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Genetic variations in E-selectin and ICAM-1: Relation to atherosclerosis

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A Study Design
B Data Collection
C Statistical Analysis
D Data Interpretation
E Manuscript Preparation
F Literature Search
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Summary

Background: This study aimed to investigate the association of both intercellular adhesion molecule-1 (ICAM-1) and endothelial cell adhesion molecule (E-selectin) polymorphisms using PCR technique and their role in the pathogenesis of atherosclerosis.

Material/Methods: The study enrolled 285 individuals, classified into 4 groups: 63 cerebrovascular atherosclerotic patients, 75 cardiovascular patients, 72 peripheral atherosclerotic patients and 75 normal healthy individuals.

Results: The frequency of the mutant AC genotype of E-selectin in peripheral, cerebral and cardiovascular atherosclerotic patients was significantly higher than in control subjects (29.17%, 28.53% and 28% *vs.* 8%, respectively). However, no significant difference was observed in the frequency of mutant CC allele between all atherosclerotic patients and control groups. The frequency of the mutant EE homozygotes of ICAM-1 in peripheral, cerebral and cardiovascular atherosclerotic patients was significantly higher compared to controls (45.8%, 42.9% and 36% *vs.* 12%, respectively). The frequency of EK of ICAM-1 showed no significant difference between atherosclerotic patients and the control group. The frequency of the mutant E allele of ICAM-1 was significantly higher in peripheral, cerebral and cardiovascular patients compared to controls (58.3%, 54.8% and 54% *vs.* 26%, respectively).

Conclusions: Ser 128Arg of E-selectin and the K469E of ICAM-1 polymorphisms may be involved in predisposition to atherosclerosis.

key words: **E-selectin • ICAM-1 • genetic predisposition • polymorphism**

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Background

Traditionally, atherosclerotic disease has been considered to be a typical disease of middle-age. Atherosclerosis is a life-threatening disease that affects critical organs, including the heart and brain [1]. The most common heart disease is coronary artery disease (CAD), which often appears as a heart attack [2]. The atherosclerotic process starts decades before the appearance of clinical symptoms (as myocardial infarction, cerebral vascular accident and peripheral vascular disease).The progression and severity of the atherosclerotic process are related to the presence, number, magnitude and duration of a series of risk factors [3]. Indeed, atherosclerosis is described as an inflammatory disease [4].

Atherosclerosis is a disease characterized by the accumulation of lipid-laden cells in the arterial wall. This disease results in lesions within the artery, which may grow into the lumen, restricting blood flow and, in critical cases, can rupture, causing complete, sudden occlusion of the artery, resulting in heart attack, stroke and possibly death [5].

Development and progression of atherosclerosis involves recruitment and binding of circulating leucocytes to areas of inflammation within the vascular endothelium mediated by a diverse array of cellular adhesion molecules [6].

Genetic factors appear to be important in the pathogenesis of cardiovascular and cerebrovascular disease. Adhesion molecules, such as members of the selectin family, participate in the interaction between leukocytes and the endothelium. They are also involved in the pathogenesis of atherosclerosis [7].

E-selectin is a surface glycoprotein molecule involved in adhesion of circulating leukocytes in activated endothelium and plays an important role in the inflammation process. Inflammation is one of the earliest events in the pathogenesis of atherosclerosis. A serine-to-arginine (S128R) polymorphism in E-selectin has been reported at increased frequency in patients with atherosclerosis [8].

Intercellular adhesion molecule-1 (ICAM-1) plays a crucial role in lymphocyte migration and activation and is considered important in the pathogenesis of atherosclerosis. K469E is a common polymorphism of the ICAM-1 gene, with potential functional significance [9].

ICAM-1 interacts with leukocyte integrins and promotes the atherosclerotic process at the surface of endothelial cells [10].

There is an association between ICAM-1 gene K469E polymorphism and ischemic stroke, and the E allele may be a genetic risk factor of ischemic stroke, in which the ICAM-1 E allele carriers may have up-regulated expression of ICAM-1 and thus are at higher risk of ischemic stroke [11].

Adhesion molecules mediate attachment and transendothelial migration of leucocytes as a critical step in pathogenesis of atherosclerosis.

The current study aimed to investigate the association of both ICAM-1 gene K469E polymorphism and E-selectin S128R polymorphism and their role in the pathogenesis of atherosclerosis.

