ORIGINAL ARTICLE

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High sensitive CRP and sICAM-1 can predict Major Adverse Cardiovascular Events: MELEN Study: A Large Turkish Population Based Study

ABSTRACT
Objective: We aimed to evaluate the predictors of major adverse cardiovascular events (MACE) in a prospective population based study.
Methods: This study included 153 participants aged>40 years with high and very high cardiovascular risk, and 50 participants aged>40 years with low cardiovascular risk according to the SCORE risk assessment. All the participants underwent a doppler ultrasound examination of carotid intima media thickness (CIMT), echocardiographic examination, ECG recording and various biochemical analyses. High-sensitivity C-reactive protein (hsCRP) was measured with chemiluminescent immunometric assay, serum amyloid-A (SAA) protein, soluble intercellular adhesion molecule-1 (sICAM), apolipoprotein-B (ApoB) were measured with ELISA method. eNOS single-nucleotide polymorphism was detected using polymerase chain reaction and restriction fragment length polymorphism methods. The follow-up was done 36 months after the baseline admission. MACE was defined as cardiovascular mortality or myocardial infarction or stroke.
Results: Frequency of MACE was higher in high and very high risk group according to low risk group. There were no significant differences in eNOS gene polymorphisms between the risk groups and control subjects, and no significant association between eNOS gene polymorphisms and MACE was detected. Age, ejection fraction (EF), CIMT, hsCRP, ApoB, sICAM-1, and SAA protein levels were all significantly associated with MACE in univariate logistic regression analyses. Multivariate analyses revealed that age (OR:1.08, CI:1.02-1.15, p=0.013), EF (OR:0.94, CI:0.89-0.99, p=0.016), hsCRP (OR:1.36, CI:1.12-1.67, p=0.003) and sICAM-1 (OR:81.0, CI: 1.04-6320, p=0.048) levels were the only independent predictors of MACE.
Conclusion: Higher age, hsCRP and sICAM-1 levels and lower EF were independent predictors of MACE.
Keywords*:* Major Adverse Cardiovascular Events, SCORE Risk, hsCRP, sICAM-1

Yüksek Duyarlı CRP ve sICAM-1 Major İstenmeyen Kardiyovasküler Olayları Tahmin Ettirebilir: MELEN Çalışması: Popülasyon Bazlı Geniş Epidemiyolojik Çalışma

ÖZET
Amaç: Major istenmeyen kardiyovasküler olayların (MACE) belirteçlerini prospektif popülasyon bazlı bir çalışmada değerlendirmeyi amaçladık.
Metod: Bu çalışmaya, SCORE risk değerlendirmesine göre yüksek ve çok yüksek kardiyovasküler riske sahip 40 yaş üstü 153 katılımcı ile düşük kardiyovasküler riske sahip 40 yaş üstü 50 katılımcı dahil edildi. Tüm katılımcılara karotis intima media kalınlığı (CIMT) ölçümü için bir doppler ultrasonografi, ekokardiyografi uygulandı., EKG kaydı alındı ve çeşitli biyokimyasal analizler yapıldı. Yüksek duyarlı CRP (hsCRP) kemilüminesans immünometrik yöntem ile ölçüldü, serum amyloid-A (SAA) protein, çözünebilir hücrelerarası adezyon molekülü-1 (sICAM), apolipoprotein-B (ApoB) ELISA method ile ölçüldü. eNOS tek nükleotid polimorfizmi polimeraz zincir reaksiyonu ve restriksiyon uzunluk parça polimorfizmi yöntemlerinin uygulanması ile saptandı. Çalışmaya başlangıcından itibaren 36 ay takip yapıldı. MACE kardiyovasküler mortalite ya da miyokard infarktüsü veya inme olarak tanımlandı.
Bulgular: MACE sıklığı düşük risk grubuna göre yüksek ve çok yüksek risk grubunda daha yüksekti. eNOS gen polimorfizmleri açısından risk grupları ve kontrol grubu arasında anlamlı fark yoktu, eNOS gen polimorfizmleri ve MACE arasında anlamlı bir ilişki saptanmadı. Yaş, ejeksiyon fraksiyonu (EF), CIMT, hsCRP, ApoB, sICAM-1 ve SAA protein düzeyleri ile MACE arasında tek değişkenli lojistik regresyon analizinde anlamlı ilişki bulunmuştur. Çok değişkenli analizde yaş (OR: 1.08, CI: 1.02-1.15, p=0.013), EF (OR: 0.94, CI: 0,89-0,99, p=0.016), hsCRP (OR: 1.36, CI: 1,12-1,67, p=0.003) ve sICAM-1 (OR: 81.0, CI: 1,04-6320, p=0.048) düzeyleri MACE için bağımsız belirteç olduğu gösterildi.
Sonuç: Yüksek yaş, hsCRP, sICAM-1 seviyeleri ve düşük EF MACE için bağımsız belirteçlerdir.
Anahtar Kelimeler: Major İstenmeyen Kardiyovasküler Olaylar, SCORE risk, Yüksek Duyarlı CRP, sICAM-1

