Impact of serum erythropoietin level on collateral vessel development in patients with coronary artery disease

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Abstract

Objective: Experimental data have shown that Erythropoietin (EPO) stimulates angiogenesis and neovascularization which may result in improved collateral development. The aim of this study was to investigate the association between serum EPO levels and the extent of coronary collaterals. Patient characteristics possibly related with coronary collaterals were also sought.

Methods: A total of 256 patients with high grade coronary stenosis or occlusion were evaluated for the extent of coronary collaterals using Rentrop classification. Patients with grade 0 or 1 collaterals were grouped as poor collaterals, while grade 2 or 3 collaterals were grouped as good collaterals.

Results: Mean age of the study population was 63 years, 77% were males. Subjects with good collaterals were significantly more likely to have anemia (p=0.038) and stable angina pectoris as clinical presentation (p=0.40). Serum EPO levels were not different among good and poor collateral groups (10.4±9.4 mU/mL vs. 9.7±11 mU/mL, p=0.397). The prevalence of all other cardiovascular risk factors, medications, and angiographic characteristics were similar between the two groups. After adjusting for age, gender, and clinical presentation with stable angina pectoris, presence of anemia persisted to be a significant correlate of the good collateral formation (OR: 1.95; 95%; CI: 1.07–3.54, p=0.029).

Conclusion: There has been conflicting results from trials studying the effects of serum EPO on coronary collateral development. The present study, with the largest patient population studying this topic, suggests that presence of anemia, but not serum EPO level, is associated with good collateral development. *(Anatol J Cardiol 2017; 17: 386-91)*

Keywords: coronary collateral, erythropoietin, anemia

Introduction

Collateral vessels serve as conduits that bridge severe stenoses or connect a territory supplied by one epicardial coronary artery with that of another. Well developed coronary collateral vessels are known to have protective impacts, such as limiting the size of myocardial infarction, and reducing the incidence of heart failure and ventricular aneurysm, leading to fewer cardiovascular events during follow-up and better survival rates (1–3). However, the existence of well developed collaterals is highly variable even among patients with similar patterns of coronary disease. Although researchers, including we, had demonstrated some potential determinants of collateral development (4–8), it is still not fully understood why some patients are capable of developing sufficient collateral circulation while others are not.

Erythropoietin (EPO) is a hypoxia induced hematopoietic hormone, mainly produced in kidneys; also has extensive non-hematopoietic effects. Experimental animal trials have shown that EPO stimulates the myocardial expression of vascular endothelial growth factor (9), increases the number and function of circulating endothelial progenitor cells (10), and enhances the structural remodeling and growth of preexisting collateral arterioles (11). However, human studies showing EPO receptor protein was undetectable in human heart, had resulted in contradiction (12, 13). Therefore, there is still an unclear relationship between EPO levels and collateral formation in clinical research area. The current study was undertaken to assess whether the serum EPO concentration was associated with the extent of angiographically visible coronary collaterals in patients with high-grade coronary artery stenosis or occlusion.

Methods

Study population

Our study had a cross-sectional design. During the year 2014, all patients undergoing coronary angiography with at least one major coronary occlusion (100% stenosis), or a stenosis of ≥95% with thrombolysis in myocardial infarction (TIMI) grade 0–1 anteg-

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Accepted Date: 02.01.2017 **Available Online Date:** 09.03.2017

DOI:10.14744/AnatolJCardiol.2017.7419

rade flow, were screened for eligibility. Among those, 256 patients were included in our study. Study population constituted of patients having coronary angiography for stable or unstable angina and for positive ischemic test results. Exclusion criteria were age less than 18 years, pregnancy, therapeutic use of erythropoietin, recent acute myocardial infarction (past 30 days), troponin I levels above upper normal limit, known malignancy, chronic renal failure (glomerular filtration rate <60 mL/min), New York Heart Association class IV heart failure, chronic obstructive pulmonary disease, and pulse oxymeter saturation reading below 90%. Patients with history of coronary bypass surgery were excluded if the distal aspect of the severely stenosed or occluded artery was supplied by the patent bypass graft. All patients gave written informed consent and local ethics committee approved the study protocol.