Material and Methods

The current study included 285 individuals classified into 4 groups (Table 1). All patients were recruited from Kasr El-Aini Hospital from June 2008 to June 2010. For all atherosclerotic patients, complete medical history and clinical data were recorded, including family history of atherosclerosis, smoking behavior, hypertension and diabetes mellitus status. Seventy-five age- and sex-matched individuals were enrolled as normal controls. Patients were proven to have CAD angiographically or by history of myocardial infarction; cerebral ischemic event was documented by computerized tomography (CT) scan or magnetic resonance imaging (MRI) of the brain; and patients with chronic ischemic rest pain, ulcers or gangrene attributable to objectively proven peripheral occlusive arterial disease (PAOD) were considered to have critical limb ischemia (CLI).

Morning blood samples were collected after 12 hours of fasting; 5 ml venous blood samples were withdrawn from each participant in the study and divided into 3 tubes under aseptic conditions after obtaining patient written consent. Two milliliters of blood were added to sodium fluoride and centrifuged; the plasma was separated and used for determination of fasting plasma glucose (FPG). Two ml of blood were left to stand for 10 minutes to clot and then were centrifuged; the serum was separated for determination of lipid profile, total cholesterol, HDL-C, LDL-C and triglycerides. One ml of blood was added to EDTA and stored at -80^{0}C to be used for detection of polymorphism of E-selectin and ICAM-1 genes.

DNA was extracted from whole blood using DNA extraction kit (Bio Basic Inc., Canada) and stored at –80°C in aliquots until required. Detection of polymorphism in both E-selectin and ICAM-1 genes was carried out using polymerase chain reaction (PCR) amplification using Taq polymerase enzyme and T-Gradient thermal cycler (Biometra, Germany). The sequence of primers used for amplification of E-selectin at codon 128 was 5'ATGGCACTCTGTAGGACTGCT3' (forward) and 5'GTCTCAGCTCACGATCACCAT3' (reverse). The sequence of primers used for amplification of ICAM-1 was forward primer, 5'GGAACCCATTGCCCGAGC3' and reverse primer, 5'GGTGAGGATTGCATTAGGTC3'.

Analysis of S128R polymorphism in codon 128 of the E-selectin gene

The Ser128Arg polymorphism is an adenine-to-cytosine substitution for cDNA position 561, resulting in an amino acid replacement of serine by arginine at position 128. The Ser128Arg polymorphism was determined by PCR amplification. Denaturation was carried out at 95°C for 5 minutes and PCR reaction was repeated for 35 cycles under the following conditions: denaturation at 95°C for 1 minute, annealing at 54°C for 45 seconds and extension at 72°C for 1 minute. Then final extension cycle was carried out at 72°C for 7 minutes. The resulting 357 bp amplification product was digested with the Pst I restriction enzyme. DNA from heterozygous individuals was cleaved into 3 fragments of 357 bp, 219 bp, and 138 bp. The products of the digestion process were separated by electrophoresis on an agarose gel and visualized by ethidium bromide staining (Figure 1).

Table 1. The demographic data of different studied groups.

Parameter		Control (n=75)	Peripheral (n=72)	Cerebral (n=63)	Cardiovascular (n=75)
Age (years)	Range	48–66	45–69	48–68	41–68
	Mean ±SE	59.52±1.01	58.21±1.38	58.62±1.39	56.68±1.37
Sex	Female	42 (56%)	30 (41.7%)	33 (52.4%)	24 (32%)
	Male	33 (44%)	42 (48.3%)	30 (47.6%)	51 (68%)
HTN	No	66 (88%)	36 (50%)	48 (76.2%)	18 (24%)
	Yes	9 (12%)	36 (50%)*	15 (23.8%)*	57 (76%)*
FH	No	75 (100%)	39 (54.2%)	36 (57.1%)	42 (56%)
	Yes	0 (0%)	33 (45.8%)*	27 (42.9%)*	33 (44%)*
Smoking	No	60 (80%)	27 (37.5%)	30 (47.6%)	33 (44%)
	Yes	15 (20%)	45 (62.5%)*	33 (52.4%)*	42 (56%)*

Data were expressed as mean ± standard error or number (%). HTN – hypertension; FH – family history.

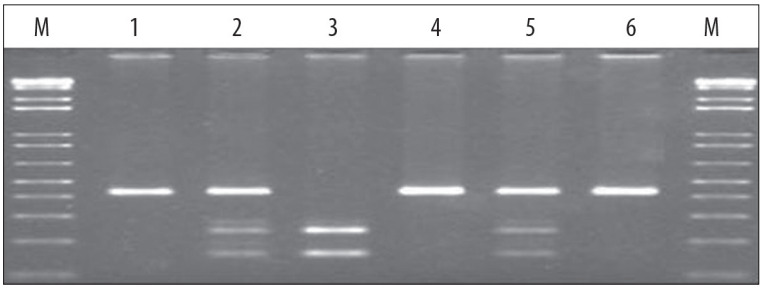

Figure 1. PCR amplification of E-Selectin followed by Pst I restriction enzyme digestion. Lane M – Molecular DNA marker; Lanes 1,4,6 – CC genotype showing positive band at 357 bp; Lanes 2, 5 – AC genotype showing 3 bands at 357, 219, and 138 bp; Lane 3 – AA genotype showing positive bands at 219 and 138.