INTRODUCTION

Atherosclerotic cardiovascular disease (CVD) is a chronic disorder developing insidiously throughout life, and usually progressing to an advanced stage by the time symptoms occur. In apparently healthy persons, CVD risk is most frequently the result of multiple interacting risk factors (1). The overall risk is affected by a broad spectrum of parameters including demographic, clinical, genetic and environmental factors. Several non-invasive tools such as echocardiography, ultrasonography and electrocardiography (ECG) as well as new biochemical markers were shown to be applicable to predict cardiovascular events (2-5).

To the best of our knowledge, inflammation plays a key role at all stages of the atherosclerotic disease process (1). High-sensitivity C-reactive protein (hsCRP) was shown as a risk factor integrating multiple metabolic and low grade inflammatory factors underlying the development of unstable atherosclerotic plaques, with a magnitude of effect matching that of classical major risk factors (1). Soluble intracellular adhesion molecule-1 (sICAM-1) has been shown to be significantly higher in patients with stable and unstable angina compared to healthy controls (6). Serum amyloid A levels (SAA) predict adverse outcomes and acute events in patients with coronary atherosclerosis (7). It has been shown that SAA is a prothrombotic and proinflammatory mediator in acute coronary syndrome (ACS) which may contribute to atherogenesis and related complications (8). The molecular advances bequeathed Apolipoprotein B100 (ApoB) which has been suggested as a new biomarker for risk assessment, offering a stronger association with CHD and CVD than cholesterol content measures (9,10). ApoB was superior to LDL-C and non-HDL-C as a marker of CV risk in a meta-analysis (11).

Epidemiologic investigation of genetic factors have revealed that some polymorphisms increase the risk of coronary artery disease (CAD) (12,13). Nevertheless, the expression of gene encoding eNOS plays an important role in physiopathological process of cardiovascular system. Since nitric oxide (NO) availability is regulated at the level of synthesis, the eNOS gene is prone to be a candidate for CVD.14 Liu et al, demonstrated that C allele of eNOS−786TNC polymorphism could increase CAD risk compared to T allele.15 Nevertheless, due to the polygenic and multifactorial determinants of the most common CVDs, the impact of any single polymorphism remains rather modest (1).

No doubt that the minaciously accelerating incidence of cardiovascular risk parallels with rising rate of carotid intima media thickness (CIMT); thus individuals without known CVD with increased CIMT simply bear increased risk for cardiac events and stroke (1). The current joint European Guidelines on CVD prevention in clinical practice recommend the use of the SCORE system since it is based on large, representative European cohort data sets.

The aim of the present study was to evaluate the predictors of major adverse cardiovascular events in a prospective population based study, along with the use of inflammatory markers, eNOS gene polymorphism, echocardiography, carotid ultrasonography and electrocardiography according to the SCORE risk assessment.

METHODS

***Patients*:** The result of this manuscript is a part of the prospective MELEN study carried out in year 2010. The rationale, methodology and basic results of the MELEN study were published before (17-21). The baseline measurements were conducted on 2230 participants (1427 women, 803 men with a mean age of 49). This study included 153 participants aged >40 yrs with high and very high cardiovascular risk (5% and >5% on the scale of SCORE), and 50 randomly selected participants aged > 40 years with low cardiovascular risk (≤%1 on the scale of SCORE) served as the control. The participants underwent a doppler ultrasound examination of CIMT, echocardiographic examination, ECG recording, bioimpedance analysis of body composition, and various biochemical analyses.

