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Gender Differences in 10-Year Mortality in Patients with Coronary Artery Disease with Elevated Lipoprotein(a): In Search of Invisible Guardian Angel

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Abstract

The objective of our study was to examine the contribution of elevated Lp(a) to 10-year mortality in 140 patients with coronary artery disease (CAD), depending on gender, baseline lipid levels, ApoA-I, ApoB, and the *ApoA1*, *ApoE*, and *ApoB* gene polymorphisms.

***Methods and Results*:** The study included 140 patients (75 men and 65 women) of the Uzbek population, hospitalized with diagnosis "CAD, unstable angina (IIB class, E. Braunwald et al., 1989)" in the period between January 2009 and February 2011. The endpoints at 10-year follow-up were death from cardiovascular causes (fatal myocardial infarction and sudden cardiac death). In the studied cohort of CAD patients, Lp(a) distribution was skewed to the right, the median was 16.9 mg/dL, and the mean was 34.3 mg/dL. At the same time, Lp(a) value greater than the 75th percentile was >41 mg/dL. In this regard, we compared the baseline values in 2 groups of patients: Group 1 (Lp(a)<41mg/dL) and Group 2 (Lp(a)>41 mg/dL). The 10-year cardiovascular mortality rate was higher significantly in Group 2 (RR=3.67; 95% CI: 1.67-8.11; *P*=0.0013). Cardiovascular mortality was significantly higher (*P*<0.001) in men of Group 2 than in men of Group 1 (RR=5.31; 95% CI: 2.03-13.88; *P*<0.001), while in women, the 10-year mortality in the compared groups did not differ significantly. Cardiovascular mortality was lower in patients with ApoA-I≥140 mg/dL, the majority of whom were women. The *ApoA1* A-carriers had significantly higher RR for cardiovascular mortality than non-A-carriers (*P*=0.0445).

Conclusion: In the absence of targeted Lp(a) therapy, long exposure to a level of Lp(a) of >41mg/dL is a factor that increases 10-year mortality in CAD patients. (**International Journal of Biomedicine. 2022;12(2):209-217.**)

Highlights

- 10-year cardiovascular mortality was higher in CAD patients with Lp(a) >41 mg/dL.
- However, the increase in mortality due to high Lp(a) was statistically significant in men than in women.
- The ApoA-I level was higher in women than in men.
- Cardiovascular mortality was lower in patients with ApoA-I ≥140 mg/dL, most of whom were women.

Key Words: lipoprotein(a) • apolipoprotein • coronary artery disease

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Abbreviations

ASCVD, atherosclerotic cardiovascular disease; **Apo**, apolipoprotein; **CI**, confidence interval; **CAD**, coronary artery disease; **CM**, cardiovascular mortality; **DM**. diabetes mellitus; **DBP**, diastolic blood pressure; **HDL-C**, high-density lipoprotein cholesterol; **HR**, hazard ratio; **hsCRP**, high-sensitive C-reactive protein; **LDL-C**, low-density lipoprotein cholesterol; **Lp(a)**, lipoprotein(a); **MI**, myocardial infarction; **NS**, not significant; **Non-HDL-C**, non-high-density lipoprotein cholesterol; **RR**, relative risk; **SBP**, systolic blood pressure; **TC**, total cholesterol; **TG**, triglycerides.

Introduction

Recognition of the leading role of the high levels of low-density lipoprotein cholesterol (LDL-C) in the onset and progression of atherosclerotic cardiovascular disease (ASCVD) is a key provision of the current recommendations of the European Society of Cardiology and the European Society of Atherosclerosis.[1] However, despite the effectiveness of step-down, lipid-lowering therapy in achieving the target level of LDL-C, patients with coronary artery disease (CAD) still have a sufficiently high residual risk of cardiovascular complications, which dictates the necessity of searching for new markers that affect the clinical course and prognosis of the disease.

The results of numerous epidemiological, clinical, experimental and genetic studies[2-5] have led to the official "inauguration" of Lp(a) as a risk factor for ASCVD in Recommendations of American College of Cardiology/ American Heart Association 2018 and the European Society of Cardiology/European Atherosclerosis Society 2019.[6,7] However, unlike LDL-C, therapeutic strategies for lowering Lp(a) for primary and secondary prevention of ASCVD are still under development and their impact on disease outcomes, including "hard" endpoints, also requires further study.

The objective of our study was to examine the contribution of elevated Lp(a) to 10-year mortality in 140 CAD patients, depending on gender, baseline lipid levels, ApoA-I, ApoB, and the *ApoA1*, *ApoE*, and *ApoB* gene polymorphisms.

Materials and Methods

Study subjects

A total of 140 patients (75 men and 65 women) of the Uzbek population, hospitalized with diagnosis "CAD, unstable angina (IIB class, E. Braunwald et al., 1989)" (main group) in the period between January 2009 and February 2011, were randomized in the prospective longitudinal study "Development of methods of differentiated pharmacotherapy and risk stratification in CAD patients, taking into account genetic polymorphism."

