Clinical Trial/Experimental Study

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Erythropoietin therapy improves endothelial function in patients with non-dialysis chronic kidney disease and anemia (EARNEST-CKD)

A clinical study

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Abstract

Background: This study investigated whether administering erythropoiesis-stimulating agents (ESAs) improves endothelial function in patients with non-dialysis chronic kidney disease (CKD) and anemia.

Methods: This single-center, prospective, single-arm comparison study enrolled patients with non-dialysis CKD (stages 4-5) and hemoglobin levels <10 g/dL. ESA administration followed the Kidney Disease: Improving Global Outcomes guideline. The primary endpoint was the change in flow-mediated dilatation after ESA administration in individual patients. The secondary endpoints were changes in 6-minute walk test results, blood pressure, New York Heart Association class, and echocardiographic parameters. The echocardiographic parameters examined included chamber quantification, Doppler parameters, and systolic and diastolic function parameters.

Results: Initially, 13 patients were screened, but 2 discontinued due to either heart failure or voluntary withdrawal. The mean flow-mediated dilatation values significantly increased by 10.59% (from 1.36% ± 1.91% to 11.95% ± 8.11%, $P = .001$). Echocardiographic findings showed that the left ventricular mass index decreased by 11.9 g/m^2 (from 105.8 ± 16.3 to 93.9 ± 19.5 g/m^2, $P = .006$), and the left atrial volume index decreased by 10.8 mL/m^2 (from 50.1 ± 11.3 to 39.3 ± 11.3 mL/m^2, $P = .004$) after 12 weeks of ESA administration. There were no significant differences between pre- and post-ESA treatment 6-minute walk test results. No significant side effects were observed during the study period.

Conclusions: This is the first clinical study to demonstrate that an ESA improves endothelial dysfunction, left ventricular hypertrophy, and left atrial volume in patients with non-dialysis CKD. Thus, ESAs may be considered as adjunctive therapy for reducing cardiovascular risk in these patients.

Abbreviations: ASE = American Society of Echocardiography, CKD = chronic kidney disease, DTI = Doppler tissue imaging, eGFR = estimated glomerular filtration rate, ESA = erythropoiesis-stimulating agents, FMD = flow-mediated dilation, Hb = hemoglobin, LA = left atrium, LAVI = left atrial volume index, LV = left ventricle, LVEDD = left ventricular end diastolic dimension, LVEF = left ventricular ejection fraction, LVESD = left ventricular end systolic dimension, LVH = left ventricular hypertrophy, LVMI = left ventricular mass index, NYHA = New York Heart Association, RVSP = right ventricular systolic pressure.

Keywords: anemia, chronic kidney disease, endothelial dysfunction, erythropoiesis-stimulating agents

Editor: Doaa Attia.

HY and SJH contributed equally to this work.

This research was supported by Medical Research Promotion Program through the Gangneung Asan Hospital funded by the Asan Foundation.

This study protocol was approved by the Gangneung Asan Hospital ethical committee of Ulsan University (GNAH IRB number 2017-05-002-007). Informed consent was obtained from all participating patients.

The authors have no conflicts of interest to disclose.

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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How to cite this article: Lim J, Yu CJ, Yu H, Ha SJ. Erythropoietin therapy improves endothelial function in patients with non-dialysis chronic kidney disease and anemia (EARNEST-CKD): a clinical study. Medicine 2021;100:42(e27601).

Received: 14 April 2021 / Received in final form: 30 August 2021 / Accepted: 1 October 2021

http://dx.doi.org/10.1097/MD.0000000000027601

1. Introduction

Cardiovascular disease is a common complication and the most common cause of death in patients with chronic kidney disease (CKD), especially those undergoing dialysis.[1] After stratification by age, race, sex, and presence of diabetes, mortality due to cardiovascular disease is 10 to 20 times higher in patients undergoing dialysis than in the general population. This is difficult to explain using only cardiovascular risk factors.[2,3] Therefore, a number of studies have examined the relationship between dialysis and cardiovascular disease, showing that hemodialysis impairs endothelial function.[4–6] Recently, several studies have demonstrated that even patients with non-dialysis CKD show endothelial dysfunction,[5,7] which may worsen arterial vessel elasticity and cause left ventricular hypertrophy (LVH).[8]

