BJMG 26 (2), 2023 • 27-34
DOI: 10.2478/bjmg-2023-0022
sciendo
ORIGINAL ARTICLE

ASSOCIATION BETWEEN THE POLYMORPHISM OF ANGIOTENSIN-CONVERTING ENZYME GENE AND INTERLEUKIN-1 BETA GENE AND THE RESPONSE TO ERYTHROPOIETIN THERAPY IN DIALYSIS PATIENTS WITH ANEMIA

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ABSTRACT

Introduction

The polymorphism of the angiotensin-converting enzyme (ACE) gene and interleukin-1 beta (IL-1b) gene could be associated with resistance in the treatment of anemia in dialysis patients with recombinant human erythropoietin (rHuEPO). The aim of the study was to evaluate the association between the polymorphism of the ACE and IL-1b genes and the response to rHuEPO therapy in dialysis patients with anemia.

Material and methods

The study investigated 69 patients on dialysis with anemia treated with recombinant human erythropoietin for 12 months. Genotyping of ACE and IL-1b polymorphism was done in all study patients at the initiation of the study. The patient's demographic characteristics, dialysis vintage, and laboratory parameters were also evaluated as factors associated with rHuEPO resistance. The erythropoietin resistance index (ERI) was calculated as the weekly rHuEPO dose per kg of body weight, divided by the hemoglobin (Hb) concentration in g/dl.

Results

The Hb ≥ 110 g/l was registered in 37 (53.6%) patients. Patients with Hb ≥ 110 g/l were characterized by significantly higher serum levels of albumin, cholesterol, and iron than those with Hb < 110 g/l. The serum level of the CRP, the weekly dose of rHuEPO, and ERI were significantly higher in patients with Hb < 110 g/l compared to patients with Hb ≥ 110 g/l. The ERI value of ≥ 10 IUkg/weekly/g/dl was present in 27 (39.1%) patients. The serum levels of ferritin and CRP, and weekly dose of rHuEPO were significantly higher in patients with ERI value ≥ 10 IU kg/weekly/g/dl compared with the patients with ERI value < 10 IUkg/weekly/g/dl. There was no significant association between the ERI and polymorphism of the ACE and IL-1b genes in study patients.

Conclusion

The polymorphism of the ACE and IL-1b genes was not significantly associated with the response to erythropoietin therapy in dialysis patients with anemia. Iron deficiency, malnutrition, and inflammation were factors associated with anemia and resistance to erythropoietin therapy in dialysis patients.

***Keywords*:** anemia, dialysis, erythropoietin, gene, polymorphism, therapy

INTRODUCTION

Recombinant human erythropoietin (rHuEPO) has been used as a treatment for anemia in patients with chronic kidney disease for more than 30 years. Chronic kidney disease is characterized by decreased secretion of endogenous erythropoietin from kidneys (1-3). The treatment of anemia with rHuEPO in dialysis patients lowers the blood transfusions, increases the patient's quality of life, and reduces the risk of cardiovascular morbidity and mortality (4-7). Based on current recommendations, the treatment of anemia with rHuEPO in patients on hemodialysis begins when the value of hemoglobin is lower than 100 g/l (6). On average, 85% of dialysis patients receive rHuEPO for

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correction of anemia to achieve a target value of hemoglobin up to 110-120 g/l (8, 9). The initial dose of rHuEPO is 50-100 IU/kg body weight/three times per week, with subcutaneous or intravenous administration.

Resistance or reduced response to treatment with rHuEPO in dialysis patients might be caused by several factors, such as iron deficiency, vitamin B12/folic acid deficiency, hypothyroidism, infection-inflammation, inadequate dialysis, hyperparathyroidism, malnutrition, bleeding, and malignancy. Irreversible factors are hemoglobinopathies and bone marrow diseases (8). The erythropoiesis resistance index (ERI) evaluates the rHuEPO responsiveness and is calculated as the weekly rHuEPO dose per kg of body weight, divided by the hemoglobin (Hb) concentration in g/dl (10). Resistance to human erythropoietin is encountered in 5-10% of patients on hemodialysis (11).

