, both low serum Se (<10.5 $\mu\text{g/dl}$) and iron deficiency significantly affected the response to ESA. Conversely, serum Se levels were significantly different among groups when patients were divided according to ERI quartiles. The association of low serum Se with ESA hyporesponsiveness persisted after adjustment of confounding variables.

Conclusion: Serum Se levels are associated with the response to ESAs and can predict ESA resistance independently of iron status in Japanese hemodialysis patients. These data open the possibility to test whether Se supplementation reduces ESA demand.

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KEYWORDS: anemia; end-stage kidney disease; erythrocyte senescence; erythropoiesis; micronutrients; selenium

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Although the management of renal anemia has markedly been improved by the advent of ESAs, the reduced response to ESAs is a clinically significant problem observed in a subpopulation of patients with chronic kidney disease (CKD). The importance of the ESA hyporesponsiveness has been highlighted by the fact

that it is associated with increased mortality and cardiovascular disorders in maintenance hemodialysis patients.^{1–3} Resistance to ESAs is associated with multiple factors, including chronic inflammation, malignancy, hematological disorders, and inadequate nutritional status.^{4,5} Previous studies have demonstrated that factors such as dialysis vintage, sex, TSAT, and serum albumin are independent predictors of ESA hyporesponsiveness in hemodialysis patients.⁶ As for the mechanisms that provide link between ESA resistance and poor mortality, both low hemoglobin (Hb) levels and high doses of ESAs are proposed to be involved⁷; however, the detailed molecular basis is still unclear.

Correspondence: Shigeru Shibata, Division of Nephrology, Department of Internal Medicine, Teikyo University School of Medicine, 2-11-1 Kaga, Itabashi-ku, Tokyo 173-8605, Japan. E-mail: shigeru.shibata@med.teikyo-u.ac.jp

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Se is one of the microminerals (trace elements) that are essential to maintain numerous biological activities.⁸ Although the amount of Se in humans is approximately 20 mg in total,⁹ its deficiency results in a number of disorders, such as cardiomyopathy, osteoarthropathy, thyroid disorder, nail changes, and impaired erythrocytosis.^{10,11} It has also been found that low serum Se levels are associated with increased mortality risk both in non-CKD and CKD populations.^{12–15} In the body, Se is incorporated as selenocysteine at the active center of selenoproteins and critically regulates their function. Human genome contains at least 25 genes encoding selenoproteins, which include glutathione peroxidases, thioredoxin reductases, selenoprotein P, and selenoprotein W.^{8,13,16} Among the diverse functions of Se and selenoproteins in humans, recent studies have identified their key roles in stress erythropoiesis, in which erythrocyte production is stimulated at extramedullary sites to counteract anemic stress.^{17–19} It has also been found that selenoproteins with antioxidant property protect erythrocytes from oxidative stress and cell senescence, thereby increasing the half-life.^{20,21}

In hemodialysis patients, several studies demonstrated that serum Se levels are lower than those in healthy controls.^{22,23} However, the clinical significance of Se in erythropoietic response in hemodialysis patients remains unclear. In this cross-sectional study, we determined the levels of serum Se in hemodialysis patients and evaluated their association with anemia and the response to ESAs.

METHODS

Patients

We performed a cross-sectional study of 173 hemodialysis patients at 4 dialysis facilities (Teikyo University Hospital [T], Shiki Ekimae Clinic [S], Nerima Hikarigaoka Hospital [N], and Tokyo-Kita Medical Center [K]). Patient inclusion criteria were at least 20 years of age and were receiving hemodialysis therapy (thrice weekly) for at least 2 months at the time of recruitment to the study. The study was approved by the Teikyo University School of Medicine Ethics Committee (number 19-090) and by the review board at participating institutions, and written informed consent was obtained. Patients included in this study received a stable dose of an ESA and had a steady level of Hb. None of the patients had acute hemorrhagic diseases, such as gastrointestinal bleeding, at the time of the study. ESAs were used according to the third edition of the guidelines for renal anemia from the Japanese Society for Dialysis Therapy.²⁴ Treatment of renal anemia was started when the Hb level was <10 g/dl in blood samples collected before the initiation of the first dialysis session of the week, and the dose was adjusted to maintain in the range of 10 to

