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Original Article

Circulating levels of erythropoietin and its relation to arterial stiffness in patients with hypertension

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Abstract: Background: Emerging experimental and clinical data indicates that erythropoietin (EPO) have significant roles in the cardiovascular system. But the relationship between endogenous EPO levels and arterial stiffness remains unknown. We investigated the EPO levels in relation to arterial stiffness parameters in patients with never treated newly diagnosed hypertension (HT). Methods: We studied 42 (47.8 ± 10 years) never treated HT patients and age and gender-matched 40 (47 ± 8.6 years) normotensive individuals. Serum EPO levels were determined in all subjects using the chemiluminescence immunoassay kit. We evaluated heart rate-corrected augmentation index (AIx@75), a marker of wave reflections and aortic pulse wave velocity (PWV) as indices of elastic-type aortic stiffness using applanation tonometry (Sphygmocor). Results: The levels of EPO were not significantly different in hypertensive patients and the controls (10.6 ± 5 vs. 11.6 ± 9, mIU/mL, p = 0.5). Aortic PWV (10.3 ± 2.3 vs. 8.7 ± 1.6 m/s, p = 0.0001) and AIx@75 (22.7 ± 10 vs. 15 ± 11, %, p = 0.002) were significantly higher in hypertensive patients than the controls. EPO levels were not correlated with brachial and central pressures. Also EPO level was not significantly correlated with AIx@75 (r = -0.15, p = 0.17) and aortic PWV (r = -0.16, p = 0.13). Conclusion: Results from this study indicate that endogenous EPO levels may not be a factor in development of increased arterial stiffness.

Keywords: Erythropoietin, arterial stiffness

Introduction

Arterial stiffness is a potential risk factor for increased cardiovascular events in patients with hypertension (HT) and healthy individuals [1, 2]. Arterial stiffness and wave reflections are increasingly used in the clinical assessment of patients with HT [1]. Pulse wave velocity (PWV) and the aortic augmentation index (AIx) are the main methods for assessing arterial stiffness [3]. That is, arterial stiffness itself is a complex phenomenon consisting of several distinct processes which include structural elements within the arterial wall, vascular smooth muscle tone, chronic low-grade inflammation, and impaired endothelial function [4]. Therefore, there is an increasing interest in factors mediating development of increased arterial stiffness.

Erythropoietin (EPO) is a hypoxia-inducible hormone that is essential for normal erythropoiesis [5]. The discovery of widespread expression of EPO receptors in the cardiovascular system and nonhematopoietic tissues indicates that the function of EPO may involve extra-hematopoietic systems [6-8].

Administration of recombinant human EPO is an efficient therapeutic approach to anemia associated with chronic renal failure [9]. But, a rise in blood pressure or a need for augmentation of antihypertensive medications is noticed in approximately one third of Epo-treated patients [10-12]. Yet, it has been implicated that EPO causes vasoconstriction, mainly in the small resistance vessels [13].

The role of endogenous EPO on the arterial stiffness in hypertensive patients remains unknown. Hence, we investigated the EPO level and its relation to aortic PWV and AIx in newly diagnosed untreated hypertensive patients.

Materials and methods

Study population

In this study, 42 consecutive newly diagnosed hypertensive individuals (47.8 ± 10 years) were

Table 1. Clinical and biochemical characteristics of the study groups

	Control (n = 40)	Hypertension (n = 42)	p
Age (years)	47 ± 8.6	47.8 ± 10	0.7
Male (%)	72	57	0.1
Body mass index (kg/m^2)	29.1 ± 4.1	30 ± 5	0.35
Smoking (%)	25	28	0.45
Systolic BP (mmHg)	119 ± 10	160 ± 15	0.0001
Diastolic BP (mmHg)	77 ± 8	94 ± 10	0.0001
Pulse Pressure (mmHg)	42 ± 6	64 ± 14	0.0001
Heart rate (bpm)	68 ± 9	72 ± 12	0.13
Total cholesterol (mg/dL)	197 ± 37	212 ± 37	0.08
Triglyceride (mg/dL)	176 ± 92	191 ± 154	0.6
LDL cholesterol (mg/dL)	128 ± 31	150 ± 81	0.1
HDL cholesterol (mg/dL)	47 ± 15	53 ± 17	0.1
Glucose (mg/dL)	95.3 ± 28	92 ± 15	0.6
Haemoglobin (g/dL)	14.2 ± 1.2	14 ± 1.7	0.7
Creatinine (mg/dL)	0.87 ± 0.1	0.83 ± 0.1	0.3
Erythropoietin (mIU/mL)	11.6 ± 9	10.6 ± 5	0.5