The polymorphism of certain genes could be associated with erythropoietin resistance during the treatment of anemia in patients on dialysis (12, 13). Every polymorphism has a rsID number ("rs" followed by a number), a unique label used by researchers and databases to identify a specific polymorphism. Polymorphism (rs1799752) of the angiotensin-converting enzyme (ACE) gene is characterized by the presence (insertion, I) or absence (deletion, D) of a 287-bp sequence of DNA (ACE I/D) in intron 16 of the ACE gene located on the chromosome 17 (17q23) (14). The angiotensin-converting enzyme is a key enzyme in the creation of angiotensin II. Additionally, angiotensin II stimulates the proliferation of erythroid precursors, which is proven by in vitro models, i.e. it affects erythropoiesis (15, 16). The genetic cluster for interleukin 1 (IL-1), located on chromosome 2 (2q14.1), is presented with IL-1a, IL-1b, and IL-1RN genes that provide genetic information on the synthesis of cytokines IL-1alpha, IL-1beta, and endogenous receptor antagonist IL-1 (17). IL-1beta suppresses the endogenous secretion of erythropoietin (18). The polymorphism of the IL-1b gene is IL-1B-511 C/T (rs 1143627) (19).

AIM OF THE STUDY

Our study aimed to evaluate the association between the polymorphism of the ACE and IL-1b genes and the response to erythropoietin therapy in dialysis patients with anemia.

PATIENTS AND METHODS

The study included 69 patients with stage 5 chronic kidney disease on maintenance hemodialysis or peritoneal dialysis. All patients signed an informed consent for participation in the study. The study was approved by the ethical commission of the Faculty of Medicine, Ss. Cyril and Methodius University in Skopje, Skopje, RN Macedonia. The design of the study was a prospective, longitudinal study, with a duration of 12 months.

Inclusive criteria for study patients:
- older than 18 years,
- treatment with dialysis for at least 3 months,
- treatment of anemia with recombinant human erythropoietin.

Exclusion criteria for study patients:
- bleeding diagnosed before involvement in the study,
- the persistence of malignant disease,
- hemoglobinopathies and diseases of the bone marrow.

The patients were recruited from the hemodialysis unit and peritoneal dialysis unit at the University Hospital of Nephrology in Skopje and the Department of Nephrology and Dialysis at the General City Hospital "8^{mi} Septemvri" in Skopje. The medical histories of the patients were used to determine demographic characteristics, etiology of kidney disease, dialysis vintage, and total weekly dose of erythropoietin. The laboratory data were obtained from the routine laboratory analyses of dialysis patients during the study period of 12 months. The total red blood cell count, hematocrit, hemoglobin (Hb), total protein, albumin, alkaline phosphatase, calcium, phosphorus, C-reactive protein (CRP), iron, and total iron binding capacity (TIBC) were analyzed monthly. The transferrin saturation index (TSAI) was calculated using the following equation: (serum Fe/TIBC) x 100% (20). The serum concentration of ferritin was determined once in three months, with a target value of more than 500 ng/ml, but not exceeding 800 ng/ml (20). The serum concentration of intact parathyroid hormone (iPTH) and cholesterol was determined once in six months. The erythropoietin resistance index (ERI) was calculated monthly as the weekly rHuEPO dose per kg of body weight, divided by the hemoglobin concentration in g/dl (10).

Genotyping of ACE and IL-1b polymorphism was done in all study patients at the initiation of the study in the Center for Biomolecular Pharmaceutical Analysis at the Institute of Pharmaceutical Chemistry at the Faculty of Pharmacy in Skopje. Genomic DNA from all study participants was isolated from peripheral blood using the MagCore Genomic DNA Whole Blood Kit (RBC Bioscience), following the manufacturer's instructions. The ACE polymorphism (rs1799752) was genotyped by

fluorescent PCR followed by fragment analysis on 3500 Automated Genetic Analyzer (Thermo Fisher Scientific), using the following primers: ACE_I/D_F:5'-CTGGAGACCACTCCCATCCTTTCT-3' and ACE_I/D_R: 6FAM-5'-GATGTGGCCATCACATTCGTCAGAT-3'. A total of 100 ng of DNA was amplified in 25μL final volume including 2 mM Mg^{2+}, 0.2 mM of each dNTPs, 0.5μM of each primer and 1U HOT FIREPol® DNA Polymerase (Solis Bio-Dyne), using the following program: initial denaturation at 95°C for 10 minutes; 35 cycles of 30 seconds at 95°C, 30 seconds at 58°C and 30 seconds at 72°C; and final elongation at 72°C for 10 minutes. The IL-1b gene (rs1143627) polymorphism was genotyped by allele discrimination PCR on a Stratagene Mx3005P (Agilent Technologies) real-time PCR system using TaqMan® SNP genotyping assay (reference ID: C___1839944_10; Thermo Fisher Scientific). The genotypes were determined in a reaction mix containing 20 ng DNA in a total volume of 25 μL, according to the manufacturer's recommended protocol. Positive and negative controls were included on each plate and reproducibility was checked by re-genotyping 10% of the cases.

