K469E polymorphism of the intercellular adhesion molecule-1 gene in Egyptians with coronary heart disease

Amal A. Mohamed,¹ Laila Rashed,² Hoda Amin,³ Manal Abu-Farha,³ Soheir Abu El Fadl,³ Sameh Pahkhoumd⁴

From the ¹Department of Clinical and Chemical Pathology, ²Department of Biochemistry, ³Department of General Medicine, ⁴Department of Cardiology, Faculty of Medicine, Cairo University, Egypt

Correspondence: Amal A. Mohamed · Department of Clinical and Chemical Pathology, Faculty of Medicine, Cairo University, Egypt · T: +2 0102412882 · amal_abd_elwahab@yahoo.com · Submitted: January 2010 Accepted: June 2010

Ann Saudi Med 2010; 30(6): 432-436
PMID: 20940515 DOI: 10.4103/0256-4947.71061

BACKGROUND AND OBJECTIVES: The initial step in atherosclerosis is the adhesion of leukocytes to activated endothelial cells mediated by intercellular adhesion molecule-1 (ICAM-1). This study aimed to investigate the association of K469E polymorphism of the ICAM-1 gene and soluble ICAM-1 (sICAM-1) serum level with coronary heart disease (CHD) in Egyptian subjects.

PATIENTS AND METHODS: Using a case-control design, we studied 100 patients with CHD, including 73 patients with acute myocardial infarction (MI) and 27 with unstable angina (UA). The control group consisted of 50 healthy subjects with normal left ventricular function. All participants were genotyped for the ICAM-1 polymorphism by the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. Serum sICAM-1 was measured by enzyme-linked immunoassay (ELISA).

RESULTS: In CHD patients, the frequencies of K genotype (KK and EK) were significantly higher when compared to controls (P<.001) and were associated with an increased risk of disease development (OR=3.8, 95% CI: 1.7 to 8.5; P=.001). K genotype frequencies in patients with MI showed no significant difference when compared to patients with UA (P=.121). Serum sICAM-1 levels were comparable between CHD patients and controls (P=.37) and between MI and UA patients (P=.23). There were no significant differences in sICAM-1 levels among patients with different genotypes (P=.532). Men presented with higher sICAM-1 levels than women (P=.004).

CONCLUSION: ICAM-1 gene polymorphism in codon 469 is associated with a risk for CHD development in Egyptian subjects. Serum sICAM-1 is not influenced by this polymorphism and is not necessarily elevated in CHD.
The K469E polymorphism of the ICAM-1 gene, which involves a change from glutamine (E) to lysine (K) in the ICAM protein in exon 6 codon 469, has been found to be related to inflammatory diseases and atherosclerosis. The aim of this study was to investigate the association of K469E polymorphism of the ICAM-1 gene and sICAM-1 serum level with coronary heart disease (CHD) in Egyptian subjects.

**PATIENTS AND METHODS**

The study population consisted of 100 patients with acute CHD, including 73 patients with acute myocardial infarction (MI) and 27 patients with unstable angina (UA) (mean [standard deviation] age: 52.6 [8.29] years; and male/female ratio: 87/13). They were recruited from the Department of Internal Medicine and critical care unit of Cairo University Hospitals in the period from October 2008 to June 2009. The diagnosis of CHD was based on medical history, physical examination, typical electrocardiographic changes, increases in serum cardiac enzymes activities and coronary angiography. Fifty healthy subjects with normal left ventricular function constituted the control group (mean [SD] age: 53.9 [6.2] years, and male/female ratio: 38/12). Subjects with any inflammatory condition were excluded. Blood samples were collected after a fast of 12 to 14 hours from all subjects after obtaining oral consent. Serum and EDTA samples were stored at –20°C until assay time.

Assay of serum total cholesterol, HDL and triglycerides was performed on automated analyzer, Hitachi 917; commercial kits were supplied by Roche Diagnostics (Boehringer Mannheim, GmbH D-68298, Mannheim, Germany). The LDL level was calculated using the Friedwald equation. Soluble ICAM-1 levels in serum were measured by solid-phase sandwich enzyme-linked immunoassay (ELISA) kit supplied by Thermo Scientific Life Science, USA.

