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P-, E-, and H-cadherins differ in their relationships with coronary stenosis, cardiovascular outcomes, and unplanned recurrent revascularization

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ABSTRACT

Background and aims: Cadherins are adhesion proteins, and their dysregulation may result in the development of atherosclerosis, plaque rupture, or lesions of the vascular wall. The aim of the present study was to detect the associations of cadherins-P, –E, and —H, with atherosclerosis and pathological cardiovascular conditions.

Methods and results: The present study with 3-year follow up evaluated atherosclerosis and fasting levels of P-, E-, and H-cadherins in the serum samples of 214 patients in a hospital setting. Coronary lesions were assessed by coronary angiography as Gensini score. Serum proteomic profiling was performed using antibody microarrays. The contents of P-, E-, and H-cadherins in the serum were measured using indirect ELISA. High levels of P- and E-cadherins and low levels of H-cadherin were associated with severity of atherosclerosis. High levels of P- and E-cadherins were associated with higher incidence of nonfatal cardiovascular outcomes. E-cadherin was associated with higher incidence of recurrent revascularization during 3 year follow-up. The results of Spearman rank correlation analysis revealed various associations of the three cadherins with lipid, endothelial, and metabolic biomarkers.

Conclusions: The data indicated that classical and atypical cadherins were associated with atherosclerosis progression. Elevated levels of P-cadherin were associated with coronary atherosclerosis. The data indicated that various lipid, endothelial, and metabolic biomarkers may influence the levels of cadherins. Thus, P-, E-, and H-cadherins may be promising markers for the assessment of cardiovascular risk.

1. Introduction

Cardiovascular diseases resulting from atherosclerosis are the leading cause of death all over the world [1]. Dysregulation of various processes involving proteins participating in cellular adhesion is pivotal for the development of vascular diseases, such as atherosclerosis and restenosis after angioplasty.

Endothelial shear stress and the corresponding disruptions in laminar blood flow in conjunction with various cellular interactions, including vascular smooth muscle (VSM) and immune cells at certain specific regions of the vascular bed are the main factors driving the onset and progression of atherogenesis and plaque formation [2]. Adherens junctions formed between multiple cell types at susceptible vascular wall sites are one of the main factors responsible for the maintenance of tissue stability and dynamic cell movement, and alterations in adherens junctions caused by shear stress may lead to the development of vascular lesions [3].

Stability, formation, and rearrangements of adherens junctions are regulated by numerous proteins and their structural modifications. In particular, cadherins are important proteins of adherens junctions present in various cell types [4]. Cadherins of type I and II are encoded by numerous genes to produce multiple cadherin species. In general, cadherins are the cell-cell and cell-matrix adhesion molecules essential for adhesion, migration, proliferation, contraction, differentiation, and apoptosis of VSM and other adjacent cells. All these processes are linked to vascular homeostasis, angiogenesis, and vessel reparation after injury [5].

Regulatory roles of cadherins are mediated at the levels of

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transcription, translation, and trafficking in addition to phosphorylation, proteolysis, or other modifications of these proteins and their interactions with their binding partners within adherens junctions, including catenins [6], to regulate endothelial cells [7]. For example, translocation of VE-cadherin to cellular periphery has been shown to facilitate the closure of gaps in vascular endothelium to prevent endothelial barrier leakage [8]. Specifically, cadherin-11 is known to mediate the interactions of macrophages within the atherosclerotic plaque tissue to influence macrophage trafficking and T-cell recruitment [9] and is essential for local inflammatory reactions in the plaque [10]. Moreover, expression of certain cadherin genes in VSM is associated with remote effects of myocardial infarction on the vascular wall to alter myocardial repair [11].

Thus, atherogenesis, plaque stability, and functional alterations of the vascular wall may result from abnormal interactions between individual cells, which are regulated by cadherins. However, specific roles of cadherins in these processes remain poorly understood. Involvement of cadherins in these events suggests that they may be used as functionally relevant biomarkers of cardiovascular diseases. Hence, the aim of the present study was to detect the associations of representative cadherins, including cadherins-P, −E, and —H, with pathological cardiovascular conditions, such as severity of coronary stenosis, incidence of nonfatal cardiovascular outcomes, and occurrence of unplanned recurrent revascularization, during 3 year follow-up after revascularization.

2. Materials and methods

The study included serum samples from the patients over 25 years of age treated at the National Research Center for Preventive Medicine (NRCPM), Russian Ministry of Health Care, Moscow, Russia. All participants underwent coronary angiography based on European Society of Cardiology guidelines [12]. Indications for angiography included positive exercise test, positive stress echocardiography, arrhythmia, symptoms of advanced angina pectoris, pathological changes in electrocardiogram with physical inability to perform exercise or stress tests, or high Duke score. Angiography was performed and Advantage Workstation software version 4.4 was used to evaluate the extent of stenosis and calculate the Gensini score as described previously [13,14]. The phone survey of the participants was conducted after a 3-year follow-up.

