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ORIGINAL ARTICLE

Erythropoietin resistance in patients with regular hemodialysis in Sohag university hospital

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ABSTRACT

Background: A definite anemia [hemoglobin (HB) 10-12 g/dl] or the need for significantly higher Erythropoietin (EPO) dosages of epoetin alfa are symptoms of EPO resistance, respectively. The recommended restorative goal is to maintain HB levels between 11 and 12 g/dl. Fe deficiency, concurrent inflammation, inadequate dialysis, hyperparathyroidism, hemolysis, vitamin B12, and folate deficiency are the main causes of EPO resistance. The objective of this study was to evaluate patients who receive frequent hemodialysis for EPO resistance.

Methods: This study was performed at Sohag University Hospital on 50 hemodialysis patients compared to 40 healthy adult subjects from June 2021 to January 2022. Serum EPO was analyzed by enzyme-linked immunosorbent assay and Angiotensin Converting Enzyme (*ACE)* rs1799752 polymorphism was assessed using the Genomic TaqMan genotyping test.

Results: This study found insignificant relation between EPO Resistance Index (ERI) and diverse *ACE* genotype groups and para thyroid hormone. A further significant direct proportional relationship was found between ERI and Ferritin. EPO and parathyroid hormone did not show any significant relationship.

Conclusion: Considering the non-critical connection between ERI and our components, it is vital to enhance the treatment of anemic patients with chronic kidney diseases to recognize the potential causes of resistance and ponder other variables for resistance before proposing an expanded EPO-stimulating agent administration.

Keywords: Erythropoietin, hemodialysis, erythropoietin resistance index, erythropoietin stimulating agents.

Introduction

Erythropoietin (EPO) is a glycoprotein hormone. During mammal development, EPO formation in mice begins in the neural crest cells during mid-gestation. Then it stimulates the yolk sac for primitive erythropoiesis for oxygen transport in mid-stage embryos (1-3). As development advances, EPO formation and subsequent erythropoiesis shift to the liver (4,5). By the final trimester of development in mammals, EPO formation shifts to the kidneys, which become the primary site of EPO generation in the developing blood cells. Then, erythropoiesis shifts from the fetal liver to bone marrow, the site of future hematopoiesis (6). EPO synthesis is stimulated by decreased oxygen tension at the renal sensor and renal tubular and interstitial cells. This includes many causes of hypoxia, such as pneumonia (resulting in less oxygen uptake), arteriovenous shunts mixing arterial and venous blood through a right to left shunt, high attitudes, low renal blood flow, etc. Others causes include breathing a gas mixture under high oxygen pressure, hypothyroidism, and hypopituitarism. The oxygen sensor is near the EPO generation site and may comprise a heme-containing protein (7). In spite of the fact that the decrease in oxygen is the essential physiologic controller of EPO synthesis, other factors exist, such as androgenic steroids, anabolic steroids, and cobalt chloride, stimulating EPO synthesis by an obscure mechanism. Protein deprivation and inflammatory cytokines, e.g., interleukin-6 and tumor necrosis factor-α, cause decreased EPO synthesis (8,9)

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and may play a role in EPO resistance. Recombinant human EPO (rHuEPO) resistance is characterized by persistent anemia (hemoglobin <10-12 g/dl) that needs an exceptionally high EPO dose (300 IU/kg/week subcutaneously or 450 IU/kg/week intravenously). Alpha EPO should be taken at a dosage of 50-100 IU/kg subcutaneously, one to three times a week. The treatment objective is to attain a weekly increment in hemoglobin levels by 0.3 g/dl. After 4 weeks of treatment, if the hemoglobin (HB) level remains below 11 g/dl, the dosage should be expanded by 25%, and if the hemoglobin level exceeds 13 g/dl, EPO should be discontinued. The target is to maintain hemoglobin levels from 11 to 12 g/dl or hematocrit values from 33% to 36% (10). EPO resistance index (ERI) is characterized by a weekly weight-adjusted αEPO dosage (U/kg/week) divided by the HB level (g/dl), and it is calculated month to month to examine resistance to αEPO treatment (11). The causes of EPO resistance in dialysis patients are mainly iron insufficiency. However, after satisfactory iron supplementation, few patients may remain anemic, possibly due to concomitant inflammation, intense or chronic disease, lack of nutritional sustenance, inadequate dialysis, severe hyperparathyroidism, aluminum intoxication, malignancy, hemolysis, vitamin B12 and folate insufficiencies, pure red cell aplasia, and myelosuppressive operators. Dialysis methodology and biocompatibility of dialysis machines are essential contributing factors (12). Antihypertensive intake, e.g., angiotensin-converting enzyme inhibitors (ACEIs) and angiotensin receptor blockers (ARBs), can diminish the hematopoietic reaction to EPO stimulating agents (ESA), where the former can lead to a high level of negative erythropoiesis control (13). Angiotensin Converting Enzyme (*ACE*) gene polymorphisms may affect ACE serum levels and pharmacological actions (14). This way, a few patients may be more vulnerable to ESA resistance upon utilizing ACE and ARB inhibitors (15).