BP: Blood pressure, LDL: Low-density lipoprotein, HDL: High-density lipoprotein.

enrolled and study parameters were obtained before initiation of antihypertensive therapy. A total of 40 age and gender-matched normal subjects (47 ± 8.6 years) were also studied. The diagnosis of HT was established according to the JNC seventh report [14]. Those patients with associated hemolytic, hepatic and renal diseases, diabetes mellitus, heart failure, valvular heart disease, and ejection fraction less than 50%, history of coronary artery disease or acute coronary syndromes, pregnancy, hypertrophic cardiomyopathy were excluded. Written informed consent was obtained from each subject, and the Institutional Ethics Committee approved the study protocol.

Measurement of erythropoietin levels

Blood samples for measuring EPO levels was drawn from an antecubital vein. Serum was immediately obtained by centrifugation of the blood at 3000 g for 10 min at +4°C and then stored at -20°C until assayed. Serum EPO levels was measured in all samples by automated two-site sandwich immunoassay with chemiluminescent detection kits (IMMUNLITE 2000, Diagnostic Products Corporation, Los Angeles, CA).

Blood pressure measurement

Brachial artery blood pressure was measured with a mercury sphygmomanometer in an office setting; the first and fifth phases of Korotkoff sounds were used for systolic and diastolic blood pressure. Appropriate cuff sizes were chosen for each subject's arm circumference. In each subject, brachial artery blood pressure was measured in at least three separate days after 15 min of comfortably sitting and the average of the measurements was recorded. According to guidelines from the JNC 7 report, HT was defined as a systolic BP of ≥ 140 mmHg or diastolic BP of ≥ 90 mmHg [14].

Measurement of pulse wave velocity

Aortic PWV was determined with the foot-to-foot method using the SphygmoCor system (AtCor Medical, Sydney, Australia) [3]. Consecutive registrations of the carotid and femoral artery pulse waves, which are electrocardiogram gated and thus, the time shift between the appearance of wave at the first and the second sites were calculated. The distance between the two sites was measured on the body surface; to determine aortic PWV in meters/second (m/s). We used the total distance between the carotid and femoral sites of measurement. The average of measurements over a period of 8 s (9-10 cardiac cycles) was calculated after the exclusion of extreme values.

Pressure waveform analysis

Assessment of arterial wall properties and wave reflection characteristics was performed noninvasively using the SphygmoCor system (Sydney, Australia). Radial artery pressure waveforms were recorded at the wrist, using applanation tonometry with a high-fidelity micromanometer (Millar Instruments, Houston, Texas). After 20 sequential waveforms had been acquired and averaged, a validated generalized mathematical transfer function was used to synthesize the corresponding central aortic pressure waveform [15]. AIx and augmentation pressure (AP) were derived from this with the technique of pressure waveform analy-

Table 2. Pulse wave analysis and velocity in the study groups

	Normotensive (n = 40)	Hypertension (n = 42)	p
Central Aortic Pressure			
Systolic (mmHg)	108 ± 11	140 ± 15	0.0001
Diastolic (mmHg)	78 ± 8	93 ± 11	0.0001
Pulse pressure (mmHg)	30 ± 6	48 ± 13	0.0001
AP (mmHg)	6 ± 4.3	12.2 ± 7.8	0.0001
Augmentation index (%)	18 ± 11	24 ± 11	0.02
AIx@75 (%)	15 ± 11	22.7 ± 10	0.002
Aortic-PWV (m/s)	8.7 ± 1.6	10.3 ± 2.3	0.0001