Exclusion criteria were as follows: any oncological disease, myocardial infarction within 6 months of admission; any acute inflammatory disease; chronic kidney failure stage III and higher with rate of glomerular filtration below 60 mL/min/1.73 m^2; decompensated diabetes mellitus type I or type II with levels of glycated hemoglobin over 7.5 %; left ventricular ejection fraction below 40 %; familial hypercholesterolemia; any hematological disease; and immune and autoimmune diseases. Additional details have been provided in our previous publications [15,16].

All patients signed a written informed consent to participate in the present study. The protocol of the study was approved by the Independent Ethics Committee of NRCPM according to Helsinki declaration (approval no. 09–05/19).

2.1. Blood sampling

Blood was withdrawn from the cubital vein at the baseline of the study. The serum and citrate plasma were obtained by centrifugation at 1000*g* for 15 min at 4°C.

2.2. Blood tests

Routine tests were performed in NRCPM to provide sufficient standardization of the tested parameters using specific guidelines approved by Center for External Quality Control of Clinical Laboratory Testing of Russian Federation (www.fsvok.ru). The methods for the assays of total cholesterol, high density lipoprotein (HDL)-cholesterol, low density lipoprotein (LDL)-cholesterol, C-reactive protein (CRP), fibrinogen, glucose, and insulin have been described in our previous publication [14]. The level of LDL-cholesterol (mmol/L) was calculated according to the Friedwald equation in the samples with serum triglycerides below 4.5 mmol/L. CRP (mg/L) was assayed by high sensitivity quantitative immunoturbidimetric method (universal range 0.3–350 mg/L; highly sensitive range 0.05–20 mg/L) using kits from DiaSys (Germany) by an Architect C 8000 analyzer (Abbot, USA). The level of fibrinogen (g/L) in the plasma was determined by the Claus method kits (Hemosil, USA) using an automatic coagulometer ACL Elite (USA) (CV < 6 %). Glucose (mmol/L) in the plasma was assayed by the hexokinase method according to WHO recommendations by kits from DiaSys (Germany) using a Sapphire-400 automatic analyzer (Japan). Insulin (μIU/mL) was measured by an immunoassay kit (Abbott Diagnostics, USA). Insulin resistance index HOMA-IR was calculated according to the equation: [glucose, mmol/L] x [insulin, μIU/mL]/22.5.

Leptin, endothelin, and adiponectin were assayed using commercial ELISA kits [12]. Leptin was assayed by a kit from Diagnostics Biochem Canada, Inc. (detection limit 0.5 ng/mL; CV% 5.8–6.8 %); endothelin (1−21) was assayed by a kit from Invitrogen, Thermofisher Scientific, Austria (detection limit 0.05 pg/mL; CV % 5 %); and adiponectin was assayed by a kit from Invitrogen, Thermofisher Scientific, Austria (detection limit 26 ng/mL; CV% 6.3–7.0 %).

Concentrations of nitrate and nitrite (NOx) were measured in the serum of patients who were under a diet in a hospital setting. The assays were performed using Griess reaction after reduction with vanadium (III) chloride [17] as described previously [18]. Reagents for the NOx assay were from Sigma-Aldrich (St Louis, MO, USA). Each sample was analyzed in duplicate.

2.3. Proteomic analysis using microarrays

Proteomic analysis using antibody microarrays with 656 antibodies per slide in two replicates (ASB 600, Full Moon BioSystems, USA) was performed in nine serum samples of patients without coronary stenosis assessed based on the Gensini score (group A) and in nine serum samples of patients with coronary stenosis with high Gensini scores (group B) as described previously [19,20]. All serum samples were selected from the study cohort. Each sample was analyzed in duplicate.

2.4. ELISA of human E-cadherin and H-cadherin

Human E-cadherin was assayed using commercial ELISA kits according to manufacturer instructions (sensitivity 0.024–0.126 ng/mL, intra-assay precision CV < 2.1 %, inter-assay precision CV < 6.2 %; Biotechne R&D systems, USA, Minneapolis, MN). H-Cadherin was assayed using commercial ELISA kits according to manufacturer instructions (sensitivity 0.312 ng/mL, intra-assay precision CV < 8 %, inter-assay precision CV < 10 %; MyBioSource, Huissen, The Netherlands). Each sample was analyzed in duplicate.

2.5. Indirect in-house ELISA of P-cadherin

Serum samples were adjusted to the same protein concentration as described previously [20]. The microplates were coated with serum, blocked with BSA and washed. Rabbit polyclonal anti-P-cadherin antibodies (100 μL; 0.5 μg/mL; Wuhan Fine Biotech, China) were added and incubated for 1 h at 37 °C. Then, the wells were washed, incubated with a secondary anti-rabbit antibody conjugated to horseradish peroxidase (Invitrogen, Thermofisher; 100 μL of 1:300,000 dilution), and washed. The signal was detected using ELISA as described previously [20,21]. Absorbance was measured at 450 nm using a Tecan Infinite 200 PRO microplate reader (Switzerland). For construction of the calibration curve, the wells of a microtiter plate were coated with serum samples at a protein concentration of 10 μg/mL in 100 μL of coating buffer, and

these samples were spiked with various concentrations of recombinant human P-cadherin (Wuhan Fine Biotech, China) at the concentrations from 0.5 to 40 pg/mL. The calibration curves were calculated by Magellan software (Tecan, Switzerland). Each sample was analyzed in duplicate, and the experiments were repeated tree time.