Erythropoiesis-stimulating agents (ESAs) are primarily used as erythropoietic growth factors in patients with CKD and anemia. Some studies have shown that ESAs reduce plasma-oxidative stress in dialysis patients.[9–12] This evidence suggests that ESAs play an important role in protecting cells by reducing oxidative stress. Other studies have demonstrated that erythropoietin directly induces heme oxygenase-1 expression, indirectly depletes iron, increases the number of circulating young red blood cells, and shows an antioxidant effect.[11,13–16] In a clinical setting, ESAs have been shown to improve endothelial dysfunction in patients with coronary artery disease.[17] Furthermore, many clinical trials have demonstrated the beneficial effects of ESAs, including decreasing LVH, reducing cardiac remodeling, and improving functional capacity.[18–22]

Several studies investigated improving endothelial dysfunction in patients with CKD, and some have reported success when patients receive vitamin D and omega-3 polyunsaturated fatty acids.[23–25] Briet et al[26] suggested that ESA administration induces impaired endothelial function as a result of endothelin-1 secretion and oxidative stress. However, a study published in 2015 showed that endothelial dysfunction was improved when ESAs were administered to rats with CKD.[27] Except for this study in rats, the beneficial effects of ESA administration to patients with CKD have not been definitively proved and it could be very controversial.

In the present study, we hypothesized that ESA administration might prevent endothelial dysfunction in patients with non-dialysis CKD and anemia. Therefore, this study evaluated the effects of ESA administrations on endothelial dysfunction and clinical outcomes in this patient population.

2. Methods

2.1. Study design and participants

This was a single-center, prospective study involving patients attending the Gangneung Asan Hospital (South Korea). Patients with non-dialysis CKD, stages 4 to 5, having estimated glomerular filtration rate (eGFR) of <30 mL/min/1.73 m^2 and with hemoglobin (Hb) levels of <10 g/dL were included; eGFR was calculated using the Chronic Kidney Disease Epidemiology Collaboration equation.

Patients were excluded from the study if they had symptoms and signs of heart failure (dyspnea more than New York Heart Association (NYHA) grade II, orthopnea, paroxysmal nocturnal dyspnea, pitting edema, pulmonary edema or pulmonary effusion), brain natriuretic peptide levels of >35 pg/mL, significant valvular heart disease (e.g., more than moderate grade severity), history of coronary artery syndrome within 60 days, significant arrhythmia (ventricular fibrillation, ventricular tachycardia, or atrioventricular block), uncontrolled hypertension, history of taking ESAs within 12 weeks before recruitment, abnormal liver function (total bilirubin >3 mg/dL or albumin <2.8 mg/dL), active bleeding requiring transfusion, pregnancy, or other causes of dyspnea.

Participating patients were scheduled to visit the hospital monthly for 12 weeks. Blood pressure measurements and laboratory tests, including Hb and creatinine levels and eGFR, were performed during each visit. Echocardiography, flow-mediated dilation (FMD), and 6-minute walk tests were performed at the start of the study and after 12 weeks of treatment. The NYHA class, determined before and after ESA administration, was also recorded.

This study protocol was approved by the Gangneung Asan Hospital ethical committee of Ulsan University (GNAH IRB number 2017-05-002-007). The study protocol was conducted in accordance with the Declaration of Helsinki. Informed consent was obtained from all participating patients.

2.2. Drug administration and dosing

The ESA used in this study was methoxy polyethylene glycol-epoetin beta (Mircera, F. Hoffmann-La Roche, Basel, Switzerland). The ESA was administered by subcutaneous injection according to the following protocol. ESA administration was initiated when the patient's Hb was ≤ 10 g/dL, with a starting dose of 0.6 μg/kg every 4 weeks. If a patient's Hb level exceeded 11 g/dL, that week's treatment was skipped; if the Hb level did not increase by >1 g/dL after 4 weeks of therapy, the dose was increased by 25%.