The exclusion criteria for the main group were MI in the previous 3 months, type 2 diabetes requiring insulin therapy, arterial hypertension grade II-III, atrial fibrillation, life-threatening ventricular arrhythmias, chronic obstructive pulmonary disease, chronic heart failure above functional class I (NYHA), chronic renal and hepatic insufficiency, preceding long-term use of lipid-lowering drugs, premenopausal hormone therapy.

The comparison group for assessing the prevalence of genetic polymorphisms consisted of 58 healthy individuals without diagnostic signs of CAD (exercise stress testing), comparable to patients by gender and age, without burdened family CAD history.

Basic therapy included double antiplatelet therapy (aspirin and clopidogrel; with aspirin intolerance - only clopidogrel, with clopidogrel intolerance - only aspirin); beta-blockers (bisoprolol in 100% of cases) in individually selected doses; ACE inhibitors (in 90% of cases); long-acting nitrates (in 60% of cases). Atorvastatin were prescribed for all patients at a dose of 20-40-80 mg/day in order to achieve target LDL-C level of <70 mg/dL. The lipid-lowering effect was assessed in 3 and 6 months after the initiation of therapy. Further control examinations were carried out at least 1-2 times a year. In the event of discontinuation of therapy, recommendations were given to resume taking atorvastatin. In case of repeated destabilization, coronary angiography was performed with hospitalization. The endpoints at 10-year follow-up were death from cardiovascular causes.

Functional and biochemical tests

All patients underwent the following examinations: 12-lead ECG, Echocardiography (EchoCG), ultrasound examination of the carotid arteries, 24-hour Holter monitoring, treadmill test, coronary angiography (in case of repeated destabilization with hospitalization), and blood tests.

Blood levels of TC, TG, HDL-C, LDL-C, and VLDL-C were determined in the venous blood using automatic biochemical analyzer Daytona (RANDOX, United Kingdom) and RANDOX test systems by the enzymatic colorimetric method. The content of LDL-C was calculated according to Fridvald's formula.

hsCRP in the blood was determined by a highly sensitive method of latex immunoturbidimetry on biochemical automatic analyzer Daytona. The blood levels of Apo-I and ApoB were determined on biochemical autoanalyzer Daytona by immunoturbidimetry, using monospecific antibodies to human ApoB and ApoA-I. The level of Lp(a) in the blood serum was determined by the latex immunoturbidimetry method using automatic biochemical analyzer Daytona and original commercially available kits.

Isolation of DNA and Genotyping of *ApoA1, ApoB,* and *ApoE* polymorphisms

Genomic DNA samples were isolated from the peripheral blood leukocytes by using the Diatom™ DNA Prep 200 Kit (Isogen Laboratory LLC, Moscow, Russia) according to manufacturer`s protocol. The quantity and quality of DNA were determined on a NanoDrop 2000 spectrophotometer (Thermo Scientific™ Wilmington, DE, USA).

The *ApoA1* (G-75A), *ApoB* (-516C/T), and *ApoE* (ε2/ε3/ε4) gene polymorphisms were identified by the PCR-RFLP method. PCR was performed on the GeneAmp®9700 thermocycler (Applied Biosystems Inc., Foster City, CA, USA). The reaction mixture (20 mL) for PCR contained of 10 ng of genomic DNA, 20 pmol of each primer, 0.5 mM of each dNTP, 50 mmol/L KCl, 1.5 mmol/L $MgCl_2$, 10 mmol/L Tris·HCl (pH 8.8 at 25°C), 2% DMSO, and 1.0U of Taq DNA polymerase. Digested fragments of each gene were size-fractionated in 3% UltraPure™ Agarose (Thermo Scientific™ Wilmington, DE, USA) gel with ethidium bromide staining, and visualized on UV transilluminator.

The *ApoA1* G-75A SNP

A 433-bp fragment of the ApoA1 gene was amplified by using the following forward (5'–AGG GAC AGA GCT GAT CCT TGA ACT CTT AAG–3' and reverse (5'–TTA GGG GAC ACC TAG CCC TCA GGA AGA GCA–3') primers.[8] The PCR was conducted according to the following cycling program: initial denaturing at 94°C for 4 minutes, then 35 cycles of denaturation for 30 seconds at 94°C, annealing at

55°C for 30 seconds, and elongation for 30 seconds at 72°C, and then a final elongation step of 72°C for 5 minutes.

Amplified products were digested with 10U of endonuclease MspI at 37°C overnight. The presence of the restriction site at positions -75(G allele) resulted in four fragments of 209 bp, 113 bp, 66 bp, and 45 bp. The absence of the restriction site at -75(A allele) resulted in three fragments of 209 bp, 179 bp, and 45 bp.

The *ApoB* -516C/T SNP

A 422 bp fragment of the ApoB (-516C/T) gene was amplified by using the following forward (5'–GCT GGG GTT TCT TGA AGA CA–3') and reverse (5'–CAA GCG TCT TCA GTG CTC TG–3') primers.[9] Amplification was performed by an initial denaturing at 94°C for 3 minutes, then by 35 cycles of denaturation for 30 seconds at 94°C, annealing at 63°C for 30 seconds, and elongation for 30 seconds at 72°C, and then by a final elongation step of 72°C for 5 minutes. The resulting 422-bp PCR product was then digested for 2 hours at 37°C with the restriction enzyme EarI, and resulted in the following genotype-specific fragments: homozygotes for the C variant, the uncut fragment of 422 bp; C/T heterozygotes, 3 fragments of 422, 306, and 116 bp; and homozygotes for the T variant, 2 fragments of 306 and 116 bp.