2.6. Statistical analysis

Statistical evaluation was performed by Statistica software version 8.0 and SPSS IBM statistics version 23. Normality of the distributions was tested by Kolmogorov-Smirnov criterion. The data are shown as the mean and standard deviation. The groups were compared using two-tailed non-parametric analysis of variance (Kruskal-Wallis and Mann-Whitney tests). Receiver operating characteristic (ROC) analysis was performed using the area under the curve (AUC) with 95 % confidence interval (CI). Youden's J index was calculated as follows: J = sensitivity + specificity – 1. The maximum value of J is used to determine an optimal cut-off of numerical variables to assess significance of the differences between false positives and false negatives. Spearman rank correlation coefficients were used to evaluate the associations of various parameters. The null hypothesis was that all correlations are zero. *P* values <0.05 were considered significant.

3. Results

The cohort included 214 patients (63 ± 10.9 years of age, 54 % men) with coronary stenosis quantified as Gensini score (57.0 ± 38.4 in the total cohort) based on the data of coronary angiography. Gensini score varied from 0 to 197 with the following borders and values of the quartiles (Q, mean ± SD): QI, 0–0, (0.0 ± 0.0); QII, 0–2 (0.63 ± 0.59); QIII, 2–40 (14.01 ± 15.79); and QIV, 41–197 (75.23 ± 36.49). There were 76 patients (35.5 %) without coronary lesions (Gensini score = 0) and 138 patients with any degree of coronary lesions (Gensini score > 0). General characteristics of the cohort are presented in Table 1.

A total of 176 (82.2 % of all enrolled participants) participants were followed up by a phone survey, and 38 participants were unavailable for follow up. The survey included cardiovascular (CV) events (*N* = 99, 56 %): CV death (*N* = 4); ischemic stroke (N = 4); acute myocardial infarction (*N* = 1); unplanned recurrent revascularization (*N* = 45) or coronary angiography (*N* = 8) at least three months after discharge from hospitalization due to deterioration of the symptoms, including coronary artery bypass grafting or percutaneous coronary angioplasty, and hospitalization related to a CV condition (*N* = 37). CV events (*N* = 95), excluding CV-death, were combined as CV-outcomes for further evaluation. CV-death was excluded due to low number of incidents, and other participants with CV-outcomes remained alive at the time of follow up.

Serum proteome profiling was performed using antibody based microarray ASB600 in small groups A (*N* = 9) and B (N = 9) with and without coronary stenosis validated by coronary angiography, respectively. These small groups A and B were selected from the total cohort and validated by us previously [12]. Antibody matrix of the ASB600 microarrays can discriminate between the P-cadherin, E-cadherin, and Pan-cadherin signals to account for different types of cadherins. The content of P-cadherin (CDH3; UniProtKB P22223) was significantly increased in group A compared with that in group B (Fig. 1). The differences in E-cadherin (CDH1; UniProtKB Q9UII7) and Pan-cadherin signals between these two groups were modest (Table 2; Fig. 1). H-Cadherin (CDH13; UniProtKB P55290) was not present in the ASB600 microarray. Thus, H-cadherin was selected as a representative of atypical cadherins. Concentrations of P-, E-, and H-cadherins were measured in all serum samples of the total cohort using indirect in-house ELISA or commercial kits. Serum concentrations of P-, E-, and H-cadherins in the total cohort were 4.02 ± 2.88 pg/mL, 429.0 ± 187.25 ng/mL and 15.08 ± 6.47 ng/mL, respectively. E- and H- cadherins were associated with age, with Spearman correlation coefficient $r = 0.25$; $P = 0.001$ and $r = 0.18$; $P = 0.015$. Only P-cadherin was inverse correlated with sex −0.27;

Table 1
General characteristics of the participants and the levels of biochemical markers in the total cohort (*N* = 214).