STATISTICAL ANALYSIS

The statistical analysis of the data was performed by using SPSS 17. The parametric variables were presented as an average value ± standard deviation (X ± SD). The parametric variables between two groups were compared with Student's t-test for independent samples, and between three different groups with the ANOVA test. Fisher's exact test was used to compare proportions. The obtained P value less than or equal to 0.05 was considered statistically significant.

RESULTS

The study included 69 patients, 63 patients on maintenance hemodialysis, and 6 patients on peritoneal dialysis. Among the patients, 48 (69.5%) were male, and 21 (30.5%) were female. The etiology of kidney disease in most of the patients was chronic glomerulopathy (n = 21, 30.5%). The other etiological causes for kidney disease were: obstructive nephropathy (n = 16, 23.2%), nephroarteriolosclerosis (n = 14, 20.3%), autosomal dominant polycystic kidney disease (n = 7, 10.1%), and unknown cause (n = 11, 15.9%). Demographic characteristics, dialysis vintage, laboratory parameters, and erythropoietin resistance index (ERI) of all study patients are presented in Table 1.

The mean hemoglobin value in all patients was 109.4 ± 11.1 g/l, achieved by the application of an average of 6909 IU of erythropoietin per week. The mean value of TSAI (%) was 29.4 ± 7.6%, and the mean value of the ferritin was 468.8 ± 343.1 μg/l. The mean value of calculated erythropoietin resistance index (ERI) was 9.6 ± 6.2 IU kg/week/dl, Table 1.

Table 1. Demographic characteristics, dialysis vintage, laboratory parameters, and erythropoietin resistance index (ERI) of all study patients.

	X	SD
Age (years)	62.1	16.3
Weight (kg)	72.3	16.0
Dialysis vintage (months)	94.8	88.7
Hemoglobin (g/l)	109.4	11.1
Red blood cell count (10^{12}/l)	3.6	0.4
Hematocrit (rv)	0.34	0.04
Alkaline phosphatase (U/L)	102.3	58.3
Total protein (g/l)	69.5	5.2
Albumin (g/l)	40.2	4.2
Calcium (mmol/l)	2.3	0.2
Phosphates (mmol/l)	1.6	0.5
Iron (μmol/l)	10.9	2.9
TIBC (μmol/l) *	37.6	8.2
TSAI (%)**	29.4	7.6
CRP (mg/l) ***	12.1	14.2
Ferritin (ng/ml)	468.8	343.1
iPTH (pg/ml)****	499.3	490.3
Erythropoietin (IU/per week)	6909.6	3465.7
ERI (IU kg/week/g/dl)*****	9.6	6.2

*Total iron binding capacity (TIBC),
**Transferrin Saturation Index (TSAI),
***C reactive protein (CRP),
**** Intact parathyroid hormone (iPTH),
***** Erythropoietin resistance index (ERI)

The concentration of hemoglobin ≥ 110 g/l was registered in 37 (53.6%) patients, Table 2. Patients with hemoglobin ≥ 110 g/l were characterized with significantly higher serum levels of albumin, cholesterol, and iron compared to the patients with hemoglobin < 110 g/l. The serum levels of the CRP, the weekly dose of rHuEPO, and ERI were significantly higher in patients with hemoglobin < 110 g/l compared to patients with hemoglobin ≥ 110 g/l, Table 2.

The ERI ≥ 10 IU/kg/week/g/dl was detected in 27 (39.1%) patients. Patients with ERI ≥10 IU/kg/week/g/dl had significantly lower levels of hemoglobin, cholesterol, iron, and TSAI% compared to patients with ERI< 10 IU/kg/week/g/dl. The serum levels of ferritin and CRP, and a weekly dose of erythropoietin were significantly higher in patients with ERI ≥ 10 IU/kg/week/g/dl compared to patients with ERI <10 IU/kg/week/g/dl, Table 3.

