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Original Research Article

Effectiveness of erythropoietin supplementation against chronic heart failure with anemia, and its effect on serum hypersensitive C reaction protein, homocysteic acid and B-type natriuretic peptide

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Abstract

Purpose: *To study the effectiveness of exogenous erythropoietin (EPO) against chronic heart failure (CHF) with anemia, and its effect on serum hypersensitive C reaction protein (hs-CRP), homocysteic acid (Hcy) and B-type natriuretic peptide (BNP).*
Methods: *A total of 136 patients suffering from CHF with anemia from June 2015 to June 2017 were randomly divided into observation group (n = 68) and control group (n = 68). On the basis of conventional anti-heart failure therapy, the control group received oral ferrous sulfate tablets, while the observation group received oral ferrous sulfate tablets combined with EPO subcutaneous injection. Blood indices, cardiac function and serology were determined and tested in all patients before treatment, and at 4 months after treatment.*
Results: *After treatment, hemoglobin (Hb), hematocrit (HCT), red blood cell (RBC), blood platelet count (PLT) and serum iron were significantly higher than those before treatment in the two groups; the levels in the observation group were significantly higher than those in control group ($p < 0.05$). Following treatment, left ventricular ejection fraction (LVEF), and 6-minute walking distance in the observation group were significantly higher than those in the control group, while end-diastolic dimension (LVEDD), end-systolic dimension (LVESD) and cardiac functional grading in the observation group were significantly lower than those in the control group ($p < 0.05$). After treatment, hs-CRP, Hcy and BNP were significantly lower than pre-treatment values in the two groups, while the values for the observation group were significantly lower than those of control group ($p < 0.05$). Correlation analysis showed that LVEF and Hb were negatively correlated with hs-CRP, Hcy and BNP ($p < 0.05$).*
Conclusion: *Serum hs-CRP, Hcy and BNP are involved in the occurrence and progression of CHF with anemia. Exogenous EPO can effectively improve anemia and cardiac function in these patients.*

Keywords: *Erythropoietin, Chronic heart failure, Anemia, C-reaction protein, B-type natriuretic peptide*

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INTRODUCTION

Chronic heart failure (CHF) is a common clinical cardiovascular disease which often occurs in middle-aged or elderly people, with serious effects on quality of life. It manifests in changes in cardiac structure and function due to primary cardiomyopathy, cardiac volume overload or long

-term ventricular compression, resulting in defects in cardiac filling [1,2]. Varied degrees of anemia often appear in most end-stage CHF patients, which lead to further exacerbation of clinical symptoms and significant increases in fatality [3]. Therefore, it is important to eliminate anemia while treating the disease. At present, the clinical therapies for anemia include blood transfusion, iron supplementation, and use of erythropoietin-stimulating agents (ESA) [4]. Erythropoietin effectively promotes the differentiation and proliferation of erythroid progenitor cells and slows down their rate of aging while increasing the hemoglobin levels of patients [5,6].

In 1989, the first recombinant human erythropoietin agent (rhEPO) was used in clinical practice, and subsequently new EPO drugs have been constantly upgraded and increasingly widely used in the treatment of anemia because they improve the treatment outcomes of anemic patients [7,8]. In the present study, CHF patients with anemia were treated by exogenously-supplemented erythropoietin, and changes in serum levels of hs-CRP, Hcy and BNP were determined before and after treatment. This was aimed at studying the clinical effectiveness and mechanisms of action of erythropoietin in the treatment of CHF patients with anemia so as to provide a new theoretical basis for treatment of this disease.

METHODS

Collection of general data for patients

A total of 136 patients with CHF combined with anemia in the Cardiology Department of Feng Hua People's Hospital of Zhe Jiang Province, from June 2015 to June 2017 were selected for the study. The inclusion criteria were as follows: (1) Patients aged between 40 and 80, (2) patients diagnosed with heart failure in conformity with the diagnostic criteria of *Guidelines for Diagnosis and Treatment of Heart Failure in China* [9]; (3) patients in Grade III to Grade IV under the cardiac functional grading criteria of New York Heart Association (NYHA); and (4) patients diagnosed with anemia according to the diagnostic criteria in *Internal Medicine* (Eight-year textbook; Second edition) [10] (adult male: Hb<120ng/L, RBC<4.5×10^{12}/L; adult female: Hb<110g/L, RBC<4.0×10^{12}/L.