General characteristics	Mean (SD) default
Sex (1, men; 2, women)	1.39
Age (years)	63.2 (10.9)
Smoking status	
current smokers (N;%)	100 (46.7)
former smokers (N;%)	24 (11.2)
never smoked (N;%)	90 (42.1)
Medication at baseline	
statins (N;%)	171 (79.9)
antihypertensive drugs (N;%)	186 (86.9)
Coronary stenosis status	
Gensini score	23 (35.6)
absence of coronary lesions (N;%)	76 (35.5)
presence of coronary lesions (N;%)	138 (64.5)
Gensini score from 1 to 41 corresponding to the borderline for the upper quartile (N;%)	83 (38.8)
Gensini score higher than 41 (N;%)	55 (25.7)
Biochemical markers	
NOx, μM	41.29 (25.30)
Endothelin-1, pg/mL	1.68(0.63)
TC, mmol/L	4.13 (1.08)
Triglycerides, mmol/L	1.58 (0.90)
LDL-cholesterol, mmol/L	2.33 (0.90)
HDL-cholesterol, mmol/L	1.10 (0.31)
Glucose, mmol/L	6.32 (1.79)
Insulin, μIU/mL	12.90 (9.82)
CRP, mg/L	9.46 (22.23)
Fibrinogen, g/L	5.03 (1.40)
Adiponectin, μg/mL	8.86 (5.01)
Leptin, ng/mL	34.00 (42.50)
Cadherin family members	
P-cadherin (CDH3), pg/mL	4.02 (2.88)
E-cadherin (CDH1), ng/mL	429.0 (187.25)
H-cadherin (CDH13), ng/mL	15.08 (6.47)

NOx, total concentration of nitrate and nitrite ions; TC, total cholesterol; LDL, low density lipoproteins; HDL, high density lipoproteins; CRP, C-reactive protein.

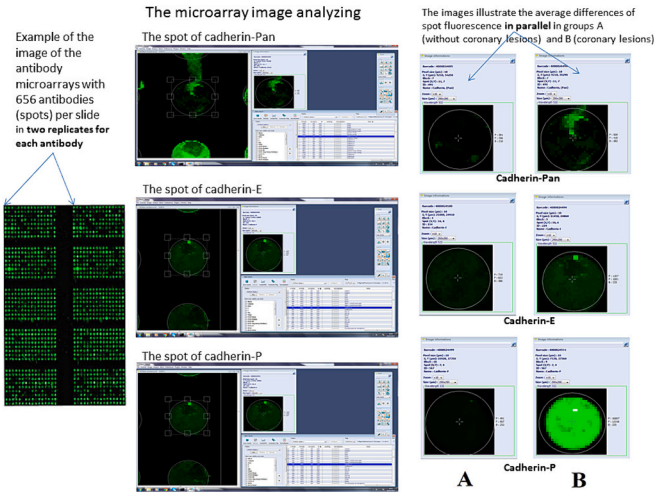

Fig. 1. Image of the Explorer antibody microarrays (ASB600, Full Moon Biosystems, USA) with 656 antibodies per slide in two replicates for each antibody (left). Labeled load of serum proteins was 500 ng; scan settings: velocity: 20 l/s; laser power: 5.0; detector gain: 100; pixel size: 10; wavelength: 532 nm. Enlarged images (on right) illustrate the examples of the differences in the levels of cadherins (Pan), *E*-cadherin, and P-cadherin in the serum of patient (total *N* = 18) with and without coronary lesions (*N* = 9) (A) and with coronary stenosis (N = 9) (B). Differences in the levels of cadherins were analyzed using Mann-Whitney *U* test. Quantification of the images is shown in Table 2 to indicate that the *P* values <0.05 were detected for E- and P-cadherins. Each sample was analyzed in duplicate.

Table 2
Serum proteins in group A with unobstructed coronary arteries and in group B with coronary atherosclerosis. The levels of serum proteins are expressed in pixels normalized (%) to positive controls in each microarray.

Microarray ASB600	Without stenosis (A) N = 9	With stenosis (B) N = 9		
	Mean (% pixels of control proteins) (SD)		Ratio B/A	P
Cadherin, (Pan)	1.7 (1.38)	2.7 (2.04)	1.6	0.10
Cadherin-E	1.7 (0.92)	5.3 (5.31)	3.1	0.008
Cadherin-P	4.0 (2.32)	83.9 (92.12)	21.0	0.0008

P values were calculated by Mann-Whitney U test.

$P = 0.0001$ (data not shown). Evaluation of the associations of these cadherins with smoking status (current and former smokers and patients that never smoked) and therapy (Table 4) indicated that only H-cadherin was inversely associated with the smoking status. Only E-cadherin was associated with statin therapy, and both H- and E-cadherins were associated with antihypertensive therapy (Table 4). Unlike E-cadherin, H-cadherin demonstrated inverse association with the use of antihypertensive medications. P-cadherin was not associated with these factors.