12 g/dl. Recombinant human erythropoietin was administered 3 times per week, and darbepoetin was administered once per week (at first session in S, at midweek session in N, and at third session in T and K). Epoetin beta pegol was used only in 2 facilities (T and S), which was administered once or twice per month. All the ESAs were administered i.v. through the dialysis circuit. Intravenous iron therapy was started if patients were not able to maintain target Hb levels despite the use of an ESA and had serum ferritin level <100 ng/ml or TSAT <20%, according to the guidelines.²⁴

Data Collection

Data on demographics (e.g., age, sex, primacy cause of end-stage kidney disease, height, weight, diabetes mellitus, blood pressure, past medical history, and ESA dosage) and clinical values (e.g., Hb, serum albumin, ferritin, and TSAT) were collected by medical record abstraction. Serum C-reactive protein levels were measured by enzyme-linked immunosorbent assay, and zinc levels were determined by colorimetric method (SRL, Tokyo, Japan). We used ERI as the value to determine the response to ESA.²⁵ ERI was defined as the weight-adjusted weekly ESA dose divided by Hb levels (U/kg/week/g/dl). The dose conversion ratio was recombinant human erythropoietin to darbepoetin to epoetin beta pegol of 1:200:250, in accordance with previous studies.^{26,27}

Determination of Serum Se Concentrations

Serum Se concentrations were measured by inductively coupled plasma mass spectrometry (ICP-MS; iCAP Q, Thermo Fisher, Waltham, MA).²⁸ Blood samples collected before the initiation of first dialysis session were used to determine serum Se levels. Serum Se levels and iron parameters were determined in the same samples or samples obtained within 1 month. To avoid trace metal contamination, all the glassware and Teflon vessels were washed with ultrasonic cleaner and then soaked and kept in trace metal-grade concentrated nitric acid for 3 days. They were then rinsed with ultrapure water and dried. Serum samples (100 µl) were mixed with nitric acid (1000 µl) and mineralized using a microwave digestion system (ETHOS 1, Milestone SRL, Sorisole, Italy). After centrifugation at 1500 revolution per minute for 1 minute, the samples were diluted with 10 ml of ultrapure water. After the internal calibration, Se standard stock solution (Fujifilm, Tokyo, Japan) was diluted with blank into 5 standard concentrations (range, 0.4–40 µg/dl). All samples were measured in duplicate using iCAP Q.

Statistical Analysis

The data are summarized as mean ± SD or median and interquartile ranges for continuous variables and as

absolute numbers and percentages for categorical values. We used the χ^2 test, Student *t* test, and Mann-Whitney *U* test to compare patient characteristics between low Se group (<10.5 $\mu\text{g/dl}$, which is a lower limit of normal serum Se levels in adult Japanese subjects²⁹) and normal Se group. Correlation between parameters was analyzed by Pearson's correlation test. Logarithmic transformation was applied for ERI, TSAT, and ferritin before correlation analysis because these variables had right-skewed distribution.

Multiple regression analysis was used to evaluate the independent association of Se with ERI after adjustment of potential confounders. In model 1, we included demographics (age, sex, and dialysis vintage), TSAT, and Se levels. In addition to these factors, we included ferritin, a history of cardiovascular diseases, albumin, and C-reactive protein in model 2. In model 3, we included intact parathyroid hormone, zinc, dialysis modality, and dialysis dose (single-pool Kt/V; spKt/V).^{6,30,31} Variables in the regression model were evaluated for collinearity using the variance inflation factor. To determine the factors that predict high ESA resistance associated with poor outcome, we used multivariable logistic analysis. We defined high ESA resistance as $\text{ERI} \geq 9.44$ based on previous studies.³² Among the previously described factors associated with reduced ESA response,^{6,30,31} all variables associated with ESA resistance in the univariate analysis ($P < 0.20$) were entered into a multivariable model. We also included age, sex, and dialysis vintage in the analysis.

To compare the difference in ERI among the 4 groups divided by serum Se levels and iron status, data were analyzed by Kruskal-Wallis analysis of variance by ranks test followed by Dunn's *post hoc* test. Iron deficiency in the study subjects was defined as $\text{TSAT} < 20\%$ or ferritin $< 100 \text{ ng/ml}$.^{24,33} Given the difference in target iron levels in hemodialysis patients between Japan and Western countries,^{5,24,34,35} we also divided the patients by distinct threshold of iron parameters ($\text{TSAT} \leq 30\%$ and ferritin $\leq 500 \text{ ng/ml}$) according to Kidney Disease: Improving Global Outcomes Clinical Practice Guideline for Anemia.⁵ To compare the difference in Se levels divided by ERI quartiles, data were analyzed by analysis of variance followed by Dunnett's *post hoc* test. All analyses were performed using JMP version 14.3.0 (SAS institute, Cary, NC) and GraphPad Prism software, version 7.05 (GraphPad Software Inc., San Diego, CA), with 2-sided significance set at 0.05.

