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Kardiyoprotektif ve anjiyojenik bir biyobelirteç olan ghrelin düzeyi koroner kolateral gelişimini ve koroner aterosklerozun ciddiyetini öngördürebilir mi?

As cardioprotective and angiogenic biomarker, can ghrelin level predict coronary collateral development and severity of coronary atherosclerosis?

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ÖZET

Amaç: Ghrelin ateroskleroz ilerleyişini engelleyerek, damar enflamasyonunu baskılayarak ve yeni damar oluşumunu uyararak kardiyovasküler sistem üzerinde koruyucu bir etki yaratmaktadır. Bu nedenle, bu çalışmanın amacı ciddi koroner arter hastalığı olan hastalarda serum ghrelin düzeyinin koroner kollateral gelişimi ve SYNTAX skoru üzerindeki etkisini incelemektir.

Yöntemler: En az bir büyük koroner arterinde >%90 darlık olan toplam 91 hasta ileriye dönük olarak bu kesitsel-gözlem- sel çalışmaya alındı. Kollateral derecelendirilmesi Rentrop-Cohen sınıflamasına göre yapıldı. Grade 2 veya 3 kollaterali olan hastalar iyi kollateral grubuna, grade 0 veya 1 kollaterali olan hastalar ise kötü kollateral grubuna dahil edildi. Serum ghrelin ve vasküler endotelyal büyüme faktörü A (VEGF-A) düzeyleri radyoimünoanaliz ve ELISA kitleri kullanılarak ölçüldü.

Bulgular: Serum ghrelin ve VEGF-A düzeyleri iyi kollateral grubunda anlamlı derecede daha yüksekti. Üstelik, Spearman korelasyon analizinde ghrelin ile SYNTAX skoru (r=-0.348, p=0.001) arasında anlamlı ters bir korelasyon vardı. Çok-değişkenli lojistik regresyon analizinde ghrelin (odds oranı [OO]: 1.004; %95 güven aralığı [GA]: 1.001-1.006; p=0.023), VEGF-A ve kronik tam tıkanma varlığının iyi koroner kollateral gelişiminin bağımsız öngördürücüleri olduğu gösterildi. ROC (Receiver operating characteristic) eğrisi analizinde ise serum ghrelin kestirim değeri >781 pg/mL alındığında, iyi koroner kollateral gelişimini %73.1 duyarlılık ve %67.7 özgüllük ile öngördürdüğü saptandı.

Sonuç: Ghrelinin antioksidan ve antienflamatuvar özellikleri sayesinde endotel fonksiyonlarını koruyarak ve yeni damar oluşumunu uyararak iyi koroner kollateral gelişimini sağlarken aynı zamanda koroner ateroskleroz gelişimini engellediği sonucu çıkarılabilir.

ABSTRACT

Objective: Ghrelin is expressed in the cardiovascular system and exerts protective effects, including inhibiting progression of atherosclerosis, suppression of vascular inflammation, and stimulating angiogenesis. The aim of this study was to investigate the effect of serum ghrelin on coronary collateral development and SYNTAX score in patients with severe coronary artery disease.

Methods: A total of 91 patients who had >90% stenosis in at least one major coronary artery were prospectively included in this cross-sectional, observational study. Collateral degree was graded according to Rentrop-Cohen classification. Patients with grade 2 or 3 collateral degree were allocated to Good Collateral Group and patients with grade 0 or 1 collateral degree were included in Poor Collateral Group. Ghrelin and vascular endothelial growth factor A (VEGF-A) levels were measured using radioimmunoassay and ELISA kits.

Results: Serum ghrelin and VEGF-A levels were significantly higher in Good Collateral Group. Furthermore, ghrelin level showed significant inverse correlation with SYNTAX score (r=-0.348; p=0.001). In multivariable regression analysis, ghrelin (odds ratio, 1.013; 95% confidence interval, 1.011-1.017; p=0.013), VEGF-A, fasting plasma glucose and presence of chronic total occlusion were independent predictors of good collateral development. In receiver operating characteristic curve analysis, ghrelin value cut-off point of >781 pg/mL predicted good collateral development with sensitivity of 73.1% and specificity of 67.7%.