AP: Augmentation pressure, AIx@75: Heart rate-corrected augmentation index, PWV: Pulse wave velocity.

sis [3]. The merging point of the incident and the reflected wave (the inflection point) was identified on the generated aortic pressure waveform. AP was calculated by the maximum systolic pressure minus pressure at the inflection point. The AIx was defined as the AP divided by pulse pressure and expressed as a percentage. Larger values of AIx indicate increased wave reflection from the periphery or earlier return of the reflected wave as a result of increased pulse wave velocity (attributable to increased arterial stiffness). AIx is dependent upon the elastic properties of the entire arterial tree (elastic and muscular arteries). In addition, because AIx is influenced by heart rate, an index normalized for heart rate of 75 bpm (AIx@75) was used in accordance with Wilkinson et al [16].

Only high-quality recordings, defined as an in-device quality index of > 80% (derived from an algorithm including average pulse height, pulse height variation, diastolic variation, and the maximum rate of rise of the peripheral waveform) and acceptable curves on visual inspection, were included in the analysis. All measurements were performed by the same person with the patient in the supine position in a quiet temperature-controlled room after a brief rest period of at least 5 min.

Statistical analyses

Continuous data are expressed as the mean ± SD. Comparison between two groups was performed using the unpaired *t* test or nonparametric means test (Mann-Whitney *U* test) for continuous variables, and using the Fisher exact test for categoric variables. Correlations between serum EPO levels and other variables were evaluated by the Pearson correlation test. A p value of < 0.05 was considered statistically signifiant. Statistical analyses were performed using SPSS software (Version 10.0, SPSS, Inc., Chicago, IL).

Results

Patients characteristics

Baseline clinical and demographic characteristics of the study population are shown in **Table 1**. There were no significant differences in age, gender, cigarette smoking status, heart rate, body mass index, fasting glucose, hemoglobin, serum creatinine and lipid profiles between the groups (**Table 1**). Serum EPO level was similar between in patients HT and controls (10.6 ± 5 vs. 11.6 ± 9, mIU/mL, $p = 0.5$) (**Table 1**). As expected, brachial blood pressures were higher in patients with HT than in normotensive participants (**Table 1**).

Pulse wave analysis and velocity

The indices of arterial stiffness and wave reflections of the study population are presented in **Table 2**. Central aortic systolic, diastolic and pulse pressures were significantly higher in patients with HT than control subjects (**Table 2**). Augmentation pressure, AIx, AIx@75 and aortic PWV were significantly higher in patients with HT than control subjects (**Table 2**).

Relationship between serum erythropoietin level and arterial stiffness

In bivariate correlation analysis, no significant correlation was observed between erythropoietin and brachial pressures, central aortic pressures, AIx@75 and aortic PWV (**Table 3**). In bivariate correlation analysis, AIx@75 was significantly associated with ($r = 0.37$, $p = 0.0001$) hemoglobin level ($r = -0.29$, $p = 0.006$), brachial systolic blood pressure ($r = 0.42$, $p = 0.0001$), brachial diastolic blood pressure ($r = 0.36$, $p = 0.001$). Aortic-PWV was significantly correlated with age ($r = 0.36$, $p = 0.001$), glucose ($r = 0.25$, $p = 0.01$), brachial systolic blood pressure ($r = 0.49$, $p = 0.0001$), brachial diastolic blood pressure ($r = 0.28$, $p = 0.008$).

Table 3. Bivariate correlation analysis erythropoietin level and arterial stiffness

	Correlation Analysis	
Variables	r	p
Brachial Blood Pressure		
Systolic (mmHg)	-0.16	0.15
Diastolic (mmHg)	-0.1	0.1
Pulse pressure (mmHg)	-0.1	0.1
Central Aortic Pressure	-0.14	0.2
Systolic (mmHg)	-0.04	0.6
Diastolic (mmHg)	-0.1	0.1
Pulse pressure (mmHg)	-0.1	0.1
AP (mmHg)	-0.13	0.22
AIx (%)	-0.11	0.3
AIx@75 (%)	-0.15	0.17
Aortic-PWV (m/s)	-0.16	0.13

AP: Augmentation pressure, AIx: Augmentation index, AIx@75: Heart rate-corrected augmentation index, PWV: Pulse wave velocity.