The *ApoE* ε2/ε3/ε4 polymorphism

A 227 bp fragment of the *ApoE* gene was amplified by using the following forward (5'-TCC AAG GAG CTG CAG GCG GCG CA-3') and reverse (5'-ACA GAA TTC GCC CCG GCC TGG TAC ACT GCC A-3') primers.[10,11] Amplification was performed by an initial denaturing at 94°C for 3 minutes, then by 40 cycles of denaturation for 30 seconds at 94°C, annealing at 68°C for 10 seconds, and elongation for 1 minute at 72°C, and then by a final elongation step of 72°C for 5 minutes. The resulting 227 bp PCR product was digested with *Hha* I (isoschizomer *Cfo* I) enzyme and the ApoE genotypes were determined as described previously.[11] Briefly, genotype ε2/ε2 (112 Cys and 158 Cys) identified by 91 bp and 81 bp fragments, ε3/ε3 (112 Cys and 158 Arg) by 91 bp and 48 bp, ε4/ε4 (112 Arg and 158 Arg) by 72 bp and 48 bp, ε2/ε3 by 91 bp, 81 bp and 48 bp, ε3/ε4 by 91 bp, 72 bp and 48 bp, and ε2/ε4 by 91 bp, 81 bp, 72 bp, and 48 bp fragments. The restriction also produced shorter fragments, which were not accounted for, because they were not informative for genotyping.

Statistical analysis was performed using the Statistica 10.0 software package (Stat-Soft Inc., USA). The normality of distribution of continuous variables was tested by the Kolmogorov-Smirnov test with the Lilliefors correction and Shapiro-Wilk test. For descriptive analysis, results are presented as mean ± standard deviation (SD), median (Me), interquartile range (IQR; 25th to 75th percentiles). For data with normal distribution, inter-group comparisons were performed using Student's t-test. Differences of continuous variables departing from the normal distribution, even after transformation, were tested by the Mann-Whitney U-test. Kruskal-Wallis test was used to compare differences between 3 or more independent groups. The Wilcoxon criterion was used to compare the differences between the paired samples. Group comparisons with respect to categorical variables are performed using chi-square tests with Yates correction or, alternatively, Fisher's exact test when expected cell counts were less than 5. The Cox proportional hazards regression analysis was applied to assess the effect of various variables on the risk of CVD development. Hazard ratio (HR), RR (relative risk), and 95% confidence interval (CI) were calculated. A probability value of $P<0.05$ was considered statistically significant.

The study protocol was approved by the Ethics Committees of the Republican Specialized Center of Cardiology and the State Committee on Science and Technology of the Republic of Uzbekistan.

Results

In the studied cohort of CAD patients, Lp(a) distribution was skewed to the right, the median was 16.9 mg/dL, and the mean was 34.3 mg/dL. At the same time, Lp(a) value greater than the 75th percentile was >41 mg/dL (Table 1), and did not differ significantly between men and women.

Table 1.

Baseline clinical, hemodynamic and biochemical parameters of CAD patients depending on Lp(a) level

Parameters	Total (n=140)	Group 1 Lp(a)<41mg/dL) (n=105)	Group 2 (Lp(a)>41mg/dL) (n=35)
Men/Women, n (%)	75/65 (53.6/46.4)	56/49 (53.3/46.7)	19/16 (54.3/45.7)
Age, years	54.8±8.5	53.9±8.9	56.3±8.1
Hypertension, n (%)	124 (88.6)	92 (87.6)	32 (91.4)
Current smoker, n (%)	11 (7.8)	8 (7.6)	3 (8.6)
DM, n (%)	31 (22.1)	24 (22.8)	7 (20.0)
History of MI, n (%)	21 (15.0)	13 (12.4)	8 (22.8)
Previous PTCA, n (%)	11 (7.8)	7 (6.7)	4 (11.4)
Heart rate, bpm	77.9±12.7	78.1±13.2	77.1±11.2
SBP, mmHg	134.1±19.5	133.3±18.8	136.7±21.5
DBP, mmHg	85.4±11.1	85.3±10.4	85.4±13.1
TC, mg/dL	228.4±43.4	229.3±44.7	225.6±39.5
LDL-C, mg/dL	141.1±37.0	138.9±38.1	147.6±33.2
HDL-C, mg/dL	39.3±8.5	38.8±8.5	40.7±8.4
TG, mg/dL	185.0 (129.5-277.5)	192.0 (131-300)	181.0 (120-239)
Non-HDL-C, mg/dL	190.0±44.1	193.5±48.1	179.7±27.1
ApoA-I, mg/dL	136.4±33.9	137.5±37.1	133.0±21.8
ApoB, mg/dL	101.0±23.3	100.0±21.8	104.3±27.3
ApoB/ApoA-I	0.78±0.26	0.77±0.25	0.81±0.25
Lp(a), mg/dL	16.9 (8.8-41.2)	12.1 (6.9-19.7)	85.9 (59.0-123.5)**
Glucose, mmol/L	5.0 (4.5-5.7)	5.0 (4.5-5.8)	4.8 (4.4-5.2)
hsCRP, mg/L	4.6 (2.2-9.0)	4.7 (1.9-9.0)	4.5 (2.9-8.7)
Endpoints, n (%)	20 (14.3)	9 (8.6)	11 (31.4)*
RR	-	RR=3.67; 95% CI: 1.67-8.11; P=0.0013	