RESULTS

Patient Characteristics and Serum Se Levels

A total of 173 patients were included in the study. Clinical characteristics of the study participants were

found in Table 1 and Supplementary Table S1. The mean age was 67 years, 77% were male, and 73% of the patients received hemodiafiltration. Median dialysis vintage was 49 months, and mean single-pool Kt/V was 1.43. Mean serum Se levels in these patients were $10.8 \pm 2.9 \mu\text{g/dl}$, which was consistent with previous reports by others in hemodialysis patients (average 10.3 $\mu\text{g/dl}$).²³ In our patients, 86 (50%) had the serum Se levels of $<10.5 \mu\text{g/dl}$, a lower limit of normal serum Se levels in adult Japanese subjects.²⁹ In addition, only 33 patients (19%) had 12.2 $\mu\text{g/dl}$ or above, which is found to be associated with minimal mortality.¹³

Patients were divided into 2 groups according to serum Se levels. Overall, the clinical characteristics of patients with low serum Se levels (those with $<10.5 \mu\text{g/dl}$) were similar to those with serum Se levels of 10.5 $\mu\text{g/dl}$ or higher, including age, sex, and Hb levels (Table 1). However, the proportion of patients that were free of ESAs was nonsignificantly fewer in the low serum Se group; the number of patients who were not receiving ESAs was 18 (21%) in the normal Se group, whereas 10 patients (12%) were free of ESAs in the low Se group. We did not find difference in body mass index and serum albumin, the indicators of nutritional status, between the 2 groups. Iron status and the proportion of patients receiving i.v. iron therapy were similar (Table 1). In those receiving iron therapy, the median iron dose was 160 mg per month in both groups ($P = 0.54$).

Association of Serum Se Levels With the Response to ESAs

To determine whether serum Se levels are associated with ESA responsiveness, we next focused on 145 patients who were receiving ESAs and calculated ERI for each patient (see the Methods section). Characteristics for these 145 patients are found in Table 2. In this population, ERI tended to be higher in patients with $<10.5 \mu\text{g/dl}$ than those with $\geq 10.5 \mu\text{g/dl}$ ($P = 0.06$; Table 2). Other variables are not different between the 2 groups, including body mass index, albumin, TSAT, and ferritin.

Next, we evaluated association of serum Se levels with ERI and Hb. As illustrated in Figure 1a, there was no significant correlation between serum Se levels and Hb levels. However, we found significant inverse association between Se levels and ERI ($r = -0.32$, $P < 0.001$; Figure 1b).

We also analyzed the relationship between Se and iron, another trace element that determines the response to ESA. As expected, both TSAT and ferritin levels significantly correlated with ERI ($r = -0.27$ and $r = -0.27$, respectively; Figure 2a and b). However, we

Table 1. Baseline characteristics of 173 hemodialysis patients

Characteristics	All	Se < 10.5 µg/dl (n = 86)	Se ≥ 10.5 µg/dl (n = 87)	P value
Age	67.0 (12.7)	67.2 (12.9)	66.8 (12.5)	0.82
Male	134 (77%)	70 (81%)	64 (74%)	0.22
Body mass index, kg/m ²	23.0 (3.9)	22.8 (3.9)	23.1 (3.9)	0.73
Time on dialysis, mo	49 (22–97)	49 (22–92)	55 (21–102)	0.90
HDF	127 (73%)	62 (72%)	65 (75%)	0.70
Dialysis dose (spKt/V)	1.43 (0.24)	1.42 (0.26)	1.44 (0.23)	0.56
Not receiving ESA	28 (16%)	10 (12%)	18 (21%)	0.10
Selenium, µg/dl	10.8 (2.9)	8.7 (1.3)	12.8 (0.2)	<0.001
Hb, g/dl	11.1 (1.1)	11.2 (1.1)	11.1 (1.0)	0.58
TSAT, %	23.7 (15.7–32.5)	23.7 (15.4–32.6)	23.9 (17.3–32.4)	0.84
Ferritin, ng/ml	70 (25–127)	68 (25–121)	75 (24–139)	0.82
Intravenous iron therapy	20 (12%)	11 (13%)	9 (10%)	0.62

Hb, hemoglobin; HDF, hemodiafiltration; IQR, interquartile range; spKt/V, single-pool Kt/V; Se, selenium; TSAT, transferrin saturation. Full list of baseline characteristics is available in [Supplementary Table S1](#). Data are n (%), mean (SD), or median (IQR).

found no correlation between Se levels and TSAT nor ferritin levels ([Figure 2c](#) and [d](#)).