Conclusion: Findings suggested that ghrelin has antioxidant and antiinflammatory properties that protect endothelial functions and also stimulate angiogenesis, which results in development of good coronary collateral and inhibition of progression of coronary atherosclerosis.

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Abbreviations:

ADMA	Asymmetric dimethyl arginine
CLI	Critical limb ischemia
GHSR	Growth hormone-releasing hormone receptor
CAD	Coronary artery disease
KKG	Koroner kollateral gelişimi
MI	Myocardial infarction
NO	Nitric oxide

Coronary angiogenesis, and development of coronary collaterals (CCD) are anastomotic connections developed between different parts of the same coronary arteries or between different coronary arteries as a chronic adaptive response against atherosclerosis so as to provide blood supply to ischemic myocardial regions.[1] In patients who developed acute occlusion or those with serious coronary artery disease, coronary collaterals limit left ventricular remodeling, and extent of infarction to allow long-term maintenance of myocardial viability, to prevent major cardiovascular events (MACE) and to exert favourable effects on prognosis, and survival.[1-4] Severity of ischemia developed secondary to coronary artery stenosis has been accepted as the most important triggering factor for the development of coronary collaterals..[5] However extent of collateral development demonstrates differences even among patients with the same level of coronary artery stenosis.. Angiogenesis which is defined as *de novo* formation of capillary vessels is one of the important processes in the development of good coronary collaterals.[6] It has been reported that conditions which induce myocardial ischemia, and increase vascular endothelial growth factors VEGF) stimulate coronary angiogenesis with development of coronary collaterals.[S,7]

Ghrelin which was firstly isolated from human, and rat stomach in the year 1999 was described as an endogenous ligand for growth- hormone- releasing receptor (GHSR [8] Recently performed studies have shown that ghrelin, and GHSR stimulate angiogenesis under both in *in vivo* and in *in vitro* conditions.[910] In addition, it is known that ghrelin improves endothelial function, prevents endothelial injury, increases blood flow via its vasodilatory effect, inhiibits proliferation of smooth muscle cell, and apoptosis, and demonstrates protective effects against development of atherosclerosis thanks to its anti-inflammatory effects on cardiovascular system.[1112] However as far as we know , any study cited in the literature has not investigated the effects of serum ghrelin on the development of ncoronary artery collaterals in coronary artery disease (CAD), and the extent, and complexity of coronary atherosclerosis as determined by SYNTAX score.

Therefore the objective of this study is to investigate if a correlation exists between coronary collateral, and SYNTAX score and serum levels of ghrelin which is a cardioprotective biomarker

METHODS

Patient group

Consecutive 91 patients consulted our cardiology outpatient clinic with the indication of stable angina pectoris, and positivity of noninvasive tests (exercise stress test or myocardial perfusion scanning) between November 2014, and August 2015 with more than 90% narrowing of at least one major artery as detected on coronary angiography (CAG).were included in this cross-sectional study. Thirty-nine patients in the study group were included in the poor collateral circulation (Rentrop 0-1) and 52 patients in good collateral circulation (Rentrop 2-3) groups. Exclusion criteria of the study encompassed conditions as acute coronary syndrome, decompensated heart failure, history of coronary artery revascularizationi moderate-severe cardiac valve disease, chronic renal disease (eGFR <60 mL/min/1.73m^2), chronic pulmonary disease, autoimmune disease, active of chronic infection, hematologic diseases, and malignancies.

Basic demographic, and clinical characteristics of the patients were recorded. Hypertension was defined as the use of antihypertensive drug or blood pressure >140/90 mmHg. Diabetes mellitus was defined as active antidabetic drug or insulin use or blood sugar measurement at any time more than 126 mg/dL Family history was defined as the presence of angina, myocardial infarction (MI), CAD or coronary revascularization in male and female family members younger than 55, and 65 years of age, respectively. The patients who were still smoking were accepted as smokers. Before this study undersigned enlightened consent forms were obtained from all participants. Study protocol was approved by the local ethics committee.