The study protocol was approved by the Ethics Committee of Duzce University (***Decision no: 2011/138, date: 13/04/2011***).

Blood sample assay

In all participants, venous blood samples were collected for routine biochemical tests, eNOS gene polymorphism detection, and determination of serum levels of SAA protein, ApoB, sICAM-1. hsCRP was detected with chemiluminescent immunometric assay using an IMMULITE® 1000 Autoanalyzer (Siemens, Erlangen,Germany). Invitrogen Human SAAELISA kit (Camarillo, CA, USA) was used for SAA protein, human sICAM-1 ELISA kit (eBioscience, Vienna, Austria) for sICAM, and AssayMax Human ApoB ELISA Kit (AssayPro, St. Charles, MO, USA) for ApoB. Microplate autoreader (Micro ELISA Autoreader, BioTek Instruments, Inc., Burlington, VT) were used for the reading of the ELISA. Standards both had r^2 values above 0.99.

Determination of three eNOS (Promoter 2786T/C (rs2070744), exon 894G/T (rs1799983) and intron G10T (rs7830)) Polymorphisms

Total DNA was isolated from peripheral blood samples of the patients via phenol–chloroform extraction methods. The eNOS single-nucleotide polymorphism was detected using polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) methods. Couples of primers were used (forward, 5'- ATG CTC CCA

CCA GGG CAT CA -3' and reverse, 5'- GTC CTT GAG TCT GAC ATT AGG G-3' for promoter -786T/C), (forward, 5'- CAT GAG GCT CAG CCC CAG AAC -3', and reverse, 5'- AGT CAA TCC CTT TGG TGC TCA C-3' for exon 894 G/T), and (forward, 5'- CCC CTG AGT CAT CTA AGT ATT C -3', and reverse, 5'- AGC TCT GGC ACA GTC AAG -3' for intron G10T). Restriction products were determined by electrophoresis on 2.0% agarose gel. Genotypes were identified according to Ngo-MIV (TT: 236 bp; TC: 236, 203, and 33 bp; and CC: 203 and 33), MboI (GG: 206 bp; GT: 206, 119, and 87 bp; and TT: 119 and 87), Hind II digestion results (GG: 577 and 99 bp; GT: 577, 374, 203, and 99 bp; and TT: 374, 203, and 99 bp), (Figure 1).

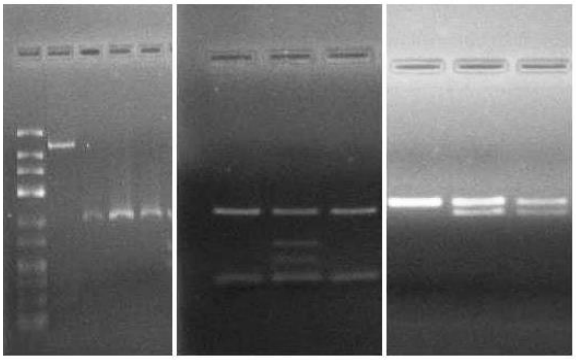

Figure 1. Agarose gel electrophoresis. Picture illustrating different eNOS gene genotypes by RFLP

***Follow-up*:** The follow-up was done 36 months after the baseline admission via telephone call. The participants were asked whether they had MI or stroke during the follow-up period. Mortality data was collected from first degree relatives. Major adverse event was defined as cardiovascular mortality or MI or stroke.

Statistical analysis: Statistical Package for Social Sciences software (SPSS® v.12, Chicago, IL, USA) was used for analysis. Descriptive parameters were shown as mean±standard deviation or in percentages. Two-sided t-tests and Pearson's chi-square tests were used to analyze the differences in means and proportions between groups. Abnormally distributed variables were compared using Mann-Whitney U test. Multiple regression analysis was used to determine the independent predictors of major cardiac adverse events. A p value of <0.05 was considered significant.