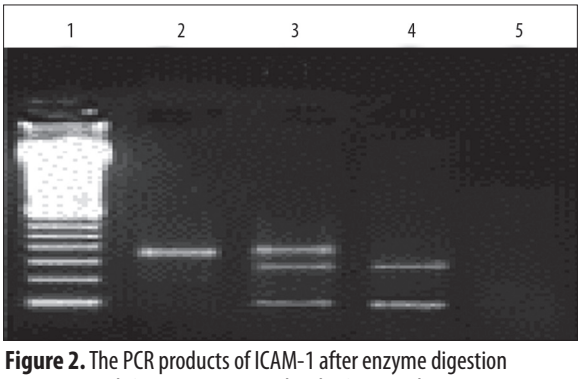

Figure 2. The PCR products of ICAM-1 after enzyme digestion with BstUI. Lane 1 – Molecular DNA marker; Lane 2 – KK genotype showing positive band at 223 bp; Lane 3 – KE genotype showing 3 bands at 223, 136, and 87 bp; Lane 4: EE genotype showing positive bands at 136 and 87 bp; Lane 5: negative control (no DNA).

Analysis of K469E polymorphism in exon 6 of the ICAM-1 gene

The presence of the C→T conversion at codon 469 of the ICAM-1 gene in exon 6 was determined by PCR amplification. Denaturation was carried out at 95°C for 5 minutes and PCR reaction was repeated for 35 cycles under the following conditions: denaturation at 95°C for 1 minute, annealing at 54°C for 45 seconds and extension at 72°C for 1 minute. Then final extension cycle was carried out at 72°C for 7 minutes. The resulting 223 bp amplification product was digested with the Bst U I restriction enzyme. The wild-type allele K had no cleavage site, whereas the PCR product was cleaved into 2 fragments of 136 bp and 87 bp in the presence of the mutant E allele. DNA from heterozygous individuals was 3 fragments 223 bp, 136 bp, 87 bp. The products of the digestion process were separated by electrophoresis on an agarose gel and visualized by ethidium bromide staining (Figure 2).

Statistical analysis

The SPSS version 12 statistical package was used in data analysis. Descriptive statistics in the form of mean and standard error for parametric data were used, while frequency and percentage were used to describe non-parametric data. Variables were described as odds ratio (OR) with 95% confidence interval (CI). P value less than or equal to 0.05 was considered significant and less than 0.01 was considered highly significant.

Results

Table 1 showed the characteristics of all studied groups. Regarding the mean serum level of lipid profile, the total cholesterol, triglycerides (TG) and low-density lipoprotein (LDL-C) were significantly higher in the cerebral and cardiovascular patients than in the controls, while that of TG

Table 2. Comparison between the mean values of FPG and lipid profile among different studied groups (mean ±SE).

Parameter	Control (n= 75)	Peripheral (n=72)	Cerebral (n=63)	Cardiovascular (n=75)
FPG (mg/dl)	90.28±2.04	182.75±19.84*	151.67±14.47*	117.16±6.98
Cholesterol (mg/dl)	174.72±5.96	186.38±9.82	199.62±11.35*	248.52±6.80*
Triglyceride (mg/dl)	98.40±5.09	145.0±16.12*	164.86±12.24*	189.72±7.99*
HDL-C (mg/dl)	61.96±3.74	39.21±1.10*	32.57±1.05*	30.76±1.13*
LDL-C (mg/dl)	85.0±5.43	125.66±9.30*	144.05±5.69*	102.68±2.51*

* Significant difference from control group at $p<0.05$. FPG – fasting plasma glucose; LDL-C – low density lipoprotein cholesterol; HDL-C – high density lipoprotein cholesterol.

Table 3. Genotype and allele frequencies of ICAM-1 in different studied groups.