Coronary angiography

Coronary angiography was realized routinely using 6-French left, and right coronary catheters, and Judkins technique. Left anterior descending artery (LAD), and circumflex artery (Cx) were evaluated from at least four, and right coronary artery from two views. Angiograms

were evaluated by two different experienced interventional cardiologists. The degree of coronary artery stenosis was decided based on the projection which showed the most severe stenosis. Coronary collateral circulation was evaluated using Rentrop-Cohen[13] method, and classified according to the uptake of contrast material by the occluded artery (Stage 0: None of the collaterals could totally uptake the contrast material; Stage 1: Visualization of side branches of the artery without filling of epicardial segment; Stage 2: Partial filling of the epicardial segment with collateral vessels; Stage 3: Total filling of the epicardial segment with collateral vessels). The patients were divided into 2 groups as Rentrop stage 0-1 (poor collateral group) and stage 2-3 (good collateral group) based on the degree of collateral development. For all patients SYNTAX scores which is used to evaluate extent, and complexity of CAD. were calculated. For the calculatiosn of SYNTAX score a program (www.syntaxscore.com) especially designed for this scoring was used. based on this scoring system,narrowings ≥ 50% were considered as significant, and lesion less than 50% stenosis were not included in the calculation. All patients underwent transthoracic echocardiography, and left ventricular ejection fraction (LVEF) was calculated based on modified Simpson method

Biochemical measurements

Biochemical, and hematological parametres were measured from venous blood samples drawn after 12 hours of fasting. Aprotonin (an enzyme inhibitor) was added to the blood samples obtained, and centrifuged for 15 minutes at 1600 rpm. Serum samples were allocated into separate tubes for the measurement of ghrelin, vascular endothelial growth factor (VEGF)-A, asymmetric dimethyl arginine (ADMA), nitric oxide (NO), and high-sensitive C-reactive protein (hs-CRP) , and preserved at -70 °C till the time of measurement. Serum ghrelin concentrations were measured in compliance with the directives of the manufacturing firm (DIAsource Immuno Assays, Louvain-la- Neuve, Belgium) using a RIA kit. For the measurements of serum nitric oxide (NO) nitrate/nitrite col. Assay kit (Cayman Chemical, Michigan, USA) and serum ADMA, ELISA kit (DLD Diagnostika GMBH, Hamburg, Germany) were ıused. VEGF-A was measured using platinum ELISA kit (Bioscience). Serum hs-CRP level was merasured using an immunoturbidimetric method. Values of other laboratory parametres were determined using standard methods.

Statistical Analysis

Data were evaluated as for their fitness to normal distribution using Kolmogorov-Smirnov test. Numerical values with normal distribution (parametric) were indicated as mean ± standard deviation, those without normal distribution (non-parametric) as median (+ interquartile range), and categorical variables as percentages. For the analysis of numerical variables Student t-test or Mann-Whitney U-test, and for categorical variables (*chi*-square test : x^2) test were used. Correlation between serum ghrelin level, and SYNTAX score was displayed using Spearman correlation analysis. ROC curve was used to determine the most suitable cut-off value of ghrelin in the prediction of good coronary collateral development. To determine independent predictors of development of good coronary collateral uni-, and multivariate logistic regression analyses were performed. As a result of univariate statistical analyses, variables with level of significance $p< 0.25$ based on Hosmer and Lemeshow formula were included in multivariate logistic regression analyses (Backward LR method). $P<0.05$ was considered as the level of statistical significance. Statistical analyses were performed using SPSS 21.0 (Statistical Package for Windows, Chicago, Illinois) program

RESULTS

Demographic, clinical, and angiographic characteristics of the study groups are summarized in Table 1.