Associations of these cadherins with the degree of coronary stenosis were assessed using Spearman rank correlation coefficients and ROC analysis. P- and E-cadherins directly correlated with Gensini score (numerical variable) corresponding to the degree of coronary stenosis with the Spearman correlations coefficients of approximately 0.2 ($P < 0.05$), while H-cadherin was inversely correlated with the severity of coronary stenosis ($r = -0.2$, $P = 0.004$) (Table 3). These observations were confirmed by the results of ROC analysis. The data indicated that P-cadherin had the strongest associations with coronary stenosis at the threshold of the Gensini score = 0 (absence of stenosis versus presence of any degree of coronary stenosis), with AUC = 0.69 (95 % CI 0.61–0.77; $P = 0.0001$) (Table 4; Fig. 2). Associations between E-cadherin and coronary stenosis at the threshold of Gensini score = 0 were weaker (AUC = 0.59; 95%CI 0.51–0.68; $P = 0.031$) (Table 3; Fig. 2). The data of

Table 3
Spearman correlations between the Gensini score (degree of stenosis), smoking status, medication status, and concentrations of cadherins in the total cohort (N = 214).

Cadherins	R	P
Gensini score (coronary stenosis degree)		
H-cadherin, ng/mL	−0.21	0.004*
P-cadherin, pg/mL	0.25	0.0001*
E-cadherin, ng/mL	0.23	0.002*
Current smokers		
H-cadherin, ng/mL	0.16	0.064
P-cadherin, pg/mL	0.10	0.2
E-cadherin, ng/mL	−0.11	0.16
Former smokers		
H-cadherin, ng/mL	0.005	0.95
P-cadherin, pg/mL	0.007	0.94
E-cadherin, ng/mL	−0.046	0.65
Never smoked		
H-cadherin, ng/mL	−0.11	0.12
P-cadherin, pg/mL	−0.07	0.27
E-cadherin, ng/mL	0.05	0.49
Statin therapy		
H-cadherin, ng/mL	−0.102	0.18
P-cadherin, pg/mL	0.051	0.47
E-cadherin, ng/mL	0.15	0.046*
Antihypertensive therapy		
H-cadherin, ng/mL	−0.17	0.025*
P-cadherin, pg/mL	0.065	0.36
E-cadherin, ng/mL	0.18	0.016*

* $P < 0.05$.

Table 4
ROC analysis of the associations of the concentrations of various cadherins (numerical variables), Gensini score (binary variable), nonfatal cardiovascular events (binary variable), and recurrent revascularization (binary variable).

Cadherins	AUC	P	95% Confidential Interval (CI)	
			Low border	Upper border
Coronary stenosis at the threshold of Gensini score = 0 (N=76) corresponding to the absence of coronary stenosis in the total cohort				
H-cadherin, ng/mL	0.34	0.001	0.26	0.42
P-cadherin, pg/mL	0.69	0.0001	0.61	0.77
E-cadherin, ng/mL	0.59	0.031	0.51	0.68
Coronary stenosis at the threshold of Gensini score = 41 (N=53) corresponding to the upper quartile with the highest Gensini score versus all other participants (N=161) in the total cohort				
H-cadherin, ng/mL	0.48	0.796	0.37	0.59
P-cadherin, pg/mL	0.57	0.122	0.48	0.67
E-cadherin, ng/mL	0.63	0.008	0.54	0.72
Nonfatal cardiovascular events* (N=95) in 176 patients that underwent the phone survey				
H-cadherin, ng/mL	0.51	0.76	0.41	0.61
P-cadherin, pg/mL	0.62	0.02	0.52	0.71
E-cadherin, ng/mL	0.61	0.03	0.51	0.70
Recurrent revascularization** (N=45) in 176 patients that underwent the phone survey				
H-cadherin, ng/mL	0.47	0.68	0.34	0.61
P-cadherin, pg/mL	0.61	0.05	0.50	0.73
E-cadherin, ng/mL	0.64	0.02	0.53	0.76
Smoking status				
Current smokers				
H-cadherin, ng/mL	0.446	0.032	0.51	0.68
P-cadherin, pg/mL	0.598	0.45	0.44	0.62
E-cadherin, ng/mL	0.534	0.23	0.35	0.53
Former smokers				
H-cadherin, ng/mL	0.499	0.985	0.355	0.642
P-cadherin, pg/mL	0.517	0.818	0.382	0.652
E-cadherin, ng/mL	0.452	0.515	0.308	0.596
Never smoked				
H-cadherin, ng/mL	0.432	0.136	0.343	0.520
P-cadherin, pg/mL	0.478	0.625	0.386	0.569
E-cadherin, ng/mL	0.514	0.765	0.423	0.604
Statin therapy				
H-cadherin, ng/mL	0.431	0.130	0.342	0.519
P-cadherin, pg/mL	0.558	0.203	0.468	0.649
E-cadherin, ng/mL	0.600	0.029	0.512	0.687
Antihypertensive therapy				
H-cadherin, ng/mL	0.364	0.031	0.253	0.475
P-cadherin, pg/mL	0.563	0.320	0.435	0.690
E-cadherin, ng/mL	0.656	0.013	0.539	0.774

*Nonfatal events after 3-year follow-up for cardiovascular events were assessed in patients by phone survey to include ischemic stroke; acute myocardial infarction; unplanned revascularization at least 3 months after discharge from hospitalization; coronary artery bypass grafting; and hospitalization related to cardiovascular events.
**Recurrent revascularization was assessed after 3-year follow-up by phone survey as an unplanned intervention.
P < 0.05 is shaded gray.