RESULTS

A total of 153 participants (50 women; mean age 69.1±8.0 years) with high and very high cardiovascular risk, and 50 control subjects with low cardiovascular risk (42 women; mean age 55.5±5.4 years) were included. Table 1 shows comparison of demographic, clinical characteristics and frequencies of polymorphism of subjects according to SCORE risk. There were no significant differences between eNOS (rs2070744), (rs1799983) and (rs7830) polymorphisms and low risk score and high risk score groups.

Comparison of variables measured with ECG, echocardiography, ultrasonography, bioimpedance analysis and laboratory parameters among subjects with low risk SCORE and high risk SCORE were shown in Table 2. Systolic blood pressure (144.3±27.9 vs 133.3±21 mmHg, p=0.004), heart rate (72.3±12.4 vs 77.6±12.7 bpm, p=0.01), left ventricle (LV) mass (251.4±78.0 vs 220.8±51.4 gr, p=0.01), CIMT (0.78±0.30 vs 0.69±0.13 mm, p=0.038), hsCRP (2.65±2.58 vs 1.92±1.56mg/L, p=0.042), QRS wave duration (97.5±17.9 vs 87.3±8.5 ms, p<0.001), creatinine (0.91±0.26 vs 0.79±0.19 mg/dL, p=0.002), uric acid (4.6±1.4 vs 4.1±1.1 mg/dL, p=0.026), SAA protein (165.3±98.7 vs 114.7±86.6 µg/mL, p=0.001), ApoB (135.4±51.5 vs 94.3±43.8 mg/dL, p<0.001), sICAM-1 (0.142±0.098 vs 0.115±0.042 ng/mL, p=0.031) were significantly higher in the high and very high risk group than low risk group.

During the follow-up of 36 months, 26 major cardiac adverse events occurred. Six participants died, 10 had stroke and only one had MI during the follow-up. Frequency of MACE was higher in high and very high risk group compared to low (25 vs 1, p=0.008).

Comparison of demographic and clinical characteristics of subjects with and without MACE was shown in Table 3. Mean age (64.8±9.0 vs 72.3±10.1 years, p<0.001) and SCORE risk (5.84±4.47 vs 7.00±2.64, p=0.018) were significantly higher in participants with MACE than in participants with no MACE. eNOS (rs2070744), (rs1799983) and (rs7830) polymorphisms were not significantly different between the two groups.

Comparison of variables measured with ECG, echocardiography, ultrasonography, bioimpedance analysis and of biochemical variables among subjects with and without major cardiac adverse events were shown in Table 4. HsCRP (4.67 ±4.70 vs. 2.14 ±1.61 mg/L p=0.005), CIMT (0.96±0.53 vs 0.73±0.18 mm, p<0.001), SAA protein (202.2±86.2 vs 145.6±97.8 µg/mL, p=0.006), ApoB (155.6±50 vs 120.8±51.6, mg/dL, p=0.021), sICAM-1 (0.181±0.076 vs 0.129±0.089 ng/mL, p=0.002) were significantly higher in participants with MACE than in participants with no MACE. Ejection fraction (EF) (56.5±11.8 vs 60.7±7.6, p=0.016) was significantly lower in participants with MACE (Table 4).

Age (p<0.001), EF (p=0.023), CIMT (p=0.004), hsCRP (p<0.001), ApoB (p=0.002), sICAM-1 (p=0.03), SAA protein (p=0.007) levels were significantly associated with MACE in univariate logistic regression analyses. Multivariate analyses disclosed that age (OR:1.08, CI:1.02-1.15, p=0.013), EF (OR:0.94, CI:0.89-0.99, p=0.016), hsCRP (OR:1.36, CI:1.12-1.67, p=0.003) and sICAM-1 (OR:81.0, CI: 1.04-6320, p=0.048) levels were the only independent predictors of MACE (Table 5).