The exclusion criteria were as follows: (1) patients with unstable angina pectoris and acute myocardial infarction in the previous three months, (2) patients with severe hemorrhagic tendency; (3) patients with severe diseases in other critical organs such as brain, lung and kidney; (4) patients with anemia due to other determinate etiologies. This study was approved by the Ethics Committee of Feng Hua People's Hospital of Zhe Jiang Province (approval no. FH2015022), and followed the guidelines of Declaration of Helsinki [11]; all patients signed the informed consent form. Patients with CHF and anemia were randomly divided into observation group and control group (68 cases/group).

Treatment methods

Patients in the two groups received conventional anti-heart failure therapies including treatment of primary diseases, adoption of low-fat and low-salt diets, use of the medications such as β-blocker agents, angiotensin converting enzyme inhibitors, diuretic agents and vasodilating agents. Patients with aggravated symptoms received digitaloid drugs and nitrate-based medicines. Patients in the control group received oral ferrous sulfate tablets (one tablet 3 times a day) (Shanghai Huanghai Pharmaceutical Co., Ltd.; National Medical No. H31021752, 0.3g/tablet). The patients in the observation group received oral iron supplement agents (one tablet 3 times a day), in addition to subcutaneous injection of EPO agents (300 IU/kg 3 times a week) (Chengdu Di'ao Jiuhong Medicinal Manufacture; National Medical No.: S20050704). Treatment in both groups lasted for four months.

Assessment of treatment outcomes

Before treatment, and 4 months after treatment, indices such as blood, cardiac function and serology were determined in all patients, and the correlations were analyzed between left ventricular ejection fraction, hemoglobin and serological indices i.e. hs-CRP, Hcy and BNP.

Assay of blood indices: Venous blood (3 mL) was collected and subjected to assay of Hb, HCT, RBC, PLT, and serum iron were determined by using a hematology analyzer.

Cardiac function test: The test was performed by using a SC-2000 color Doppler cardiac ultrasonic detector with probe frequency of 2.5 MHz and scanning rate of 50 mm/s (SIEMENS, Germany). Data for LVEF, LVEDD and LVESD were recorded by left ventricular parasternal long-axis views and apical four-chamber views, and mean values were obtained after measurement of three cardiac cycles. The maximum walking distances of patients were recorded in 6-minute walking test.

Determination of serological indices

Early morning fasted venous blood (3mL) was collected from patients in both groups and then centrifuged at 3000 rpm for 10 min. The levels of hs-CRP and BNP were determined by electro-chemiluminescence immunologic techniques using Elecsyo2010 electrochemical automatic immune-analyzer (German Roche Company). Levels of Hcy were determined by fluorescence polarization immunoassay with automatic immune-analyzer (Abbott Laboratories (America).

Statistical analysis

Statistical analysis was performed using SPSS 21.0 statistical software. Measurement data were expressed as mean ± standard deviation (mean ± SD), and differences between the two groups were compared with paired *t*-test. Numerical data were expressed as percentage (%), and compared using χ^2 test. The correlations between LVEF, Hb and serological indices were determined with Pearson's correlation coefficient. Differences were considered statistically significant at p <0.05.

RESULTS

Demographic and general data

A total of 136 cases showed no significant difference in general data such as gender, age, body mass index (BMI) and so on between the two groups (p > 0.05). The results are shown in Table 1.

Hematological indices

Before treatment, there were no significant differences in levels of Hb, HCT, RBC, PLT and serum iron between the two groups (p >0.05). The levels of Hb, HCT, RBC, PLT and serum iron after treatment were significantly higher than their levels before treatment in the two groups, but after treatment, their levels in the observation group were significantly higher than those in the control group (p <0.05). These results are shown in Table 2.

Cardiac function

Before treatment, there were no significant differences in LVEF, LVEDD, LVESD, cardiac functional grading, and 6-min walking distance between the two groups (p >0.05).

Table 1: Demographic and general data for the patients {n (%), (mean ± SD)}

Group	Cases	Gender		Age (years)	BMI (kg/m^2)	Heart function grading		Basic disease		
		Male	*Female*			*Grade III*	*Grade IV*	*Diabetes*	*Hypertension*	*Hyperlipidemia*
Observation	68	32(47.06)	36(52.94)	58.41±7.52	21.36±2.19	45(66.18)	23(33.82)	17(25.00)	21(30.88)	24(35.29)
Control	68	30(44.12)	38(55.88)	57.63±7.31	20.80±2.37	49(72.06)	19(27.94)	14(20.59)	19(27.94)	26(38.24)
χ^2/t		0.119		0.613	1.431	0.551		0.396		
p		0.731		0.541	0.155	0.458		0.820		