Table 2. Comparison of demographic characteristics, dialysis vintage, laboratory parameters, and erythropoietin resistance index (ERI) between patients with hemoglobin value <110 g/l and ≥ 110 g/l.

	Hb < 110 g/l, (N=32)	**Hb ≥ 110 g/l, (N=37)**	**P**
	X ± SD	**X ± SD**	
Age (years)	58.6 ± 18.3	65.1 ± 138	0.099
Weight (kg)	70.8 ± 16.9	73.5 ± 15.3	0.486
Dialysis vintage (months)	84.0 ± 79.7	104.1 ± 95.9	0.351
Hemoglobin (g/l)	99.9 ± 9.2	116.9 ± 4.8	0.000
Red blood cell count (10^{12}/l)	3.3 ± 0.4	38. ± 0.2	0.000
Hematocrit (rv)	0.31 ± 0.03	0.36 ± 0.02	0.000
Alkaline phosphatase (U/L)	114.4 ± 96.5	103.1 ± 53.1	0.539
Total protein (g/l)	68.1 ± 6.5	70.3 ± 3.8	0.086
Albumin (g/l)	38.6 ± 5.9	40.8 ± 2.7	0.044
Calcium (mmol/l)	2.3 ± 0.2	2.4 ± 0.3	0.282
Phosphates (mmol/l)	1.6 ± 0.5	1.6 ± 0.5	0.494
Cholesterol (mg/dl)	3.5 ± 0.8	4.1 ± 0.8	0.005
Iron (µmol/l)	9.7 ± 3.0	11.7 ± 2.6	0.005
TIBC (µmol/l) *	35.6 ± 9.4	38.1± 7.6	0.229
TSAI (%)**	27.8 ± 7.9	31.4 ± 7.2	0.051
Ferritin (ng/ml)	566.6 ± 529.5	437.0 ± 299.7	0.210
iPTH (pg/ml) ***	560.7 ± 550.5	442.9 ± 410.8	0.317
CRP(mg/l) ****	21.8 ± 34.3	8.3 ± 9.9	0.025
Erythropoietin (IU/per week)	8714.6 ± 2982.4	5234.6 ± 2836.1	0.000
ERI (IU kg/week/g/dl)*****	13.5 ± 6.1	6.2 ± 3.4	0.000

*Total iron binding capacity (TIBC), **Transferrin Saturation Index (TSAI), ***Intact parathyroid hormone (iPTH),
**** C reactive protein (CRP), ***** Erythropoietin resistance index (ERI).

Table 3. Comparison of demographic characteristics, dialysis vintage, and laboratory parameters between patients with ERI ≥ 10 and ERI < 10 IU/kg/week/g/dl.

	ERI ≥ 10, (N =27)	**ERI < 10, (N = 42)**	**P**
	X ± SD	**X ± SD**	
Age (years)	59.1 ± 19.7	63.9 ± 13.5	0.234
Weight (kg)	68.6 ± 14.1	74.6 ± 16.9	0.131
Dialysis vintage (months)	70.6 ± 56.4	110.3 ± 102.0	0.070
Hemoglobin (g/l)	100.6 ± 11.4	114.4 ± 6.9	0.000
Red blood cell count (10^{12}/l)	3.4 ± 0.4	3.7 ± 0.3	0.000
Hematocrit (rv)	0.32 ± 0.04	0.35 ± 0.03	0.000
Alkaline phosphatase (U/L)	106.6 ± 66.2	109.5 ± 82.4	0.879
Total protein (g/l)	69.1 ± 5.8	69.5 ± 4.9	0.762
Albumin (g/l)	39.5 ± 4.9	40.0 ±4.37	0.639
Calcium (mmol/l)	2.3 ± 0.3	2.3 ± 0.2	0.672
Phosphates (mmol/l)	1.6 ± 0.5	1.6 ± 0.5	0.940
Cholesterol (mg/dl)	3.5 ± 0.7	3.9 ± 0.9	0.045
Iron (µmol/l)	9.2 ± 2.7	11.8 ± 2.6	0.000
TIBC (µmol/l) *	36.3 ± 9.3	37.4 ± 8.0	0.609
TSAI (%)**	26.4 ± 7.5	31.8 ± 7.1	0.003
Ferritin (ng/ml)	630.4 ± 555.4	412.3 ± 290.4	0.038
CRP(mg/l)***	24.2 ± 36.8	8.5 ± 9.9	0.010
iPTH (pg/ml)****	518.5 ± 511.6	483.1 ± 464.3	0.770
Erythropoietin (IU/per week)	9392.1 ± 2496.0	5213.4 ± 2811.9	0.000

* Total iron binding capacity (TIBC), **Transferrin Saturation Index (TSAI), ***C reactive protein (CRP),
**** Intact parathyroid hormone (iPTH), ***** Erythropoietin resistance index (ERI).