Parameter	Genotypes			Alleles	
	KK	EK	EE	K	E
Control (n=75)	45 (60%)	21 (28%)	9 (12%)	111 (74%)	39 (26%)
Peripheral (n=72)	21 (29.2%)[a]	18 (25%)	33 (45.8%)[a]	60 (41.7%)[aa]	84 (58.3%)[aa]
Cerebral (n=63)	21 (33.3%)[b]	15 (23.8%)	27 (42.9%)[b]	57 (45.2%)[bb]	69 (54.8%)[bb]
Cardiovascular (n=75)	21 (28%)[c]	27 (36%)	27 (36%)[c]	69 (46%)[cc]	81 (54%)[cc]

All symbols were relative to control group.
[a] χ^2=7.481; p=0.006; OR=0.273; CI 95%=0.091–0.817. [b] χ^2=5.812; p=0.016; OR=0.296; CI 95%=0.097–0.908.
[c] χ^2=5.812; p=0.016; OR=0.296; CI 95%=0.097–0.908.
[aa] χ^2=10.522; p=0.001; OR= 0.251; CI 95%=0.107–0.589. [bb] χ^2=7.928; p=0.005; OR=0.290; CI 95%=0.121–0.697.
[cc] χ^2=8.167; p=0.004; OR=0.299; CI 95%=0.129–0.695.

and LDL-C were significantly high in peripheral patients. High-density lipoprotein (HDL-C) was significantly lower in patients than in controls. Moreover, the mean fasting plasma glucose (FPG) level was significantly higher in peripheral and cerebral patients than in controls.

The distribution of ICAM-1 genotypes and alleles in different studied groups is shown in Table 2. The frequency of the mutant EE genotype was significantly higher in the peripheral, cerebral and cardiovascular patients (45.8%, 42.9% and 36%, respectively) than in controls (12%). On the other hand, the frequency of EK genotype failed to show any significant difference between patients and controls.

Moreover, the frequency of the mutant E allele was significantly higher in peripheral, cerebral and cardiovascular patients (58.3%, 54.8% and 54%, respectively) than in controls (26%).

Table 3 shows the distribution of E-selectin genotypes and alleles in different studied groups. The frequency of the heterozygous AC genotype was significantly higher in peripheral, cerebral and cardiovascular patients (29.17%, 28.53% and 28%, respectively) in relation to controls (8%), while the frequency of the CC genotype failed to show any statistically significant difference between the patients and controls.

Moreover, the frequency of the mutant C allele was higher in all studied groups – peripheral, cerebral and cardiovascular – as it reached 18.75%, 19.05% and 18%, respectively, compared to control subjects (4%).

The different clinical and biochemical values of the studied patients were categorized according to different ICAM-1 genotypes; KK, EK and EE (Table 4). According to the sex distribution, the frequency of the mutant EE (65.5%) was significantly higher in relation to KK (52.4%) in male atherosclerotic patients. As regards hypertension, the frequency of the mutant EE (65.5%) was significantly higher in hypertensive atherosclerotic patients as compared to KK (33.3%). Regarding smoking behavior and family history, the frequency of the mutant EE genotypes were significantly higher in atherosclerotic patients in relation to both EK and KK genotypes (75.9% *vs.* 30% and 42.9%, and 62% *vs.* 35% and 28.6%, respectively).

The mean serum level of cholesterol increased significantly in patients carrying EE and EK genotypes compared to KK (232.28, 218.15 *vs.* 179.95 mg/dl, respectively). The mean serum levels of LDL-C and triglycerides increased significantly in EE genotype as compared to KK genotype, while there was no significant difference in the mean serum level of HDL-C in different ICAM-1 genotypes.

The different clinical and biochemical values of the studied patients are described in Table 5 according to different E-selectin genotypes; AA and AC + CC. Regarding smoking,

Table 4. Genotype and allele frequencies of E-selectin in different studied groups.

Parameter	Genotypes			Alleles	
	AA	AC	CC	A	C
Control (n=75)	69 (92%)	6 (8%)	0 (0%)	144 (96%)	6 (4%)
Peripheral (n=72)	48 (66.7%)[a]	21 (29.17%)[a]	3 (4.13%)	117 (81.25%)[aa]	27 (18.75%)[aa]
Cerebral (n=63)	42 (66.7%)[b]	18 (28.53%)[b]	3 (4.77%)	102 (80.95%)[bb]	24 (19.05%)[bb]
Cardiovascular (n=75)	51 (68%)[c]	21 (28%)[c]	3 (4%)	123 (82%)[cc]	27 (18%)[cc]

All symbols were relative to control group.
[a] χ^2=5.098; p=0.04; OR=5.750; CI 95%=1.076–30.720. [b] χ^2=4.805; p=0.028; OR= 5.750; CI 95%=1.044–31.669.
[c] χ^2=4.758; p=0.029; OR=5.412; CI 95%=1.017–28.791.
[aa] χ^2=4.542; p=0.033; OR=4.800; CI 95%=0.964–23.901. [bb] χ^2=4.290; p=0.038; OR=4.800; CI 95%=0.940–24.517.
[cc] χ^2=4.255; p=0.039; OR=4.571; CI 95%=0.919–22.730.