Independent Association of Serum Se Levels With ERI

Next, we performed multiple regression analyses to determine whether serum Se levels are associated with ERI after adjustment of potential cofounders. Consistent with previous studies,⁶ the results demonstrated significant association of ESA hyporesponsiveness with

TSAT, being female, and time on hemodialysis ([Table 3](#), model 1). Along with these factors, we found that serum Se levels were highly significantly associated with ERI ([Table 3](#), model 1). In addition to these factors, we included ferritin, serum albumin (a surrogate of nutritional status), a history of cardiovascular diseases, and C-reactive protein (surrogate of chronic inflammation), based on the previous literature.⁶ The association between Se and ERI was significant also in this model (model 2). The association between the 2

Table 2. Characteristics of patients receiving ESAs

Characteristics	All	Se < 10.5 µg/dl (n = 76)	Se ≥ 10.5 µg/dl (n = 69)	P value
Age	67.2 (12.8)	67.3 (12.9)	67.0 (12.8)	0.88
Male	111 (77%)	62 (82%)	49 (71%)	0.13
Body mass index, kg/m ²	22.9 (4.0)	22.8 (3.8)	23.1 (4.2)	0.96
Time on dialysis, mo	49 (21–94)	50 (21–93)	49 (20–94)	0.81
HDF	106 (73%)	56 (74%)	50 (72%)	0.87
Dialysis dose (spKt/V)	1.43 (0.24)	1.41 (0.24)	1.45 (0.24)	0.38
SBP, mm Hg	154.3 (24.8)	155.1 (24.6)	153.3 (25.3)	0.67
DBP, mm Hg	80.3 (14.6)	81.0 (14.1)	79.6 (15.2)	0.55
HR, /min	76.8 (13.3)	76.2 (13.1)	77.5 (13.5)	0.55
Smoking history	25 (17%)	15 (20%)	10 (14%)	0.40
Diabetes mellitus	78 (54%)	42 (55%)	36 (52%)	0.71
Cardiovascular diseases	55 (38%)	28 (37%)	27 (39%)	0.78
Antihypertensive medication	110 (76%)	56 (74%)	54 (78%)	0.52
ERI	8.04 (3.63–14.71)	9.30 (4.87–15.81)	6.81 (3.26–13.72)	0.06
Selenium, µg/dl	10.8 (3.0)	8.7 (1.2)	13.0 (2.7)	<0.001
Hb, g/dl	11.0 (1.0)	11.1 (1.0)	10.9 (0.9)	0.33
Albumin, g/dl	3.7 (0.4)	3.6 (0.4)	3.7 (0.3)	0.32
Fe, µg/dl	58 (45–80)	58 (46–79)	59 (43–81)	0.71
TIBC, µg/dl	265 (226–305)	265 (214–303)	265 (230–309)	0.64
TSAT, %	23.7 (16.1–32.2)	23.9 (15.7–32.6)	23.4 (17.7–31.9)	0.87
Ferritin, ng/ml	73 (25–125)	69 (24–120)	83 (25–141)	0.67
iPTH, pg/ml	156 (107–215)	167 (117–213)	153 (95–221)	0.5
Zinc, µg/dl	62 (13)	61 (11)	64 (14)	0.15
CRP, mg/dl	0.11 (0.04–0.33)	0.09 (0.04–0.38)	0.12 (0.05–0.28)	0.88

CRP, C-reactive protein; DBP, diastolic blood pressure; ERI, erythropoiesis resistance index; ESA, erythropoiesis-stimulating agent; Fe, iron; Hb, hemoglobin; HDF, hemodiafiltration; HR, heart rate; iPTH, intact parathyroid hormone; IQR, interquartile range; SBP, systolic blood pressure; Se, selenium; spKt/V, single-pool Kt/V; TIBC, total iron binding capacity; TSAT, transferrin saturation.

Data are n (%), mean (SD), or median (IQR).