2.3. Study endpoints

The primary endpoint of this study was the FMD change, in each patient, following ESA administration. The secondary endpoints were the changes in each patient's echocardiographic parameters (left ventricular mass index [LVMI], left atrial [LA] diameter, LA volume index [LAVI], right ventricular systolic pressure, E wave to e' [E/e'] ratio, left ventricular end systolic dimension [LVESD], left ventricular end diastolic dimension [LVEDD] and left ventricular ejection fraction [LVEF]), 6-minute walk test result, systolic blood pressure, diastolic blood pressure, and NYHA class following ESA administration.

2.4. Echocardiographic and Doppler measurements

Each patient was assessed using 2-dimensional transthoracic echocardiography before and after ESA administration. Standard 2-dimensional pulsed-wave Doppler and pulsed-wave Doppler tissue imaging (DTI) echocardiographic parameters were collected from parasternal and apical acoustic windows based on American Society of Echocardiography (ASE) guidelines, using an IE33 (Phillips, Andover, MA) instrument.[28] During the assessment, each patient was positioned in the left lateral supine position under electrocardiographic monitoring. M-mode tracings obtained just below the mitral valve leaflets were acquired using the parasternal short-axis view. We measured LVEDD, LVESD, interventricular septal wall thickness, posterior wall thickness, and LA diameter. LVE diastolic and LVE systolic

volumes were acquired from apical 2- and 4-chamber views, using the biplane modified Simpson rule; the LVEF was calculated according to ASE recommendations.[29] The LAVI was also measured using the biplane Simpson method. Left ventricle (LV) mass was calculated using the Devereux formula and indexed to body surface area calculated using the Mosteller formula. LV filling variables were determined from pulsed-wave Doppler recordings of transmitral flow velocity. The sample volume was allocated at the tips of the mitral valve leaflets and the Doppler velocity recordings of 3 cardiac cycles, at a paper speed of 100 mm/s, were digitized and the variables averaged. LV diastolic function was evaluated using pulsed-wave Doppler and pulsed-wave DTI recordings, based on ASE/European Association of Cardiovascular Imaging recommendations.[30] Transmitral flow was used to gather peak early (E) and atrial (A) flow velocities. We used the mean peak early diastolic (e') velocity obtained from the septal side of the mitral annulus in the 4-chamber view with appropriate DTI settings. Systolic and late diastolic velocity and isovolumic relaxation time were computed utilizing pulsed-wave DTI at the septal insertion sites of the mitral leaflets in the apical 4-chamber view. The E/e' ratio was calculated to determine LV filling pressures. Finally, the tricuspid regurgitation pressure gradient was measured using color-flow Doppler imaging and the parasternal right ventricle inflow view.

2.5. Measurement of brachial artery diameters and flow mediated dilation

Patients were asked to rest for 10 minute in the supine position before each exam. Brachial artery images were acquired using a commercially available system (Vivid 7, GE Vingmed, Horten, Norway) equipped with a 14-MHz linear array transducer. For FMD measurements, the baseline brachial artery diameter was averaged from 6 separate images taken at 5-second intervals. Subsequently, a pneumatic cuff, placed on the forearm, was inflated to 250 mm Hg for 5 minute. Following cuff deflation, the brachial artery diameter was reexamined and averaged from 6 separate images taken at 5-second intervals. The FMD was calculated as a percentage of the maximum increase in arterial diameter. The brachial artery diameter was semiautomatically calculated from the trailing edge of the intima-blood interface to the leading edge, using a modified version of ImageJ software (National Institutes of Health, Bethesda, MD) and custom-designed software. The cutoff value for normal endothelial function assessed by FMD of the brachial artery is 7.1%.[31]

2.6. Statistical analysis

Data were stored and analyzed using SPSS version 19.0 software (IBM, Armonk, NY). Continuous variables were expressed as mean ± standard deviation, and categorical variables were expressed as frequency and percentage. Continuous variables following a normal distribution were analyzed using Student *t* test, and non-normal data were analyzed using a Mann–Whitney *U* test. In all cases, $P < .05$ was considered statistically significant.