Table 1. Comparison of demographic, clinical characteristics and frequencies of polymorphism of subjects according to SCORE risk

	Low risk score (n=50)	**High risk score (n=153)**	p
Mean age, years	55.5±5.4	69.1±8.0	**<0.001**
Female, n (%)	42 (84)	50 (32.7)	**<0.001**
BMI, kg/m^2	28.9±5.6	32.1±5.1	**0.001**
Active smoker, n (%)	7 (14)	84 (54.9)	**<0.001**
Hypertension, n (%)	34 (68)	115 (75.2)	0.320
Type II Diabetes Mellitus, n (%)	29 (58)	79 (51.6)	0.434
Obesity, n (%)	62 (40.5)	32 (64)	**0.004**
eNOS promoter (-786T/C) genotypes			
TT, n (%)	25 (50)	66 (43.1)	0.475
TC, n (%)	19 (38)	73 (47.7)	
CC, n (%)	6 (12)	14 (9.2)	
eNOS exon (894 G/T) genotypes			
GG, n (%)	28 (56)	103 (67.3)	0.116
GT, n (%)	20 (40)	49 (32)	
TT, n (%)	2 (4)	1 (0.7)	
eNOS intron (G10T) genotypes			
GG, n (%)	48 (96)	140 (91.5)	0.368
GT, n (%)	2 (4)	13 (8.5)	

BMI, body mass index; eNOS, endothelial nitric oxide synthase.

Table 2. Comparison of variables measured with ECG, echocardiography, ultrasonography, bioimpedance analysis and laboratory parameters among subjects with low risk score and high risk score

	Low risk score (n=50)	**High risk score (n=153)**	p
Systolic blood pressure, mmHg	133.3±21	144.3±27.9	**0.004**
Diastolic blood pressure, mmHg	82.2±12	84.3±14.8	0.482
(Logarithmic transformed value)	(1.91±0.06)	(1.99±0.07)	
Heart rate, bpm	77.6±12.7	72.3±12.4	**0.010**
LV mass, gr	220.8±51.4	251.4±78.0	**0.010**
Total cholesterol, mg/dL	181.9±37.9	193.1±42.7	0.093
LDL cholesterol, mg/dL	103.6±33.4	115.8±40.4	0.078
(Logarithmic transformed value)	2.00±0.12	2.04±0.14	0.074
HDL cholesterol, mg/dL	41.7±11.2	41.8±12.0	0.984
Triglycerides, mg/dL	214.2±159.2	201.0±121.3	0.917
(Logarithmic transformed value)	2.24±0.28	2.24±0.24	
CIMT, mm	0.69±0.13	0.78±0.30	**0.038**
(Logarithmic transformed value)	-0.18±0.08	-0.13±0.12	
High CIMT (≥0.83), n (%)	11 (22)	59 (38.6)	**0.032**
High sensitive CRP, mg/L	1.92±1.56	2.65±2.58	**0.042**
(Logarithmic transformed value)	0.148±0.360	0.272±0.375	
Ejection Fraction, %	61.9±8.0	59.6±8.4	0.089
(Logarithmic transformed value)	1.79±0.68	1.78±0.78	0.145
PR wave duration, ms	152.4±18.7	160.3±26.2	0.074
QRS wave duration, ms	87.3±8.5	97.5±17.9	**<0.001**
(Logarithmic transformed value)	1.94±0.14	2.00±0.71	
Corrected QT wave duration,ms	407±23.9	406±24.4	0.927
White blood cell	6.5±1.7	6.4±1.5	0.614
Hemoglobin, g/dl	13.1±1.1	13.2±1.5	0.452
Neutrophil/Lymphocyte ratio	1.77±0.84	1.92±0.96	0.296
(Logarithmic transformed value)	0.205±0.193	0.241±0.196	0.252
Creatinine, mg/dL	0.79±0.19	0.91±0.26	**0.002**
BUN, mg/dL	34.8±10.1	37.1±10.9	0.194
Uric acid, mg/dL	4.1±1.1	4.6±1.4	**0.026**
SAA protein, µg/mL	114.7±86.6	165.3±98.7	**0.001**
Apolipoprotein B, mg/dL	94.3±43.8	135.4±51.5	**<0.001**
(Logarithmic transformed value)	1.89±0.39	2.09±0.26	
sICAM-1, ng/mL	0.115±0.042	0.142±0.098	**0.031**
(Logarithmic transformed value)	-1.023±0.427	-0.917±0.240	
MACE, n (%)	1 (2)	25 (16.3)	**0.008**