Table 2: Hematological indices for the patients (mean ± SD)

Group	Time	Hb(g/L)	HCT (%)	RBC (×10^{12} /L)	Serum iron (μmol/L)	PLT (×10^9/L)
Observation (n = 68)	Before treatment	83.25±9.51	28.35±5.67	1.89±0.32	7.64±1.40	107.54±9.23
	After treatment	107.13±13.49	36.28±4.80	3.32±0.45	16.58±2.72	154.33±12.46
t		11.931	8.802	21.356	24.099	24.883
p		0.000	0.000	0.000	0.000	0.000
Control (n = 68)	Before treatment	82.76±9.64	27.62±5.54	1.92±0.35	7.80±1.29	107.61±9.18
	After treatment	93.49±12.21[a]	33.40±4.52[a]	2.46±0.41[a]	14.38±2.39[a]	121.47±10.27
t		5.688	6.666	8.260	19.979	8.297
p		0.000	0.000	0.000	0.000	0.000

[a]P< 0.001, post-treatment comparison between the two groups: t_{Hb}=6.168, t_{HCT}=3.602, t_{RBC}=11.649, t_{PLT}=16.782, $t_{serum\ iron}$=5.010

However, in the observation group after treatment, LVEF and 6-minute walking distance were significantly higher than those before treatment; LVEDD, LVESD and cardiac function grading level were significantly lower than corresponding levels before treatment, and their degrees of improvement were significantly higher than those in the control group (p <0.05). There were no significant differences in LVEF, LVEDD, LVESD, cardiac function grading, and 6-min walking distance in the control group before and after treatment (p >0.05). These results are depicted in Table 3.

Serological indices

Before treatment, there were no significant differences in levels of hs-CRP, Hcy and BNP between the two groups (p >0.05). The levels of hs-CRP, Hcy and BNP after treatment were significantly lower than those before treatment in the two groups, and their levels in the observation group after treatment were significantly lower than those in the control group (p <0.05, Table 4).

Correlation between LVEF, Hb and serological indices

Table 5 shows the results of correlation analysis, which reveal that LVEF and Hb were negatively correlated with hs-CRP, Hcy and BNP (p <0.05).

Table 5: Correlation between LVEF, Hb and serological indices

Index	LVEF (%)		Hb(g/L)	
	r	*p*	*r*	*p*
hs-CRP	-0.503	0.000	-0.465	0.000
Hcy	-0.482	0.000	-0.376	0.002
BNP	-0.651	0.000	-0.528	0.000

DISCUSSION

Anemia is one of the major complications of CHF patients. Approximately one-fifth to one-third of CHF patients are anemic, the incidence rate of which may be as high as 50 % in severe cases [12]. Renal ischemia and anoxia caused by CHF-induced renal hypo-perfusion usually contribute to reduced synthesis and hypo-secretion of EPO, causing a decrease in EPO level which then results in anemia [13]. Therefore, in addition to conventional anti-heart failure treatments, it is particularly important to actively treat anemia.

Table 3: Cardiac function indices (mean ± SD)

Group	Time	LVEF (%)	LVEDD (mm)	LVESD (mm)	Heart function grading	6-min walking distance (m)
Observation(n = 68)	Before treatment	37.38±4.84	60.24±5.87	50.18±4.33	3.67±0.96	314.29±51.32
	After treatment	54.74±5.64	49.63±4.61	37.62±5.24	2.86±0.77	410.33±73.25
t		19.262	11.722	15.237	5.427	8.860
p		0.000	0.000	0.000	0.000	0.000
Control (n 6 8)	Before treatment	36.75±4.27	60.65±5.45	50.22±4.51	3.41±0.54	327.52±48.65
	After treatment	37.25±4.81[a]	59.81±5.04[a]	48.82±6.67	3.25±0.49[a]	342.46±63.2[a]
t		0.641	0.933	1.741	1.144	1.540
p		0.522	0.352	0.125	0.254	0.125

[a]P < 0.05; post-treatment comparison between the two groups: t_{LVEF} =19.457, t_{LVEDD} =12.290, t_{LVESD} =10.888, $t_{cardiac\ function\ grading}$ =2.915, $t_{6\text{-}min\ walking\ distance}$ =5.787

