

## Factors associated with erythropoiesis-stimulating agent hyporesponsiveness anemia in chronic kidney disease patients

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### Abstract

Anemia is one of the most common problems in chronic kidney disease (CKD). Despite comprehensive investigations in several cases, definite causes of anemia frequently remain unknown. Our study aimed to analyze the factors that possibly affect anemia in CKD patients who were referred for hematology consultation. A total of 87 patients were retrospectively included in the cohort. Forty-four cases were excluded, including 30 cases with unavailable intact parathyroid hormone (iPTH) data, 11 cases with bone marrow diseases (8 Pure red cell aplasia, 3 Myelodysplastic syndrome) and 3 cases with thalassemia. In total, 43 patients were analyzed. Patients with high iPTH had a significantly lower Hemoglobin (Hb) level and required a higher dose of erythropoiesis stimulating agents (ESAs) compared with the normal iPTH group (Hb 8.29 vs 9.24 mg/dL,  $P=0.032$  and ESAs dose of 16,352.94 vs. 12,444.44 U/week,  $P=0.024$ ). Univariate, followed by stepwise multivariate analysis was performed and determined that serum phosphate (PO<sub>4</sub>) was significantly associated with lower Hb level ( $P=0.01$  and  $P=0.013$ , respectively). In addition, Hb level was inversely correlated with iPTH and serum phosphate (PO<sub>4</sub>) level ( $r=-0.54$ ,  $P<0.001$  and  $r=-0.47$ ,  $P=0.005$ ; respective-

ly). Mineral disequilibrium is an important factor associated with anemia in ESA hyporesponsive CKD. Also, hyperphosphatemia and secondary hyperparathyroidism are significantly correlated with low Hb. As a result, we strongly suggest correction of mineral disequilibrium factors prior to performing bone marrow study.

### Introduction

Anemia in chronic kidney disease (CKD) is a common problem in CKD patients, particularly in hemodialysis dependent end-stage renal disease (ESRD). Previous study showed higher mortality rate in CKD patients with anemia.<sup>1</sup> Erythropoietin deficiency is the major essential mechanism of anemia in CKD patients.<sup>2</sup> The erythropoiesis stimulating agent (ESA) is the primary treatment of anemia in CKD. Clinical practice guidelines, Kidney Disease: Improving Global Outcomes (KDIGO) 2012, recommended keeping target goals for hemoglobin levels at 11-12 g/dL in CKD patients with ESA usage.<sup>3</sup> Despite adequate ESA treatment, some patients remain anemic or eventually lose response to ESA, defined as ESA hyporesponsiveness. There are many plausible causes of anemia in ESA hyporesponsiveness conditions including nutritional deficiency, co-existing bone marrow diseases, and secondary hyperparathyroidism.<sup>2,4</sup> Secondary hyperparathyroidism is also a major cause of ESA hyporesponsiveness. Pathogenesis of anemia in secondary hyperparathyroidism is still unclear but could be attributed to decreased red blood cell (RBC) production from calcitriol deficiency or the inhibition of erythropoiesis from parathyroid hormone associated with bone marrow fibrosis and interference with red cell survival.<sup>5</sup> Previous studies in hemodialysis patients revealed a significant relationship between ESA hyporesponsiveness and higher intact parathyroid hormone (iPTH) level,<sup>6,7</sup> and the inverse relationship between iPTH and hemoglobin level.<sup>8</sup> One particular study demonstrated that serum iPTH level above 122 pg/mL had 67% sensitivity and 75% specificity to predict hemoglobin level below 10.0 g/dL.<sup>9</sup> Herein, we attempted to explore the causes of anemia in CKD with ESA hyporesponsiveness, in order to analyze the factors that could affect hemoglobin levels in CKD patients referred to the Hematology clinic for anemia evaluation.

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### Materials and Methods

#### Study participants

Our study retrospectively reviewed the data of all CKD patients referred to the Hematology clinic at King Chulalongkorn Memorial Hospital for anemia consultation from January 2007 to June 2017. All CKD patients who were deemed anemic despite

receiving erythropoiesis stimulating agents and underwent bone marrow study were included in this analysis. Subjects with iron deficiency anemia were excluded by iron study prior to the bone marrow study.

### Study design

Baseline demographic data, underlying diseases, and ESA dose per week were collected. Laboratory data within 6 months from date of bone marrow study including hemoglobin, reticulocyte count, ferritin, transferrin saturation, blood urea nitrogen (BUN), creatinine, creatinine clearance, intact parathyroid hormone, calcium, phosphate, albumin, alkaline phosphates were also recorded.