Discussion

In the present study, we investigated the erythropoietin levels in relation to arterial stiffness in patients with newly diagnosed hypertensive patients. We have found that serum EPO levels of hypertensive patients and normotensive subjects were comparable. Moreover, no significant relationship was detected between the EPO levels and the values of AIx@75 and aortic PWV.

Erythropoietin is a hematopoietic hormone produced primarily in the kidneys in response to hypoxia [5]. Experimental studies have shown protective effect of exogenous EPO treatment against hypoxic insult in different tissues [17-19]. It has also been implicated that EPO may be beneficial in cardiovascular disease [8]. Previous studies showed that, normalization of hemoglobin levels with EPO in congestive heart failure patients was associated with improved LV ejection fraction and enhanced exercise capacity [20, 21]. It has been suggest that high endogenous EPO levels may be responsible from the smaller infarct size in patients with acute myocardial infarction [22]. On the other hand the restoration of anemia by EPO is often associated with a rise in blood pressure [10-12].

The measurement of PWV is generally accepted as the most simple, non-invasive and highly reproducible method for determination of arterial stiffness. A number of conditions, including advanced age, smoking, hypertension, metabolic syndrome, hypercholesterolemia, and type II diabetes are associated with increased PWV [3]. In accordance with this, we also determined significant correlation between AIx@75 and age, hemoglobin level, brachial systolic blood pressure and brachial diastolic blood. Additionally the aortic-PWV was significantly correlated with age, blood glucose, brachial systolic blood pressure and brachial diastolic blood pressure. Previous studies have shown that arterial stiffness increases in hypertensive individuals [3]. In accordance, compared with normotensive individuals, aortic PWV and AIx@75 were higher in our hypertensive subjects, indicating deterioration in arterial stiffness and wave reflections.

The rationale of investigating EPO levels in association with increased arterial stiffness, relies on the overlap of the reported vascular effects of the EPO and physiopathology of arterial stiffness. EPO may have a direct vasopressor effect through muscle contraction at the level of the small resistance vasculature [13]. In an in vitro study it has been shown that application of EPO have been shown to induce contractions of rat aortic smooth muscle cells [23]. It is well-known that increased vascular tonus leads to impaired arterial compliance [4]. Briet et al demonstrated that recombinant human EPO therapy impairs endothelial function in patients with chronic kidney disease [24]. Also they found that EPO-induced endothelial dysfunction was significantly associated with carotid stiffness [24]. It is also well known that endothelial dysfunctions can lead to alteration of aortic-PWV and AIx [25]. On the other hand Bartels et al reported that EPO treatment did not affect the aortic PWV [26]. In our study we failed to find any significant correlation between the EPO levels and aortic PWV and AIx@75. And, serum EPO levels of patients with HT and normotensive subjects were comparable. Additionally serum EPO levels were not significantly associated with brachial and central aortic pressure in our study.

The hypertension secondary to the EPO treatment in chronic kidney disease is mainly suggested to be due to its high-dose [27]. Additionally, in a secondary analysis of cardiovascular events in trials of anemia correction of

chronic kidney disease by EPO it was determined that the increased lethal CV events were due to its high dose [28]. Our finding of normal levels of endogenous EPO and lack of significant correlation of EPO levels with AIx@75 and aortic PWV in newly diagnosed untreated hypertensive patients is in accordance with the above-mentioned high dose related exogenous EPO effects. So, the levels of endogenous EPO in our patients were not related in the present hypertension and arterial stiffness.

Limitations of study

Small number of patients is a potential limitation of this study. Considering our finding of normal levels of EPO in hypertensive patients may be due to the fact that our population was consisted of newly diagnosed untreated hypertensive patients. For a more conclusive statement on the possible role of endogenous EPO levels and arterial stiffness there is a need for further comprehensive studies.