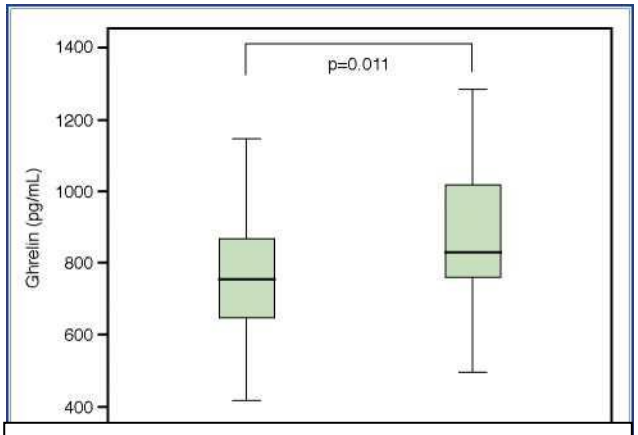


Figure 1. Serum ghrelin levels in good, and poor coronary collateral groups

(p values 0.011; 0.042 and 0.043, respectively . However serum

Table 1. Basic demographic, clinical and angiographic characteristics of the study groups (n=91)

Parametres	Poor collateral group (n=39)	Good collateral group (n=52)	*P*
Age, year	65.7±8.9	62.2±10.7	0.094
Gender, n (%)			0.230
Male	32 (82.1)	37 (71.2)	
Female	7 (17.9)	15 (28.8)	
Hypertension , n (%)	32 (82.1)	34 (65.4)	0.078
Diabetes mellitus, n (%)	17 (43.6)	12 (23.1)	0.038
Body mass index(kg/m^2)	27.0±2.3	26.9±2.5	0.864
Smoking n (%)	11 (28.2)	21 (40.4)	0.229
Family history of coronary artery disease	8 (20.5)	13 (25.0)	0.615
Drugs usedr, n (%)			
Renin-anjigiotensin system blockers	19 (48.7)	23 (44.2)	0.671
P-blockers	10 (25.6)	15 (28.8)	0.735
Calcium channel blockers	9 (23.1)	9 (17.3)	0.494
Statins	5 (12.8)	12 (23.1)	0.214
Aspirin	12 (30.8)	24 (46.2)	0.137
Oral anti-diabetics	13 (33.3)	7 (13.5)	0.023
Left ventricular ejection fraction , %*	57 (47-63)	59 (43-66)	0.216
Duration of ischemic symptoms, months	7.4±4.6	9.7±5.1	0.110
Chronic total occlusion, n (%)	9 (23.1)	35 (67.3)	<0.001
Severe coronary lesion, n (%)			
Left anterior descending artery	22 (56.4)	27 (51.9)	0.671
Left circumflex artery	7 (17.9)	19 (36.5)	0.052
Right coronary artery	16 (41.0)	30 (57.7)	0.116
Number of vessels with severe lesions	1.15±0.4	1.46±0.6	0.011

Data are given as mean ± standard deviation, median or percentages (%) . 'Median (interquartile range).

Poor collateral group contained 39 (mean age: 65.7±8.9 years, male: 82.1%), and good collateral group 52 patients hasta (mean age: 62.2±10.7 years, male, 71.2%). Any intergroup difference was not detected as for age, gender, body mass index (BMI), smoking status, family history of CAD, LVEF, and drug treatments used (excl. oral antidiabetics) (p>0.05). However hypertension, and diabetes mellitus were more frequently detected in the poor collateral group. P value for hypertension (p=0.078) could not reach a statistically significant value, while p value for diabetes mellitus (p=0.038) was statistically significant. Besides vessels with chronic total occlusion , and also severely stenotic vessels were more frequently observed in the good collateral group (p<0.001 , and p=0.011, respectively).

Laboratory data of the study groups are shown in Table 2. Serum levels of ghrelin and VEGF-A were significantly higher in the good collateral group (Figure), while fasting blood glucose was significantly increased in the poor collateral group

However NO level was higher in the good collateral group, and hs-CRP level was increased in the poor collateral group without any statistically significant intergroup difference (p>0.05). In the Spearman correlation analysis a significantly inverse correlation existed between serum ghrelin level, and SYNTAX score (r=- 0.348, p=0.001) (Figure 2).