CKD was defined by having a glomerular filtration rate (GFR) <60 mL/min/1.73 m<sup>2</sup> for 3 months or more.<sup>10</sup> Patients with anemia were defined by having a hemoglobin level below 10 g/dL. ESAs were administered for at least 3 months. High parathyroid group was defined as having an iPTH level above 65 pg/mL according to the reference range of serum iPTH level at King Chulalongkorn Memorial Hospital.

### Bone marrow study

Bone marrow assessments were conducted by both a hematologist and a hematopathologist. Assessments were made from aspirate smears, core biopsies and reticulin stains. Bone marrow fibrosis was semiquantitatively determined by reticulin and trichrome staining. Reticulin was graded according to the World Health Organization (WHO) as 0-3.<sup>11</sup>

### Statistical analysis

For descriptive analysis, we used IBM SPSS statistics 22 program to calculate con-

tinuous data including percentage, mean and standard deviation for various characteristics. To compare the normal and high parathyroid groups, we analyzed differences by conducting independent t-test with a significant P-value at 0.05 using IBM SPSS statistics 22 program. Moreover, univariate analysis using a Pearson correlation coefficient and multivariate analysis were used for correlation between hemoglobin levels and other variable data including age, gender, body mass index (BMI), iPTH, serum calcium, phosphate, serum albumin, transferrin saturation ratio, ferritin, hemodialysis and non-hemodialysis cases, using Strata version 15.1 program.

## Results

### Patient's characteristics

A total of 87 patients were included in the study and all patients were performed a bone marrow study to investigate the causes of anemia. Eleven patients (12.6%) were diagnosed as having bone marrow disease [8 pure red cell aplasia (PRCA) and 3 myelodysplastic syndromes (MDS)], while three cases had thalassemia diseases. Because serum iPTH were not available in 30 patients, only 43 cases could be evaluated for mineral status.

Among the 43 CKD patients, the mean age was 63 (range 33-88) years old and 54.5% were male. The common coexisting diseases included hypertension (n=30; 69.8%), diabetes mellitus (n=16; 37.2%) and dyslipidemia (n=13; 30.2%). Underlying causes of chronic kidney disease were diabetic nephropathy, chronic glomerulonephritis, hypertensive nephropathy in 14 (32.6%), 4 (9.3%) and 1 (2.3%)

cases, respectively. Thirty-six (83.7%) patients were on chronic hemodialysis. Evaluation for bone marrow fibrosis by reticulin staining was performed in 42 patients with chronic kidney disease (Table 1). Most of the patients (29/42) showed fibrosis grade 1. In addition, 4 patients showed fibrosis grade 3-4 and grade 2. Three patients showed fibrosis grade 0.

### Potential factors associated with ESA hyporesponsiveness

The mean hemoglobin (Hb) level from the 43 CKD patients was 8.49 g/dL ( $\pm$ 1.19). Thirty-four (79.1%) patients showed high intact parathyroid (iPTH) level (iPTH  $\geq$ 65 pg/mL). Mean iPTH levels were found to be 361.73 pg/mL (22.9-3,362). Clinical characteristics of patients with high and normal iPTH level are shown in Table 2. Patients with high iPTH levels were older than those with normal iPTH levels (71 vs. 60 years old; P=0.02). Comparing normal and high iPTH groups, a significantly lower hemoglobin level (8.29 vs 9.24 mg/dL; P=0.032) and a higher dose of erythropoietin usage (16,352.94 vs 12,444.44 unit/week; P=0.024) were demonstrated in the high iPTH group (Table 3). In addition, serum calcium was found to be significantly lower in patients with high iPTH compared to normal iPTH levels (9.33 vs. 10.25 mg/dL;

**Table 1. Fibrosis grading by reticulin staining.**

Fibrosis grade	Number (total N=42)
0	3
1	29
1-2	4
2	4

**Table 2. Clinical characteristics of whole, normal iPTH and high iPTH groups.**

Variables	All patients	Normal iPTH (<65 pg/mL)	High iPTH (>65 pg/mL)	P
No. Patients	43 (100%)	9 (20.9%)	34 (79.1%)	-
Age (years)	63.0 (33.0-88.0)	70.22 (60.0-86.0)	61.09 (33.0-88.0)	0.02
Sex (%)				-
Male	24 (55.8)	5 (55.6)	19 (55.9)	
Female	19 (44.2)	4 (44.4)	15 (44.1)	
BMI (Kg/m <sup>2</sup> )	21.29 (14.20-26.45)	22.30 (21.6-23.2)	21.18 (14.2-26.45)	0.54
Underlying disease (%)				-
Diabetes	16 (37.2)	4 (44.4)	12 (35.3)	
Hypertension	30 (69.8)	8 (88.9)	22 (22)	
Dyslipidemia	13 (30.2)	3 (33.3)	10 (29.4)	
No underlying	6 (14.0)	1 (11.1)	5 (14.7)	
ESRD on HD (%)	36 (83.7)	8 (88.9)	28 (82.4)	-