Subjects and Methods

This prospective study was performed at Sohag University Hospital, Sohag, Egypt. Fifty Egyptian adult patients on regular hemodialysis were enrolled, and 40 healthy adult subjects served as the negative control. This study lasted from June 2021 to January 2022. We used a sample size of 90 respondents (50 cases and 40 controls) to attain an 80% power and a 5% confidence interval of significance upon using a two-tailed test (type 1 error). Before performing the study, ethical committee approval was gained from the Sohag faculty of medicine. All participants signed written agreements and consented to participate in the study. All individuals were informed about the biochemical blood tests, and their clinical evaluations were performed before the study. Serum levels of parathyroid hormones (parathormone, PTH) and ferritin were assayed using commercially available kits. Other data were obtained by history. Inclusion criteria included patients having chronic renal failure and receiving hemodialysis for 3 months or more; age >18 years or more, and receiving EPO for anemia due to kidney diseases; and individuals with persistent blood loss that requires a blood transfusion, intense kidney disease, blood diseases, people having malignancy, and intercurrent infections.

About 3 ml of venous blood was withdrawn from the patients in the early morning after an overnight fast before the dialysis sessions. Another 2 ml of blood was collected in plain tubes for serum analysis, and 1 ml was collected in ethylene diamine tetraacetic acid (EDTA) tubes for genomic DNA extraction. Blood samples were centrifuged, and serum was collected and stored at (-80) until the biochemical assays. DNA extraction was done using QIAamp blood pack (cat.nos.51104 and 51106) according to the manufacturer's instructions. Enzyme-linked immunosorbent assay (ELISA) was used to assess the serum level of EPO using a human EPO ELISA Pack (Glory Science Co., China) according to the manufacturer's instructions. TaqMan single nucleotide polymorphism (SNP) genotyping test was used for ACE rs1799752 SNP genotyping by Step One real-time polymerase chain reaction (PCR) (Biosystems, USA). A total reaction volume of 25 µl was prepared using 12.5 µl TaqMan® Genotyping master mix and 1.25 µl specific TaqMan® SNP genotyping test) with its forward and reverse primers and two TaqMan® MGB tests; one VIC® color for the first allele, one FAM™ color for the second allele , and 5 µl (20 ng) of DNA. The temperature was raised to 95 for 10 minutes and then by 40 amplification cycles. Each cycle included denaturation at 95 for 15 seconds, annealing of primers, and then extension at 60 for 60 seconds. PCR outcomes were analyzed using TaqMan® Genotyper™ Program. ERI is defined as the weekly adjusted EPO dose (U/kg/week) divided by the HB level (g/dl), and it is calculated to investigate the resistance to EPO treatment. The patients enrolled in this study received alpha EPO (epoetin alpha, Pharco, Egypt) 4,000 IU twice weekly according to this formula (11):

$$\text{ERI} = \frac{\text{dose of ESA (IU/ wk)}}{\text{HB (g /dl)} \times \text{body weight (kg)}}.$$

Statistical Package for the Social Sciences computer program version 25 was used to assess the study's data. The quantitative results were presented as means ± SD and median. Qualitative results were presented as numbers and percentages. Tests of significance such as the chi-square test, independent *t*-test, and one-way ANOVA test were utilized for comparing the different parameters in the allocated participants. A value of $p < 0.05$ was chosen as the significance level in all the statistical tests in our consideration. Also, bivariate correlation analysis was done among clinical and laboratory parameters. For correlation analyses, r (Pearson's correlation coefficient) was used ($r < 0.2$ means negligible correlation, 0.2-0.4 means mild correlation, 0.4-0.6 means moderate correlation, and $r = 0.6$-0.8 means high correlation, and >0.8 means excellent correlation).

