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Association of calcium channel blocker use with lower hemoglobin levels in chronic kidney disease

M.A. CIKRIKCIOGLU, C. KARATOPRAK, M. CAKIRCA, M. KISKAC, M. ZORLU, G. CETIN[1], K. YILDIZ, R. ERKOC[2], M. ALAY[2], S. ERKAL[3], S.N. ERKAL[4], S. DOGAN[5], R. KAZANCIOGLU[2]

Department of Internal Medicine, Bezmialem Vakif University, Faculty of Medicine, Fatih, Istanbul, Turkey
[1]Division of Hematology, Department of Internal Medicine, Bezmialem Vakif University, Faculty of Medicine, Fatih, Istanbul, Turkey
[2]Division of Nephrology, Department of Internal Medicine, Bezmialem Vakif University, Faculty of Medicine, Fatih, Istanbul, Turkey
[3]Department of Biochemistry, Kanuni Sultan Suleyman Training and Research Hospital, Halkali, Kucukcekmece, Istanbul, Turkey
[4]Intern Doctor, Istanbul University, Cerrahpasa Faculty of Medicine, Cerrahpasa, Fatih, Istanbul, Turkey.
[5]Private Ahenk Biochemistry Laboratory, Sisli, Istanbul, Turkey

Abstract. – AIM: To search whether calcium channel blockers (CCBs) are associated with lower hemoglobin levels in chronic kidney disease (CKD) patients who are not on renal replacement therapy (RRT), vitamin D and antianemic treatment.

PATIENTS AND METHODS: CKD patients were classified into two groups. Patients on CCBs treatment (103 patients) and patients not using CCBs (104 patients) were compared cross-sectionally regarding clinical findings, complete blood count (CBC), biochemistry and regular medication use. Patients with polycystic kidney disease, comorbidities that could influence CBC other than iron deficiency of obscure origin, patients receiving RRT, erythropoietin (EPO), vitamin D, phosphate binders and drugs that could influence CBC were excluded. Under dependent variable of CCB use, all significantly different independent variables were subjected to multivariate binary logistic regression analysis (MBLRA).

RESULTS: Lower hemoglobin, lower bilirubinemia, higher serum EPO, higher systolic blood pressure were observed in CCB users. Two groups were similar concerning age, gender, BMI, CKD etiology, CKD stage, pretibial edema prevalence, cardiothoracic index, diastolic blood pressure, corrected reticulocyte count, BUN, creatinine, eGFR, proBNP, parathormone, alkaline phosphatase, phosphorous, corrected calcemia, sCRP, relative EPO deficiency and prevalence of relative EPO deficient patients. Groups were comparable regarding comorbidities, types and usage frequencies of all antihypertensive medications other than CCBs. Higher systolic blood pressure and lower hemoglobin were significantly associated with CCB use after MBLRA.

CONCLUSIONS: Hemoglobin was significantly lower in CCB users compared to non-users, among CKD patients who did not receive RRT, EPO, phosphate binders, vitamin D, iron, vitamin B12 and folic acid.

Key Words:
Calcium channel blockers, Anemia, Hemoglobin, Chronic kidney disease, Erythropoietin.

Introduction

Erythropoietin (EPO) is an essential hormone in erythropoiesis, and is produced mainly in kidneys. EPO stimulates the differentiation and proliferation of erythroid precursor cells[1]. After stimulation of the erythroid precursor cell by EPO, intracellular free calcium and cyclic AMP increase, and protein kinases are activated, followed by cell differentiation and proliferation[1]. In order that erythropoietin can exhibit its proliferative activity, it is imperative that the molecule should increase free calcium concentration within the erythroid precursors[1]. EPO was shown to increase intracellular free calcium concentration in healthy individuals through calcium influx from extracellular fluid to erythroid precursors *in vitro*; whereas nifedipin impeded the calcium influx and blocked the effect of erythropoietin[1]. Therefore, in erythropoietin-stimulated cells, calcium ion influx rather than intracellular Ca^{2+} mobilization was responsible for increased intracellular free calcium[1].