CIMT:Carotid Intima-Media Thickness; HDL:High-density lipoprotein; LDL:Low-density lipoprotein; LV: Left ventricular; MACE: Major adverse cardiac events; SAA: Serum amyloid A; sICAM: Soluble intercellular adhesion molecule-1

Table 3. Comparison of demographic and clinical characteristics of subjects with and without major cardiac adverse events

	No MACE (n=177)	**MACE (n=26)**	**p**
Mean age, years	64.8±9.0	72.3±10.1	**<0.001**
Female, n (%)	82 (46.3)	10 (38.5)	0.452
BMI, kg/m2	29.8±5.8	28.1±4.2	0.129
Active smoker, n (%)	79 (44.7)	12 (46.2)	0.968
Hypertension, n (%)	130 (73.4)	19 (73.1)	0.968
Type II Diabetes Mellitus, n (%)	98 (55.4)	10 (38.5)	0.107
Obesity, n (%)	86 (48.6)	8 (30.8)	0.089
SCORE risk	5.84±4.47	7.00±2.64	**0.018**
(Logarithmic transformed value)	0.612±0.407	0.808±0.208	
eNOS promoter (-786T/C) genotypes			
TT, n (%)	79 (44.6)	12 (46.2)	0.536
TC, n (%)	82 (46.3)	10 (38.5)	
CC, n (%)	16 (9)	4 (15.4)	
eNOS exon (894 G/T) genotypes			
GG, n (%)	115 (65)	16 (61.5)	0.555
GT, n (%)	60 (33.9)	9 (34.6)	
TT, n (%)	2 (1.1)	1 (3.8)	
eNOS intron (G10T) genotypes			
GG, n (%)	163 (92.1)	25 (96.2)	0.735
GT, n (%)	14 (7.9)	1 (3.8)	

BMI, body mass index; eNOS, endothelial nitric oxide synthase.

Tablo 4. Comparison of variables measured with ECG, echocardiography, ultrasonography, bioempedance analysis and of biochemical variables among subjects with and without major cardiac adverse events

	No MACE (n=177)	**MACE (n=26)**	**p**
Systolic blood pressure, mmHg	141.6±27.3	141.3±23.3	0.956
Diastolic blood pressure, mmHg	84.1±14.3	81.5±13.1	0.384
(Logarithmic transformed value)	1.92±0.07	1.91±0.08	0.397
Heart rate, bpm	73.2±12.3	76.5±14.9	0.218
LV mass, gr	272.0±94.9	239.8±69.1	0.097
Total cholesterol, mg/dL	184.1±44.3	191.7±41.2	0.462
LDL cholesterol, mg/dL	103.3±39.5.2	113.3±39.4	0.118
(Logarithmic transformed value)	1.98±0.16	2.03±0.13	
HDL cholesterol, mg/dL	39.7±12.3	42.0±11.6	0.565
Triglycerides, mg/dL	201.0±121.3	213.9±135.9	0.917
(Logarithmic transformed value)	2.23±0.24	2.24±0.27	
CIMT, mm	0.73±0.18	0.96±0.53	**<0.001**
(Logarithmic transformed value)	-0.15±0.10	-0.06±0.17	
High CIMT (≥0.83), n (%)	54 (30.5)	16 (61.5)	**0.003**
High sensitive CRP, mg/L	2.14 ±1.61	4.67 ±4.70	**0.005**
(Logarithmic transformed value)	0.212±0.347	0.432±0.494	
Ejection Fraction, %	60.7±7.6	56.5±11.8	**0.016**
(Logarithmic transformed value)	1.78±0.07	1.74±0.11	
PR wave duration, ms	156.7±23.0	169.3±32.8	0.071
QRS wave duration, ms	94.3±16.7	99.3±15.8	0.109
(Logarithmic transformed value)	1.97±0.67	1.99±0.69	
Corrected QT wave duration, ms	406±24.0	410±26.1	0.393
White blood cell	6.4±1.6	6.5±1.6	0.876
Hemoglobin, g/dL	13.3±1.4	12.9±1.5	0.232
Neutrophil/Lymphocyte ratio	1.8±0.89	2.0±1.1	0.395
Creatinine, mg/dL	0.89±0.26	0.90±0.16	0.662
BUN, mg/dL	36.2±10.9	38.3±9.8	0.354
Uric acid, mg/dL	4.4±1.3	4.9±1.7	0.067
SAA protein, µg/mL	145.6±97.8	202.2±86.2	**0.006**
Apolipoprotein B, mg/dL	120.8±51.6	155.6±50	**0.021**
(Logarithmic transformed value)	2.01±0.32	2.17±0.15	
sICAM-1, ng/mL	0.129±0.089	0.181±0.076	**0.002**
(Logarithmic transformed value)	-0.96±0.31	-0.78±0.18	