3. Results

3.1. Baseline characteristics

Initially, 13 patients were screened, but 2 patients were excluded from the analyses. One was excluded due to a diagnosis of heart failure, based on clinical signs, biomarker level (brain natriuretic peptide = 959.9 pg/mL), and an echocardiographic exam (LVEF = 41%); the other voluntarily withdrew from the study. Of the remaining 11 patients, the average age was 62 ± 14 years. Six males and five females participated. Five patients had an eGFR ranging from 15 to 30 mL/min/1.73 m^2 and 6 had an eGFR <15 mL/min/1.73 m^2. The average Hb levels were 8.7 g/dL, respectively (Table 1); Hb levels were below 12 g/dL upon completion of the 12 weeks of treatment.

Table 1

Baseline characteristics of study population.

	Total patients (n = 11)
Age (yrs), mean (SD)	62 ± 14
Male	6 (55)
Diabetes mellitus	6 (55)
Hyperlipidemia	5 (45)
BMI (kg/m^2)	23.4 ± 3.5
Hb (g/dL)	8.7 ± 0.9
Cr (mg/dL)	4.16 ± 1.27
eGFR (mL/min/1.73 m^2)	14.45 ± 5.71
SBP (mm Hg)	141.5 ± 19.4
DBP (mm Hg), mean (SD)	71.6 ± 13.9
LVEF (%), mean (SD)	66.5 ± 5.1

Data are presented as means ± SD or n (%).
BMI = body mass index, Cr = creatinine, DBP = diastolic blood pressure, eGFR = estimated glomerular filtration rate, Hb = hemoglobin, LVEF = left ventricular ejection fraction, SBP = systolic blood pressure, SD = standard deviation.

3.2. Effects of ESAs on endothelial function and heart functions

The mean FMD showed a significant increase of 10.59% (from 1.36% ± 1.91% to 11.95% ± 8.11%, $P = .001$) after 12 weeks of ESA administration as shown in Figure 1 (Table 2). Additionally, the mean LVMI was reduced by 11.9 g/m^2 (from 105.8 ± 16.3 g/m^2 to 93.9 ± 19.5 g/m^2, $P = .006$), and the mean LAVI was reduced by 10.8 mL/m^2 (from 50.1 ± 11.3 mL/m^2 to 39.3 ± 11.3 mL/m^2, $P = .004$) after 12 weeks of ESA injection (Fig. 2, Table 3). However, the right ventricular systolic pressure, E/e', LVESD, LVEDD, and LVEF did not show significant changes following the intervention (Table 3).

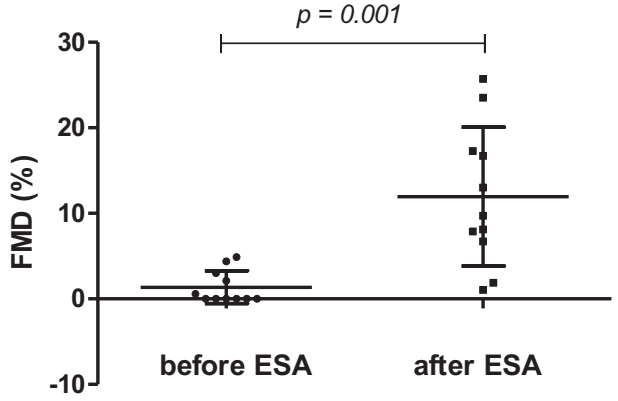

Figure 1. FMD before and after ESA administration. (n = 11). Data are reported as means ± SD. ESA = erythropoiesis-stimulating agents, FMD = flow-mediated dilatation, SD = standard deviation.

Table 2

Laboratory and clinical outcomes.

	Baseline (n = 11)	After 12 wk (n = 11)	*P* value
Hb (g/dL)	8.7 ± 0.9	10.7 ± 0.8	<.001
Cr (mg/dL)	4.16 ± 1.27	4.83 ± 1.71	.003
eGFR (mL/min/1.73 m^2)	14.45 ± 5.71	12.27 ± 5.72	.005
SBP (mm Hg)	141.5 ± 19.4	145.7 ± 19.7	.79
DBP (mm Hg)	71.4 ± 13.9	71.1 ± 15.7	.89
FMD (%)	1.36 ± 1.91	11.95 ± 8.11	.001
6MWT (m)	378.0 ± 117	395.8 ± 133.4	.437
NYHA (class)	1.00 (1-1)	1.00 (1-1)	.317

Data are reported as means ± SD = except NYHA class data which are reported as median (IQR). 6 MWT = 6-min walk test, Cr = creatinine, DBP = diastolic blood pressure, eGFR = estimated glomerular filtration rate, FMD = flow-mediated dilatation, Hb = hemoglobin, IQR = interquartile range, NYHA = New York Heart Association, SBP = systolic blood pressure, SD = standard deviation.