Table 5. Clinical values in different atherosclerotic patients with different ICAM-1 genotypes.

Parameter		KK (n=63)	EK (n=60)	EE (n=87)
Sex	F	30 (47.6%)	27 (45%)	30 (34.5%)
	M	33 (52.4%)	33 (55%)	57 (65.5%)*
Age		59.33±1.47	57.85±1.49	56.62±1.22
Hypertension	No	42 (66.7%)	36 (60%)	30 (34.5%)
	Yes	21 (33.3%)	24 (40%)	57 (65.5%)*
Smoking	No	36 (57.1%)	42 (70%)	21 (24.1%)
	Yes	27 (42.9%)	18 (30%)	66 (75.9%)*,**
Family history	No	45 (71.4%)	39 (65%)	33 (37.9%)
	Yes	18 (28.6%)	21 (35%)	54 (62.1%)*

* Significant difference from KK at p<0.05; ** significant difference from EK at p<0.05.

Table 6. The biochemical values in all studied patients with different ICAM-1 genotypes.

Parameter	KK (n=63)	EK (n=60)	EE (n=87)
FPG (mg/dl)	147.76±14.40	159.85±18.88	144.83±14.12
Cholesterol (mg/dl)	179.95±8.04	218.15±13.32*	232.28±8.49*
Triglyceride (mg/dl)	133.48±10.30	162.15±14.89	194.45±10.79*
HDL (mg/dl)	33.95±1.54	33.85±1.68	34.62±0.98
LDL (mg/dl)	117.84±7.43	116.51±7.84	131.14±6.57*

* Significant difference from KK at p<0.05.

hypertension and family history, the frequencies of the mutant genotypes were significantly higher in patients as compared to AA genotypes. The mean serum levels of cholesterol and triglycerides were significantly higher in AC+CC genotypes (233.65 *vs.* 201.11, and 190 *vs.* 155.64, respectively) as compared to AA genotype.

Multiple logistic regression analysis was performed to evaluate the interaction between ICAM-1, E-selectin genotypes and other classical risk factors in determination of predictors of atherosclerosis (Tables 6–9). This analysis showed that independent risk factors were smoking (OR=0.135, 95%CI=0.05–0.34), hypertension (OR=0.59, 95% CI=0.47–0.72), total cholesterol (OR=0.059, 95% CI=0.008–0.442), triglycerides(OR=0.023,

Table 7. Comparison between ICAM-1 genotypes and laboratory data in peripheral, cerebral and cardiovascular groups.

Parameter	Peripheral (72)			Cerebral (63)			Cardiovascular (75)		
	KK (n=21)	EK (n=18)	EE (n=33)	KK (n=21)	EK (n=15)	EE (n=27)	KK (n=21)	EK (n=27)	EE (n=27)
FPG (mg/dl)	179.71±31.84	182.17±45.05	185.00±32.14	162.43±19.87	191.2±47.93	121.33±10.91	101.14±9.79	127.56±12.59	119.22±12.36
Cholesterol (mg/dl)	163±11.61	175.67±20.97	207.09±15.13*	160.71±10.32	205.4±34.86	226.67±10.51*	216.14±8.77	253.56±9.87*	268.67±9.31*
TG (mg/dl)	101±13.99	139.17±42.69	176.18±22.57*	117.43±12.59	162.8±33.62	202.89±8.24*	182±9.79	177.11±4.98	208.33±19.54
HDL (mg/dl)	41.71±1.82	42.0±1.65	36.09±1.51*,**	35.22±0.70*	31.0±3.03	30.29±1.74	29.86±1.50	30.0±1.8	32.22±2.39
LDL (mg/dl)	116.23±19.25	105.7±15.13	142.55±13.2	136.29±8.3	160.4±11.51	141.0±9.29	101.0±2.99	99.33±3.42	107.33±5.60

* Significant difference from KK at $p<0.05$; ** significant difference from EK at $p<0.05$.

Table 8. The biochemical values in all studied patients with different E-selectin genotypes.