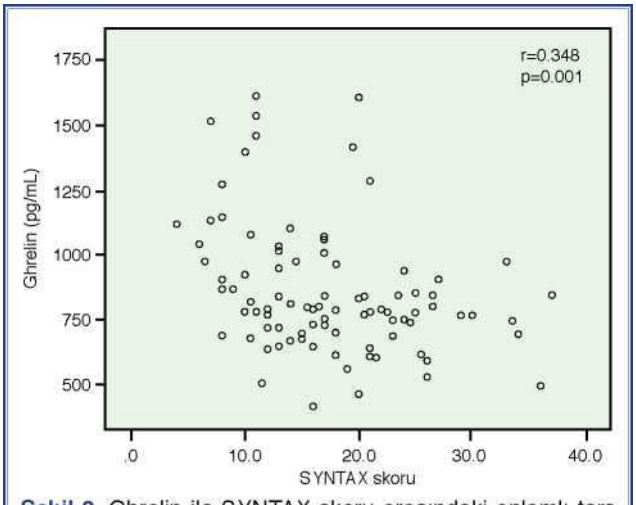
Results of uni-, and multivariate analyses performed to determine independent predictors of good coronary collateral group are shown in Table 3. In the multivariate logistic regression analysis ghrelin (odds ratio [OO]: 1.013; 95 % confidence interval [CI]: 1.011-1.017; p=0.013), VEGF-A (OR: 1.006; 95 %CI: 1.002-1.011; p=0.042), fasting blood sugar (OR: 0.973; 95% CI: 0.951-0.997; p=0.025) , and the presence of chronic total occlusion (OR: 6.023; 95% CI: 2.12316.167; p<0.001) were demonstrated to be independent predictors of development of good coronary collaterals.

Table 2. Comparison of laboratory parametres of the study groups

Parametres	Poor collateral group (n=39)	Good collateral group (n=52)	*P*
Hemoglobin (g/dL)	14.1±1.2	14.3±1.6	0.609
Platelet (10^3/mm^3)	222±57	237±59	0.244
Mean platelet volume (fL*)	8.8±1.2	8.6±1.1	0.385
White blood cell (10^3/mm^3)	7.5±1.6	7.2±1.5	0.416
Fasting blood sugar (g/dL*)	100 (93-134)	96 (86-105)	0.043
Creatinine (mg/dL)	0.92±0.2	0.86±0.2	0.118
Total cholesterol (mg/dL)	193±37	207±41	0.092
HDL-cholesterol (mg/dL)	39±8	44±15	0.191
LDL-cholesterol (mg/dL)	125±35	134±41	0.315
Triglyceride(mg/dL)	152±53	154±51	0.915
Ghrelin, (pg/mL*)	757 (647-871)	830 (756-1028)	0.011
VEGF-A (pg/mL)	928±318	1167±424	0.042
Nitric oxide (uM*)	6.6 (4.0-10.5)	7.7 (4.5-16.4)	0.256
ADMA (umol/L)	0.43±0.1	0.38±0.1	0.074
hs-CRP (mg/L*)	4.9 (1.3-11.2)	3.2 (1.8-6.4)	0.164

Data are giiven as mean ± standard deviation, median or percentage (%). HDL: High-density lipoprotein ; LDL: Low-density lipoprotein; ADMA: Asymmetric dimethyl arginine; hs-CRP: High-sensitive -C-reactiveprotein; VEGF: Vascular endothelial growth factors. 'Median (interquartile range).

However in ROC curve analysis serum ghrelin cut-off value of >781 pg/mL has been detected to predict development of good coronary collaterals with 73.1 % sensitivity, and 67.7% specificity (area under curve [AUC]: 0.721 [95 %CI: 0.613-0.828, p< 0.001]) (Figure 3). Besides in the ROC analysis for ghrelinin >781 pg/mL, positive, and negative predictive values were 76.9%, and 71.8 %, respectively



Şekil 2. Ghrelin ile SYNTAX skoru arasındaki anlamlı ters korelasyon grafiği.

DISCUSSION

Basic findings of the study were as follows: 1. As far as we know, ours is the first study which demonstrated that ghrelin is an independent predictor of good collateral development; 2. Besides it firstly displayed the presence of a significant inverse correlation between serum ghrelin level, and SYNTAX score..