** - P<0.01 between Groups 1 and 2; ** - P<0.001 between Groups 1 and 2*

In this regard, we compared the baseline values in 2 groups of patients: Group 1 (Lp(a)<41mg/dL) and Group 2 (Lp(a)>41mg/dL). The 2 groups of patients did not differ in gender, age, the severity of the clinical condition, or hemodynamic and biochemical parameters; however, despite the standard statin therapy, the 10-year cardiovascular mortality rate was significantly higher in Group 2 (P=0.0013) (Table 1).

In the cohort of CAD patients, men and women were represented nearly equally: 54% and 46%, respectively (Table 2). Men and women did not differ significantly in age, cardiovascular risk factors, or severity of the clinical condition. However, when comparing lipid parameters, the levels of HDL-C (P=0.021) and ApoA-I (P=0.016) were higher in women than in men. Mortality from cardiovascular causes (fatal myocardial infarction and sudden cardiac death) during 10-year follow-up was recorded in 14(18.7%) cases among men and in 6(9.2%) cases among women (P=0.15). However, gender differences were observed: cardiovascular mortality was significantly higher (P<0.001) in men of Group 2 (Subgroup 2[M]) than in men of Group 1 (Subgroup 1[M]), while in women, the 10-year mortality in the compared subgroups (Subgroup 2[W]) and Subgroup 1[W]) did not differ significantly. At the same time, in women in both subgroups, the level of ApoA-I was significantly higher than in men, although HDL-C did not differ significantly. When constructing a Cox regression model, among the most significant determinants (gender, MI, DM, Lp(a), ApoA-I) affecting mortality, only Lp(a) value reached a significant degree in the whole group and in men (Table 3).

Taking into account the identified differences in the level of ApoA-I among the surveyed patients (Tables 1 and 2) and the possible effect of the cluster of ApoA-I-related lipid parameters on 10-year mortality, we compared the main clinical and biochemical markers and endpoints in patient groups with ApoA-I levels above and below 140 mg/dL (Table 4). In our study, this value was the median of distribution among women who had lower mortality rates with high Lp(a) levels than in men who had median ApoA-I of 125 mg/dL. Patients with ApoA-I ≥140 mg/dL had additional bonuses in the form of high HDL-C (P<0.001), low ApoB/ApoA-I ratio (P<0.001), and reduced cardiovascular mortality (P=0.031). Unfortunately, only 55(39.3%) patients, the majority of whom (63.6%) were women, had ApoA-I≥140mg/dL (Table 4)**.**

The distribution of polymorphic markers of the *ApoE* (ε2/ε3/ε4), *ApoA1* (G-75A), and *ApoB* (-516C/T) genes in CAD patients and healthy people were in Hardy-Weinberg equilibrium. An analysis of the frequency distribution of alleles and genotypes of the *ApoA1* G-75A SNP showed that the carriage of the allele A was more predominant in the CAD patients than in the healthy people (P=0.0016). We did not observe significant gender differences in the distribution of the studied genetic polymorphisms (Table 5).

Table 2.

Baseline clinical, hemodynamic and biochemical parameters of CAD patients depending on gender and (Lp(a) level