Results

In this study, ERI was not significantly associated with diverse *ACE* genotype groups (*p*-value between DD &II = 0.184; *p*-value between DD &II = 0.837; *p*-value between ID &II = 0.184) (Figure 1). There was a non-critical contrast between *ACE* genotypes and utilizing ACEI treatment or not (*p*-value = 0.153) (Table 1). ERI

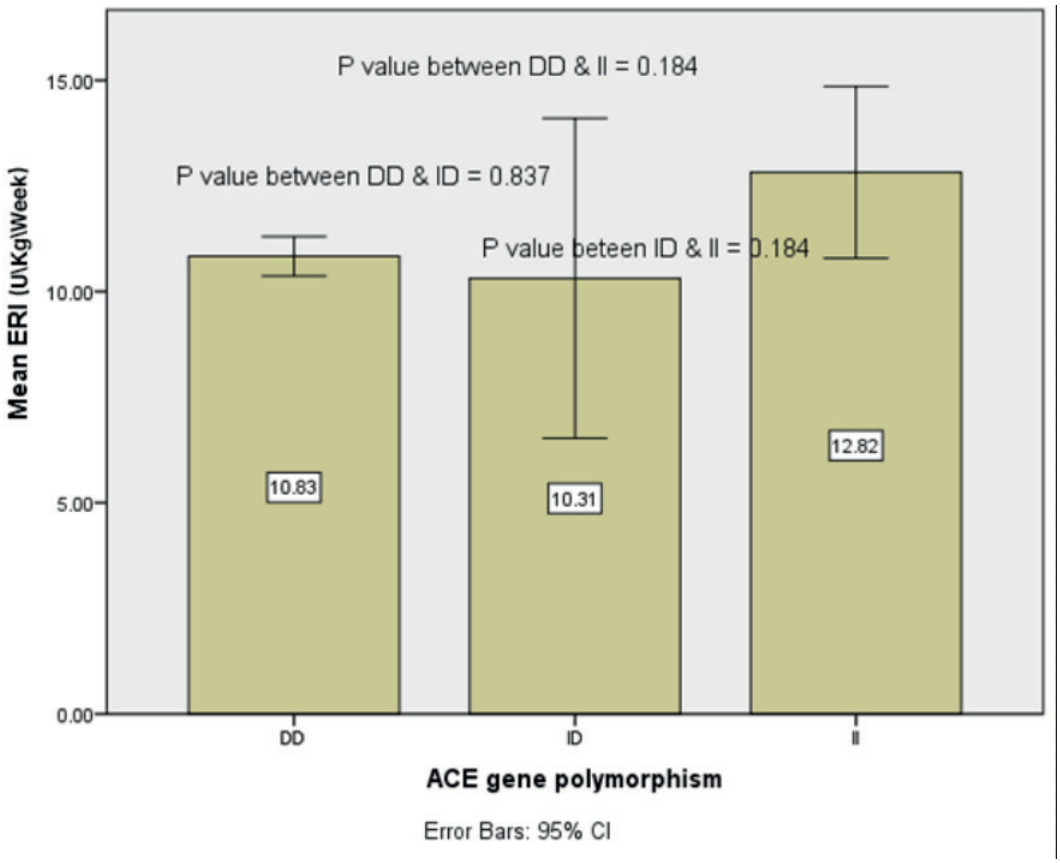

Figure 1. *Relation between ERI with ACE gene polymorphism.*

Table 1. *ACE inhibitor and ACE gene polymorphism cross-tabulation.*

			ACE gene polymorphism		Total	*p*-value
			DD	ID + II		
ACE inhibitor	(Non-using ACEI)	Number of participants	65	17	82	0.153 NS
		(% within *ACE* gene polymorphism)	89.0%	100.0%	91.1%	
	(Using ACEI)	Number of participants	8	0	8	
		(% within *ACE* gene polymorphism)	11.0%	0.0%	8.9%	
Total		Number of participants	73	17	90	
		(% within *ACE* gene polymorphism)	100.0%	100.0%	100.0%	

and ferritin have a significant proportional relationship (p-value < 0.0001) (Table 2 and Figure 2). The relationship between ERI and PTH was found non-significant (p-value = 0.085) (Figure 3). There was a non-significant correlation between EPO and PTH (p-value = 0.554, r = 0.063) (Figure 4). The relationship was significant upon using ACEI therapy (p-value 0.012, r = −0.825) (Table 3). There was a non-significant correlation between EPO and length of dialysis (p-value = 0.582, r = 0.059) (Figure 5). Further, the genotype distribution of *ACE* rs1799752 showed no deviation from Hardy Weinberg Equilibrium (p = 0.242 > 0.05).