Corresponding Author: Mehmet Ali Cikrikcioglu, MD; e-mail: malicikrikcioglu@yahoo.com

Anemia may develop early in the course of CKD and is nearly universal in patients with CKD stage 5[2]. The main causes of anemia in CKD are EPO deficiency, EPO resistance, and shortened red cell span, with EPO deficiency being the most prominent one[3,4]. CCBs are frequently used to control hypertension in CKD patients. In CKD patients with EPO deficiency, CCB use may additionally impede EPO activity and, thereby, further diminish hemoglobin (Hb) and hematocrit (Htc) levels. Therefore, we planned our study with the hypothesis that CCB use in CKD patients who are not on renal replacement treatment (RRT) on treatments of EPO, vitamin D3 and/or phosphate binders is associated with lower levels of hemoglobin. To our best knowledge, there is no published study conducted to test this hypothesis.

Materials and Methods

Study Design

Patients using CCBs and patients not using CCBs in stage 2-5 CKD were compared cross-sectionally regarding clinical findings, complete blood count (CBC), biochemistry analysis and regular medication use.

Study Population

A total of 207 adult (110 female) CKD patients meeting the inclusion and exclusion criteria and who gave written informed consent were selected for the study among all CKD patients consecutively seen and treated in the Outpatient Clinics of Internal Medicine Department of Bezmialem Vakif University Faculty of Medicine between December 2011 and December 2012 were enrolled in the study. Diagnostic criteria and staging for CKD were made in accordance with the NKF KDOQI Guidelines[5]. Patients with eGFR values of 89-60 mL/min, 59-30 mL/min, 29-15 mL/min and < 15 mL/min during the previous 3 months or more duration, with or without kidney injury were defined as stage 2-5 CKD patients, respectively. eGFR was calculated using MDRD (modification of Diet in Renal Disease) formula[5].

Inclusion Criteria

Stage 2-5 adult CKD patients of any etiology except polycystic kidney disease were recruited in the study. Those patients in whom the dose or type of the antihypertensive medication was not changed during the period of minimum 3 months before enrolment were eligible for the study. Patients who had iron deficiency of obscure origin, patients with chronic obstructive pulmonary disease (COPD) in remission period or with controlled type 2 diabetes mellitus, as well as patients using essential amino acid tablets, or receiving anti-acidosis (NaHCO3 capsule) or anti-potassium (calcium polystyrene sulphate granule) treatment were also included in the study.

Exclusion Criteria

Exclusion criteria were as follows: renal replacement therapy, polycystic kidney disease, heart failure, uncontrolled diabetes mellitus, COPD in exacerbation period, malign solid tumours, patients undergoing chemotherapy or radiotherapy, hematologic malign diseases, myelodysplastic syndrome (MDS), hemolytic anemias, portal hypertension, collagen tissue diseases, chronic liver diseases, chronic immunosuppressive treatment, pituitary or adrenal hormonal dysfunction, hypothyroidism, hyperthyroidism, chronic and acute infections, diabetic foot, recurrent epistaxis, menometrorrhagia, marked persistent hematuria, positive fecal occult blood test, history of hematemesis, melena, hematochezia, gastrectomy, vegetarian diet, major surgery, blood transfusion, and treatment with vitamin D, phosphate binders, EPO, Vitamin B12, folic acid and iron.

Patients were not recruited in the study unless they gave informed consent. Those receiving antihypertensive treatment for a duration shorter than three months were not included, either. Furthermore, any change in the antihypertensive therapy during the last three months was also a reason for exclusion.

Detailed information on regular drugs use during at least 3 months prior to enrolment in the study, disease history, past medical history and family history were obtained from all patients. A systematic physical examination was performed. Blood pressures of patients were taken fasting in the morning prior to blood sample collection, after 30 minutes of rest in sitting position. Three consecutive arterial blood pressure measurements were done at the right arm with 3-minute intervals and the value at the second measurement was used in the statistical analysis.