Parameters	Men	Women	Men		Women	
			Subgroup 1[M] (Lp(a)<41mg/dL)	Subgroup 2[M] (Lp(a)>41mg/dL)	Subgroup 1[W] (Lp(a)<41mg/dL)	Subgroup 2[W] (Lp(a)>41mg/dL)
n (%)	75 (53.6)	65 (46.4)	56	19	49	16
Average age, years	52.5±8.8	57.4±7.6	51.7±8.9	55.3±7.4	56.9±7.6	57.6±8.9
Hypertension, n (%)	67 (89.3)	57 (87.7)	49 (87.5)	18 (94.7)	43 (87.8)	14 (87.5)
Current smoker, n (%)	8 (10.7)	3 (4.6)	7 (12.5)	1 (5.3)	1 (2.0)	2 (12.5)
Diabetes, n (%)	14 (18.7)	17 (26.2)	10 (17.8)	4 (21.1)	14 (28.6)	3 (18.8)
History of MI, n (%)	12 (16.0)	9 (13.8)	6 (10.7)	6 (31.6)*	7 (14.3)	2 (12.5)
Previous PTCA, n (%)	7 (9.3)	4 (6.2)	4 (7.1)	3 (15.8)	3 (6.1)	1 (6.25)
TC, mg/dL	221.9±41.2	235.9±44.9	223.3±43.2	217.8±35.1	236.3±45.9	234.9±43.4
LDL-C, mg/dL	139.3±35.5	143.1±38.8	137.2±36.8	145.7±31.4	140.9±39.8	149.8±36.3
HDL-C, mg/dL	37.7±8.8	41.0±7.7^	37.4±9.2	38.6±7.9	40.3±7.4	43.3±8.5
TG, mg/dL	186.0 (132.0-257.0)	184.0 (128.0-299.0)	199.5 (141-292)	145 (118-201)	184.0 (123-354)	218.0 (141-295)
Non-HDL-C, mg/dL	188.2±45.6	192.2±42.7	191.3±49.4	179.2±31.2	196.1±47.0	180.3±22.3
ApoA-I, mg/dL	128.0±25.2	145.9±39.9^	130.8±27.2	120.0±15.8	145.1±44.9[&]	148.4±17.5[#]
ApoB, mg/dL	97.9±21.9	104.7±24.5	95.4±17.9	105.2±30.2	105.1±24.8[&]	103.3±24.3
ApoB/ApoA-I	0.79±0.22	0.77±0.29	0.75±0.20	0.89±0.25	0.79±0.3	0.71±0.2[#]
Lp(a), mg/dL	19.5 (8.6-42.2)	14.7 (9.3-39.0)	15.6 (7.0-21.4)	90.4 (63-124)**	10.8 (6.3-17.0)	80.0 (58-123)**
Glucose, mmol/L	4.9 (4.5-5.8)	5.0 (4.5-5.5)	5.0 (4.5-5.9)	4.6 (4.3-5.1)	5.0 (4.5-5.8)	5.0 (4.6-5.3)
hsCRP, mg/L	4.7 (2.5-9.0)	4.5 (2.0-9.0)	4.8 (2.4-8.8)	4.5 (3.6-9.0)	4.0 (1.8-10.4)	4.5 (2.8-5.5)
Endpoints, n (%)	14 (18.7)	6 (9.2)	5 (8.9)	9 (47.4)**	4 (8.2)	2 (12.5)
RR	RR=2.02; 95% CI: 0.83-4.96; P=0.15		RR=5.31; 95% CI: 2.03-13.88; P<0.001		RR=1.53; 95% CI: 0.31-7.60; NS	

*^ - P<0.05 - between men and women; *, **- P<0.05, P<0.01- between Subgroup 1[M] and Subgroup 2[M], and between Subgroup 1[W] and Subgroup 2[W]; [&]- P<0.05 between Subgroup 1[W] and Subgroup 1[M]; [#]- P<0.01 - between Subgroup 2[W] and Subgroup 2[M].*

Table 3.

Relationship between the influence of some lipid and clinical parameters on CM using Cox's multiple regression analysis adjusted for age in the whole group and in men

Parameters	Total (n=140)		Men (n=75)	
	HR (95% CI)	*P*	HR (95% CI)	*P*
Male sex	1.18 (0.79-1.76)	0.41	-	-
Lp(a)	1.01 (1.00-1.01)	0.039	1.01 (1.00-1.02)	0.015
DM	1.23 (0.77-1.95)	0.38	1.23 (0.57-2.65)	0.59
MI	1.17 (0.79-1.71)	0.42	1.13 (0.65-1.95)	0.67
ApoA-I	0.99 (0.98-1.01)	0.64	1.00 (0.96-1.10)	0.46

CM- cardiovascular mortality

Table 4.

Baseline clinical and biochemical parameters of patients depending on ApoA-I level

Parameters	ApoA-I<140mg/dL (n=85)	ApoA-I≥140mg/dL (n=55)
Gender, Men /Women, n (%)	55/30 (64.7/35.3)	20/35 (36.4/63.6)
Average age, years	53.9±8.2	56.0±9.1
DM, n (%)	17 (20.0)	14 (25.4)
History of MI, n (%)	15 (17.6)	6 (10.9)
Previous PCI, n (%)	7 (8.2)	4 (7.3)
TC, mg/dL	227.9±46.9	229.2±37.7
TG, mg/dL	181 (123-239)	204 (163-2180)
Non-HDL-C, mg/dL	189.1±44.3	191.6±44.2
HDL-C, mg/dL	37.0±7.5	42.8±8.8**
LDL-C, mg/dL	142.9±39.0	138.3±33.8
ApoA-I, mg/dL	115.4±15.5	168.7±28.8**
ApoB, mg/dL	98.8±25.0	104.5±20.0
ApoB/ApoA-I	0.88±0.27	0.63±0.15**
Lp (a), mg/dL	17 (9-39)	16 (8-49)
hsCRP, mg/L	5.24 (2.6-9.7)	3.72 (2.0-6.4)
Endpoints, n (%)	17 (20.0)	3 (5.4)*
RR	RR=3.67; 95% CI: 1.13-11.9; *P*=0.031	

*,** - *P*=0.031, *P*<0.001 - between groups

The SNPs of the *ApoA1* (G-75A) and *ApoB* (-516C/*T)* genes did not affect significantly the level of lipids and apolipoproteins in the blood in the CAD patients. Only, the *ApoE* ε4-carriers had significantly higher ApoB concentration (*P*=0.047) and ApoB/ApoA-I ratio (*P*=0.001). In addition, ε4-carriers had a tendency to decrease the level of ApoA-I (*P*=0.056). The *ApoA1* A-carriers had significantly higher RR for CM than non-A-carriers (*P*=0.0445) (Table 6).