Discussion

EPO lack is the leading cause of anemia due to chronic kidney diseases (CKD). Upon treatment with EPO stimulators, few dialysis patients presented with manifest resistance to ESA, which may increase the mortality hazards due to kidney infections (15). ESAs are commonly utilized to treat anemia related to CKD. ESA resistance or hypo-responsiveness is defined as patients who don't accomplish the specified HB levels despite the higher-than-normal doses of ESAs or who require progressively higher ESA doses to preserve a specific HB level (16). To slow down the lethal results of EPO resistance, observation programs should maintain the nutritional supplements (iron and folate stores), minimize oxidative stress-induced hemolysis, treat hyperparathyroidism, dodge catheter disease, and improve urea clearance (17). Our study revealed that ERI was not significantly associated with the *ACE* genotype, which agrees with a previous report where no notable impact of the *ACE* I/D polymorphism was on EPO resistance (18). Moreover, a recent study reported

Table 2. *Relationship between ERI and ferritin level.*

Item	Group A ERI 15 (30%)	Group B ERI 25 (50%)	Group C ERI 10 (20%)	*p*-value
Ferritin (ng/ml)	56.13 ± 8.64	113 ± 16.83	169.4 ± 17.46	<0.0001* significant

p-value compares the three groups $p < 0.05$ statistically significant.
(Group A) ERI = <9 U/kg/week/g/100 ml.
(Group B) ERI = 9-12 U/kg/week/g/100 ml (Group C) ERI = >12U/kg/week/g/100 ml.

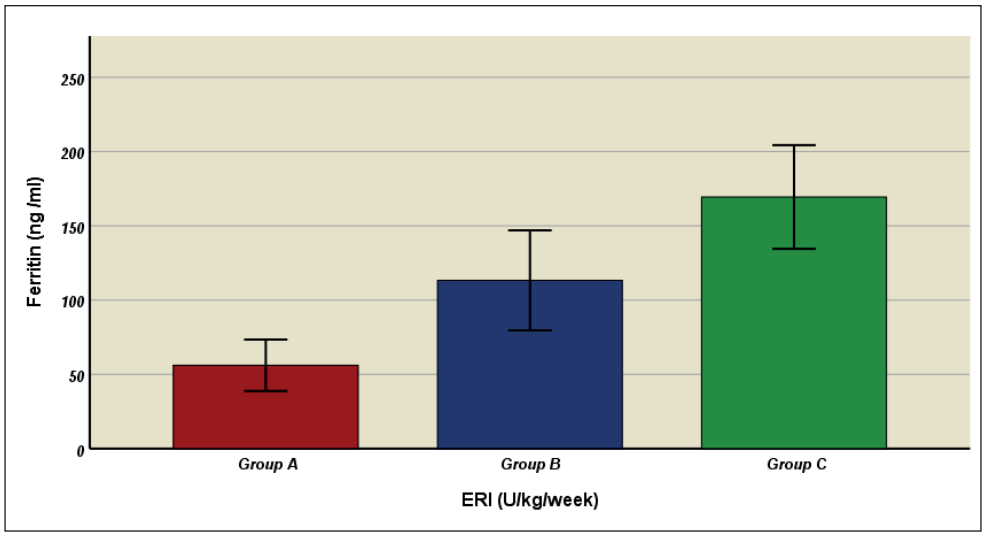

Figure 2. *Relationship between ERI and serum ferritin level using one-way ANOVA test (Group A); ERI ≤9 U/kg/week/g/100 ml; Group B ERI = 9-12 U/kg/week/g/100 ml; Group C ERI ≥12 U/kg/week/g/100 ml; p < 0.05 was set for statistically significant results; Error bars showed ± 2 SD).*