Parameter	AA (n=141)	AC+CC (n=69)
FBS (mg/dl)	153.55±11.71	142.74±13.25
Cholesterol (mg/dl)	201.11±7.59	233.65±10.77*
Triglyceride (mg/dl)	155.64±8.74	190.00±13.02*
HDL (mg/dl)	34.02±1.02	34.57±1.10
LDL (mg/dl)	122.17±5.28	124.60±6.95

* Significant difference from AA at $p<0.05$.

Table 9. Comparison between E-selectin genotypes regarding to laboratory data in peripheral, cerebral, cardiovascular patients.

Parameter	Peripheral		Cerebral		Cardiovascular	
	AA (n=48)	AC+CC (n=24)	AA (n=42)	AC+CC (n=21)	AA (n=57)	AC+CC (n=24)
FBS (mg/dl)	179.25±26.76	189.75±28.26	165.14±19.96	124.71±13.67	119.82±8.66	111.50±12.28
Cholesterol (mg/dl)	182.75±11.15	193.63±20.18	190.14±15.63	218.57±11.77	244.00±7.02	258.13±15.35
TG (mg/dl)	133.25±12.32	168.50±19.02*	147.86±16.18	198.86±8.63*	183.12±4.80	203.75±23.03
HDL (mg/dl)	40.50±1.36	36.63±1.60*	31.43±1.43	34.86±0.86*	30.06±1.18	32.25±2.55
LDL (mg/dl)	121.75±11.44	133.48±16.69	150.00±6.79	132.14±9.41	99.65±2.02	113.14±6.25*

* Significant difference from AA at $p<0.05$.

95% CI=0.003–0.165), EE genotype of ICAM-1 (OR=0.204, 95% CI=0.103–0.403) and AC+CC genotype of E-selectin (OR=0.087, 95%CI=0.02–0.36). However, presence of diabetes and serum LDL and HDL were insignificant variables for atherosclerosis.

Discussion

Atherosclerosis is considered to be a chronic inflammation process. Systematic and local inflammation participates in the whole process of atheroma occurrence, progression and erosion [12].

An initial finding in this study is the prevalence of atherosclerotic risk factors, including hypertension, smoking and family history in the studied groups – peripheral, cerebral and cardiovascular atherosclerotic patients – as compared to controls. These results agree with others [9,13] who observed that hypertension and smoking were significantly

more frequent in peripheral atherosclerotic patients and ischemic cerebrovascular patients than in controls.

Moreover, our study revealed a significant increase in lipid profile of patients compared to controls. These results are in agreement with other studies reporting significantly more hypercholesterolemia in cerebrovascular patients as compared to controls [11,13].

In addition, the present study showed a significant decrease in HDL-C level in patients as compared to controls. This finding is in agreement with other studies that reported significantly lower levels of HDL-C in coronary artery disease patients [14–16].

In the present study the frequency of the mutant heterozygous AC genotype of the E-selectin gene was significantly higher in patients than in control. Our findings are in concordance with the earlier studies of Wenzel et al., [17] that investigated the E-selectin polymorphism (Ser 128 Arg) in a German population and found a higher Arg 128 prevalence among 97 patients ⩽50 years old with severe coronary and peripheral atherosclerosis than among control subjects.

Our findings could be explained on the basis that the relationship between the E-selectin S128R polymorphism and atherosclerotic disease might reflect an amplified inflammatory response resulting from the action of altered selectin molecules containing the serine-arginine mutation [18].

Mechanistic studies have indicated that the substitution of arginine for serine exhibits dramatically decreased binding specificity while increasing affinity for additional ligands, resulting in a 2- to 3-fold increase in cellular adhesion. The E-selectin 128R allele may thus increase leukocyte adherence to activated endothelium in areas susceptible to atherosclerotic plaque formation, thereby contributing to the progression of atherosclerosis and CAD [15].

Another possible mechanism for the participation of E-selectin polymorphism as a potential risk factor for genetic susceptibility to myocardial infarction has been investigated by Yoshida et al., [19] who suggested the possibility that Ser128Arg mutation in the E-selectin may play a functional role through constitutive oligomerization or via induction of a conformational change. Notably, polymorphic S128R-E-selectin exhibited constitutive phosphorylation without leukocyte adhesion, which indicates that a potential conformational change of E-selectin attributable to the S128R polymorphism may influence the intracellular signaling pathway of E-selectin. Thus, the constitutive activation observed may be a result of dysregulation of E-selectin-dependent signaling in endothelial cells, which may play a role in the pathogenesis of MI.

These results were supported also by Yoshida et al., [19] who reported significantly higher frequencies of AC genotype in Japanese patients as compared to controls, which indicated that S128R polymorphism was associated with myocardial infarction and might determine susceptibility to CAD. This could be attributed to the polymorphism, which can functionally alter leukocyte-endothelial interactions. Similarly, Shaker et al. [20] found that the distribution of the mutant AC genotype was significantly higher in peripheral atherosclerotic patients than in controls in an Egyptian population.