**Molecular analysis of K469E polymorphism of ICAM-1 gene**

DNA extraction was performed using the standard salting-out technique. Genotype analysis was performed using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. The sense primer was 5’-GGA ACC CAT TGC CCG AGC -3’, and the antisense primer was 5’- GGT GAG GAT TGC ATT AGG TC -3’. PCR amplification of a 223-bp fragment of exon 6 was performed in 50-µL reaction mixture consisting of 67 mM Tris-HCl (pH, 8.8), 1 mM magnesium chloride, 10 mM dNTPs, 5 pmol of each primer, 100 ng of extracted DNA and 2.5 units of Taq DNA polymerase (Amersham Pharmacia Biotech.).

Thermal cycling was carried out with an initial 95°C denaturation step for 10 minutes, followed by 35 cycles of denaturation for 1 minute at 95°C, annealing for 1 minute at 58°C, extension for 1 minute at 72°C and a final extension step at 72°C for 10 minutes. The PCR product was then digested with the restriction enzyme BstU I (Amersham Pharmacia Biotech., USA) for 12 hours at 37°C. The fragment length of the KK genotype was 223 bp; the fragment lengths of the EE genotype were 136 and 87 bp; the fragment lengths of the EK genotype were 223, 136 and 87 bp. The products of the digestion process were separated by electrophoresis on a 2.5% agarose gel, followed by ethidium bromide staining.

Data were statistically described in terms of mean and standard deviation (SD), frequencies (number of cases) and relative frequencies (percentages). Comparison of quantitative variables between the study groups was done using the t test and one-way ANOVA test for independent samples. For comparing categorical data, chi-square test was performed. Risk analysis was performed by calculating the odds ratio (OR) and the 95% confidence interval (CI). A P value less than .05 was considered statistically significant. All statistical calculations were done using computer programs Microsoft Excel version 7 (Microsoft Corporation, NY, USA) and SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) version 15 for Microsoft Windows.

**RESULTS**

This study included 100 CHD patients, 27 of whom had unstable angina (UA) (22 men and 5 women, mean [SD] age, 54.9 [8.7] years), and 73 patients had acute myocardial infarction (MI) (65 men and 8 women, mean [SD] age, 50.3 [7.9] years). The control group consisted of 50 healthy subjects. There were no significant differences in age and gender between CHD patients and controls. Patients with hypercholesterolemia and smokers were more frequent in the CHD group when compared with controls (Table 1).

**Distribution of allele and genotype frequencies**

The genotype frequencies were 43% EE, 37% EK and 20% KK in patients with CHD, thus differing from those in the control subjects, which were 74% EE, 22% EK and 4% KK. The genotype frequencies were 38.3% EE, 38.3% EK and 23.3% KK in patients with MI and 55.6% EE, 33.3% EK and 11.1% KK in patients with UA, showing no significant difference (Table 2).
Table 1. Characteristics of coronary heart disease (CHD) patients (n=100) and controls (n= 50).

| Variable                      | CHD patients | Controls | P  |
|-------------------------------|--------------|----------|----|
| Age (years)*                  | 52.6 (8.29)  | 53.9 (6.2) | .34|
| Gender (male /female)         | 87 /13       | 38 /12   | .23|
| Hypertension (%)              | 71           | 68       | .70|
| Smoking (%)                   | 65           | 39       | .002|
| Diabetes mellitus (%)         | 30           | 26       | .61|
| Hypercholesterolemia (%)      | 79           | 52       | .001|
| ICAM level (pg/mL)*           | 838.79 (153.93) | 802.55 (144.68) | .37|

*Values are mean (standard deviation).

Table 2. Frequencies of the K469E variants of ICAM-1 gene in CHD (n=100), unstable angina (UA) (n=27), myocardial infarction (MI) (n=73) and controls (n= 50).

| ICAM-1 Genotype n (%)         | CHD | UA   | MI   | Controls |
|-------------------------------|-----|------|------|----------|
| EE                            | 43  | 15   | 28   | 37       |
| EK                            | 37  | 9    | 28   | 11       |
| KK                            | 20  | 3    | 17   | 2        |
| KK+EK                         | 57  | 12   | 45   | 13       |

*Significantly increased as compared to controls.