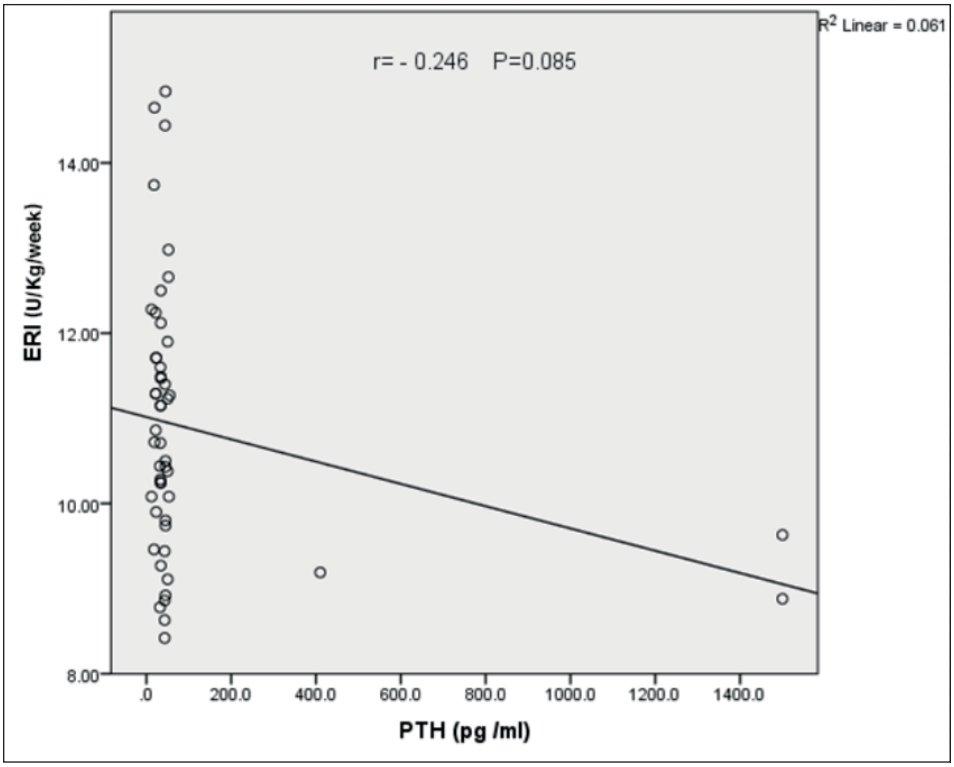

Figure 3. *Correlation between ERI and PTH level (p < 0.05 denotes statistically significant values).*

a decreased EPO resistance in patients with the D/D genotype compared to other genotypes (14). Our data found that the ID genotype had less EPO resistance, and II had more EPO resistance. Among hemodialysis patients in Korea, the D/D genotype had less EPO resistance than other genotypes (19). The *ACE* DD genotype lowers the