Since ApoA-I is the inverse regulator in ApoB/ApoA-I ratio, it was interesting to compare the lipids and apolipoproteins in the *ApoE* ε4-carriers depending on the *ApoA1* G-75A SNP (Table 7). Patients with combined carriage of the *ApoE* ε4 allele and the GG genotype *of* the *ApoA1* G-75A SNP of the promoter region of the *ApoA1* gene showed a significantly higher level (*P*=0.01) of ApoA-I in the absence of a significant increase in HDL-C than carriers of the ε4+A alleles. This may indicate a higher level of primarily monomeric forms of ApoA-I in them, present in the plasma in the form of so-called "lipid-poor" ApoA-I or pre-β1HDL.

Discussion

Lp(a) is an independent risk factor for cardiovascular disease, and its inclusion in known risk scales (Framingham Risk Score and Reynolds Risk Score) improves the prediction of adverse cardiovascular events.[12,13] However, in contrast to the results for men (n=5161) in the framework of the JUPITER study, which showed a strong association of Lp(a) with the development of cardiovascular diseases (myocardial infarction, ischemic stroke, coronary revascularization, and cardiovascular death), including with low TC, the results of 3 cohort studies in women demonstrated its limited effect on cardiovascular risk.[14]

In Women's Health Study (WHS, n=24,558, a median of follow-up of 10.2 years), a case-cohort sample from the study of Women's Health Initiative (WHI, n=1,815, a median of follow-up of 9.9 years), and in women in JUPITER study (n=2569, an average of follow-up of 1.9 years, endpoints also included hospitalizations additionally due to angina destabilization), a high Lp(a) did not affect the development of endpoints independently, but only in combination with the level of TC >220 mg/dL.

Table 5.

Distribution of polymorphic markers of the ApoE (ε2/ε3/ε4), ApoA1 (G-75A), and ApoB (-516C/T) genes in CAD patients and healthy individuals in the Uzbek population

Genes	"Damaging" alleles	Healthy (n=58)	CAD patients (n=140)	Women (n=65)	Men (n=75)
ApoE (ε2/ε3/ε4)	ε4-carriers / vs not-ε4	7/51 ε4/ε2 -1 ε3/ε4 - 6	35/105 ε4/ε4 -1, ε4/ε2 -1, ε3/ε4 -33	14/51 ε3/ε4 -14	21/54 ε4/ε4 -1, ε4/ε2 -1, ε3/ε4 -19
RR		RR=2.07; 95% CI: 0.98-4.39; NS		RR=1.17; 95% CI: 0.84-1.62; NS	
ApoA1 (G-75A)	A-carriers / vs GG	9/49 GA-9	60/80 AA-2, GA-58	29/36 AA-1, GA-28	31/44 AA-1, GA-30
RR		RR=2.76; 95% CI: 1.47-5.19; *P*=0.0016		RR=0.96; 95% CI: 0.66-1.40; NS	
ApoB -516C/T	T- carriers / vs CC	25/33 TT-1, TC-24	48/92 TT-1, TC-47	19/46 TT-1,TC-18	29/46 TC-29
RR		RR=0.80; 95% CI: 0.55-1.16; NS		RR=1.32; 95% CI: 0.82-2.12; NS	

Table 6.

Lipid metabolism parameters in CAD patients depending on polymorphic markers of the ApoE (ε2/ε3/ε4), ApoA1 (G-75A), and ApoB (-516C/T) genes in the Uzbek population

Parameters	*ApoE* ε2/ε3/ε4		*ApoA1* G-75A		*ApoB* -516 C/T	
	ε4-carriers (n=35)	non-ε4 carriers (n=105)	A-carriers (n=61)	non-A carriers (n=79)	T-carriers (n=48)	non-T carriers (n=92)
TC, mg/dL	234.1±41.2	226.5±44.1	230.4±45.1	226.8±42.2	227.5±46.8	228.9±41.7
TG, mg/dL	192 (125.0-250.0)	184.0 (131.0-295.0)	201 (135-284)	183 (123-269)	182.5 (128-242)	196 (134-314)
Non-HDL-C, mg/dL	196.6±40.9	187.9±45.1	189.8±43.6	190.2±44.8	185.9±41.1	192.2±45.7
HDL-C, mg/dL	37.5±7.0	39.8±8.8	39.2±8.6	39.3±8.4	39.9±9.4	38.9±8.0
VLDL-C, mg/dL	38.0 (25.0-46.0)	39.0 (26.0-63.0)	41 (27-59)	37 (26-54)	37 (24-49)	40 (27-67)
LDL-C, mg/dL	148.1±37.0	138.8±36.9	138.0±38.2	143.5±36.1	148.8±39.7	137.1±35.1
ApoA-I, mg/dL	126.9±29.4	139.5±34.8	131.8±37.1	139.9±31.0	134.7±35.2	137.2±37.4
ApoB, mg/dL	107.8±27.3	98.8±21.4*	97.5±19.8	103.8±25.4	105.0±23.0	98.9±23.3
ApoB/ApoA-I	0.90±0.3	0.74±0.2**	0.78±0.26	0.77±0.25	0.82±0.27	0.76±0.25
Lp(a), mg/dL	25.7 (12.9-37.1)	15.9 (7.6-51.5)	19.7 (6.3-40.2)	16.0 (9.2-44.0)	17.1 (9.1-32.1)	16.9 (8.5-43.1)
Glucose, mmol/L	4.9 (4.3-5.2)	5.0 (4.5-5.8)	5.1 (4.6-6.1)	4.9 (4.3-5.5)	4.9 (4.3-5.2)	5.1 (4.6-5.8)
hsCRP, mg/L	5.3 (3.6-8.9)	4.5 (2.0-9.0)	4.5 (1.8-8.5)	4.8 (2.3-10.4)	5.0 (1.5-9.3)	4.3 (2.3-4.8)
Endpoints, n (%)	5 (14.3)	15 (14.3)	13 (21.3)	7 (8.9)^	7/ (14.6)	13 (14.1)
RR	RR=1.00; 95% CI: 0.39-2.55; NS		RR=2.4; 95%CI: 1.02-5.66; *P*=0.0445		RR=1.03; 95% CI: 0.44-2.42; NS	