Table 4. The frequency of the ACE I/D genotypes (rs1799752) in all patients, and the mean value of ERI in the patients with different genotypes of ACE I/D.

ACE I/D* rs1799752			**ERI**
	Number of patients	%	X ± SD
II	10	14.5	11.87 ± 6.56 [a, b]
ID	41	59.4	8.42 ± 5.84 [a, c]
DD	18	26.1	10.92 ± 6.56 [b, c]

*Angiotensin-converting enzyme gene with insertion (I) or deletion (D) of a 287-bp sequence of DNA,
[a] P=0.237, [b] P=0.914, [c] P=0.307

Table 5. The frequency of genotypes of the IL-1B 511 C/T (rs1143627) in all patients and the mean value of ERI in patients with different genotypes of IL-1B 511 C/T.

IL-1B 511 C/T* rs1143627			**ERI**
	Number of patients	%	X ± SD
CC	7	10.1	8.34 ± 3.64 [a, b]
CT	35	50.7	9.83 ± 6.75 [a, c]
TT	27	39.1	9.56 ± 5.68 [b, c]

*Polymorphism of IL-1b gene is IL-1B 511 C/T with bases cytosine (**C**)/ thymine (**T**)
[a] P=0.826, [b] P=0.886, [c] P=0.983

The polymorphism of the ACE gene (ACE I/D, rs1799752) is presented by three genotypes: ACE II, ACE ID, and ACE DD. The ACE ID genotype was with the highest frequency, at 59.4%, detected in 41 patients. There was no significant difference in ERI between the three genotypes of the ACE I/D, Table 4.

The polymorphism of the IL-1b gene (IL-1B-511 C/T, rs1143627) is presented with three different genotypes: IL-1B CC, IL-1B CT, and IL-1B TT. The IL-1B CT genotype was with the highest frequency of 50.7%, detected in 35 patients. There was no significant difference in ERI between the three genotypes of the IL-1B-511 C/T, Table 5.

DISCUSSION

The study included patients with stage 5 chronic kidney disease on dialysis with anemia treated with recombinant human erythropoietin. The mean hemoglobin level of 109.4 ± 11.1 g/l was achieved in study patients with an average dose of erythropoietin of 6909 IU per week. The target value of hemoglobin in dialysis patients is 110-120 g/l (8,9). The mean level of TSAI (%) in the study patients was 29.4 ± 7.6%, and the mean level of ferritin was 468.8 ± 343.1 μg/l. KDIGO (Kidney Disease: Improving Global Outcomes) guidelines for anemia in chronic kidney disease recommend not exceeding a TSAI of 30% and a serum ferritin level of 800 ng/ml (20). The mean value of iPTH in study patients was 499.3 ± 490.3 pg/ml. KDIGO guidelines recommended maintaining iPTH levels 2- to 9-fold the upper normal limit, corresponding to a range of 130–600 pg/mL (21). The mean value of the ERI was 9.6 ± 6.2 IU/kg/week/g/dl. In the study of Santos EJF et al. with a total number of 99 patients with anemia treated with erythropoietin, the mean value of ERI was 15.3 ± 9.0 IU/kg/week/g/dl (23). The mean ERI value of 7.0 ± 4.4 IU/kg/week/g/dl was noted in 60 patients on peritoneal dialysis in the study performed by Kaneko S et al. (24).