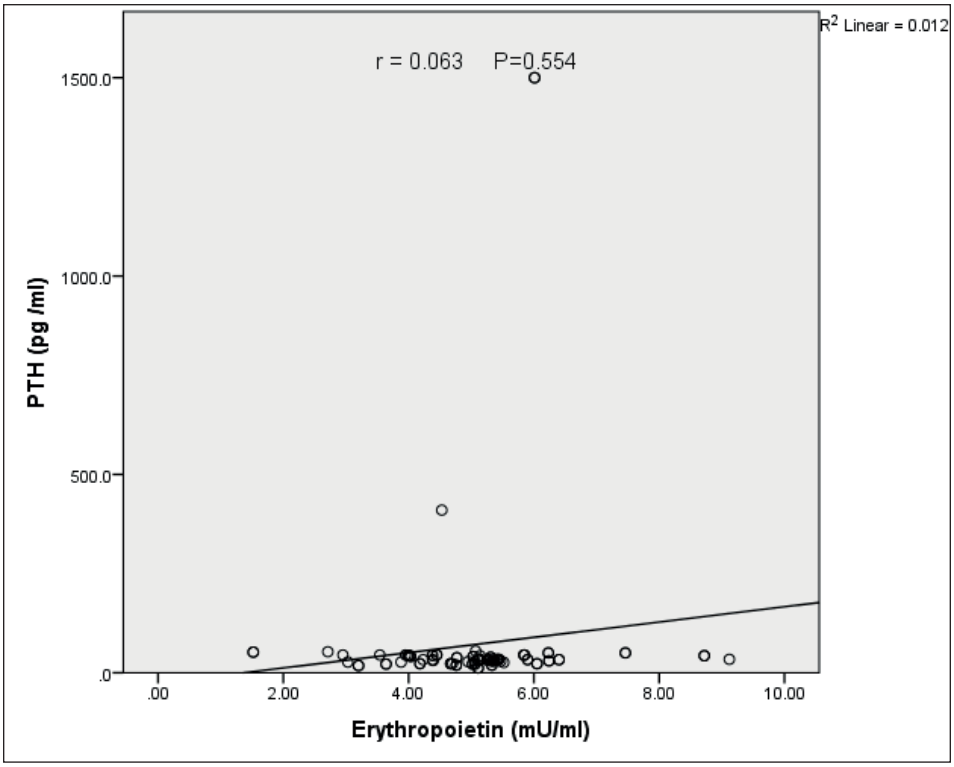

Figure 4. *Correlation between EPO and PTH (p < 0.05 denotes statistically significant values).*

Table 3. *Correlation between EPO and PTH in patients using ACEI versus patients not using ACEI (p < 0.05 denotes statistically significant values).*

Correlation between EPO and PTH	Correlation coefficient	*p*-value
Overall	0.063	0.544
Not using ACE inhibitors	0.161	0.149
Using ACE inhibitors	-0.825	0.012

EPO requirement due to the differing allele frequencies between races. The dispersion of patients with ACEI treatment among genotype bunches within the referred ponders may well be potential clarifications for our disparity as (6 out of 45 hemodialysis patients with DD genotype used ACEI therapy; 8 from the total of 73 DD participants used ACEI therapy (Figure 1, Table 1) as ACEIs piece post transplantation erythrocytosis. ACEIs did not essentially alter the erythropoietic reaction to rHUEPO (20). Patients on Continuous Ambulatory Peritoneal Dialysis reported that the *ACE* II/ID genotypes appear to be related to decreasing EPO responses (21,22). A significant correlation existed between ERI and serum ferritin level ($p = 0.0001$). High ferritin level was associated with high ERI, which agreed with the findings on patients whose ERI had the highest ferritin values (23), an indicator of active inflammatory status. Chronic aggravation and cytokines can decrease anemia by decreasing the life span of erythrocytes, enhancing erythroid antecedent's apoptosis, and specifically repressing the proliferation of erythrocytes progenitors (24,25) (Table 2 and Figure 2). Our study reported a non-significant correlation between PTH and ERI ($p = 0.085$). Similarly, there were no critical differences in ERI between the subgroups of patients classified according to the serum levels of parathyroid hormones (26) (Figure 3). The correlation between levels of PTH and EPO was insignificant ($p = 0.554$ (Figure 4). Also, there was a significant correlation between PTH and EPO upon using ACEIs (Table 3). Similarly, patients with ESA responsiveness had more cruel PTH levels than ESA hypo responders (27). In another cohort study in hemodialysis patients, there was a modest but significant relationship between higher PTH levels and decreased erythropoiesis (28). Decreased ESA responsiveness was related to more prominent serum PTH and alkaline phosphatase levels (29). However, the most impressive ESA responsiveness was associated with moderate to low PTH levels (150-300 pg./ml) and low-normal alkaline phosphatase value. Moreover, higher PTH levels were independently related to decreased ESA responsiveness. Hyperparathyroidism may specifically cause ESA hypo-responsiveness by reducing the endogenous EPO, diminishing bone marrow erythroid cells, and shortening the erythrocyte's life span. That impacts the effects of renal osteodystrophy on bone marrow fibrosis as confirmed by the increase in reestablished bone marrow space and increments in serum EPO levels after parathyroidectomy. Subsequently, ESA hypo responsiveness could be adjusted through treatment with active vitamin D (calcitriol) and/or