Because of rapid urbanization, unnecessarily higher amounts of energy intake, and sedentary life style, coronary artery disease is an increasingly important public health problem. In patients with serious CAD, revascularization performed with percutaneous coronary intervention (PCI) or coronary artery bypass surgery is an acceptable treatment method. However, unfortunately, in some patients with serious CAD methods of revascularization has not been performed.. In patients with serious CAD collateral vessels supply extra blood support to ischemic areas to aid in preservation of myocardial viability, and ventricular functions.[14] Good collateral vessels decrease mortality at a rate of 36 % when compared with poor collaterals.[2]

Table 3. In uni-, and multivariate logistis regression analyses independent relationship between the development of coronary collaterals, and clinical, angiographic, and laboratory data (Backward LR metodu)

	Univariate analysis		Multivariate analysis	
Variables	Odds ratio (95% CI)	*P*	Odds ratio (95 %GI)	*P*
Age	0.963 (0.922-1.007)	0.098		
Male gender	0.540 (0.196-1.488)	0.233		
Hypertension	0.413 (0.152-1.120)	0.082	0.262 (0.070-0.985)	0.067
Diabetes mellitus	0.388 (0.157-0.958)	0.040		
Smoking	1.724 (0.708-4.202)	0.231		
Left ventricular ejection fraction	1.006 (0.969-1.044)	0.752		
Presence of chronic total occlusion	6.863 (2.671-17.634)	<0.001	6.023 (2.123-16.167)	0.001
Number of vessels with severe lesions	3.076 (1.219-7.758)	0.017	3.022 (0.906-10.079)	0.072
Fasting blood sugar	0.980 (0.963-0.997)	0.022	0.973 (0.951-0.997)	0.025
Creatinine	0.175 (0.019-1.572)	0.120		
Total cholesterol	1.009 (0.998-1.021)	0.096		
High-density lipoprotein-cholesterol	1.025 (0.986-1.066)	0.218		
Ghrelin	1.006 (1.004-1.009)	0.008	1.013 (1.011-1.017)	0.013
ADMA	0.058 (0.002-1.792)	0.104		
Vascular endothelial growth factors	1.011 (1.010-1.012)	0.047	1.006 (1.002-1.011)	0.042
High-sensation -C-reactive protein	0.949 (0.894-1.007)	0.084		

ADMA: Asymmetric dimethylarginine; CI: confidence interval

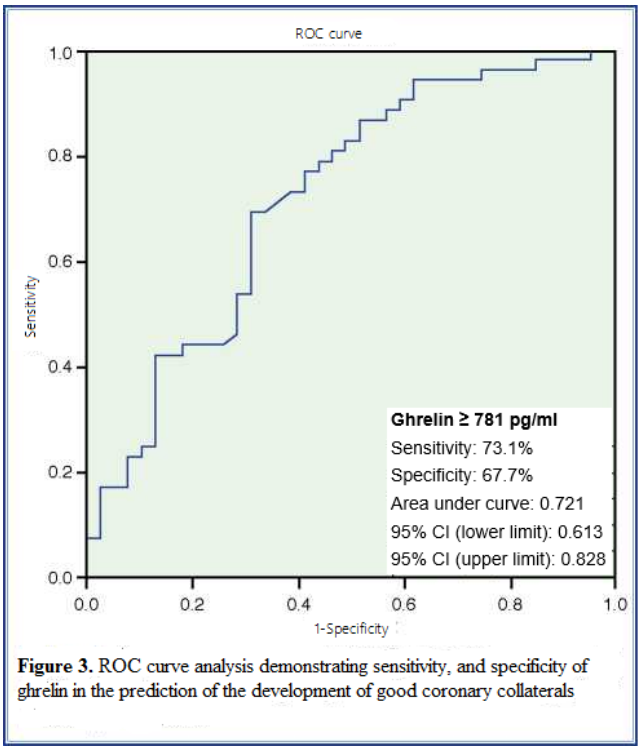


Figure 3. ROC curve analysis demonstrating sensitivity, and specificity of ghrelin in the prediction of the development of good coronary collaterals

Even though pathophysiology of development of coronary collaterals has not been fully elucidated, some clues have been obtained thanks to advances in molecular biology, and genetics.