Patients who used CCBs regularly during minimum 3 months prior to enrolment in the study were accepted as CCB users. Patients who did not use CCBs during minimum 3 months prior to enrolment were accepted as CCB nonusers.

Laboratory

Samples: Blood samples for complete blood count (CBC), extensive biochemistry, thyroid hor-

mones, pro-brain natriuretic peptide (Pro-BNP), parathormone (PTH), erythropoietin (EPO), 25 (OH) vitamin D3, erythrocyte sedimentation rate (ESR), sensitive C-reactive protein (sCRP) and venous blood gas analysis were collected early in the morning after an overnight fast. Fresh urine samples were collected in the morning for urinalysis and urinary protein/creatinine. Feces samples were also taken for fecal occult blood test.

Measurements: Urinary ultrasonographic examination was performed on each patient. Chest x-rays were taken in patients with pretibial edema. CBCs were measured with the Roche Sysmex 1800i Hemogram device (Kobe, Japan). Reticulocyte counts were done manually. Biochemistry analyses were done with the Roche Cobas e601 auto analyser (Tokyo, Japan) using Roche reagents (Mannheim, Germany).

25 (OH) vitamin D3 was assayed using Dia Source brand RIA kit (4000 Sart Tilman Liège, Belgium) at Iso-Data 20/20 Gamma Counter (RIA) (Iso-Data Inc., Palatine, IL, USA). Ferritin, intact parathyroid hormone, pro-BNP and erythropoietin were assayed with Siemens Immulite 2000 Hormone device (Flanders, NJ, USA) using Siemens reagents (Llanberis, Gwynedd, UK).

Calculation of relative EPO deficiency: The expected EPO value of patients based on hemoglobin (Hb) value was calculated with the "log (expected EPO) = 4.46 – (0.274 × Hb)" formula[6]. Expected EPO was found by taking the antilogarithm of the resultant value. If the observed EPO/expected EPO ratio was less than 1, this was accepted as relative EPO deficiency. If this ratio was equal to or greater than 1, it was considered that there was no EPO deficiency. Percentage of relative EPO deficiency in each patient was found with the following formula "[1-observed EPO/expected EPO] × 100".

Statistical Analysis

Numerical variables are presented as means with standard deviations, and nominal variables in ratios. Patients were divided into 2 groups as CCB users and nonusers. Nominal and continuous (numerical) independent variables were compared between the two groups. Chi-square test was used to compare nominal independent variables. Shapiro-Wilk test was done to see if the continuous independent variables were normally distributed. Normally distributed independent continuous variables were compared with the Student's *t* test; whereas non-normally distributed independent continuous variables were compared with the Mann-Whitney U test. Under dependent variable of CCB use, all significantly different independent variables between CCB users and CCB nonusers were subjected to multivariate binary logistic regression analysis (MBLRA). In addition, multivariable linear regression analysis with dependent variable of hemoglobin value was performed to show the significant association between CCB use and lower hemoglobin values in CKD, after controlling for the impact of other independent variables which might be potentially associated with lower hemoglobin values. Bivariate correlations were also sought among several selected variables. A two-tailed p value of <0.05 was considered to be statistically significant.

Ethics

The study was approved by the Ethics Committee of the Bezmialem Vakif University Medical Faculty and written informed consents were obtained from patients. The study was conducted according to the Helsinki Declaration of 2009.

Results

252 patients were screened for the study. Totally 45 patients were eliminated due to exclusion criteria. Thus, the study was conducted on 207 patients.

Mean age of study patients was 68.6 ±10.7 (range 30-95) years. Among the 110 female patients, 95.5% were post-menopausal. CCB user (103 subjects) and non-user (104 subjects) patients were similar in terms of age, gender, BMI and frequency of women in fertile age (3 women in the CCB user group and 2 women in the CCB non-user group) (Tables I, III).