Coronary angiography and collateral scoring

Coronary angiography was routinely performed by the Judkins method without the use of nitroglycerin. Coronary angiographies were performed through the radial route in 68% of the patients. Percentage diameter stenosis was measured using computerized quantitative angiography in a biplane mode (Philips DCI, Eindhoven, Netherlands). Two experienced interventional cardiologists, blinded to patient characteristics, reviewed the angiograms on the day of angiography and graded the coronary collaterals according to Rentrop classification (14). In subjects with >1 collateral supplying the distal aspect of the diseased artery, the higher collateral grade was used. In subjects with >1 severely diseased vessel with collaterals, the vessel with the higher collateral grade was chosen for the analysis.

Intra- and inter-observer agreement levels of coronary collateral grades were determined from a random sample of 50 coronary angiograms (Kappa values were 0.930 for intra-and 0.890 for inter-observer agreement, $p<0.001$ for both). Disagreements were resolved by an additional joint reading. Patients were then divided into two groups according to their collateral grades. Group 1 consisted of patients with grade 0 or grade 1 collaterals (poor collateral group) and group 2 consisted of patients with grade 2 or grade 3 collaterals (good collateral group).

Risk factor assessment

Information about smoking, alcohol consumption and physical activity status were collected by interviewing the patients. Being a current smoker was defined as smoking at least one cigaret per day in the last 6 months or longer, whereas daily alcohol consumption was defined as having at least one drink per day in the last 6 months or longer. Regular physical activity information was gathered by interviewing the patients and it was arbitrarily defined as exercising (at least 30 minutes of brisk walking) three or more times a week. Medical records were reviewed for previous myocardial infarction. All medicines being used for at least two weeks at the time of admission were recorded. Body mass index was calculated by dividing weight (kg) by square of height (m^2). Hypertension was defined as systolic blood pressure ≥140 mm Hg, or diastolic blood pressure ≥90 mm Hg, or use of antihypertensive medication in the previous 2 weeks. Diabetes mellitus was defined as fasting blood glucose ≥126 mg/dL or use of antidiabetic medication in the previous 2 weeks. There was no separate group as prediabetics. Median blood glucose levels were also demonstrated in the table, as the mean glucose levels were at the high range due to one third of the patients being diabetic. Diagnosis of unstable angina pectoris was made according to guidelines established by the European Society of Cardiology 2015 guidelines (15). Left ventricular ejection fraction was calculated by contrast venticulography using a standard area-length formula (16) (n=186) or estimated by echocardiography when ventriculography was not performed (n=70). Severe stenosis was defined as quantitatively measured diameter stenosis of ≥95% in any of the major coronary arteries with ≤1 TIMI grade anterograde flow. Occlusion was defined as 100% stenosis in any of the major coronary arteries.

Laboratory assays

Venous blood samples for EPO measurement were collected immediately following coronary angiography (10±6 min) from each patient, so that the blood loss during the procedure could not have any effect on the EPO levels. Coded plasma samples were stored a –80°C and analyzed at the end of the study. Erythropoietin was measured in duplicate using a commercially available enzyme-linked immunosorbent assay (ELISA) (Biomerica Inc, Newport, CA, USA; sensitivity 1.2 mU/mL). The mean of the two assays was used in the analysis. Blood samples for other parameters were obtained from participants at the time of admission to the hospital before the coronary angiography, after a 12 hour fasting and 10 minute rest. Markers for anemia, such as transferrin saturation, total iron binding capacity, and ferritin levels were not measured, as they were not part of the routine preprocedural assays. Anemia was defined according to World Health Organization (WHO) criteria as a hemoglobin concentration below 12 g/dL in women and below 13 g/dL in men (17).