A hemoglobin level above 110 g/l with erythropoietin therapy was achieved in 53.6% of the study patients. Patients with hemoglobin ≥ 110 g/l were characterized by significantly higher serum values of albumin, cholesterol, and iron compared to patients with hemoglobin <110 g/l. The serum values of CRP, weekly dose of rHuEPO, and ERI were significantly higher in patients with hemoglobin < 110g/l, compared to patients with hemoglobin ≥ 110g/l. Malnutrition and inflammation in dialysis patients were associated with anemia and resistance to erythropoietin therapy (25, 26). Radić J et al. studied 101 patients on peritoneal dialysis, divided into two groups, a group of 60 patients (59.4%) with hemoglobin ≥ 110 g/l and a group of 41 patients (40.6%) with hemoglobin<110 g/l. The serum value of the albumin was significantly higher in the group of patients with the level of hemoglobin ≥ 110 g/l compared to the group of patients with the level of hemoglobin <110 g/l, (44.2 ± 8.5 *vs.* 39.9 ± 8.5, P = 0.003). Also, the serum value of CRP was significantly higher in the group with hemoglobin <110 g/l compared to the group with hemoglobin ≥ 110 g/l, (7.8 ± 7.9 *vs.* 3.5 ± 6.3, P = 0.005) (26).

Patients involved in the study with an ERI ≥10 IU/kg/week/g/dl had significantly lower serum values of iron and TSAI% and significantly higher serum values of ferritin and CRP compared to patients with an ERI <10 IU/kg/g/dl. Iron deficiency and inflammation were the most common causes of reduced erythropoietin response during the treatment of anemia in dialysis patients (27). Ferritin and CRP are well-known acute-phase proteins of inflammation (28,29).

The frequency of polymorphism of the ACE I/D gene in the study patients was: 14,5% with ACE II, 59,4% with ACE I/D, and 26,1% with ACE DD. A study by Jeong KH et al. included 167 patients on hemodialysis, with a similar frequency of polymorphism of the ACE I/D gene: 25.1% with ACE II, 54.5% with ACE I/D, and 20.4% with ACE DD (19). The frequency of polymorphism of the IL-1B C/T gene in the study patients was 10.1% with IL-1B CC, 50.7% with IL-1B CT, and 39.1% with IL-1B TT. The distributions of IL-1B C/T polymorphism in the study of Jeong KH and al. with 167 hemodialysis patients was 21.6% with IL-1B CC, 43.1% with IL-1B CT, and 35.3% with IL-1B TT (19). In the same study, the ACE DD and IL-1B CC genotypes were associated with significantly lower values of ERI compared to other genotypes (ACE II: 13.2 ± 5.5 *vs.* ACE I/D: 13.9 ± 7.6 *vs.* ACE DD: 10.0 ± 5.1, P = 0.038, and IL-1B CC: 9.6 ± 5.1 *vs*. IL-1B CT: 15.2 ± 7.5 vs. IL-1B TT: 12.2 ± 5.7, P = 0.004) (19). The association of ACE DD with lower ERI value was also confirmed in a group of 50 patients with chronic kidney disease and anemia in a study by Nand N et al. (13). The same study did not confirm the association of polymorphism of the IL-1b gene with ERI (13). A study by Varagunam M et al. included 46 patients on peritoneal dialysis with anemia, treated with erythropoietin, and showed that genotype ACE DD was associated with lower total weekly doses of erythropoietin compared to genotypes ACE II and ACE I/D (22). Kiss Z et al. evaluated 660 hemodialysis patients with anemia treated with erythropoietin and the patients with ACE DD genotype had significantly higher ERI compared to the patients with ACE II (P = 0.046) (30). In our study, there was no significant association of polymorphism of the ACE and IL-1b genes with rHuEPO responsiveness in dialysis patients. Several published studies did not find a significant association between genetic polymorphism and erythropoietin treatment response in dialysis patients. The pro-inflammatory cytokine polymorphism was not associated with rHuEPO responsiveness in a study with 112 patients on peritoneal dialysis (12). Hatano M et al. did not find a significant association between the ACE polymorphism and the rHuEPO dose in 91 hemodialysis patients (31). The polymorphism of the ACE was genotyped in 70 Iraqi patients on hemodialysis and there was no significant effect of polymorphism on hemoglobin levels (32).

Our study has several limitations: the small sample size, no measurement of serum angiotensin II levels, and erythropoietin levels in studied patients, which might be a possible explanation for rHuEPO responsiveness. Many other ACE gene polymorphism could affect the response to rHuEPO as rs4343, rs429, and rs4341, which may be in linkage disequilibrium with studied rs1799752.

In conclusion: iron deficiency, inflammation, malnutrition, and hyperparathyroidism are factors associated with anemia and resistance to erythropoietin therapy in dialysis patients. The genetic polymorphism have been identified as possible causes of resistance to erythropoietin in dialysis patients. Studies with a larger sample size should be performed to confirm the association of polymorphism with erythropoietin responsiveness.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest related to the publication of this manuscript.