*,** - *P*=0.047, *P*=0.001 - between ε4-carriers and non-ε4 carriers; ^- *P*<0.05 - between A-carriers and non-A-carriers

Table 7.

The levels of lipids and apolipoproteins in the ApoE ε4-carriers depending on the ApoA1 G-75A SNP

Genes, Parameters	*ApoE*, ε4 carriers (n=35)		*ApoE*, ε4 non-carriers (n=105)	
	ApoA1 (GA,AA) (n=15)	*ApoA1* (GG) (n=20)	*ApoA1* (GA,AA) (n=45)	*ApoA1* (GG) (n=60)
Men/Women, n (%)	7/8 (46.7/53.3)	14/6 (70/30)	24/21 (53.3/46.7)	30/30 (50/50)
TC, mg/dL	234.1±52.7	234.1±31.4	230.1±43.0	223.8±45.1
TG, mg/dL	184.0 (125.0-234.0)	195.0 (145-253.5)	204.0 (135-295)	235.1 (123.5-299.5)
Non-HDL-C, mg/dL	195.7±53.4	197.3±30.0	188.6±40.6	187.3±48.6
HDL-C, mg/dL	38.4±8,2	36.8±6.2	39.6±8.9	40.0±8.9
LDL-C, mg/dL	138.2±42.2	155.5±31.7	138.2±37.7	139.2±36.6
ApoA-I, mg/dL	112.7±22.5	137.7±29.9*	138.8±38.9	140.0±31.8
ApoB, mg/dL	100.3±22.2	113.5±29.9	96.3±19.3	100.6±22.9
ApoB/ApoA-I	0.94±0.33	0.86±0.30	0.73±0.21	0.75±0.23
Lp(a), mg/dL	19.8 (12.0-29.3)	27.9 (13.7-40.6)	19.7 (6.3-55.0)	14.4 (8.3-45.2)
Glucose, mmol/L	4.9 (4.2-5.1)	5.0 (4.7-5.6)	5.1 (4.8-6.8)	4.9 (4.3-5.4)
hsCRP, mg/L	3.6 (1.8-8.9)	5.5 (4.0-16.2)	4.5 (1.5-8.0)	3.9 (2.2-9.7)
Endpoints, n (%)	4 (26.7)	1 (5.0)	9 (20.0)	6 (10.0)
RR	RR=5.33; 95% CI: 0.66-42.9; NS		RR=2.00; 95% CI: 0.77-5.21; NS	

* - *P=0.01 – between ApoA1 (GA, AA) and ApoA1 (GG)*

Though women had a significant but small change in C-statistic (0.790–0.797; *P*=0.035), such as in the WHS test sample, this did not make a significant contribution to improving the risk-stratification scale.[14] WHS and WHI did not show a significant effect of hormone therapy or race on the risk of cardiovascular outcomes, and JUPITER did not include women taking postmenopausal hormone therapy. These results indicate a different impact of high Lp(a) on cardiovascular risk in men and women.

Also, some other studies, including Cardiovascular Health Study in the elderly (2375 women and 1597 men),[15] Stanford Five-City Project,[16] and Framingham Heart Study (n=3121),[17] confirmed the role of Lp(a) as an independent cardiovascular risk factor in men, in the absence of a significant association in women. In our study, in the cohort of 140 patients with coronary artery disease, 10-year mortality from cardiovascular causes in the group of patients with Lp(a) level >41mg/dl exceeded significantly (*P*=0.002) mortality in the group with Lp(a) <41 mg/dL (Table 1).

However, as we noted in the above studies, the increase in mortality with high Lp(a) was most significant in men (*P*<0.001), with no significant association in women. At the time of randomization in the study, men and women did not differ in average age or the severity of the clinical condition (Table 2); however, women had significantly higher levels of HDL-C (*P*<0.05) and ApoA-I (*P*<0.01). In addition, only ApoA-I concentration remained higher among women than in men, regardless of the Lp(a) level (>41mg/dL or <41mg/dL).