Statistics

Data were expressed as numbers and percentages for discrete variables and as means±SD. for continuous variables. For data with asymmetrical distribution interquartile range and median values were used. Chi-square test with Yates correction was used to assess the significance of difference between discrete variables. Continuous variables were compared by student t-test or the Mann–Whitney U rank sum test. Multivariable logistic regression analysis was used to adjust for the characteristics that were significant correlates of collateral development in the univariate analysis (with $p<0.1$) plus terms for age and gender due to their well-established association with coronary heart disease. All two-way interactions among significant main effects were examined. Correlations between two variables were represented with Pearson correlation coefficient, r (–1, +1). Statistical analyzes were performed using SPSS software package

Table 1. Demographics of patients according to collateral classification

	Poor collateral n=108	Good collateral n=148	*P*
Age, years	62.5±11	63.1±11	0.691
Sex, male, n (%)	86 (80)	111 (75)	0.385
Body mass index, kg/m^2	28.0±4.6	27.8±4.6	0.726
Diabetes mellitus, n (%)	37 (34)	50 (34)	0.937
Systemic hypertension, n (%)	73 (68)	110 (74)	0.239
Current smoking, n (%)	38 (35)	52 (35)	0.993
Alcohol, n (%)	5 (4.6)	7 (4.7)	0.970
SAP and/or + stress test, n (%)	51 (47)	89 (60)	0.040
Unstable angina pectoris, n (%)	57 (53)	59 (40)	0.091
Previous myocardial infarction, n (%)	52 (48)	73 (49)	0.853
Coronary by-pass history, n (%)	23 (21)	25 (17)	0.373
Statin, n (%)	76 (70)	104 (74)	0.554
Beta-blocker, n (%)	80 (74)	101 (68)	0.311
ACE-I or ARB, n (%)	78 (72)	116 (78)	0.256
Asetylsalicylic acid, n (%)	82 (76)	120 (81)	0.213
Nitrate, n (%)	61 (57)	86 (58)	0.795
Calcium channel blocker, n (%)	0 (0)	3 (2)	0.265
Total cholesterol, mg/dL	177±45	180±47	0.580
Low-density lipoprotein, mg/dL	104±38	107±40	0.509
High-density lipoprotein, mg/dL	40±11	40±11	0.709
Triglyceride, mg/dL	164±98	160±89	0.724
Ejection fraction, %	45±12	47±14	0.409
Glucose, mg/dL*	122±55 97 (86–138)	118±56 98 (85–127)	0.638
Creatinine, mg/dL	1.04±0.2	1.05±0.3	0.774
Hemoglobin, g/dL	13.8±1.6	13.5±1.9	0.144
Hematocrit, %	41±4.6	40±5.2	0.099
Anemia, n (%)	23 (21)	49 (33)	0.038
Erythropoietin, mU/mL*	9.7±11 6.8 (4.1–9.9)	10.4±9.4 7.5 (4.5–12.8)	0.397

*Data is presented as mean ± standard deviation and median (interquartile range).
ACE-I - angiotensin converting enzyme inhibitor; ARB - angiotensin receptor blocker; SAP - stable angina pectoris

(SPSS Inc., Chicago, Illinois, USA), version 11.5 for Windows and p<0.05 was considered statistically significant.

Results

Our study group comprised of 256 patients. Mean age of the study population was 63 years and 77% were males. The number of the patients with prior myocardial infarction was 125 (49%). Of the 256 patients, 148 (58%) had well-developed collaterals (51 patients had grade 3 and 97 patients had grade 2 collaterals), whereas 108 (42%) had impaired collateral development

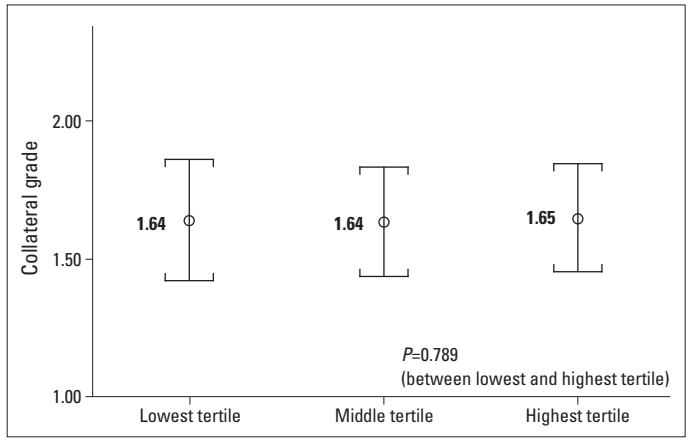

Figure 1. Mean Rentrop collateral grades did not show any difference between the highest and lowest tertiles (1.64 vs. 1.65, *P*=0.789)