FUNDING STATEMENT

The study was funded by the Faculty of Medicine, Ss. Cyril and Methodius University in Skopje, Skopje, RN Macedonia.

REFERENCES

1. Winearls CG, Oliver DO, Pippard MJ, Reid C, Downing MR, Cotes PM. Effect of human erythropoietin derived from recombinant DNA on the anaemia of patients maintained by chronic haemodialysis. Lancet. 1986; 2(8517):1175–1178.
2. Eschbach JW, Egrie JC, Downing MR, Browne JK, Adamson JW. Correction of the anemia of end-stage renal disease with recombinant human erythropoietin. Results of a combined phase I and II clinical trial. N Engl J Med. 1987 Jan 8;316(2):73-78.
3. Zins B, Drueke T, Zingraff J, Bererhi L, Kreis H, Naret C, et al. Erythropoietin treatment in anaemic patients on haemodialysis. Lancet. 1986;2(8519):1329.
4. Besarab A, Bolton WK, Browne JK, Egrie JC, Nissenson AR, Okamoto DM, et al. The effects of normal as compared with low hematocrit values in patients with cardiac disease who are receiving hemodialysis and detin. N Engl J Med. 1998; 339(9):584–590.
5. Locatelli F, Pisoni RL, Combe C, Bommer J, Andreucci VE, Piera L, et al. Anaemia in haemodialysis patients of five European countries: association with

morbidity and mortality in the Dialysis Outcomes and Practice Patterns Study (DOPPS). Nephrol Dial Transplant. 2004; 19(1):121–132.

6. Wyatt CM, Drueke TB Higher hemoglobin levels and quality of life in patients with advanced chronic kidney disease: no longer a moving target? Kidney Int. 2016 May;89(5):971-973.
7. Okazaki M, Komatsu M, Kawaguchi H, Tsuchiya K, Nitta K. Erythropoietin resistance index and the all-cause mortality of chronic hemodialysis patients. Blood Purif. 2014;37(2):106-112.
8. KDIGO clinical practice guideline for anemia in chronic kidney disease. Chapter 3. Use of ESAs and other agents to treat anemia in CKD. Kidney Int Suppl. 2012; 2:299–310.
9. USRDS. 2013 Annual Data Report: Atlas of chronic kidney disease and end-stage renal disease in the United States, national institutes of health, and national institute of diabetes and digestive and kidney diseases. MD: Bethesda; 2013.
10. Wong HS, Chang CM, Kao CC, Hsu YW, Liu X, Chang WC, Wu MS, Chang WC. V-J combinations of T-cell receptor predict responses to erythropoietin in end-stage renal disease patients. J Biomed Sci. 2017 Jul 11;24(1):43.
11. Priyadarshi A, Shapiro JI. Erythropoietin resistance in the treatment of the anemia of chronic renal failure. Semin Dial 2006;19:273-278.
12. Sharples EJ, Varagunam M, Sinnott PJ, McCloskey DJ, Raftery MJ, Yaqoob MM. The effect of proinflammatory cytokine gene and angiotensin-converting enzyme polymorphism on erythropoietin requirements in patients on continuous ambulatory peritoneal dialysis. Perit Dial Int 2006; 26:64-68.
13. Nand N, Deshmukh AR, Joshi S, Sachdeva MP, Sakthivel. Role of ACE and IL-1β Gene Polymorphism in Erythropoeitin Hyporesponsive Patients with Chronic Kidney Disease with Anemia. J Assoc Physicians India. 2017 Feb;65(2):32-36.
14. Rigat B, Hubert C, Alhenc-Gelas F, Cambien F, Corvol P, Soubrier F. An insertion/deletion polymorphism in the angiotensin I-converting enzyme gene accounting for half the variance of serum enzyme levels. J Clin Invest 1990; 86: 1343-1346.
15. Mrug M, Stopka T, Julian BA, Prchal JF, Prchal JT. Angiotensin II stimulates proliferation of normal early erythroid progenitors. J Clin Invest 1997;100: 2310-2314.
16. Kim YC, Mungunsukh O, Day RM. Erythropoietin Regulation by Angiotensin II. Vitam Horm. 2017; 105:57-77.
17. Dinarello CA. Biologic basis for interleukin-1 in disease. Blood 1996;87: 2095-2147.
18. Jelkmann W. Proinflammatory cytokines lowering erythropoietin production. J Interferon Cytokine Res 1998; 18:555-559.
19. Jeong KH, Lee TW, Ihm CG, Lee SH, Moon JY. Polymorphism in two genes, IL-1B and ACE, are associated with erythropoietin resistance in Korean patients on maintenance hemodialysis. Exp Mol Med. 2008 Apr 30;40(2):161-166
20. Berns JS. Interpretation of the Kidney Disease: Improving Global Outcomes guidelines for iron therapy: commentary and emerging evidence. Clin Kidney J. 2017 Dec;10(Suppl 1):i3-i8.
21. KDIGO 2017 Clinical Practice Guideline Update for the Diagnosis, Evaluation, Prevention, and Treatment of Chronic Kidney Disease–Mineral and Bone Disorder (CKD-MBD). Kidney International Supplements 2017; 7: 1–59.
22. Varagunam M, McCloskey DJ, Sinnott PJ, Raftery MJ, Yaqoob MM. Angiotensin-converting enzyme gene polymorphism and erythropoietin requirement. Perit Dial Int 2003; 23:111-115.
23. Santos EJF, Hortegal EV, Serra HO, Lages JS, Salgado-Filho N, Dos Santos AM. Epoetin alfa resistance in hemodialysis patients with chronic kidney disease: a longitudinal study. Braz J Med Biol Res. 2018; 51(7):e7288.
24. Kaneko S, Hirai K, Morino J, Minato S, Yanai K, Mutsuyoshi Y, Ishii H, Matsuyama M, Kitano T, Shindo M, Aomatsu A, Miyazawa H, Ueda Y, Ito K, Ookawara S, Morishita Y. Association between carnitine deficiency and the erythropoietin resistance index in patients undergoing peritoneal dialysis: a cross-sectional observational study. Ren Fail. 2020 Nov;42(1):146-153.
25. Wu HHL, Chinnadurai R. Erythropoietin-Stimulating Agent Hyporesponsiveness in Patients Living with Chronic Kidney Disease. Kidney Dis (Basel). 2022 Jan 14; 8(2):103-114.
26. Radić J, Bašić-Jukić N, Vujičić B, Klarić D, Radulović G, Jakić M, et al. Anemia Is Correlated with Malnutrition and Inflammation in Croatian Peritoneal Dialysis Patients: A Multicenter Nationwide Study. Perit Dial Int. 2017 Jul-Aug;37(4):472-475.