Compared to CCB non-user patients, CCB user patients had lower Htc and Hb values (Table I). Corrected reticulocyte count was lower in the CCB user group without significance (Table I). The mean Hb value of CCB user patients was 0.660 g/dL lower than that of CCB non-users.

Serum EPO values were significantly higher in CCB users compared to nonusers (Table I). However, the two groups were similar concerning relative EPO deficiency (Table I). Among all patients, the prevalence of patients with relative EPO deficiency was 70.5%. EPO could not be measured in 2.9% of patients, thus only 26.6% of all patients had no such deficiency. Mean relative EPO deficiency was 66.91±24.45% in the patients with EPO deficiency. The two groups were comparable in terms of relative EPO deficient patient prevalence (Table III).

Table I. Demographics and laboratory results of chronic kidney disease patients using and not using CCBs.

Parameters	Patients using CCBs n: 103 mean ± SD		Patients not using CCBs n: 104 mean ± SD		Normal range	*p* value	Type of distribution
Age, years	68.63	10.24	68.50	11.13		0.930	NND
BMI, kg/m^2	30.45	5.43	29.68	5.38	18.5–24.9	0.307	ND
Cardiothoracic index on chest X ray, ratio, %	54.52	8.13	53.24	6.43	<0.5	0.567	ND
Systolic blood pressure, mm Hg	147.22	31.03	135.23	28.14	<135	0.004	NND
Diastolic blood pressure, mm Hg	78.75	12.94	77.76	12.38	<85	0.649	NND
proBNP, pg/mL	1633.02	3069.19	2001.36	5212.04	0-110	0.545	NND
sCRP, mg/dL	9.69	12.32	11.33	13.68	0-5	0.574	NND
TSH, mIU/L	2.65	2.52	3.09	3.11	0.27-4.2	0.421	NND
HbA1C, %	7.24	1.79	7.44	1.83	<6.5	0.295	NND
Albumin, g/dL	4.11	0.47	4.10	0.47	3.5–5.0	0.804	NND
Sodium, mEq/L	137.49	3.40	137.94	2.90	135–155	0.191	NND
Magnesium, mg/dL	1.96	0.25	1.94	0.29	1.6-2.6	0.659	ND
Potassium, mEq/L	4.88	0.68	5.02	0.67	3.5–5.5	0.137	ND
Corrected serum calcium, mg/dL	9.21	0.53	9.29	0.64	8.6-10.6	0.242	ND
Phosphorus, mg/dL	3.74	0.75	3.70	0.79	2.7-4.5	0.701	ND
Urine protein/creatinine, mg/24 h	1568.79	2556.47	1146.63	2578.57	<200 mg/24 h	0.117	NND
eGFR, ml/min	38.10	14.32	39.13	12.54	≥90	0.850	ND
Creatinine, mg/dL	1.86	0.71	1.80	0.75	<1.3	0.514	NND
Urea, mg/dL	74.48	31.31	72.62	29.07	0-50	0.767	NND
Uric acid, mg/dL	6.91	1.75	7.04	1.76	2.4-5.7	0.137	NND
Conjugated bilirubin, mg/dL	0.13	0.08	0.15	0.07	0-0.2	0.007	NND
Unconjugated bilirubin, mg/dL	0.24	0.13	0.30	0.18	0.2-0.8	0.009	NND
LDH, U/L	186.13	42.98	181.29	32.90	0-250	0.682	ND
Alkaline phosphatase, U/L	92.01	46.23	86.94	33.98	35-104	0.370	NND
HCO3 (ACT), mmol/L	26.31	4.42	26.25	4.09	21-28	0.688	ND
25 (OH) cholecalcipherol, ng/mL	27.25	13.38	26.55	12.43	30-74	0.800	NND
Serum erythropoietin, IU/L	10.28	8.69	7.71	7.05	3.5-17.6	0.012	NND
Relative erythropoietin deficiency, %	68.10	25.52	65.68	23.41		0.421	NND
Parathormone pg/mL	134.44	117.34	135.65	152.42	12-65	0.440	NND
Corrected reticulocyte, %	0.85	0.63	0.97	1.00	0.5-1.5	0.409	NND
Hemoglobin, g/dL	11.54	1.64	12.20	1.67	12.7–18.1	0.005	ND
Hematocrit, %	35.57	4.89	37.54	5.20	37.7–53.7	0.006	ND
Folic acid, ng/mL	8.70	3.48	8.93	3.55	4.6-18.7	0.650	NND
Vitamin B12, pg/mL	562.55	474.60	533.70	463.68	191-663	0.635	NND
Ferritin, ng/mL	131.79	127.73	137.42	129.68	5-148	0.465	NND
Transferrin saturation rate, %	21.24	9.0	23.30	9.05	0.15–0.40	0.121	NND