Table 3

Changes in echocardiographic characteristics between baseline and 12 wk after ESA administration.

	Baseline (n = 11)	After 12 wks (n = 11)	*P* value
LVMI (g/m^2)	105.8 ± 16.3	93.9 ± 19.5	.006
LAVI (mL/m^2)	50.1 ± 11.3	39.3 ± 11.3	.004
RVSP (mm Hg)	28.8 ± 5.1	28.5 ± 5.2	.983
E/e' ratio	13 ± 2.7	12 ± 3.0	.135
LVESD (mm)	33 ± 2.6	33 ± 1.3	.999
LVEDD (mm)	52.6 ± 1.9	51.7 ± 1.9	.067
LVEF (%)	66.5 ± 5.1	64.5 ± 3.9	.295

Data are reported as means ± SD.
E/e' = E wave to e' ratio, ESA = erythropoiesis-stimulating agents, LA diameter = left atrial diameter, LAVI = left atrial volume index, LVEDD = left ventricular end-diastolic dimension, LVEF = left ventricular ejection fraction, LVESD = left ventricular end-systolic dimension, LVMI = left ventricular mass index, RVSP = right ventricular systolic pressure, SD = standard deviation.

The 6-minute walk test was not performed in some participants for a variety of reasons, including arthralgia, difficulty walking, and others. However, 6 (54.5%) patients underwent the test prior to and after the study. The average results were 378 m before ESA administration, and 395 m after the intervention. Although the 6-minute walk test difference showed a tendency to increase following ESA administration, the change was not significant.

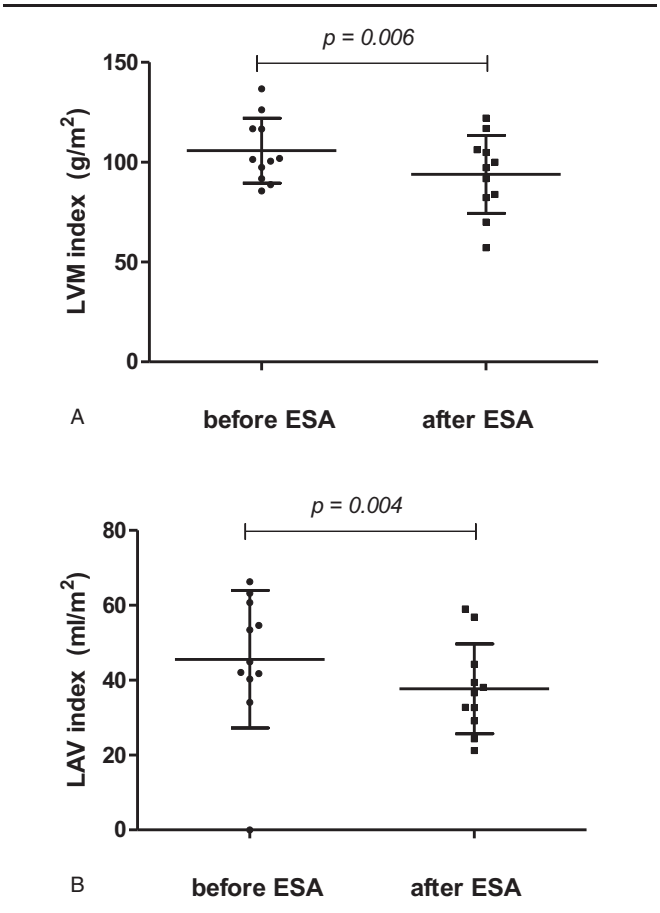

Figure 2. LVMI (A) and LAVI (B) before and after ESA administration. (n = 11). Data are reported as means ± SD. ESA = erythropoiesis-stimulating agents, LAVI = left atrial volume index, LVMI = left ventricular mass index, SD = standard deviation.