27. Weir MR. Managing Anemia across the Stages of Kidney Disease in Those Hyporesponsive to Erythropoiesis-Stimulating Agents. Am J Nephrol. 2021; 52(6):450-466.
28. Rambod M, Kovesdy CP, Kalantar-Zadeh K. Combined high serum ferritin and low iron saturation in hemodialysis patients: the role of inflammation. Clin J Am Soc Nephrol. 2008 Nov;3(6):1691-1701.
29. Karaboyas A, Morgenstern H, Fleischer NL, Vanholder RC, Dhalwani NN, Schaeffner E, et al. Inflammation and erythropoiesis-stimulating agent response in hemodialysis patients: a Self-matched Longitudinal Study of anemia management in the dialysis outcomes and Practice Patterns Study (DOPPS). Kidney Med. 2020;2:286–296
30. Kiss Z, Ambrus C, Kulcsár I, Szegedi J, Kiss I. ACEGENE-BB_HU workgroup; ACEGENE-BB HU workgroup. Effect of angiotensin-converting enzyme gene insertion/deletion polymorphism and angiotensin-converting enzyme inhibition on erythropoiesis in patients on haemodialysis. J Renin Angiotensin Aldosterone Syst. 2015 Dec;16(4):1021-1027.
31. Hatano M, Yoshida T, Mimuro T, Kimata N, Tsuchiya K, Sanaka T, Nihei H. The effects of ACE inhibitor treatment and ACE gene polymorphism on erythropoiesis in chronic hemodialysis patients. Nihon Jinzo Gakkai Shi. 2000 Oct; 42(8):632-639.
32. Al-Radeef, M. Y., Fawzi, H. A., & Allawi, A. A. ACE gene polymorphism and its association with serum erythropoietin and hemoglobin in Iraqi hemodialysis patients. The Application of Clinical Genetics. 2019; 12: 107-112.