ND: Normal distribution; NND: Nonnormal distribution.

In the group of CCB users, systolic arterial blood pressure of patients was significantly higher than that of CCB non users.

Bilirubins were significantly lower in CCB users. Serum creatinine, urea, proBNP, urine protein/creatinine were higher and eGFR was lower among the CCB using group though not statistically significant (Table I).

CCB user and non-user patients in our study were comparable in terms of sCRP, actual bicarbonate in venous blood gas, 25 hydroxycholecalciferol, serum parathormone, serum phosphorous, vitamin B12, folic acid, transferrin saturation rate and ferritin. The two groups were also similar concerning cardiothoracic index values, pretibial edema prevalence, CKD etiologies, comorbidities with a potential to influence Hb and Htc, types and usage frequencies of all antihypertensive medications other than CCBs, and regular medication use within at least 3 months before enrolment in the study (Tables I-III).

In the MBLRA done with the dependent variable CCB use versus the independent variables of serum EPO level, systolic blood pressure, hemoglobin, serum unconjugated bilirubin, eGFR, urine protein/creatinine ratio, only lower hemoglobin (OR: 1.37, 95% CI: 1.12-1.68, $p = 0.002$) and higher systolic blood pressure (OR: 1.017, 95% CI: 1.005-1.029, $p = 0.005$) were found to be

Table II. Drugs patients regularly used during at least 3 months prior to enrolment in the study.

Drugs	All patients n: 207	Group using CCBs n: 103	Group not using CCBs n: 104	*p* value
ACE inhibitors	48	26	22	0.595
Angiotensin receptor blockers	74	35	39	0.702
Beta blockers	64	31	33	0.907
Alpha blockers	24	15	9	0.236
Spironolactone	14	5	9	0.302
Furosemide	52	30	22	0.201
Thiazides	91	42	49	0.436
(NaHCO3) capsule	6	3	3	0.714
Calcium polystyrene sulphate granule	11	7	4	0.494
Essential amino acid tablet	8	6	2	0.256
Acetylsalicylic acid	68	30	38	0.323

associated with CCB use, with statistical significance. These two variables together accounted for 62.1 % of the patients in the model.

There was a positive bivariate correlation between corrected calcemia and hemoglobin (r = 0.247, $p < 0.001$). However, there was no bivariate correlation between corrected calcemia and either eGFR or creatininemia. A positive correlation was also existed between indirect bilirubinemia and Hb (r = 0.492, $p < 0.001$).

Multivariable linear regression analysis with stepwise method was performed using the dependent variable Hb value. The independent variables age ($p = 0.327$), BMI ($p = 0.700$), systolic blood pressure ($p = 0.981$), pro BNP ($p = 0.079$), corrected calcemia ($p = 0.053$), urine protein/creatinine ($p = 0.209$) were not related with Hb values. Female gender, lower eGFR, higher relative EPO deficiency and CCB use were significantly associated with lower Hb level independent of each other (Table IV).