Regarding the distribution of E-selectin alleles, the frequency of the mutant C allele was significantly higher in patients than in controls. The involvement of E-selectin gene polymorphism had been studied extensively in several ethnic groups. Abu-Amero et al. [21] in CAD patients of Saudi Arabia, Li et al. [22] in Central China, and Tripathy et al. [16] in India, who considered the C allele as a potential risk factor for myocardial infarction and CAD, as this mutation was reported to change the binding specificity of E-selectin.

Our findings are in concordance with the earlier studies of Ye et al., [23] who reported that the mutant allele in the E-selectin gene was significantly associated with CAD in white Americans. This study detected the S128R mutation based on the observation that it is due to the transversion of nucleotide A561 to C, which abolishes a pst I recognition sites. It indicated that the C allele is associated with early-onset CAD.

Similarly, Hattori et al., [13] revealed a significant association between A561C polymorphisms in the E-selectin gene and ischemic cerebrovascular disease without diabetes mellitus and hypercholesterolemia in a Japanese population, as well as the presence of the C, allele was independent of the acquired risk factors.

Adhesion molecules, like members of the selectin family, especially E-selectin, participate in the interaction between leukocytes and endothelium. The polymorphism at codon 128in the epidermal growth factor-like domain results in an adenine-to-cytosine substitution that causes amino acid exchange from serine to arginine and has a profound effect on ligand recognition and binding [6].

This could be explained on the basis that A561C polymorphism could facilitate the attachment between neutrophils and endothelial cells in the first stage of atherosclerosis and increase the expression of E-selectin in the human body, thus A561C is a genetic risk factor in atherothrombotic cerebrovascular disease [13].

Our study found an increased frequency of E-selectin S128R in the high risk patients' subgroup in analysis stratified by well-established risk factors for atherosclerosis, including presence of family history and elevated serum levels of cholesterol and triglycerides in comparison with homozygous AA genotype.

In contrast, Endler et al. [24] found no statistically significant difference between the E-selectin genotypes and CAD risk factors regarding hypertension and smoking in Austrian subjects.

The present study also found the K469E polymorphism in the ICAM-1 gene in peripheral, cerebrovascular and cardiovascular atherosclerotic patients. ICAM-1 is produced as a consequence of inflammation. ICAM-1 could mediate monocytes, macrophages, T lymphocytes and platelets to assemble and adhere to the vascular wall, which plays a key role in the pathogenesis of atherosclerosis [25]. The mechanisms of the proinflammatory effects of ICAM-1 on endothelial cells might be that ICAM-1 binds and interacts with leukocyte integrin receptors such as lymphocyte function-associated antigen-1 LFA-1. This provides an adaptive alternative in the adhesion process between circulating cells and

the endothelium, leading to the attachment of leukocytes to endothelial cells and the transendothelial migration of leukocytes into the intima and thus to the accumulation of leukocytes in the vascular wall [26].

In addition to its role in cell-to-cell adhesion by integrin-ICAM-1 interaction, ICAM-1 also serves as a receptor for soluble fibrinogen. It has been reported that fibrinogen mediates leukocyte adhesion to the endothelium through an ICAM-1 dependent pathway. ICAM-1 might therefore play a major role in the pathogenesis of endothelial dysfunction and CHD [14].

The frequency of the homozygous mutant EE genotype was significantly higher in patients than in controls. These results might be explained on the basis that ICAM-1 induces the expression of several proinflammatory cytokines, including IL-6, which in turn induces the synthesis of several acute phase proteins, thus promoting and maintaining the inflammatory phenotype [14].

Our results are strongly supported by those reported by Gaetani et al. [9] in an Italian population and Shaker et al. [20] in an Egyptian population, who found that the frequency of the mutant EE genotype of ICAM-1 gene in peripheral arterial patients was significantly higher than in controls. In addition, Pola et al. [27] reported that the frequency of the EE genotype of ICAM-1 genotypes was 2-fold higher in stroke patients than in controls.

Our study found that frequencies of the mutant E allele were significantly higher in patients than in controls. Our results are in accordance with those of others [11,22] who reported that the mutant E allele of ICAM-1 gene might be considered a genetic risk factor for ischemic stroke as well as Longoni et al. [28] who found high K469E polymorphism expression in CAD patients. Thus, an association between EE genotype and CAD indicates the proinflammatory risk factor of K469E polymorphism.