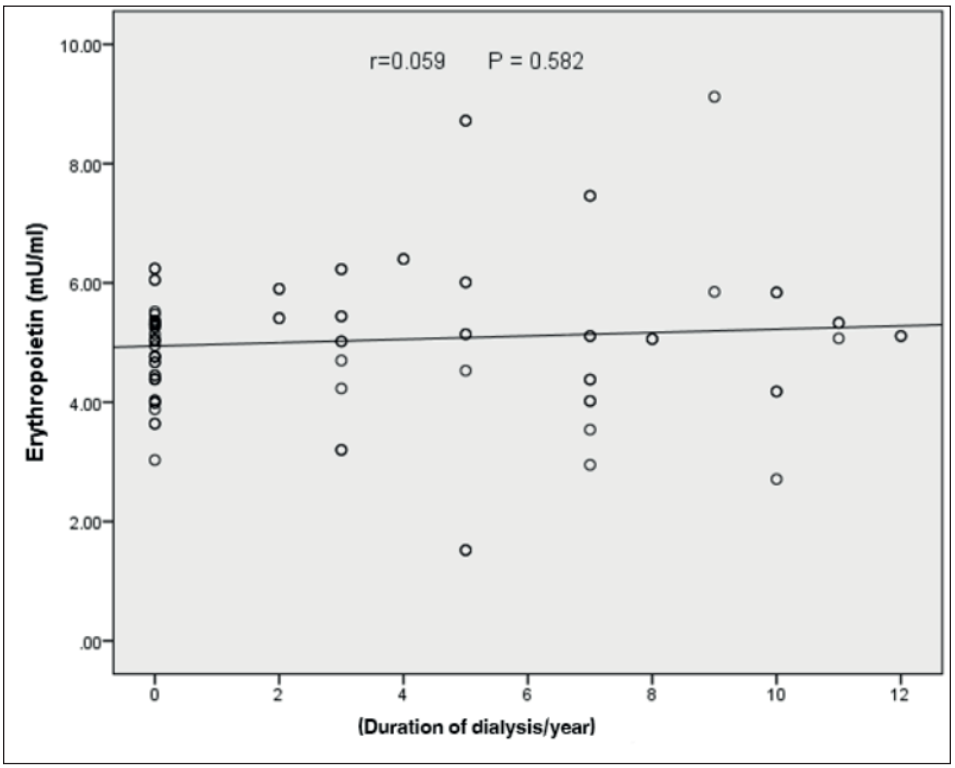

Figure 5 *Correlation between EPO level and the duration of dialysis p < 0.05 denotes statistically significant values. Correlation between EPO level and the duration of dialysis p < 0.05 denotes statistically significant values.*

calcimimetics, which diminish PTH emission and improve the high-turnover bone infection manifested as decreased serum alkaline phosphatase levels. Moreover, there is a significant correlation between the duration of dialysis and EPO level ($p = 0.582$) (Figure 5). Also, there is a significant correlation between the time of dialysis and EPO requirements in different *ACE* genotypes (21). Moreover, there is a substantial relationship between the impacts of intravenous and subcutaneous EPO and the duration of dialysis in hemodialysis patients ($p = 0.187$) (30). Erythropoiesis occurs within the bone marrow, where endogenous or exogenous EPO acts upon erythroid precursors, which undergo maturation into reticulocytes and erythrocytes. This process includes a few cytokines (IL-3, IL-12, insulin-like growth factor-1) and granulocyte-monocytecolony–stimulating growth factor, which stimulates cell multiplication. In contrast, other cytokines, e.g., IL-1, IL-6, tumor necrotic factor alpha, and interferon-gamma, can block this process. The last-mentioned cytokines may also be included in inflammation, intense or unremitting disease conditions, and malignancy. Any of these circumstances may favor erythropoiesis resistance. Moreover, comparing patients under distinctive circumstances and assessing the impact of distinctive medications on a single patient may cause this discrepancy (26).

Conclusion

Considering the non-critical connection between ERI and our components, it is vital to enhance the treatment of anemic patients with CKD to recognize the potential causes of resistance and ponder other variables for resistance before proposing an expanded EPO -stimulating agent administration.

List of Abbreviations

SPSS	Statistical Package for the Social Sciences
ACE	Angiotensin converting enzyme
ACEI	Angiotensin converting enzyme inhibitors
ARB	Angiotensin receptor blocker
EPO	Erythropoietin
ERI	Erythropoietin resistance index
ESA	Erythropoietin stimulating agents
PTH	Parathyroid hormone
rHuEPO	Recombinant human erythropoietin

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Declaration of conflicting interests
The authors of this article have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

Consent to participate
Informed consent was obtained from the patients.

Ethical approval
This study was approved by the Institutional Research Board of the Sohag Faculty of Medicine. Dated : December 2019, Approval Number: med: 23-02-2020.

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