Among the CCB user group, 69.9% were using dihydropyridine group of CCBs and 30.1% were using non-dihydropyridine group of CCBs. These two subgroups were similar in terms of Htc and Hb values.

Discussion

Levels of Hb and Htc were lower, whereas serum EPO values were higher in CCB using patients compared to non-users in our study. These findings are in favour of our study hypothesis. EPO deficiency can be seen even in stage 1-2 CKD[6];

Table III. Comparison of certain nominal variables between the two groups.

Variable	All patients n: 207	Group using CCBs n: 103	Group not using CCBs n: 104	*p* value
Gender, female/male, n	110/97	57/46	53/51	0.623
Diabetic nephropathy, n	63	32	31	0.740
Benign nefrosclerosis, n	65	36	29	0.344
Tubulointersititial disease, n	14	8	6	0.726
Chronic glomerulonephritis, n	8	3	5	0.760
Obstructive nephropthy, n	20	8	12	0.538
Unknown etiologies, n	39	16	23	0.351
Stage 2 CKD patients, n	12	5	7	0.353
Stage 3 CKD patients, n	136	64	72	0.779
Stage 4 CKD patients, n	53	31	22	0.189
Stage 5 CKD patients, n	6	3	3	0.687
Patients with pretibial edema, n	49	24	25	0.879
Type 2 DM, n	130	64	66	0.957
COPD, n	29	13	16	0.665
Patients with relative EPO deficiency, n	146	74	72	0.878

DM, diabetes mellitus; COPD, chronic obstructive pulmonary disease.

Table IV. Multivariable linear regression analysis with the dependent variable Hb.

Independent variables	Unstandardized coefficient		Standardized Coefficient		
	B	SE	β	t	*p* value
Female gender	-0.358	0.179	-0.106	-2.002	0.047
Relative EPO deficiency	-1.216	0.108	-0.616	-11.262	< 0.001
eGFR	0.021	0.007	0.164	3.063	0.003
Calcium channel blocker use	-0.589	0.169	-0.175	-3.484	0.001

F: 59.117, R square: 0.590, Adjusted R square: 0.580

however, the deficiency aggravates with declining GFR[6]. In CKD, if mGFR is > 30 mL/min, increased EPO release response to reduced Hb still continues, albeit blunted[6]. If mGFR is < 30 mL/min, the negative correlation between EPO and Hb disappears[6]. Since 72.6% of our patients had eGFR ≥ 30 mL/min, the still functional feed-back mechanism between Hb and EPO might have caused the higher EPO levels in CCB users[6]. Other than this, more EPO may be released upon blockage of EPO activity by CCB use, but increase in EPO values in CCB user patients might have fallen short to raise the Hb levels up to that of CCB non-users. Indeed, the mean relative EPO deficiency and frequency of relative EPO deficient patients were similar in the groups. It is not known whether CCB use leads to clinically lower Hb levels in subjects without CKD. CCB use by individuals without CKD may not cause a reduction in Hb and Hct, since there is no EPO resistance and no limitation on EPO release. In patients with CKD who have reduced EPO release and EPO resistance, CCB use may additionally impede EPO activity and, thereby, further diminish Hb and Htc levels.