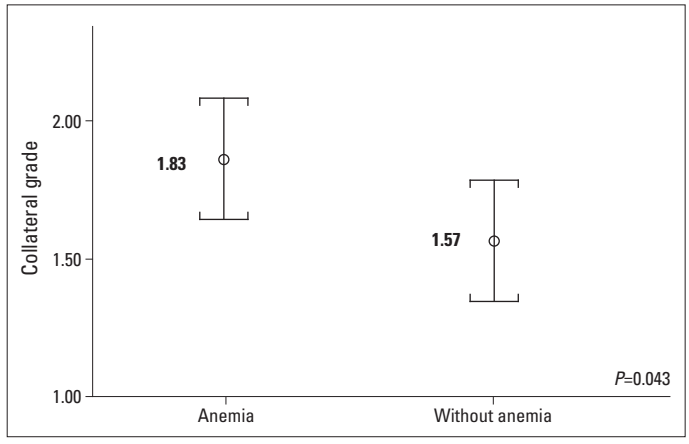

Figure 2. Mean Rentrop collateral grade in patients with anemia was significantly higher than the collateral grade in those without anemia (1.83 vs. 1.57, *P*=0.043)

(73 patients had grade 1 and 35 patients had grade 0 collaterals). During the collateral grading, there were 23 (9%) inter-observer disagreements which were resolved by additional joint readings.

Demographic, angiographic and medication characteristics of the subjects at the time of coronary angiography and comparison according to their collateral classification are shown in Table 1. EPO levels were not different among good and poor collateral groups (10.4±9.4 vs. 9.7±11, p=0.397). When patients were divided into tertiles according to their EPO levels, mean Rentrop collateral grades did not show any difference among highest and lowest tertiles (1.64 vs. 1.65, p=0.789) (Fig. 1). Subjects with well developed collaterals were significantly more likely to have anemia (p=0.038) and stable angina pectoris as clinical presentation (p=0.040). Mean Rentrop collateral grade was 1.83±0.9 in patients with anemia and 1.57±1.0 in those without anemia (p=0.043) (Fig. 2). There was no correlation between Rentrop collateral grade and hemoglobin level (r=–0.07, p=0.256). The prevalence of all other cardiovascular risk factors and medications were similar at different grades of collaterals. Multivariate analysis demonstrated anemia (OR: 1.95; 95%; CI: 1.07–3.54, p=0.029) and clinical presentation with stable angina pectoris

Table 2. Multivariate analysis for possible predictors of good collateral formation

Possible predictors	Multivariate analysis *P* value	Mutivariate OR (95%CI)
Age, years	0.970	0.99 (0.97–1.02)
Sex	0.378	1.30 (0.69–2.58)
Diabetes mellitus	0.523	1.19 (0.69–2.09)
Stable angina pectoris	0.016	1.93 (1.13–3.29)
Anemia	0.029	1.95 (1.07–3.54)
Statin use	0.613	0.86 (0.48–1.54)
ACE/ARB use	0.727	0.31 (0.39–1.34)

ACE-I - angiotensin converting enzyme inhibitor; ARB - angiotensin receptor blocker

(OR: 1.93; 95%; CI: 1.13–3.29, p=0.016) as independent predictors of collateral formation. Table 2 represents the multivariate analysis for possible predictors of good collateral formation.

Discussion

In this cohort of 256 patients who had at least one major coronary artery occlusion, or a stenosis of ≥95% with TIMI grade 0–1 antegrade flow on their angiograms, we failed to observe any association between serum EPO level and angiographically visible coronary collateral formation. However, presence of anemia was an independent predictor of good collateral development. Collateral score was significantly higher among patients with anemia than those with normal hemoglobin level. Also, our study results revealed that stable angina pectoris was another significant predictor of good collateral development. This result could easily be predicted to be so, as tissue hypoxia is the main stimulus for collateral development.