CIMT:Carotid Intima-Media Thickness; HDL: High-density lipoprotein; LDL: Low-density lipoprotein; LV:Left ventricular; MACE: Major adverse cardiac events; SAA: Serum amyloid A; sICAM: Soluble intercellular adhesion molecule-1.

Table 5. Results of univariate and multivariate Logistic regression analysis for prediction of MACE

	Univariate analysis			Multivariate analysis		
	OR	95% CI	p	OR	95% CI	p
Age	1.09	1.04-1.15	**<0.001**	1.08	1.02-1.15	**0.013**
EF	0.95	0.92-0.99	**0.023**	0.94	0.89-0.99	**0.016**
CIMT	13.2	2.29-76.5	**0.004**	2.88	0.41-20.2	0.287
HsCRP	1.39	1.18-1.65	**<0.001**	1.36	1.12-1.67	**0.003**
ApoB	1.013	1.005-1.021	**0.002**	1.01	0.99-1.02	0.073
sICAM-1	153	1.62-14609	**0.03**	81.0	1.04-6320	**0.048**
SAA	1.006	1.002-1.010	**0.007**	1.005	0.999-1.011	0.075

CIMT: Carotid Intima-Media Thickness; HDL: High-density lipoprotein; LDL: Low-density lipoprotein; LV: Left ventricular; MACE: Major adverse cardiac events; SAA: Serum amyloid A; sICAM: Soluble intercellular adhesion molecule-1.

DISCUSSION

The present study showed that higher age, hsCRP, sICAM-1 levels and lower EF were independent predictors of MACE in participants with high cardiovascular risk compared to the control subjects with low cardiovascular risk. CIMT, QRS wave duration, serum levels of hsCRP, creatinine, uric acid, SAA protein, ApoB, sICAM-1, and frequency of MACE were higher in high risk group than in low risk group according to the SCORE risk. However, no significant association between eNOS gene polymorphisms and SCORE risk and MACE was detected.

Major adverse cardiovascular events including MI and stroke are the leading causes of death worldwide. In apparently healthy persons, cardiovascular risk is the most common result of multiple interacting risk factors (1). The overall risk is affected by demographic, clinical, genetic and environmental factors. The current assessment of the risk of developing CAD, based on a variety of scoring systems, is generally predicted on classical risk factors. However, these factors have limited sensitivities and specificities. Molecular biomarkers related to the pathophysiology of atherosclerosis may be useful in re-classifying patients into more appropriate risk categories, either in addition to or in conjunction with traditional risk factors, allowing for interventions before symptoms become manifest. This would allow for earlier treatment of subclinical CAD, with the potential to reduce morbidity and mortality. A number of non-invasive tools such as echocardiography, ultrasonography and ECG were shown to be applicable to predict cardiovascular events, as well (2-5,22).

Increasing age and male sex increase CVD risk and are fixed characteristics used to stratify risk assessments (1,23). Mean age was significantly higher in participants with MACE and age was independent predictor of MACE in our study. As to the reason of older age of the participants, there was no significant difference between genders in the study. The prognostic value of EF has been well established and is used in clinical practice. EF has incremental prognostic values in predicting cardiac death, and provides clinically useful risk stratification (24). It was significantly lower in participants with MACE, and was an independent predictor of MACE. All in all, we see that the results of the present study support the concerning literature data (24,25).

The mechanism of the association between inflammation and future cardiovascular events still remains cloudy. Inflammation is suggested to play a crucial role in atherogenesis and development of atherosclerotic plaques in coronary arteries (26). Proinflammatory cytokines have been isolated in atherosclerotic lesions, and shown to have pro-atherogenic feature (27,28). Additionally, inflammation may be associated with a rupture of preexisting atherosclerotic plaques.