The clinical symptoms of the patients, as assessed using the NYHA classification, did not worsen during ESA administration.

3.3. Safety profiles of ESAs

There were no significant changes in systolic or diastolic blood pressures observed during the study period. The average systolic blood pressure before ESA administration was 141.5 ± 19.4 mm Hg, compared with 145.7 ± 19.8 mm Hg after ESA administration. The average diastolic blood pressure before ESA administration was 71.4 ± 13.9 mm Hg and 71.1 ± 15.7 mm Hg after ESA administration. When reviewing the blood pressure medications administered to patients before and after the study, 2 participants added 1 blood pressure medication and the others did not change their medications (Table 2). There were no side effects associated with ESA administration, such as uncontrolled blood pressure, stroke, seizure, or injection site infection.

4. Discussion

This study is the first to suggest that ESA administration improves endothelial dysfunction, LVMI and LAVI in patients with non-dialysis CKD. The FMD was significantly increased after 12 weeks of ESA administration, suggesting that ESAs can be considered as adjunctive therapies for improving cardiovascular risk without introducing serious side effects.

Since endothelial dysfunction is known to be a primary mechanism of cardiovascular outcome deterioration, a major problem in patients with CKD, numerous trials involving vitamin C, growth hormone, and L-arginine have been conducted to improve endothelial dysfunction in these patients.[32–34] Some studies have suggested that oxidative stress markers are consistent with endothelial dysfunction in patients with CKD; as reactive oxygen species production increases, nitric oxide bioavailability decreases and vasodilation becomes ineffective.[35,36] Several studies have explored the effects of ESAs on endothelial function, with inconclusive results. One study demonstrated that ESAs aggravate endothelial function through endothelin-1 secretion and oxidative stress.[26] That study had the advantage of directly measuring endothelium-dependent relaxation using acetylcholine, but was limited by being conducted ex vivo, without measuring clinical parameters. Additionally, a significant number of patients included in the study had histories

of cardiovascular disease and severe arterial stiffness, which likely impacted some study parameters. Conversely, Bartnicki et al[37] showed that ESA administration may decrease oxidative stress in patients with non-dialysis CKD by improving the antioxidant defense system and reducing ROS production. In a 2015 study on rats, ESA administration corrected anemia and prevented endothelial dysfunction in a rat model of CKD; the results were explained by reduced local oxidative stress and enhanced endothelial nitric oxide synthase phosphorylation.[27] Our present study showed that ESA administration resulted in clinically improved endothelial dysfunction in patients with CKD, correlating with the previous animal study results. As previously mentioned, endothelial dysfunction has been considered to be a prognostic indicator of cardiovascular and metabolic diseases, such as CKD. Currently, there are no perfect therapeutics to prevent endothelial dysfunction. However, according to our data, ESA therapy might be a promising therapeutic options for CKD with endothelial dysfunction.

Since Furchgott and Zawadzki, among others, discovered endothelium dependent relaxing factors, rapid progress has been made in evaluating endothelial function.[38] Presently, numerous methods exist to measure endothelial dysfunction, including intravascular coronary FMD, ultrasound brachial artery FMD, flow-mediated magnetic resonance imaging, and pulsed-wave analysis. Among those methods, brachial artery FMD is widely used because it provides fast, inexpensive, and noninvasive measurements that have proven reproducible and useful in many clinical studies.[39–41] Therefore, to evaluate endothelial dysfunction in this study, we used brachial FMD to measure endothelium-dependent vasodilation.

The Kidney Disease: Improving Global Outcomes (KDIGO) guideline recommends initiating ESA therapy in consideration of prior responses to iron therapy, rate of Hb concentration decline, transfusion risk, and symptoms attributable to anemia when patient Hb concentrations are <10 g/dL. Several studies have reported benefits associated with ESA administration, such as reduced mortality, reduced hospitalization risk, shortened hospitalization, fewer transfusion-related complications, and improved quality of life.[42] However, concerns exist about the safety and long-term effects of ESA administration, including increased hypertension, seizures, and stroke risk, in patients with CKD.[43,44] Thus, KDIGO and the US Food and Drug Administration recommend that Hb levels should not exceed 11.5 g/dL or 11 g/dL, respectively.