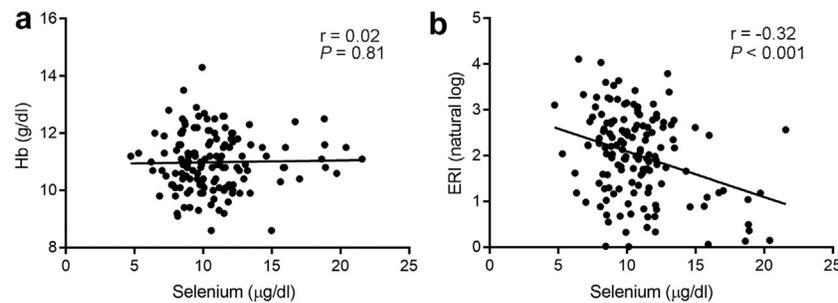


Figure 1. Low serum Se levels correlates with resistance to ESA. (a) Relationship between Se levels and Hb levels. (b) Relationship between Se levels and ERI (see Methods section). *P* values were calculated by Pearson's correlation test. ERI, erythropoiesis resistance index; ESA, erythropoiesis-stimulating agent; Hb, hemoglobin; Se, selenium.

variables was statistically significant after further adjusting with zinc, type of dialysis, dialysis dose, and intact parathyroid hormone levels (Table 3, model 3).³⁰

Previous studies have revealed that an ERI level of >15 (in European study)³⁶ or that of ≥ 9.44 (in Japanese study)³² predicts increased mortality in hemodialysis patients. In logistic regression analysis, there was a significant association of serum Se with high ERI resistance after adjustment of potential confounding factors, when the cutoff value was set at ERI > 15 (Supplementary Table S2; odds ratio, 0.74 per 1 µg/dl increase in serum Se, *P* = 0.005). This association was consistently observed when the cutoff value was set at ERI ≥ 9.44 (Supplementary Table S2; odds ratio, 0.86 per 1 µg/dl increase in serum Se, *P* = 0.03).

Se and Iron Status Predict the Response to ESA

We finally tested whether iron status and separately serum Se levels predict the response to ESAs. We

divided the patients into 4 groups according to Se levels (<10.5 µg/dl and ≥ 10.5 µg/dl) and the presence and absence of iron deficiency (TSAT < 20% or ferritin < 100 ng/ml). We then compared ERI in each group and found that there were significant differences in ERI levels across 4 groups (Table 4) (*P* = 0.009). Given that the target iron levels in patients with CKD with anemia differ between Japan and Europe or North America, we also divided the patients using different threshold (TSAT $\leq 30\%$ and ferritin ≤ 500 ng/ml, which is an indication for iron therapy in renal anemia in hemodialysis patients in Kidney Disease: Improving Global Outcomes Guideline).⁵ Again, we found significant differences in ERI across groups (Table 4) (*P* = 0.04). Conversely, when patients were divided by the quartiles of ERI, Se levels were significantly different among the 4 groups (Table 5) (*P* < 0.001).

To test whether serum Se levels affect ERI separately from iron deficiency, we performed multiple regression