$P=0.025$ ). In a univariate analysis followed by a stepwise multivariate analysis, serum phosphate was significantly associated with lower hemoglobin level ( $P=0.01$  and  $P=0.013$ , respectively, Table 4). As shown in Figure 1, serum hemoglobin level was inversely correlated with iPTH and serum phosphate level ( $r=-0.54$ ,  $P<0.001$  and  $r=-0.47$ ,  $P=0.005$ ; respectively). When classifying iPTH levels into 1 to more than 9 times of the normal level, 6 patients (14%) had a serum iPTH nine times higher than

the normal level. Patients with iPTH three times more than the normal value ( $n=4$ ) showed significantly lower hemoglobin levels than patients with iPTH three times lower than the normal value ( $n=27$ ), (Hb 7.3 g/dL vs. 9 g/dL,  $P=0.01$ ).

## Discussion

This study was done in CKD patients

with anemia who were already treated with erythropoiesis stimulating agents (ESA). Iron deficiency anemia was excluded by iron study and positive iron staining in bone marrow. Bone marrow disease was the cause of anemia in 12% (11/87) of subjects, while 3.4% (3/87) were diagnosed as thalassemia diseases. In patients with normal bone marrow study, patients with higher iPTH level had lower hemoglobin level than patients with a normal iPTH level. This result was concordant with the previous

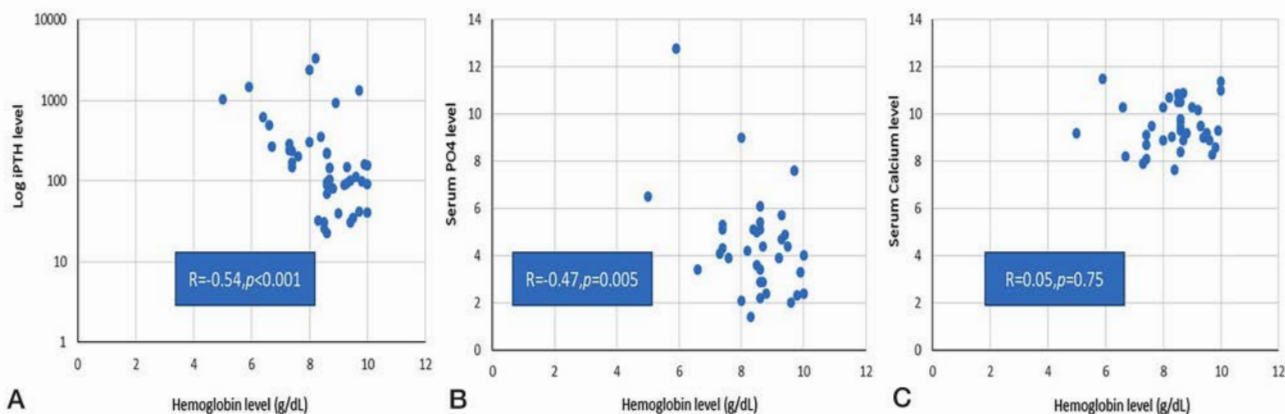
**Table 3. Biochemistry of whole, normal iPTH and high iPTH groups.**

Variables	All patients	Normal iPTH	High iPTH (<65 pg/mL)	P (>65 pg/mL)
No. Patients	43 (100%)	9 (20.9%)	34 (79.1%)	-
Hb (g/dL)	8.49 ( $\pm 1.19$ )	9.24 ( $\pm 0.61$ )	8.29 ( $\pm 1.24$ )	0.032
Iron (microgram/dL)	82.1 ( $\pm 48.26$ )	89.22 ( $\pm 60.9$ )	80.06 ( $\pm 45.12$ )	0.623
Ferritin (ng/mL)	1115.84 ( $\pm 895.21$ )	1162.32 ( $\pm 1006.0$ )	1102.77 ( $\pm 878.68$ )	0.863
Transferrin (mg/dL)	215.9 ( $\pm 65.80$ )	231.22 ( $\pm 101.08$ )	211.45 ( $\pm 53.05$ )	0.586
Transferrin saturation (%)	40.87 ( $\pm 25.96$ )	42.23 ( $\pm 28.23$ )	40.47 ( $\pm 25.74$ )	0.861
Reticulocyte count	74050 ( $\pm 35193.49$ )	69000 ( $\pm 22068.07$ )	74771.43 ( $\pm 37031.03$ )	0.79
Calcium (mg/dL)	9.50 ( $\pm 0.99$ )	10.25 ( $\pm 0.85$ )	9.33 ( $\pm 0.95$ )	0.025
Phosphate (mg/dL)	4.45 ( $\pm 2.18$ )	4.08 ( $\pm 1.58$ )	4.52 ( $\pm 2.29$ )	0.656
ALP (IU/L)	122.72 ( $\pm 85.48$ )	84.2 ( $\pm 27.04$ )	134.05 ( $\pm 93.84$ )	0.261
Albumin (mg/dL)	3.51 ( $\pm 0.61$ )	3.20 ( $\pm 0.66$ )	3.5 ( $\pm 0.58$ )	0.141
ESA dose (unit/week)	15534.88 ( $\pm 7983.16$ )	12444.44 ( $\pm 2185.81$ )	16352.94 ( $\pm 8755.54$ )	0.024
Iron supplement	16 (37.2%)	4 (44.4%)	12 (35.3%)	-