In previous studies, ESA therapy reduced the LVMI in patients with non-dialysis CKD.[45,46] Specifically, several studies have suggested that the effect of ESA administration on LVH is only evident in patients with low Hb levels (<10 g/dL) and underlying LVH.[47,48] Our findings showed that the LVMI was significantly reduced after ESA administration, consistent with previous studies, although only 36% of the patients in our study population demonstrated LVH. This might be explained by an increase in Hb leading to a decreased LV load and reduced LVH. Additionally, ESA therapy had an impact on the decrease in LA volume. This suggested that ESA administration had a positive effect on atrial remodeling. LA structural remodeling is a complicated phenotypic expression following changes in LA size,[49] shape, and architecture and alterations in the cardiomyocyte, fibroblast, and non-collagenous infiltrative compartments of the atrium.[50] LA enlargement, which is simple to measure, is the default clinical indication of structural remodeling that develops most frequently in response to LA pressure and volume overload. In the absence of mitral valvular disease, atrial fibrillation, and high cardiac output states such as thyrotoxicosis, it is an exemplary biomarker for the presence and severity of LV diastolic dysfunction.[51] Further, there were no significant changes in the functional capacities of the patients who underwent the 6-minute walk test.

Raising Hb levels through ESA administration has been performed cautiously because of the potential side effects, including exacerbation of hypertension and an increased risk of seizures.[52] Thus, we also examined the effect of ESA administration on blood pressure, as blood pressure rises can be managed if the Hb correction target is not excessively high. In the present study, significant changes in systolic and diastolic blood pressures were not observed during the study period; only 2 participants added additional blood pressure medications (1 each) during the study. Although this was a short-term study, uncontrolled hypertension, injection site infections, strokes, and seizures were not observed.

Our study has some limitations. First, this was a single-center, prospective, single-arm comparison study without a parallel control group involving a small number of patients over a mid-term study period. Due to the narrow and strict eligibility criteria that allowed for only patients with non-dialysis CKD (stages 4 to 5) and anemia to be enrolled and various tests to be performed, registering a larger cohort of patients from a single institution was difficult. Second, we could not analyze the effect of medication, sex, or lifestyle patterns due to the lack of a parallel control group. Although this study did not demonstrate correction of the LVH effect caused by anemia, a multi-center study would be expected to better evaluate whether the Hb level is a confounding factor of the LVMI. Third, although the mechanism of action of all ESAs is the same, the half-life, receptor binding affinity, and in vivo bioactivity of each ESA is different. Each ESA may show variable clinical effects due to these differences in pharmacokinetics and pharmacodynamics. In our study, only continuous erythropoietin receptor activator (Mircera) was used as an ESA, and it is necessary to proceed with research using other ESAs. Despite these limitations, this study showed that patients with non-dialysis CKD and anemia showed improved endothelial dysfunction, LVH and LA volume following ESA administration. As the number of these patients is gradually increasing and the potential impact of ESA administration was taken into consideration, we believe that large-scale, multi-center, prospective studies are needed to further investigate the effects of ESA administrations on endothelial dysfunction in patients with CKD. Finally, although it is not a long duration, our study showed the safety and efficacy of ESA for 12 weeks. We believe that it helps to demonstrate the feasibility of a large-scale long-term study in the future.

5. Conclusion

This is the first clinical study to demonstrate that an ESA can improve endothelial dysfunction, left ventricular hypertrophy and left atrial volume in patients with non-dialysis CKD and anemia. The present results suggest that ESA administration may be considered as adjunctive therapy for reducing cardiovascular risk in these patients.

Author contributions

LJA collected, analyzed, and interpreted the data and drafted the manuscript. YCJ collected, analyzed, and interpreted the data.

YH and HSJ conceptualized and conceived the study design; collected, analyzed, and interpreted the data; and edited and revised the manuscript. All authors read and approved the final manuscript.

Data curation: Jina Lim, Chung Jo Yu, Sang Jin Ha, Hoon Yu.
Supervision: Sang Jin Ha, Hoon Yu.
Writing – original draft: Jina Lim.
Writing – review & editing: Sang Jin Ha, Hoon Yu.

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