In conclusion, serum EPO levels of hypertensive patients and normotensive controls were comparable. Also the EPO levels were not a significant determinant of AIx@75 and aortic PWV, suggesting that endogenous EPO is not associated with arterial stiffness and wave reflections.

Disclosure of conflict of interest

None.

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References

[1] Laurent S, Boutouyrie P, Asmar R, Gautier I, Laloux B, Guize L, Ducimetiere P, Benetos A. Aortic stiffness is an independent predictor of all-cause and cardiovascular mortality in hypertensive patients. Hypertension 2001; 37: 1236-1241.

[2] Nürnberger J, Keflioglu-Scheiber A, Opazo Saez AM, Wenzel RR, Philipp T, Schäfers RF. Augmentation index is associated with cardiovascular risk. J Hypertens 2002; 20: 2407-2414.

[3] Laurent S, Cockcroft J, Van Bortel L, Boutouyrie P, Giannattasio C, Hayoz D, Pannier B, Vlachopoulos C, Wilkinson I, Struijker-Boudier H. European Network for Non-invasive Investigation of Large Arteries: Expert consensus document on arterial stiffness: methodological issues and clinical applications. Eur Heart J 2006; 27: 2588-2605.

[4] Laurent S, Boutouyrie P. Recent Advances in Arterial Stiffness and Wave Reflection in Human Hypertension. Hypertension 2007; 49: 1202-1206.

[5] Henry DH, Bowers P, Romano MT, Provenzano R. Epoetin alfa. Clinical evolution of a pleiotropic cytokine. Arch Intern Med 2004; 164: 262-76.

[6] Anagnostou A, Liu Z, Steiner M, Chin K, Lee ES, Kessimian N, Noguchi CT. Erythropoietin receptor mRNA expression in human endothelial cells. Proc Natl Acad Sci U S A 1994; 91: 3974-3978.

[7] Ammarguellat F, Gogusev J, Drueke TB. Direct effect of erythropoietin on rat vascular smooth-muscle cell via a putative erythropoietin receptor. Nephrol Dial Transplant 1996; 11: 687-692.

[8] Smith KJ, Bleyer AJ, Little WC, Sane DC. The cardiovascular effects of erythropoietin. Cardiovasc Res 2003; 59: 538-548.

[9] Collins AJ. Anaemia management prior to dialysis: cardiovascular and cost-benefit observations. Nephrol Dial Transplant 2003; 18 Suppl 2: ii2-ii6.

[10] Faulds D, Sorkin EM. Epoetin (recombinant human erythropoietin). A review of its pharmacodynamic and pharmacokinetic properties and therapeutic potential in anemia and the stimulation of erythropoiesis. Drugs 1989; 38: 863-899.

[11] Raine AE. Hypertension, blood viscosity, and cardiovascular morbidity in renal failure implications of erythropoietin therapy. Lancet 1988; 1: 97-100.

[12] Shimada N, Saka S, Sekizuka K, Tanaka A, Takahashi Y, Nakamura T, Ebihara I, Koide H. Increased endothelin: nitric oxide ratio is associated with erythropoietin-induced hypertension in hemodialysis patients. Ren Fail 2003; 25: 569-578.

[13] Heidenreich S, Rahn KH, Zidek W. Direct vasopressor effect of recombinant human erythropoietin on renal resistance vessels. Kidney Int 1991; 39: 259-265.

[14] Chobanian AV, Bakris GL, Black HR, Cushman WC, Green LA, Izzo JL Jr, Jones DW, Materson BJ, Oparil S, Wright JT Jr, Roccella EJ; National Heart, Lung and Blood Institute Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure; National High Blood Pressure Education Program Coordinating Committee. The seventh report of the Joint National Committee on Pre-