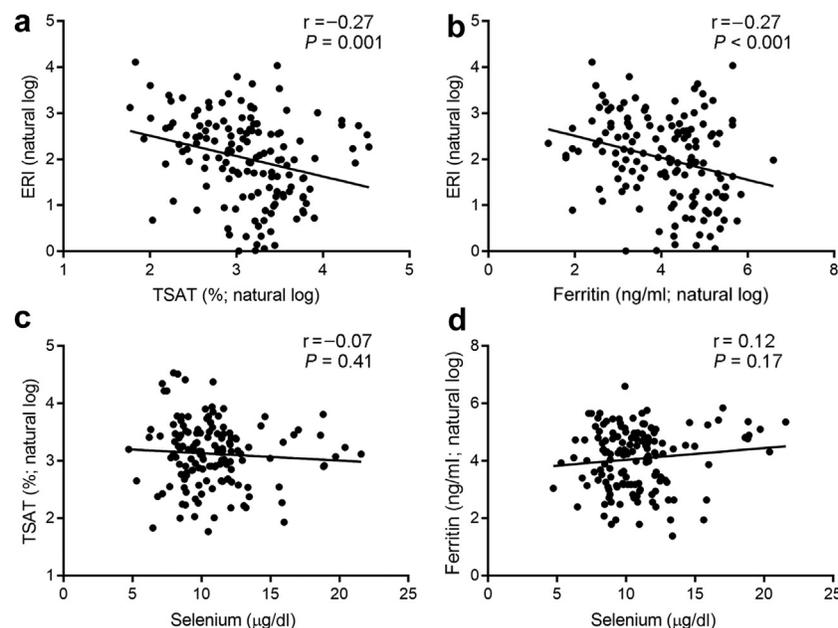


Figure 2. Relationship between serum Se levels and TSAT or ferritin levels. (a, b) Correlation analysis between (a) ERI and TSAT or (b) ferritin levels. (c, d) Correlation between (c) Se levels and TSAT or (d) ferritin levels. *P* values were calculated by Pearson's correlation test. ERI, erythropoiesis resistance index; Se, selenium; TSAT, transferrin saturation.

Table 3. Multiple regression analysis with ERI (natural log) as the dependent variable

Model 1	β	95% CI	P value
Female (vs. male)	0.60	0.30–0.91	<0.001
Age, yrs	0.008	–0.002 to 0.018	0.13
Time on dialysis (≥ 49 mo) (vs. <49 mo)	0.35	0.09–0.61	0.008
Selenium, $\mu\text{g}/\text{dl}$	–0.12	–0.16 to –0.07	<0.001
TSAT, % (natural log)	–0.52	–0.76 to –0.29	<0.001
Model 2	b	95% CI	P value
Female (vs. male)	0.59	0.27–0.91	<0.001
Age, years	0.01	–0.01 to 0.02	0.34
Time on dialysis (≥ 49 mo) (vs. <49 mo)	0.33	0.07–0.60	0.02
Selenium, $\mu\text{g}/\text{dl}$	–0.11	–0.15 to –0.06	<0.001
TSAT, % (natural log)	–0.47	–0.77 to –0.18	0.002
Ferritin, ng/ml (natural log)	–0.07	–0.23 to 0.09	0.38
Cardiovascular diseases	–0.04	–0.17 to 0.10	0.59
Alb, g/dl	–0.36	–0.77 to 0.04	0.08
CRP, mg/dl (natural log)	–0.03	–0.14 to 0.07	0.55
Model 3	b	95% CI	P value
Female (vs. male)	0.56	0.21–0.91	0.002
Age, yrs	0.00	–0.01 to 0.02	0.43
Time on dialysis (≥ 49 mo) (vs. <49 mo)	0.29	–0.01 to 0.60	0.06
Selenium, $\mu\text{g}/\text{dl}$	–0.11	–0.15 to –0.06	<0.001
TSAT, % (natural log)	–0.47	–0.77 to –0.18	0.002
Ferritin, ng/ml (natural log)	–0.07	–0.23 to 0.10	0.43
Cardiovascular diseases	–0.05	–0.19 to 0.09	0.49
Alb, g/dl	–0.34	–0.77 to 0.10	0.13
CRP, mg/dl (natural log)	–0.02	–0.13 to 0.09	0.66
iPTH, pg/ml (natural log)	0.12	–0.10 to 0.34	0.28
Zinc, $\mu\text{g}/\text{dl}$	0.00	–0.01 to 0.01	0.95
HDF (vs. HD)	0.04	–0.29 to 0.37	0.82
spKt/V	0.21	–0.50 to 0.92	0.56

Alb, albumin; CRP, C-reactive protein; ERI, erythropoiesis resistance index; HD, hemodialysis; HDF, hemodiafiltration; iPTH, intact parathyroid hormone; spKt/V, single-pool Kt/V; TSAT, transferrin saturation.

analysis. The results demonstrated that the presence of low Se levels and separately absolute iron deficiency was independently associated with higher ERI levels (Table 6).

DISCUSSION

In this study, we found that serum Se levels inversely correlate with ERI in Japanese hemodialysis patients, but they were not associated with iron status, a key determinant of ESA response. Multiple regression analysis confirmed the independent correlation between serum Se levels and ERI. In addition, we found that low serum Se levels (<10.5 $\mu\text{g}/\text{dl}$) and iron status

was useful in predicting the response to ESAs in our cohort.