ROC analysis confirmed inverse relationship of H-cadherin with coronary stenosis at the threshold of Gensini score = 0 (Table 4; Fig. 2).

The associations of all cadherins with high degree of coronary stenosis were assessed at the threshold of Gensini score = 41, corresponding to the border of the upper quartile of the Gensini score [13]. The results indicated that only E-cadherin was associated with severe coronary stenosis (Table 4; Fig. 2).

All cadherins were assessed as predictors of nonfatal cardiovascular events and recurrent revascularization during 3-year follow up in 176

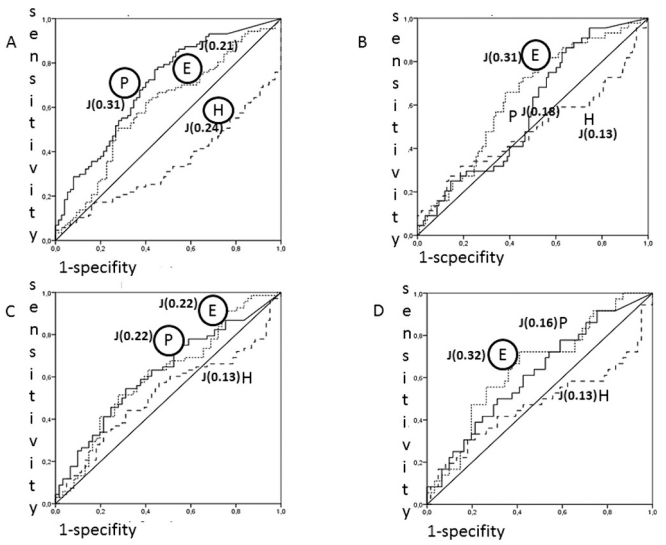

Fig. 2. ROC curves illustrate the associations of E-; P-, and H-cadherins with: (A) coronary lesions as a binary variable in the total cohort ($N = 2014$) where 0 corresponds to patients without coronary lesions (Gensini score = 0; $N = 76$; 35.5 %), and 1 corresponds to patients with Gensini score > 0 with coronary lesions ($N = 138$; 64.5 %); (B) with the severity of coronary lesions as a binary variable defined as 0 for patients with Gensini score ≤ 41 ($N = 83$; 38.8 %) versus 1 for patients with Gensini score > 41 ($N = 55$; 25.7 %); (C) with nonfatal cardiovascular events ($N = 95$ out of the total of 176 patients who responded to follow-up after 3 years); and (D) with unplanned recurrent revascularization ($N = 45$ out of 176 patients after 3 year follow-up. Circled letters correspond to $P < 0.05$. Youden's (J index) was calculated as described in the Methods section. Each sample was analyzed in duplicate. In-house ELISA for P-cadherin was performed in triplicate.

patients that underwent the phone survey. P- and E-cadherins were associated with nonfatal cardiovascular event, unlike H-cadherin (Table 4; Fig. 2), and only E-cadherin was associated with recurrent revascularization during 3-year follow up. Notably, correlations between concentrations or various cadherins were not detected (data not shown).

Associations of various lipid (total cholesterol, triglycerides, LDL-cholesterol, and HDL-cholesterol), metabolic (glucose, insulin, adiponectin, and leptin), inflammatory (C-reactive protein and fibrinogen), and endothelial functional biomarkers (NOx and endothelin) with cadherins were evaluated using Spearman rank correlation coefficients (Table 5). P-cadherin was positively correlated with endothelin-1 and glucose and inversely correlated with triglycerides. E-cadherin was positively correlated with glucose and triglycerides and inversely correlated with total cholesterol, LDL-cholesterol, HDL-cholesterol, and leptin levels. H-cadherin was inversely correlated with glucose levels. Glucose levels correlated with all tested cadherins; however, the correlations with P- and E-cadherins were positive and the correlation with H-cadherin was negative. E-cadherin had the strongest overall correlations with lipid biomarkers compared with that for other cadherins (Table 5).

Thus, our results indicated that high levels of P- and E-cadherins were associated with higher incidence of nonfatal cardiovascular outcomes. High P-and E-cadherins and low H-cadherin were associated with the severity of coronary lesions. E-cadherin was associated with higher incidence of recurrent revascularization during 3 year follow-up. The data may suggest that various parameters of the lipid profile, functional endothelium, and energy metabolism impacted cadherin expression. The levels of E- and H-cadherins may be influenced by therapy, and H-cadherin was the only cadherin inversely associated with current smoking status. These observations demonstrated that classical and atypical cadherins are involved in atherosclerosis progression.