Several studies have revealed that EPO had the potential to limit infarct size, postinfarct remodeling, and reperfusion injury (18–20). Stimulation of neovascularization was one of the proposed mechanisms that could contribute to cardioprotective effect of EPO (21). Collateral vessel formation was thought to be a complex mixture of two linked processes; angiogenesis (proliferation of capillaries within a tissue) and arteriogenesis (remodeling and growth of collateral arteries from preexisting arterioles). It had been demonstrated that EPO stimulates angiogenesis in vivo (22, 23). Endothelial progenitor cell recruitment from the bone marrow and increased myocardial expression of vascular endothelial growth factor (VEGF) were the suggested mechanisms responsible for the increased capillary formation noted in these studies (9). Erythropoetin had also been implicated in the process of arteriogenesis (24).

Our findings, therefore, seem to contradict with the results of these studies claiming a relation between EPO and collateral development. However, in recent years, trials revealing similar contradictory results have been published. Administration of EPO and analogs failed to reduce myocardial infarct size and failed to effect recovery of myocardium, they also led to unwanted side effects, such as increased thrombosis (25–28). Sinclair et al. (13) concluded that erythropoetin receptors were undetectable in cardiac cells. So, there are still ongoing debates and questions about the presence and effect of EPO on human heart. Apart from them, we have several explanations for the lack of association between EPO and angiographically visible collateral formation. First, angiographically visible collaterals represent only a fraction of the total collateral vessels because collaterals are angiographically demonstrable only when they reach 100 μm. Moreover, angiography may not detect most collaterals situated intramurally. It is possible, therefore, that the high serum EPO in selected patients of the present study might have increased neovascularization which could not be visualized by coronary arteriography. Second, enhanced collateral formation does not necessarily reflect a state of enhanced collateral blood flow. Erythropoetin induced increase in blood viscosity might even lead to decreased collateral flow. Indeed, EPO has many nonerythropoietic effects that could be hazardous, particularly at higher doses (29). EPO increases prostoglandin F2 α and thromboxane, lowers prostacyclin release in cultured human endothelial cells, and enhances platelet reactivity that can cause a pro-thrombotic state (30). More importantly, EPO dose-dependently lowers nitric oxide production, increases reactive oxygen species generation and downregulates nitric oxide synthase expression in cultured endothelial cells (31, 32). Thus, EPO can potentially contribute to endothelial dysfunction. The beneficial effects of EPO, such as stimulating VEGF and increasing the number and function of endothelial progenitor cells may be counterbalanced by the potential complications, resulting in a neutral net effect on collateral development.

In contrast to these assumptions, Şahinarslan et al. (33) demonstrated that serum EPO level was related to better coronary collateral development in a cohort of 99 patients with coronary artery stenosis of >70%. Although stenotic lesions between 70% and 90% undoubtedly can produce myocardial ischemia and anginal pain, they are only moderate degree stimulants from a hemodynamic viewpoint. They are virtually never accompanied with angiographically visible collateral circulation, and a more severe narrowing (greater than 90%) or complete obstruction is necessary to stimulate development of visible collaterals. Thus, our study patients who had total or subtotal coronary occlusion were not comparable with them. Recently, Xu et al. (34) published their trial revealing serum EPO levels were positively correlated with coronary collateral development in patients with chronic total occlusion. The study population included 31 patients with chronic total occlusion and 18 patients with normal coronary arteries. We believe the study sample was too small to conclude findings. Recent review of Jelkmann et al. (35) concluded that potential clinical benefits suggested by preclinical studies of EPO, through the mechanism of angiogenesis or progenitor cell mobilization from bone marrow, was not supported by clinical studies.