Several lines of evidence have revealed that Se and selenoproteins play critical roles in erythrocyte homeostasis. Chronic Se deficiency results in morphologic abnormalities in erythrocytes in humans,¹¹ and clinical studies have demonstrated the association between anemia and low serum Se concentrations in non-CKD populations.^{37–39} Consistently, in experimental studies, both Se-deficient diet and genetic ablation of Trsp (encoding selenocysteine transfer RNA) induce anemia in mice.^{19,40} Because iron cation in the heme protein can produce hydroxyl radical, a strong oxidant, erythrocytes express a

Table 4. ERI levels according to Se and iron status

Variable	Se ≥ 10.5 $\mu\text{g}/\text{dl}$ Iron deficiency (–)	Se < 10.5 $\mu\text{g}/\text{dl}$ Iron deficiency (–)	Se ≥ 10.5 $\mu\text{g}/\text{dl}$ Iron deficiency (+)	Se < 10.5 $\mu\text{g}/\text{dl}$ Iron deficiency (+)
ERI	3.4 (2.3–6.9) (Ref)	9.7 (3.4–16.5) ^a	8.8 (4.6–15.5) ^a	9.3 (5.6–15.3) ^b
Variable	Se ≥ 10.5 $\mu\text{g}/\text{dl}$ TSAT >30% or ferritin > 500 ng/ml	Se < 10.5 $\mu\text{g}/\text{dl}$ TSAT >30% or ferritin > 500 ng/ml	Se ≥ 10.5 $\mu\text{g}/\text{dl}$ TSAT $\leq 30\%$ and ferritin ≤ 500 ng/ml	Se < 10.5 $\mu\text{g}/\text{dl}$ TSAT $\leq 30\%$ and ferritin ≤ 500 ng/ml
ERI	5.1 (3.3–7.3) (Ref)	7.3 (3.5–12.7)	8.6 (3.2–14.4)	10.8 (6.0–21.4) ^a

ERI, erythropoiesis resistance index; IQR, interquartile range; Ref, reference; Se, selenium; TSAT, transferrin saturation.

^aP < 0.05 compared with Ref group.

^bP < 0.01 compared with Ref group.

Table 5. Serum Se levels according to ERI quartiles

Variable	ERI quartile 1 (<3.6)	ERI quartile 2 (3.6–8.1)	ERI quartile 3 (8.2–14.5)	ERI quartile 4 (>14.5)
Se (µg/dl)	12.4 ± 4.1	10.3 ± 1.8	10.7 ± 2.8	9.7 ± 2.1 ^a

ERI, erythropoiesis resistance index; Se, selenium.

^aP < 0.001 compared with quartile 1.

number of antioxidant selenoproteins, such as glutathione peroxidases and thioredoxin reductases.^{18,41} These selenoproteins prevent erythrocyte senescence and hemolysis.^{40,42–44} Besides the role as reactive oxygen species scavengers, recent studies have revealed that selenoproteins are required for the differentiation of stress erythroid progenitors.^{17,18} In hypoxic conditions, such as severe anemia and blood loss, erythrocyte production at extramedullary sites is drastically increased to facilitate the recovery (stress erythropoiesis).⁴⁵ However, the depletion of selenoproteins prevents the expansion and maturation of erythrocyte progenitor cells in response to erythropoietin, resulting in the impaired response to anemic stress. It has been found that the patients with advanced CKD have increased levels of oxidative stress.^{46,47} In addition, factors such as blood trapping in the dialysis equipment and frequent laboratory examinations can result in blood loss in hemodialysis patients.⁴⁸ Therefore, we infer that the roles of Se in preventing erythrocyte damage and in facilitating stress erythropoiesis can be relevant in counteracting the progression of anemia in hemodialysis patients. Conversely, the deficiency in Se can blunt the response of erythroid progenitors to ESAs in hemodialysis patients.

We found that lower serum Se levels were associated with higher ERI levels when patients were divided into 4 categories based on Se levels and iron status (Table 4). The difference among the 4 groups was significant after adjustment of possible confounding factors (Table 6). In patients without iron deficiency, ERI was higher in subjects with Se < 10.5 µg/dl than those with ≥10.5 µg/dl, indicating the predictive value of serum Se in ESA response in hemodialysis patients. These data are consistent with the above-mentioned experimental evidence revealing the

Table 6. Association of selenium and iron status with ERI

Se and iron status	β	95% CI	P value
Se ≥ 10.5 µg/dl and ID (–)	Reference	—	—
Se < 10.5 µg/dl and ID (–)	0.71	0.21–1.21	0.006
Se ≥ 10.5 µg/dl and ID (+)	0.63	0.19–1.06	0.006
Se < 10.5 µg/dl and ID (+)	0.78	0.34–1.21	<0.001

ERI, erythropoiesis resistance index; ID, iron deficiency; Se, selenium.