4. Discussion

Cadherins are a family of cell proteins forming cells junctions named for "calcium-dependent adhesion". Altered functions and expression of various cadherins are well established in various types of cancer. For example, loss of E-cadherin is required for tumor metastasis. Degradation of E-cadherin in response to oxidative stress was detected in metastasis of hepatocellular carcinoma [22]. E-cadherin is considered an active suppressor of the growth of many epithelial cancers [23]. Abnormal expression of P-cadherin is associated with a poor prognosis in patients with lung, ovarian and other human cancers [24,25]. H-cadherin is normally expressed in the heart and is also known as T-cadherin(CDH13). The level of H-cadherin, but not CDH13 gene expression, is elevated in early prostate cancer and promotes sensitivity of cancer cells to doxorubicin [26].

However, the role of cadherins in the progression of cardiovascular diseases is poorly understood. In general, normal tissue morphogenesis requires dynamic intercellular contacts [27]. These contacts are also important for the formation of the coronary artery wall and VSM layers. VSM cells play a major role in vascular health and pathogenesis by influencing the vascular tone. Vessel tone is regulated by shear stress, which initiates myogenic response to ensure optimal blood flow and protects vessels from mechanical injury [28]. In VSM cells, cadherins

Table 5
Spearman rank correlations of the levels of cadherin family members with lipid profile biomarkers, endothelial biomarkers, biomarkers of energy metabolism, and acute inflammation-associated proteins.

		TC, mmol/L	Triglyceride, mmol/L	LDL-cholesterol, mmol/L	HDL-cholesterol, mmol/L	NOx, μM	Endothelin-1, pg/mL	Adiponectin, μg/mL	Leptin, ng/mL	Glucose, mmol/L	Insulin, μIU/mL	CRP, mg/L	Fibrinogen, g/L
P-cadherin, pg/mL	r	0.03	-0.22**	0.09	-0.03	-0.09	0.25*	0.10	-0.01	0.15*	0.01	0.09	-0.07
	P	0.71	0.002	0.20	0.63	0.20	0.03	0.14	0.85	0.03	0.84	0.18	0.30
E-cadherin, ng/mL	r	-0.23**	0.17*	-0.25**	-0.19*	0.08	-0.18	-0.01	-0.17*	0.23**	0.12	0.03	0.14
	P	0.002	0.02	0.001	0.01	0.27	0.14	0.98	0.02	0.002	0.10	0.67	0.06
H-cadherin, ng/mL	r	-0.08	-0.019	-0.03	-0.06	0.10	-0.05	0.12	0.06	-0.19*	-0.01	-0.03	-0.01
	P	0.28	0.80	0.72	0.40	0.17	0.70	0.10	0.38	0.01	0.81	0.73	0.93

$P < 0.05$ is highlighted by shading. *$P < 0.05$; **$P < 0.01$.
TC, total cholesterol; LDL, low density lipoproteins; HDL, high density lipoproteins; CRP, C-reactive protein.

and integrins participate in the crosstalk between mechanosensitive molecules [29]. Cadherin expression is altered in VSM in vascular diseases and contributes to molecular control of VSM phenotype and functions. Transition from differentiated to dedifferentiated phenotypes of VSM typically depends on vascular injury and progression of vascular diseases [5].

Cadherins vary in tissue expression and functions [27–30]. Generally, the functions and molecular activity of the cadherin superfamily have been studied in experimental models and in vitro in the context of cardiovascular diseases. We were the first to demonstrate the associations of P-, E-, and H-cadherin with the severity of coronary atherosclerosis and certain cardiovascular outcomes, such as relative risk of cardiovascular events and unplanned recurrent revascularization in patients.

H-cadherin is known to be upregulated in VSM layer and other cells in atherosclerotic lesions [30], and the data of the present study demonstrated that serum H-cadherin was inversely associated with the severity of coronary stenosis. The present study showed that serum levels of classic P-and E-cadherins were positively associated with the severity of coronary stenosis. The exact role of H-cadherin in neointima formation and atherosclerosis development is poorly understood. H-cadherin is a special member of the cadherin superfamily because H-cadherin lacks the transmembrane and cytoplasmic domains common to other cadherins. Unlike classic cadherins, such as P- and E-cadherins, glycosylphosphatidylinositol-anchored H-cadherin is not known to be involved in cell–cell adhesion due to the lack of direct contact with the cytoskeleton. Moreover, specific signaling pathways involving H-cadherin remain to be elucidated.