vention, Detection, Evaluation, and Treatment of High Blood Pressure: the JNC 7 report. JAMA 2003; 289: 2560-2572.
[15] Pauca AL, O'Rourke MF, Kon ND. Prospective evaluation of a method for estimating ascending aortic pressure from the radial artery pressure waveform. Hypertension 2001; 38: 932-937.
[16] Wilkinson IB, MacCallum H, Flint L, Cockcroft JR, Newby DE, Webb DJ. The influence of heart rate on augmentation index and central arterial pressure in humans. J Physiol 2000; 525: 263-270.
[17] Brines ML, Ghezzi P, Keenan S, Agnello D, de Lanerolle NC, Cerami C, Itri LM, Cerami A. Erythropoietin crosses the blood-brain barrier to protect against experimental brain injury. Proc Natl Acad Sci U S A 2000; 97: 10526-31.
[18] Patel NS, Sharples EJ, Cuzzocrea S, Chatterjee PK, Britti D, Yaqoob MM, Thiemermann C. Pretreatment with EPO reduces the injury and dysfunction caused by ischemia/reperfusion in the mouse kidney in vivo. Kidney Int 2004; 66: 983-9.
[19] Grimm C, Wenzel A, Groszer M, Mayser H, Seeliger M, Samardzija M, Bauer C, Gassmann M, Remé CE. HIF-1-induced erythropoietin in the hypoxic retina protects against light-induced retinal degeneration. Nat Med 2002; 8: 718-24.
[20] Silverberg DS, Wexler D, Sheps D, Blum M, Keren G, Baruch R, Schwartz D, Yachnin T, Steinbruch S, Shapira I, Laniado S, Iaina A. The effect of correction of mild anemia in severe, resistant congestive heart failure using subcutaneous erythropoietin and intravenous iron: a randomized controlled study. J Am Coll Cardiol 2001; 37: 1775-80.
[21] Mancini DM, Katz SD, Lang CC, LaManca J, Hudaihed A, Androne AS. Effect of erythropoietin on exercise capacity in patients with moderate to severe chronic heart failure. Circulation 2003; 107: 294-9.
[22] Namiuchi S, Kagaya Y, Ohta J, Shiba N, Sugi M, Oikawa M, Kunii H, Yamao H, Komatsu N, Yui M, Tada H, Sakuma M, Watanabe J, Ichihara T, Shirato K. High Serum Erythropoietin Level Is Associated With Smaller Infarct Size in Patients With Acute Myocardial Infarction Who Undergo Successful Primary Percutaneous Coronary Intervention. J Am Coll Cardiol 2005; 45: 1406-12.
[23] Morakkabati N, Gollnick F, Meyer R, Fandrey J, Jelkmann W. Erythropoietin induces Ca2 mobilization and contraction in rat mesangial and aortic smooth muscle cultures. Exp Hematol 1996; 24: 392-397.
[24] Briet M, Barhoumi T, Mian MO, Sierra C, Boutouyrie P, Davidman M, Bercovitch D, Nessim SJ, Frisch G, Paradis P, Lipman ML, Schiffrin EL. Effects of recombinant human erythropoietin on resistance artery endothelial function in stage 4 chronic kidney disease. J Am Heart Assoc 2013; 2: e000128.
[25] McEniery CM, Wallace S, Mackenzie IS, McDonnell B, Yasmin, Newby DE, Cockcroft JR, Wilkinson IB. Endothelial function is associated with pulse pressure, pulse wave velocity, and augmentation index in healthy humans. Hypertension 2006; 48: 602-608.
[26] Bartels V, Hillebrand U, Kosch M, Hausberg M, Kisters K, Di Marco GS, Reiermann S, Pavenstaedt H, Lang D. Influence of erythropoietin on arterial stiffness and endothelial function in renal transplant recipients. Am J Nephrol 2012; 36: 355-61.
[27] Lipsic E, Schoemaker RG, van der Meer P, Voors AA, van Veldhuisen DJ, van Gilst WH. Protective effects of erythropoietin in cardiac ischemia: from bench to bedside. J Am Coll Cardiol 2006; 48: 2161-7.
[28] Szczech LA, Barnhart HX, Inrig JK, Reddan DN, Sapp S, Califf RM, Patel UD, Singh AK. Secondary analysis of the CHOIR trial epoetinalpha dose and achieved hemoglobin outcomes. Kidney Int 2008; 74: 791-8.