Table 3. Odds ratios for CHD patients heterozygous or homozygous for the -469 K variant.

| Genotype | Odds ratio (95% CI) | P  |
|----------|---------------------|----|
| EK       | 2.9 (1.2-7.8)       | .014|
| KK       | 8.8 (1.8-57.2)      | .003|
| KK+EK    | 3.8 (1.7-8.5)       | .001|

DISCUSSION

Leukocyte adherence to the vascular endothelium is one of the earliest demonstrable events in atherosclerosis. Intercellular adhesion molecule-1 (ICAM-1) mediates the interaction of activated endothelial cells with leukocytes and plays a fundamental role in the pathogenesis of coronary atherosclerosis. ICAM-1 single-base C/T polymorphism, which determines an amino acid substitution from glutamine (E) to lysine (K) in the ICAM-1
In the current study, ICAM-1 EK and KK variants represented 57% of our CHD patients and were associated with increased risk of disease development; OR=3.8 (95% CI: 1.7 to 8.5; \(P<.001\)). This agrees with the findings of Jiang et al in German patients with CHD, who suggested an important role for ICAM-1 gene polymorphism in codon 469 in the susceptibility for CHD and MI. In Han Chinese population, Zhang et al found that the presence of KK genotype of ICAM-1 codon 469 conferred an increased risk for CHD. Also, Liu et al reported that KK homozygotes had a higher risk of re-stenosis after coronary stenting and concluded that the ICAM-1 KK genotype may serve as a predictor of in-stent re-stenosis, especially in obese and hyperlipidemia patients. Similarly, Lu et al found that K allele frequency was higher in CHD patients than in controls, and K allele carriers develop myocardial infarction more easily. In Egyptian patients with peripheral arterial occlusive disease, Shaker et al found a statistical difference in the clinical history and laboratory data between the EE and EK variants among those with ischemic heart disease.

A study by Banda et al showed that mice with ICAM-1 deficiency had normal endothelial function (vasorelaxation in response to acetylcholine) after ischemia-reperfusion, whereas wild-type mice had impaired vasorelaxation in response to acetylcholine, indicating that ICAM-1 gene function may be related to impaired endothelium-dependent vasodilatation. This dysfunction of the endothelium plays a key role in all stages of atherosclerosis.

On the other hand, McGlinchey et al found no association between the ICAM-1 K469E polymorphism and CHD in a well-defined Irish population. Similarly, Aminian et al found no significant differences between CHD patients and controls as regards KK genotype and concluded that there was no strong relation between K469E polymorphisms and occurrence of CHD and MI in the studied population from Fars province, Iran. Also, a Slovenian study reported that the K469E polymorphism of the ICAM-1 gene was not associated with MI in subjects with type 2 diabetes. The discrepancy among the studies could be due to selection of patient groups from different ethnic populations, variable sample sizes or different interactions of the genetic background with environmental factors.

In the present study, the frequencies of the K genotype (KK and EK) in acute MI patients (61.6%) showed no significant difference as compared to UA patients (44.4%) (\(P=.121\)). Previous studies compared ICAM-1 K469E polymorphisms between MI and CHD group as a whole. In our work, there were no significant differences in sICAM-1 levels between CHD patients and control subjects (\(P=.37\)) and no significant differences in sICAM-1 levels among patients with different genotypes (\(P=.53\)). However, sICAM-1 level elevation among patients with coronary disease was reported by Ridker et al and Güray et al. Also, in the Han population of China, individuals with the K allele had higher plasma level of sICAM-1 than those without the K allele.

The present study showed higher levels of sICAM-1 in men than women with CHD (\(P=.004\)). However, Zakai et al studied the relation between inflammation and cardiovascular disease risk (CVD) in older adults, and they found that sICAM-1 was associated with CVD in women only. This difference may be attributed to the limited number of female patients in our study or the different ages of patients. In comparison between UA patients and acute MI patients, there was no significant difference as regards the sICAM levels (\(P=.23\)); this agrees with the findings of Güray et al and Damnjanovic et al.

In conclusion, our results suggest that the KK and EK genotypes of the ICAM-1 gene polymorphism in codon 469 are associated with the risk for CHD development in Egyptians. Circulating sICAM-1 is not influenced by this polymorphism and is not necessarily elevated in acute coronary heart disease.
REFERENCES