Classic cadherins act as membrane receptors mediating signal transduction from the extracellular matrix to intracellular small GTPases and the beta-catenin/Wnt pathway and play important roles in cytoskeleton reorganization. Cadherins are necessary for cell–cell adhesion and tissue integrity in adult organisms. The data of the present study demonstrated that elevated levels of serum P- and E-cadherins were associated with nonfatal cardiovascular events after 3-year follow-up, and serum H-cadherin was not associated with this outcome. Higher levels of serum E-cadherin were associated with unplanned recurrent revascularization during 3-year follow up. Loss of E-cadherin expression is known to be the key feature of epithelial-to-mesenchymal transition (EMT), which is also associated with ecto-domain shedding of cadherins from VSM and with mechanotransduction [31]. During endothelial-to-mesenchymal transition (EndMT), endothelial cells lose the expression of junctional proteins, including VE-cadherin, platelet endothelial cell adhesion molecules, and endothelial nitric oxide synthase (eNOS). Concurrently, transitioned endothelial cells gain mesenchymal markers, including ferroptosis suppressor protein 1 (FSP1) and alpha-smooth muscle actin. These changes correspond to the transformations from apical-basal polarized cobblestone-shaped cells into spindle-shaped, motile, and invasive ones. EndMT enhances extracellular matrix depositions of collagen I, collagen III, laminin, and fibronectin, thereby promoting vascular fibrosis and plaque formation [32].

On the other hand, EMT plays an important role in the progression of diabetic nephropathy [33]. The data of the present study indicated that glucose levels correlated with all tested cadherins. The correlations with P- and E-cadherins were positive, while the correlation with H-cadherin was negative. However, a previous study of other authors demonstrated that high glucose levels can induce a decrease in E-cadherin expression [34]. However, under pathological conditions, high glucose levels are associated with coronary atherosclerosis development according to meta-analysis [35]. Thus, our observations demonstrated a direct positive association of glucose with *E*-cadherin since both parameters were associated with the severity of coronary stenosis.

The results of the present study indicated that E-cadherin was inversely correlated with the levels of total cholesterol, LDL-cholesterol, HDL-cholesterol, and leptin and directly correlated with triglycerides. However, high total cholesterol and LDL-cholesterol are well-known valid markers of atherosclerosis, and high E-cadherin levels were shown to be associated with the severity of atherosclerosis.

P-cadherin associations with cardiovascular events have not been demonstrated previously. The data of the present study demonstrated that P-cadherin had stronger associations with atherosclerosis compared to that of other tested cadherins. Moreover, P-cadherin was directly correlated with endothelin-1 levels in the blood. Notably, endothelin-1 is a known marker of endothelial dysfunction, which is the key feature of atherosclerosis progression [36]. Thus, we suggest that increased levels of E- and P-cadherins in the blood may result from the ecto-domain shedding of these cadherins from VSM in patients with coronary heart disease and that increased levels of H-cadherin may protect coronary vessels from atherosclerosis progression.

4.1. Limitations

The present study has certain limitations due to low number of cardiovascular deaths. Thus, conclusions regarding the risk of cardiovascular mortality cannot be made.

Information about patients, which did not respond to follow-up, was missing thus introducing a possible bias. Additionally, exact timing of the events was unavailable due to some subjects being unable to accurately recall the dates of the event. Thus, Cox regression analysis was not performed.

Serum concentrations of cadherins have not been measured previously in patients in sufficiently large studies. Hence, suitable reference standards for ELISA were unavailable. To overcome these problems, the present study used recombinant cadherin species (P-cadherin specifically) as a standard. However, cadherins are complex transmembrane glycoproteins with multiple antigenic epitopes. Specific forms of cadherins shedded in the blood and detected in the serum may originate from various unknown sources and have unknown structural and antigenic properties. Thus, the reactivity of the recombinant reference standard toward antibodies may differ from the reactivity of cadherin species present in the serum, suggesting that the calibration curve and levels of cadherins (P-cadherin specifically) determined based on this curve may not accurately represent the actual levels in the serum and are provided only as an estimate.

5. Conclusions

P-, E-, and H-cadherins have variable associations with the severity of coronary stenosis, cardiovascular outcomes, and unplanned recurrent revascularization during 3-yaer follow-up. H-cadherin was inversely correlated with the severity of atherosclerosis, whereas high levels of P- and E-cadherins were directly associated with the severity of coronary lesions and higher incidence of nonfatal cardiovascular outcomes. P-cadherin demonstrated the strongest association with the severity of coronary stenosis, and E-cadherin was associated with higher incidence of recurrent revascularization during 3 year follow-up. The data of Spearman rank correlation analysis revealed the associations of these cadherin family members with lipid, endothelial, and metabolic biomarkers. Thus, classic and atypical cadherins may have different impact on atherosclerosis progression.

CRediT authorship contribution statement

Nadezhda G. Gumanova: Writing – review & editing, Writing – original draft, Validation, Software, Methodology, Investigation, Data curation, Conceptualization. **Dmitry K. Vasilyev:** Validation, Investigation, Data curation. **Natalya L. Bogdanova:** Investigation, Formal analysis. **Yaroslav I. Havrichenko:** Visualization, Validation, Methodology. **Oxana M. Drapkina:** Project administration, Funding acquisition.

Declaration of competing interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

The authors declare that they have no competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Nadezhda Gumanova reports a relationship with FGBU Nacional'nyj medicinskij issledovatel'skij centr terapii i profilakticeskoj mediciny Ministerstva zdravoohranenia Rossijskoj Federacii that includes: employment.

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