High hsCRP levels are associated with atherosclerotic plaque rupture, which may be related to ongoing inflammation within the fibrous cap of the plaque (29-31). An association of hsCRP with risk for CVD has been described in numerous studies (32,33). Otherwise, in a recent study, levels of hsCRP were not independently related to incident vascular diseases events (34). In a study from primary care settings, one single measurement of hsCRP does not significantly improve the prediction of incident cardiovascular events (35). HsCRP was significantly higher in the high risk group compared to the low risk group, and it was an independent predictor of MACE in the population based study of MELEN.

SAA has been shown to be a predictor of cardiovascular events (36,37). Whereas, some studies found that this relationship may be dependent on other risk factors (38,39). Johnson et al reported that, SAA levels were strong predictors of cardiovascular events, independent of the presence of atherosclerotic risk factors, angiographic CAD and comorbid disease (26). ApoB showed a stronger association with CHD and CVD than cholesterol content measures (9,10). Thompson et al suggested that, ApoB can be substituted for LDL cholesterol; however it does not add more to the risk assessment (40). We showed that, ApoB and SAA were significantly higher in high risk group compared to the low risk group, which were also significantly higher in MACE than in non-MACE groups. Additionally, ApoB and SAA were significantly associated with MACE in univariate logistic regression analyses and had borderline significance in the multivariate

regression analysis. We think that, if the number of MACE would have been higher, these biomarkers might have independently predicted the rate of MACE.

sICAM-1 has been shown to be significantly higher in patients with stable and unstable angina compared with healthy controls (6). In another study, sICAM-1 levels were found to be significantly elevated in patients with unstable angina pectoris than in stable angina and healthy controls. These findings indicate a possible role of sICAM-1 as a marker of ongoing inflammation in the atherosclerotic process (41). Healthy subjects with the top quartile of the reference interval of sICAM-1 are at higher risk of developing MI than those in the lowest quartile (42). sICAM-1 was significantly higher in the risk group compared to the control, and sICAM-1 was an independent predictor of MACE in the present study.

There is a gradual increase in cardiac events and stroke risk with rising CIMT (1). CIMT is a strong predictor of future vascular events (43). CIMT was significantly higher in the risk group compared to the control. CIMT was significantly associated with MACE in univariate logistic regression analyses, though it lost its significance in the multivariate assessment.

While eNOS gene polymorphisms have been in association with myocardial infarction in the study of Hibi et al. (44) showed that there was no significant increase in the risk of AMI or the severity of coronary atherosclerosis among individuals with the genotype of the eNOS polymorphism. Granath et al. (45) found no evidence of an association between eNOS gene polymorphism and the risk of CAD or MI in an Australian-Caucasian population (46). According to the present outcome, there were no significant differences in eNOS (rs2070744), (rs1799983) and (rs7830) gene polymorphisms between high and very high risk group and low risk group according to SCORE risk charts. Additionally, we found no significant association between eNOS gene polymorphisms and MACE in Turkish patients.

Conclusion

Systolic blood pressure, heart rate, LV mass, CIMT, QRS wave duration, serum levels of hsCRP, creatinine, uric acid, SAA protein, ApoB, and sICAM-1 were significantly associated with SCORE risk. Age, EF, CIMT, hsCRP, ApoB, sICAM-1, SAA protein levels were significantly associated with MACE in univariate logistic regression analyses. Higher age, hsCRP and sICAM-1 levels and lower EF were independent predictors of MACE. There were no significant differences in eNOS gene polymorphisms between high and very high risk groups and low risk group according to the SCORE risk charts, and no significant association was detected between eNOS gene polymorphisms and MACE.

A clinical benefit can be provided by inflammatory risk parameters, particularly hsCRP and SICAM-1 in high and very high risk population with MI, stroke and death. Patients with high cardiovascular risk who have increased serum levels of hsCRP and sICAM-1 may be given a more aggressive medical treatment regime and more effective lifestyle to reduce MACE rates (47).

Declaration of interest*: There is no conflict of interest.*

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