In recent years, despite the epidemiological evidence,[18,19] it is known that clinical studies have not confirmed the linear feedback of HDL-C level with the risk of developing CVD.[20,21] In addition, pharmacological interventions aimed at increasing HDL-C levels using niacin[22] and CETP inhibitors[23] have not demonstrated a positive effect on cardiovascular disease outcomes. Also, genetic studies using Mendelian randomization have not confirmed a causal nexus between HDL-C level and CAD development.[24,25] In connection with it, attention switched to the functional abilities of HDL-C,[26] primarily to HDL-C conditioned capacity of cholesterol efflux from cells, which turned out to be a strong predictor of cardiovascular events.[27]

ApoA-I is a key component of HDL and plays a major functional role in the ability of cholesterol efflux from cells,[28] which probably explains the inverse correlation between the level of ApoA-I/HDL-C and a decrease in the risk of atherosclerosis. Unfortunately, a recent Phase II randomized clinical trial using 5 weekly infusions of MDCO-216 (recombinant ApoA-I Milano) and 10 weekly infusions of CER-001 (recombinant wild-type ApoA-I, 10 weeks) did not confirm their positive influence on the regression of coronary atherosclerosis,[29,30] which was assessed by intravascular ultrasound sonography. However, the study period of 5-10 weeks may not have been long enough. A third ApoA-I product, CSL112 representing a reconstituted form of native ApoA-I from human plasma,[31] has the most favorable surrogate criteria, including an increase in the ability to promote cholesterol efflux from cells in CAD patients,[32,33] and is preparing currently for full-scale clinical randomized trials of Phase III.

Results of a recent genetic study using Mendelian randomization also have not found causal nexus between ApoA-I level and CAD development,[34,35] which coincides with the data of a Richardson et al. study,[36] conducted using data of UK BioBank, which has demonstrated once again that apoB is causally related to CAD. The association of polymorphisms of genes involved in Lipid metabolism was studied in several studies. [37-41] Our limited study of genetic polymorphisms [*ApoA1* (G-75A), *ApoE* (ε2/ε3/ε4), and *ApoB* (-516C/T)] found a connection between the *ApoE* ε4 allele with increased ApoB level and ApoB/ApoA-I ratio. However, CAD patients with combined carriage of the *ApoE* ε4 allele with the *ApoA1* GG genotype showed a higher level of ApoA-I. It is possible that in response to an increased level of ApoB and ApoB/ApoA-I in carriers of the ε4 allele, activation in the promoter region of *ApoA1* in patients with the GG genotype of the *ApoA1* G-75A SNP[38] contributes to an increase in the concentration of ApoA-I in plasma and atheroprotective effect.[42]

It is known that, along with an increase in the ability to promote cholesterol efflux from cells, HDL-C/ApoA-I has pleiotropic properties, including antioxidant, anti-inflammatory, and antithrombotic activity,[43] which perhaps help stabilize rather than reduce atherosclerotic plaques, improving cardiovascular outcomes.[31] In contrast, Lp(a) has oxidative, proinflammatory and prothrombotic properties underlying its proatherogenic action.[44,45]

Liu et al.[46] have found in a transgenic mouse model that under conditions of an increased Lp(a) level, an increase in ApoA-I level has a dominant effect on a decrease in susceptibility to atherosclerosis under various conditions, including those that are not associated with changes in plasma lipids.

At the same time, in women who have been taking statins for a long time (10 years) to lower LDL-C, long-term exposure to increased natural ApoA-I perhaps interferes with the proatherogenic effect of Lp(a). The results of a 10-year prospective SWAN study are interesting, in which an increase in total cholesterol, LDL cholesterol, and ApoB was found in 3302 women of various ethnicities a year after the onset of physiological menopause, but Lp(a) level did not change significantly.[47] Levels of HDL-C and ApoA-I increased, to the greatest extent, during the 1-year interval around physiological menopause, and 12 months after its onset, HDL-C level decreased (P=0.01), while the concentration of ApoA-I did not decrease, but remained increased.

There is no doubt that at the present stage, the problem of combating excess mortality from high Lp(a) is extremely acute. Many "weapons" will be needed in this way: from a targeted decrease in its concentration to the neutralization of the mechanism of action, risk stratification of patients, and differentiated choice of treatments.

Conclusion

The results of our study have demonstrated clearly that in the absence of targeted Lp(a) therapy, long exposure to a high level of Lp(a) is a factor that increases 10-year mortality in CAD patients. However, cardiovascular mortality was lower in patients with ApoA-I≥140mg/dL, the majority of whom were women.

Study Limitation

This study was limited to the framework of the genetic branch, which included the study of the ApoA, ApoB, and ApoE polymorphisms in 140 CAD patients that had not been studied previously in the Uzbek population. Therefore, some features in CAD courses in women with high Lp(a) deserve further in-depth study on a large clinical cohort.

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Competing Interests

The authors declare that they have no competing interests.

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