We had mentioned before that it was imperative for EPO to increase free intracellular calcium ion in order to stimulate the differentiation and proliferation of erythroid precursor cells and this was achieved through calcium ion influx from extracellular fluid, not by the mobilization of intracytoplasmic calcium ions. Though EPO causes calcium influx into the erythroblasts through voltage independent calcium channels and nifedipine is known to be a voltage dependent calcium channel blocker, nifedipine impeded calcium influx to erythroblasts and blocked the EPO activity *in vitro*[1,7]. Later on, calcium influx into erythroblasts by EPO was shown to be mediated by TRPC2 (transient receptor potential cation channel, subfamily C, member 2) and TRPC3 channels[8]. These channels are a group of calcium permeable cation channels belonging to transient receptor potential (TRP) ion channels superfamily, and expressed on non-excitable cells, taking part in numerous physiological events[9]. Notwithstanding, another study reported that anemia was not observed in TRPC2, TRPC3, TRPC2/TRPC3 knockout mice[9]. After *in vitro* EPO stimulation at equal concentrations, splenic erythroblasts of mutant mice showed less increase in intracellular calcium ions compared to their littermate wild-type controls[9]. Yet, moderate increase in calcium ion concentration occurred in the erythroblasts of TRPC2, TRPC3, TRPC2/TRPC3 knockout mice[9]. As this study suggests, there might be as yet unknown ion channels, other than TRPC2 and TRPC3, on which EPO is effective[9]. Since there is no CKD in these mutant mice, intra-erythroblastic Ca^{+2} concentration might have been brought up to an optimum level by the release of more EPO and through some Ca^{+2} ion channels yet unknown, with a consequent anemia protective activity. EPO should increase the Ca^{+2} ion influx into the erythroblasts up to an optimum level in order that it can exhibit an optimum anti-anemic activity[10]. As a matter of fact, serum EPO levels of TRCP2, TRCP3, TRCP2/TRCP3 knockout mice and their littermate wild-type controls were not compared. In addition, we do not know the *in vivo* levels of Ca^{+2} ion concentration inside the erythroblasts of these mutant mice in comparison with the control group. Again, in the above study, intracellular calcium ion levels in the splenic erythroblasts of the TRCP2, TRCP3, TRCP2/TRCP3 knockout mice and their littermate wild-type controls were not measured in a manner reflecting the *in vivo* situation as in the study[10] mentioned in the following paragraph.

It was reported in an *in vitro* study that burst forming unit-erythrocyte (BFU-E) and colony forming unit-erythrocyte (CFU-E) derived erythroblasts of anemic, continuous ambulatory peritoneal dialysis patients exhibited less proliferation and had lower intracytoplasmic free calcium before rHuEPO therapy, compared to controls[10]. *In vitro* addition of calcium to the medium surrounding the erythroblasts was

shown to increase intracellular free calcium concentration and proliferation of erythroblasts and this process was potentiated by active vitamin D[10]. When same patients received recombinant human erythropoietin (rHuEPO) treatment, the patients' anemia improved and intracellular calcium concentration turned to normal levels in their erythroblasts[10].

There was a positive correlation between corrected calcemia and hemoglobin. Higher calcemia values may have a clinically positive influence on hemoglobin levels and potentiate the effect of EPO. There was no correlation between corrected calcemia and estimated glomerular filtration rate (eGFR); no correlation existed between corrected calcemia and serum creatinine, either. In other words, the positive effect of calcemia on hemoglobin seems to be independent of the degree of renal impairment. These findings overlap with the experiment in which addition of calcium into intercellular medium enhanced erythroblast proliferation under suboptimal EPO presence in the *in vitro* study mentioned above[10].

The lower bilirubinemia observed in patients under CCB treatment might be due to the lower production/destruction cycle of hemoglobin.

Another *in vitro* study that might lend support to our study reported that nifedipin inhibited the differantiation of pluripotent stem cell into cardiomyocytes by altering calcium ion influx into the cell at the early stages of differentiation[11]. This is because calcium ion influx into the stem cell activates Ca^{+2} signaling pathway, and this contributes to numerous physiological cellular pathways[11].

As a result of MBLRA, both lower hemoglobin and higher systolic blood pressure were found to be associated with CCB use independent from each other. Higher systolic pressure may be an indicator of hidden hypervolemia, and it might be proposed that Hb was found lower in CCB users as a consequence of this. Based on MBLRA rules, the cause for decreased Hb cannot be higher systolic blood pressure; because if higher systolic blood pressure was the cause for the lower hemoglobin values observed in CCB using patients, lower hemoglobin variable would be eliminated after MBLRA, and higher systolic blood pressure would take its place. In other words, even the remaining variables after MBLRA are associated with each other; one variable cannot be assumed as a cause for the other, and the latter cannot be overlooked.