Following variables were included as covariables: gender, age, time on dialysis, cardiovascular diseases, albumin, CRP, iPTH, and zinc. Dependent variable: ERI (natural log).

role of Se in erythrocyte function independently of iron status.

In our study, approximately half of the participants had lower Se levels than the population-based reference values. Previously, several studies analyzed serum Se levels in hemodialysis patients.²⁹ In a systematic review and meta-analysis involving 128 studies, serum Se levels were lower in hemodialysis patients than in control subjects.⁴⁹ In another study involving 1041 hemodialysis patients, serum Se levels were also low compared with control subjects (multivariate-adjusted means, 10.3 µg/dl in hemodialysis patients vs. 11.7 µg/dl in control subjects).²³ Our results are in line with these reports and suggest that low Se levels are frequently observed in Asian hemodialysis patients. Another study involving 198 hemodialysis patients in Canada reported that low Se levels were less common.⁵⁰ The difference may be attributable to the difference in population, because Se status has been found to vary across the world.¹³ Factors that influence Se levels in hemodialysis patients merit further evaluation.

Previous studies have reported that the iron status and its management are significantly different between Japan and Western countries. Indeed, the median ferritin level in our patients was 70 ng/ml, which was close to the data in the Japanese dialysis registry (62 ng/ml) and was low compared with the values among dialysis patients in Western countries.^{34,35} In addition, the reported ERI levels that are associated with increased mortality are different between these regions. Taking these differences into consideration, we performed several analyses using different cutoff values and found that the inverse association between Se levels and ERI is consistent. Although these data indicate that the finding of the current study may possibly be applicable to other populations, it would be desirable to confirm the association in different cohorts.

The lack of association between Hb levels and Se levels might be because patients with reduced response to ESAs could have received higher doses to achieve target Hb levels of 10 to 12 g/dl. In one study that tested the effects of Se supplementation in hemodialysis patients, subjective global assessment score and malnutrition-inflammation score were improved, whereas Hb levels remained unaltered.⁵¹ Because the information on ESA doses was not available in that study, it was unclear whether Se supplementation reduced the required dose of ESA to maintain Hb levels. It will be of interest to address this point in future prospective analysis.

There are several limitations to this study. First, this study analyzed the cross-sectional data and does

not provide information on causal relationship. Although we speculate that either impaired stress erythropoiesis or erythrocyte senescence and hemolysis induced by oxidative stress (or both) may mediate the association between the 2 parameters, we did not evaluate markers for oxidative stress in this study and the underlying mechanisms remain undetermined. Whether Se supplementation improves the response to ESAs and reduces ESA demand to maintain target Hb levels also need further evaluation. Second, this study included patients from one region. Given the geographic differences in Se status and the management of iron status, the findings of the current study need to be tested in different populations. Third, because hypoxia-inducible factor prolyl hydroxylase inhibitors were not available at the time of data collection, we do not have information on whether Se levels relate to the response to hypoxia-inducible factor prolyl hydroxylase inhibitors, which will be an interesting issue that should be analyzed in future studies. Fourth, serum Se levels were determined at a single point in our study. The reported within-person coefficient of variance for Se is 5.1%,⁵² which is similar to the values of calcium and potassium and is lower than those of glucose, uric acid, phosphorus, and so on. Nonetheless, it would have been ideal to determine serum Se levels at several time points to minimize the influence of intraindividual variation. Fifth, it is possible that the study can potentially be confounded by unmeasured factors, although we included variables such as iron status, albumin, and zinc in multivariate analysis.

Despite these limitations, our study demonstrated the significant inverse relationship between ERI and serum Se levels, providing insights into the molecular basis of ESA hyporesponsiveness in hemodialysis patients. We also found that iron and Se can independently affect the response to ESAs. Given the evidence that low Se levels are associated with poor prognosis in hemodialysis patients and in patients without CKD, Se deficiency may be involved in the association between poor ESA response and increased mortality. Our data indicate that Se deficiency can be an underestimated cause of ESA hyporesponsiveness, which warrants further investigation.

DISCLOSURE

All the authors declared no competing interests.

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SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

Table S1. Baseline characteristics according to Se concentrations.

Table S2. Risk factors for high ERI levels using logistic regression analysis.

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