Table 4: Serological indices

Group	Time	hs-CRP (mg/L)	Hcy (μmol/L)	BNP (ng /L)
Observationv (n = 68)	Before treatment	7.06±1.13	19.68±3.40	248.94±49.52
	After treatment	4.57±0.76	9.87±2.10	169.41±34.73
t		15.078	20.243	10.843
p		0.000	0.000	0.000
Control (n = 68)	Before treatment	7.13±1.07	19.25±3.79	237.52±54.71
	After treatment	5.22±0.64[a]	14.12±3.36[a]	219.73±44.39[a]
t		12.633	8.352	2.082
p		0.000	0.000	0.039

[a]P < 0.001, post-treatment comparison between the two groups: $t_{hs\text{-}CRP}$ =5.395, t_{Hcy} =8.845, t_{BNP} =7.359

However, the use of only symptomatic supporting therapies such as treatment of primary diseases and blood transfusion cannot effectively alleviate anemia symptoms in CHF patients, and cannot maintain Hb level continuously even with increased volume of blood transfusion [14].

It has been reported that EPO can regulate erythropoiesis and promote differentiation, proliferation and maturation of erythroid progenitor cells [15]. Since 1989, recombinant human erythropoietin agent (rhEPO) has been widely used in the treatment of anemia caused by various diseases. Patients with CHF and anemia have been treated by percutaneous erythropoietin injection therapy at monthly dose of 50 IU, and the levels of Hb and EPO of patients were increased by 22.4 % and 29.3 ± 14.3 IU/ml, respectively after 6-month treatment [16].

The results of this study showed that after treatment, the levels of Hb, HCT, RBC, PLT and serum iron in the observation group were significantly higher than those in the control group, indicating that EPO adjuvant therapy can effectively improve anemia situation of patients with CHF combined with anemia.

The interaction and mutual influence of CHF and anemia set up a vicious circle, which aggravates the conditions of CHF patients. Anemia accelerates the heartbeat of CHF patients and increases their myocardial oxygen consumption, thereby aggravating work load of the heart, which then decreases cardiac function gradually [17]. A meta-analysis of 9 randomized controlled trials in which 750 patients with CHF and anemia received ESP therapies for 3-12 months showed that hospitalization risk, death rate and the adverse events were significantly decreased [18]. In a study in which 73 elderly patients with heart failure and anemia were randomly divided into control group given conventional anti-heart failure therapies, and experimental group given oral iron supplement agents, EPO subcutaneous injection as well as conventional anti-heart failure therapies, the results showed that when compared with the control group, cardiac function indices were markedly improved and the incidence of cardiovascular adverse events were significantly decreased in the experimental group [19].

The results of the present study showed that cardiac function indices i.e. LVEF, LVEDD, LVESD, cardiac function grading, and 6-min walking distance in the observation group after treatment were significantly higher than corresponding values in the control group, indicating that EPO adjuvant therapies can improve clinical outcomes in patients with CHF and anemia. However, results from other studies [20] suggest that high dose of EPO should be avoided because even if EPO is not associated with increases in death rate and cardiovascular adverse events, the risk of thrombosis is increased.

It is known that Hs-CRP is one of the most easily determined hypersensitive inflammatory markers [21]. It can release inflammatory mediators, increase production of oxygen radicals, reduce generation of nitric oxide, and cause apoptosis of myocardial cells [22]. Increased levels of BNP, a diuretic, natriuretic and vasodilating substance secreted by ventricular myocardial cells, are associated with oxidative stress [23]. Studies have shown that incidence of CHF is closely related to the increases in levels of hs-CRP, BNP and IL-6 [24]. Jian LH *et al* [25] thought that some inflammatory cytokines and intermediate metabolites produced in inflammatory reaction processes can damage cardiomyocytes and directly or indirectly inhibit hemotopoiesis and production of renal erythropoietin, resulting in anemia.

The results of this study showed that in the observation group after treatment, the levels of hs-CRP, Hcy and BNP were significantly lower than those in the control group, and the levels of LVEF and Hb were significantly negatively correlated with the levels of hs-CRP, Hcy and BNP, indicating that CHF-induced anemia may be related to inflammatory reactions.

CONCLUSION

The results obtained in this study indicate that exogenous EPO has marked clinical effects on the treatment of CHF with anemia, including effective treatment of anemia and improvement in cardiac function. Thus, hs-CRP, Hcy and BNP may be involved in the mechanisms of CHF-induced anemia.

DECLARATIONS

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Conflict of interest

No conflict of interest is associated with this work

Contribution of authors

We declare that this work was done by the author(s) named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors, all authors read and approved the manuscript for publication. Xiao-fang Liu conceived and designed the study, Jing-zhi Liang, Ke-da Zheng and Xue-fen Wang collected and analyzed the data, Xiao-fang Liu wrote the manuscript.

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