1. Albert MA. Inflammatory biomarkers, race/ethnicity and cardiovascular disease. Nutr Rev 2007;65:S234-8.
2. Albert MA, Glynn RJ, Buring JE, Ridker PM. Differential effect of soluble intercellular adhesion molecule-1 on the progression of atherosclerosis as compared to arterial thrombosis: A prospective analysis of the Women's Health Study. Atherosclerosis 2008;197:297-302.
3. Witkowska AM, Borawska MH. Soluble intercellular adhesion molecule-1 (sICAM-1): An overview. Eur Cytokine Netw 2004;15:91-8.
4. Liu ZP, Huo Y, Li JP, Zhang Y, Xue L, Zhao CY, et al. Polymorphism K469E of intercellular adhesion molecule-1 gene and restenosis after coronary stenting in Chinese patients. Chin Med J (Engl) 2004;117:172-5.
5. Harning R, Mainolfi E, Bystryn JC, Henn M, Merluzzi VJ, Rothlein R. Serum levels of circulating intercellular adhesion molecule 1 in human malignant melanoma. Cancer Res 1991;51:5003-5.
6. Sambrook J, Fritsch EF, Maniatis T. Molecular cloning: A laboratory manual Cold Spring. Vol No.1. NY: Harbor Laboratory Cold Spring Harbor; 1989. p. 1-62.
7. Jiang H, Klein RM, Niederacher D, Du M, Marx R, Horlitz M, et al. C/T polymorphism of the intercellular adhesion molecule-1 gene (exon 6, codon 469). A risk factor for coronary heart disease and myocardial infarction. Int J Cardiol 2002;84:171-7.
8. Jenny NS, Arnold AM, Koller LH, Sharrett AR, Fried LP, Psaty BM, et al. Soluble intracellular adhesion molecule-1 is associated with cardiovascular disease risk and mortality in older adults. J Thromb Haemost 2006;4:197-13.
9. Zhang SR, Xu LX, Gao QQ, Zhang HQ, Xu BS, Lin J, et al. [The correlation between ICAM-1 gene K469E polymorphism and coronary heart disease]. Zhonghua Yi Xue Yi Chuan Xue Za Zhi 2006;22:205-7.
10. Lu FH, Shang Q, Wen PE, Su GH, Wu JM, Tian Q, et al. [A study on K469E polymorphism of ICAM1 gene and ICAM1 plasma level in patients with coronary heart disease]. Zhonghua Yi Xue Yi Chuan Xue Za Zhi 2006;22:195-7.
11. Shaker O, Zahra A, Sayed A, Refaat A, El-Khaiat Z, Hegazy G, et al. Role of ICAM-1 and E-selectin gene polymorphisms in pathogenesis of PAOD in Egyptian patients. Vasc Health Risk Manag 2010;6:9-15.
12. Banda MA, Lefer DJ, Granger DN. Postischemic endothelium-dependent vascular reactivity is preserved in adhesion molecule-deficient mice. Am J Physiol 1997;273:H2721-5.
13. McGlinchey PG, Spence MS, Patterson CC, Allen AR, Murphy G, Belton C, et al. The intercellular adhesion molecule-1 (ICAM-1) gene K469E polymorphism is not associated with ischaemic heart disease: An investigation using family-based tests of association. Eur J Immunogenet 2004;31:201-6.
14. Aminian B, Abdil Ardekani AR, Arandi N. ICAM-1 polymorphisms (G241R, K469E), in coronary artery disease and myocardial infarction. Iran J Immunol 2007;4:227-35.
15. Milutinovic A, Petrovic D. The K469E Polymorphism of the Interstitial Adhesion Molecule 1 (ICAM-1) Gene Is Not Associated with Myocardial Infarction in Caucasians with Type 2 Diabetes. Folia Biol (Praha) 2006;52:79-80.
16. Redker PM, Hennekens CH, Buring JE, Ritai N. C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. N Engl J Med 2000;342:836-43.
17. Malik I, Danesh J, Whincup P, Bhatia V, Papanicola O, Walker M, et al. Soluble adhesion molecules and prediction of coronary heart disease: A prospective study and meta-analysis. Lancet 2000;356:971-6.
18. Gu¨r¨ay U, Erbay AR, Gu¨r¨ay Y, Y¨ilmaz MB, Boyaci AA, Sasmaz H, et al. Levels of soluble adhesion molecules in various clinical presentations of coronary atherosclerosis. Int J Cardiol 2004;96:235-40.
19. Zakai NA, Katz R, Jenny NS, Psaty BM, Reiner AP, Schwartz SM, et al. Inflammation and hemostasis biomarkers and cardiovascular risk in the elderly: The Cardiovascular Health Study. J Thromb Haemost 2007;5:1128-35.
20. Dami´njanovic G, Jelic M, Dindic B, Ilic S. [Serum concentration of soluble adhesion molecules in patients with different forms of coronary artery disease]. Vojnosanit Pregl 2009;66:265-70.