Our results support the hypothesis that ICAM-1 can mediate adhesion of circulating leukocytes to the activated endothelium and that it plays roles in firm attachment and in transendothelial migration of leukocytes [14].

These biological findings are supported by the hypothesis that the ICAM-1 gene polymorphism located on exon 6 and leading to amino acid substitution could alter ligand binding or the stability of the multimeric ICAM-1 on the cell surface, and therefore alter signal transduction. The mutant genotype of ICAM-1 gene is associated with greater cell expression of the ICAM-1 gene, which in turn leads to greater adhesion of leucocytes.

The E469 allele of the ICAM-1 could alter the structure and function of the ICAM-1 D5 region, which has been implicated in the ICAM-1 molecular dimerization and adhesion function, and changed the combination of ICAM-1 ligands such as LFA-1 and the complement receptor 3 (CR3). The combination of alterations contributed to leukocytes adhering to the vascular wall more easily, which caused the atherosclerosis [29].

According to our results, the frequency of the EE genotype was significantly higher than other genotypes (EK and KK) as regards atherosclerotic risk factors such as smoking, familial history and hypertension, as well as hypercholesteremia and elevated triglycerides in patients.

These results are in agreement with those reported by Sarecka-Hujar et al., [10] who found relations between ICAM-1 polymorphism and traditional risk factors of CAD that could increase the risk of the disease. Smoking is an established risk factor for CAD, myocardial infarction and sudden cardiac death. Cigarette smoking has been shown to be associated with elevated levels of circulating ICAM-1 and concentrations of other markers of endothelium dysfunction such as E-selectin [31].

This could be explained on the basis that nicotine increases the production of ICAM-1 on the surface of endothelial cells via a specific pathway involving protein kinase C (PKC) and p-38 mitogen-activated protein kinase (p38 MAPK)-mediated activation of nuclear factor (NF)-kappa B [32]. Moreover, Halvorsen et al. [33] demonstrated that smoking cessation was implicated in decreased level of ICAM-1.

However, some authors have not found any relationship between the K469E polymorphism of ICAM-1 gene and the occurrence of CHD and MI. Mc Glinchey et al. [34] found no association between the ICAM-1 K469E polymorphism and CHD in a well-defined Irish population; Aminian et al. [35] failed to find any significant differences between CHD patients and controls regarding KK genotype, despite of their high frequencies in CHD patients in a population from Fars province, Iran; and a Slovenian study reported that the K469E polymorphism of the ICAM-1 gene was not associated with MI in subjects with diabetes mellitus [36].

Other studies have reported a linkage between K469E polymorphism of ICAM-1 and coronary heart disease and found that the frequency of KK genotypes and K alleles were significantly higher than in controls, and that the K allele might serve as a genetic risk factor of CHD in a Chinese population [37]. Similarly, Lu et al. [38] concluded that the K allele, in conjunction with smoking, might be a genetic factor influencing the risk of CHD in a Han population in China.

These discrepancies may be due to different population characteristics or different interactions of the genetic background with environmental factors.

Logistic multiple regression analysis showed that the EE genotype of ICAM-1 and AC+CC genotypes of E-selectin were independent risk factors for atherosclerosis. Other independent risk factors were hypertension, smoking, family history and hypercholesterolemia.

The results of our study support the hypothesis that polymorphisms of genes encoding inflammatory molecules such as S128R of E-selectin and K469E of ICAM-1 are significantly and independently associated with atherosclerosis. Further studies are needed to validate these results and assess the relative importance of these inflammatory markers on the progression of atherosclerosis.

Conclusions

The frequencies of the Ser128Arg polymorphism of the E-selectin gene and K469E polymorphism of the ICAM-1

gene were significantly higher in peripheral, cardiovascular and cerebrovascular atherosclerotic patients compared to controls. This supports the hypothesis that the K469E polymorphism of ICAM-1 is important in the pathophysiology of vascular diseases and may be important in the pathogenesis of ischemic stroke. The E-selectin polymorphism may account for the pathogenesis of myocardial infarction and cerebrovascular diseases.

Our results suggest that both the Ser128Arg polymorphism of E-selectin gene and the K469E polymorphism of ICAM-1 may be involved in predisposition of atherosclerosis. However, these polymorphisms can explain only a small part of genetic susceptibility to atherosclerosis. Further studies are needed to characterize the molecular mechanisms by which E-selectin and ICAM-1 are involved in susceptibility to atherosclerosis.

The authors of this manuscript have certified that they comply with the Principles of Ethical Publishing in the International Journal of Cardiology [39].

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