Ghrelin is a peptide containing 28 amino acids which is released from cardiovascular (CV) system, and endothelial cells, and it exerts protective effects on CV system.[15] In an experimental study performed on rats, subcutaneous administration of ghrelin twice daily for two weeks after induction of MI, had decreased plasma norepinephrine concentration, left ventricular (LV) end-diastolic pressure, LV mass index, and LV remodeling..[16] In patients with chronic congestive heart failure it had provenly decreased peripheral vascular resistance with resultant reduced cardiac output, and cardiac index, and also increased cardiac contractility.[17] Besides, it was reported that it alleviates cardiac injury due to ischemia/reperfusion injury, and decreases the size of infarction through preventing production of reactive oxygen species .[18] However in another study it has been demonstrated that ghrelin depresses apoptosis of vascular endothelial cells, and increases NO bioreactivity, so it assumes an important role in the preservation of endothelial cell functions.[19] Under healthy conditions vascular homeostasis is regulated through contrasting effects of vasodilator, and vasoconstrictor factors of endothelial origin mainly NO, VEGF, and endothelin (ET-1). [20]

In the maintenance of normal angiogenesis, arteriogenesis, and coronary endothelial functions lead the way among key components which play roles in the maintenance of the development of coronary collaterals. In ischemic conditions (especially MI) newly formed coronary collaterals after MI are important in the maintenance, and improvement of cardiac functions. In animal experiments it is already known that ghrelin stimulates retinal angiogenesis in rats through GHSRla-mediated signal pathways, and increases VEGF inducing post-MI angiogenesis.[2122] Wang et al. demonstrated in rats with induced dşabetes, and MI , ghrelin administration resolved impaired angiogenesis.[23] Similarly in a study,Katare et al. emphasized that ghrelin administered subcutaneously for two weeks in mouse model in which critical limb ischemia was induced, triggered functional angiogenesis through activation of proangiogenic microRNAs , and so ghrelin is a promising agent in the early stage treatment of critical ischemia of extremity. [24] Results from our study demonstrate paralellism with these literature data. In our study. When compared to patients with poor collaterals, in patients with good collaterals higher serum ghrelin, VEGF-A, and NO levels were detected. Besides in the multivariate logistic regression analysis we demonstrated that ghrelin, and VEGF-A are independent predictors of improved coronary collateral development. From the results of our study one can deduce that ghrelin stimulates angiogenesis via regulating release of VEGF, and NO with resultant development of coronary collaterals.

Apart from these characteristic features, vasoactive, anti-inflammatory properties of ghrelin are already known. Ghrelin has also recognized anti-inflammatory, and antioxidant features which prevent release of cytokines as interleukin 1, beta, and tumor-necrosis factor (TNF)-a.[25] In essence, oxidative stress induces atherogenesis because of increased severity of vascular inflammation as a result of production of free radicals, and occurrence of lipid peroxidation.[2627] Therefore inflammation, and oxidative stress have been accepted as cornerstones of development, progression of atherosclerosis, and plaque rupture. Studies performed have pointed out that as a potential biomarker of atherosclerosis lower ghrelin levels are widely seen in patients with type-2 diabetes mellitus which are also related to severe carotid atherosclerosis.[28] In a study by Zhang et al. the authors emphasized that lower plasma ghrelin levels in diabetic patients with CAD were closely related to severity, and morphology of angiographically detected lesions.[29] In our study hs-CRP which is accepted as an reliable inflammatory biomarker was much higher in the group with poor collaterals. However we demonstrated an inverse correlation between serum ghrelin levels, and SYNTHAX score which demonstrates the extent, and complexity of coronary atherosclerosis.

Limitations

Results of our study should be interpreted in the light of some limitations. Priorly it is a single-center study performed with relatively limited number of patients which limits generalization of our results. However although a prospective study it is a cross-sectional study without follow-up data. In addition, because of our restricted budget, comparisons between many other biomarkers which may play a role in the pathophysiology of coronary collaterals, and cytokine and ghrelin could not be performed.

Conclusion

One may deduce that ghrelin protects endothelial functions, stimulates angiogenesis to enable improved development of coronary collaterals, and at the same time prevents development of coronary atherosclerosis. More comprehensive, multicenter prospective studies should be performed to better elucidate the relationship between ghrelin, and development of coronary collaterals.

Conflict of interest: None declared

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