We conducted multivariable linear regression analysis and demonstrated once again that CCB use was associated with lower Hb in CKD patients after controlling all the factors which might contribute to the lower Hb levels in CCB users. R^2 value of our regression model is high (Table IV). So factors are associated with lower Hb levels in CKD, which couldn't be included in our regression model, are fewer.

In a clinical study which also had a crossectional design, hemodialysis patients were classified into two groups as patients on CCB treatment and patients not using CCB. CCB treatment was shown to be associated with lower hemoglobin, consumption of a higher dose recombinant human erythropoietin/body weight (rHuEPO/BW), higher blood pressure and more frequent utilization of antihypertensive drugs[12]. After MBLRA was performed with dependent variable of CCB use, independent variable of lower hemoglobin was eliminated the other three variables, namely treatment with higher dose rHuEPO/BW, higher arterial blood pressure and more frequent utilization of antihypertensive drugs were found to be independently associated with CCB use[12]. However, the authors interpreted their findings differently than we do. They did not regard the association of CAs with lower hemoglobin values and utilization of higher amount of rHuEPO use coming up as a result of their study as a separate entity that deserves to be elucidated. Instead, the authors related their study findings to the more frequent occurrence of hypertension caused by rHuEPO use. In other words, they argued that rHuEPO was initiated because the patient was anemic; later on, CCBs and other antihypertensive medications had to be administered because hypertension occurred or deteriorated. As already mentioned above, such an argument is inaccurate because it is contradictory to the logic of MBLRA in the first instance. Secondly, it may also be possible that anemia was worsened because of CCB use, with a consequent necessity for higher rHuEPO doses, which in turn leading to a more extensive antihypertensive treatment. The authors also performed multivariable linear regression analysis with dependent variable of rHuEPO/BW. According to this statistical test, factors associated with higher dose of rHuEPO/BW treatment were CCB use, older age and female gender. Higher blood pressure, ACE inhibitors, angiotensin receptor blockers were not associated with higher dose of rHuEPO/BW treatment[12].

Studies conducted on patients on hemodialysis treatment have found that CCB use did not result in EPO resistance[13,14]. These studies were conducted to find out whether ACE inhibitors or angiotensin receptor blockers (ARBs) caused EPO resistance or not. Patients who were not on antihypertensive

treatment and who used CCBs were taken as controls in these studies, and it was concluded that ACE inhibitors and ARBs caused EPO resistance, but CCBs did not[13,14]. However, these studies included few CCB using patients; 20 subjects in one study and 10 subjects in the other[13,14]. Saudan et al[15] divided the hemodialysis patients into 5 groups based on antihypertensive medication use, and these 5 groups were found to be similar in terms of EPO resistance (Group 1: ACE inhibitors, Group 2: ARBs, Group 3: ACE inhibitor plus ARB, Group 4: other antihypertensive medications, Group 5: no antihypertensive treatment). In this study, it is not clear for how long the patients used antihypertensive medications before enrolment in the study, and it is not known how many patients in the "other antihypertensive medications" group were using CCBs.

Crossectional design could be a criticism to our study. Trials should be conducted to reach a definitive conclusion, indeed. We hope that our study may pave the way for such kind of research. Also the lower percentage (62.1%) in explaining our model with the independent variables obtained after MBLRA may be a limitation to our work. However, in our study, the prevalence of EPO deficient patients was already 70.5%.

Conclusions

CCBs stand as a good alternative in CKD patients, since ACE inhibitors or ARBs might lead to hyperpotassemia and/or excessive reduction in GFR. However the knowledge that CCBs might deteriorate anemia would impede undue efforts in diagnosis and treatment and enable us to take this into account while prescribing for patients with a potential to be influenced adversely from anemia.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

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