Previous myocardial infarction, chronic tissue hypoxia, and medications such as angiotensin pathway inhibitors and statins are thought to be the possible inducing factors for collateral

development (36). The results of our study revealed no significant relationship of good collateral development with previous infarction and the medications used. Nearly half of our patients previously had myocardial infarction, the mean ejection fraction was around 45% and nearly three quarters of the patients were on guideline advised medical therapy. However, the time period for the drug use is important at this point. We recorded the drug as being used regularly if the patient had been taking it for at least two weeks, but it takes much more time for a statin or angiotensin pathway inhibitor to stimulate new collateral growth. So, it was a limitation of our study not to detail and compare the length of the use of these specific drugs. Tissue hypoxia was shown to be an inducer for collateral development in our study, as there were increased collaterals in stable angina pectoris patients. Stable angina patients belonged to the group having typical exercise induced ischemic symptoms for at least 2 months and collateral development was induced in reply to chronic tissue hypoxia. In the unstable angina patients, who had ischemic symptoms for less than 2 months, good collateral development was not yet stimulated, as anticipated. Major finding of our study was that anemia was significantly associated with better collateral development. In 1950's a number of studies had suggested that there was an increase in intercoronary collateralization in anemia (37, 38). Most of these studies were based on anatomical observations and had used an arbitrary grading system. Although tissue hypoxia and increased flow due to decreased blood viscosity were speculated to be possible determinants of anemia induced neovascularization, the underlying mechanisms were poorly defined. In a recent trial, Kawamura et al. (39) evaluated the relation between anemia and new vessel formation in a mouse ischemic leg model. They observed increased expression of hypoxia-inducible transcription factor-1alpha and vascular endothelial growth factor and increase in both the expression and activity of Akt and endothelial NO synthase in ischemic legs. Another possible explanation for the better collateral formation in anemia was the interferation of hemoglobin with nitric oxide (40). Given the beneficial effects of nitric oxide in collateral formation, a state of relative nitric oxide deficiency secondary to increased degradation in higher hemoglobin levels may help to explain why well developed collaterals are more prevalent among anemic patients.

In the current study, blood samples for EPO measurement were collected immediately after the coronary angiography. One might therefore, argue that EPO values might not reflect the true steady-state EPO values, as they possibly could be affected by the coronary angiography procedure. However, a recent study suggested that coronary angiography did not have a significant impact on serum EPO levels.

Study limitations

The potential limitations of the present study are the relatively small sample size and the cross-sectional study design. However, to our knowledge, it was the largest sample size among the similar EPO and collateral studies. All demographic and clinical variables were collected systematically using a prespecified data collection form and the prospectively collected angiographic and laboratory data did provide us the opportunity to control for most, if not all, of the potential confounders.

It might be debated that Cohen–Rentrop classification is semiquantitative and might not represent the collaterals as precisely as fractional flow reserve (FFR) which is currently the gold standard for determining the presence and degree of collaterals. Although hemodynamic assessment is superior to angiographic assessment in grading collateral circulation, close correlation between Cohen–Rentrop classification and collateral FFR has been documented before. Based on these data we preferred to use this widely used, practical angiographic method to grade collateral circulation.

Conclusion

Our findings indicate that the presence of anemia, but not high EPO level, was associated with improved collateral formation. The possible collaterogenic effect of anemia may suggest a previously unrecognized mechanism through which correction of anemia may worsen cardiovascular outcomes.

Acknowledgements: The authors thank Mrs. Selda Yıldırım for her expert assistance as a research nurse.

Funding sources: This study was supported by a grant from Turkish Metabolic Syndrome Society, Turkey.

Conflict of interest: None declared.

Peer-review: Externally peer-reviewed.

Authorship contributions: Concept – N.Ö., Ç.Ö., S.G.; Design – N.Ö., Ç.Ö., S.G.; Supervision – Ç.E., C.T.K., A.O.; Fundings – S.G., Ç.Ö., B.C., Ç.E.; Materials – C.T.K., A.O., B.C.; Data collection &/or processing – N.Ö., B.C., A.O., C.T.K.; Analysis &/or interpretation – N.Ö., S.G., Ç.Ö., Ç.E.; Literature search – A.O., B.C.; Writing – N.Ö., Ç.Ö.; Critical review – N.Ö., S.G., Ç.Ö., Ç.E.

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