**Table 4. Univariate and multivariate analysis.**

Parameters	Univariate		Stepwise multivariate	
	Coefficient (95% CI)	P	Coefficient (95% CI)	P
Age	0.023 (0.00 to 0.046)	0.05		
BMI	0.067 (-0.09 to 0.22)	0.37		
Serum iPTH/1000	-0.53 (-1.07 to 0.002)	0.05		
Calcium	0.086 (-0.309 to 0.48)	0.66		
Serum phosphate	-0.22 (-0.38 to -0.05)	0.01	-0.22 (-0.38 to -0.049)	0.013
Albumin	-0.24 (-0.91 to 0.43)	0.47		

In multivariate adjusting for age and serum iPTH and using stepwise for selected final model.



**Figure 1. Correlation of Hemoglobin with serum iPTH (A), serum phosphate (PO<sub>4</sub>) (B) and serum Calcium (C).**



studies.<sup>12,13</sup> Association of secondary hyperparathyroidism and anemia in chronic kidney disease which could be explained from the inhibitory effect of parathyroid hormone to erythropoietin secretion corresponded with a study showing higher serum erythropoietin level after parathyroidectomy.<sup>14</sup> Besides the above-mentioned mechanisms, some studies demonstrated the inhibitory effect of parathyroid hormone on the growth of bone marrow erythroid progenitors and stimulation of fibrous formation in bone marrow.<sup>15</sup> But, in our study there was no significant bone marrow fibrosis. A previous study also showed that treatment of secondary hyperparathyroidism with cinacalcet (calcimimetic) could increase hemoglobin levels in chronic hemodialysis patients.<sup>16</sup> In non-hemodialysis CKD, optimal iPTH level is not known, but maintaining a normal iPTH level is recommended. Although the target level of intact parathyroid hormone in hemodialysis dependent ESRD patients within two to nine times above normal value is suggested,<sup>17</sup> we found that patients with a serum iPTH level higher than three times above upper normal limit showed lower hemoglobin levels than patients with elevated iPTH level less than three times above normal levels. This finding might indirectly suggest the optimal iPTH level in CKD patients in order to correct anemia associated with EPO hyporesponsiveness.

Due to evidence of worse outcome in hyperphosphatemia, the KDIGO guideline in 2017 recommended to lower phosphate levels to normal range in both non-dialysis and dialysis CKD.<sup>17</sup> Our study showed that high serum phosphate was an independent factor of low hemoglobin level. This fact supported phosphate lowering treatment in CKD patients. The strength of our study came from the fact that all patients underwent bone marrow studies to exclude bone marrow disease. However, this study had some limitations as several patients were excluded due to missing iPTH values and hepcidin, as one of the major players of anemia in CKD was not evaluated, which was one of the players in anemia in CKD. Our study demonstrated disturbance of mineral equilibrium and hyperparathyroidism as major causes of ESA hyporesponsive anemia, after excluding iron deficiency anemia and bone marrow diseases. In this study, we didn't show data of drug or treatment such as activated vitamin D and calcimimetics that could affect calcium and phosphate metabolism. By the result of serum phosphate and calcium level whether receive those treatment or not, we could implement the results of our study by promoting inten-

sive phosphate lowering treatment in order to achieve normal serum phosphate levels and lower serum iPTH levels not to be higher than three times above normal range as initial steps of anemia management in CKD before considering bone marrow study in this patient population. A prospective follow-up study might be helpful to support the hypothesis.

## Conclusions

In conclusion, this study showed that hyperphosphatemia was significantly associated with lower hemoglobin level in CKD-patients. Serum phosphate and serum iPTH were inversely correlated with hemoglobin levels. Patients with an iPTH level within three times above normal values had a higher hemoglobin level compared to CKD with an elevated iPTH more than three times above normal ranges. These findings strongly suggest clinicians to consider closely monitoring serum phosphate and iPTH levels as a part of anemia treatment in CKD patients before proceeding to perform bone marrow study.

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