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ASSOCIATION OF E-SELECTIN GENE POLYMORPHISMS IN EGYPTIANS WITH CORONARY ARTERY DISEASE

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ABSTRACT

Genetic polymorphisms of the E-selectin are associated with coronary artery disease. **Objectives:** We aimed to investigate the association between the A561C and G98T polymorphisms in E-selectin gene and coronary artery disease in Egyptian population. **Subjects & methods:** The study enrolled 251 individuals, classified into 2 groups: 152 coronary artery disease patients and 99 normal healthy individuals. A561C and G98T polymorphisms of E-selectin were analyzed by PCR-RFLP (Polymerase chain reaction - Restriction fragment length polymorphism) technique. **Results:** The frequency of C allele of the E selectin A561C polymorphism was higher in coronary artery disease patients than control group but with no statistical significant difference between the two studied groups (p=0.53). The frequency of T allele of G98T polymorphism of E-selectin was higher in coronary artery disease patients when compared to control group but with no statistical significant difference (P=0.36). **Conclusion:** A561C and G98T polymorphisms of E-selectin gene are not associated with the predisposition to coronary artery disease and appear to be protective in Egyptian population.

KEYWORDS: E-selectin, polymorphism, PCR-RFLP, CAD, Egyptians.

INTRODUCTION

Coronary artery disease (CAD) is one of the most important worldwide health problems with the highest rate of mortality and morbidity in the recent years.[1] Although, it is known that CAD is a multifactorial disease that results from the interaction between the genetic predisposition and environmental factors in pateints[2], its exact pathogenesis is not totally clear till now. E-selectin, a cell-surface membrane glycoprotein, is a member of the selectin superfamily of adhesion molecule. It is expressed on endothelial cell after activation by cytokines as interleukin-1, lipopolysaccharide, and tumor necrosis factor-alpha.[4] E-selectin has a major role in the process of leucocyte adhesion to the endothelial cells.[5] It mediates monocytes and lymphocytes activation and induces inflammation which leads to atherosclerosis and various vascular diseases.[6] Single nucleotide polymorphisms (SNPs) of the E-selectin gene are currently considered to be a high risk factor for atherosclerosis development. It has been previously identified the associations between the A561C, G98T and C1839T variations of the E-selection gene with CAD, hypertension and ischemic cerebrovascular diseases.[7-9] There were results that reported the association of the E-selectin A561C polymorphism with the angiographic severe CAD Saudi Arabs patients, however the association was lost when adjusted to CAD risk factors[10] and the significant association between the E-selectin G98T polymorphism and the development of premature CAD.[11] Although two previous studies were done to assess the association between the E-selectin A561C polymorphism in CAD and atherosclerotic Egyptian patients[12,13], here we conducted a bigger-size case-control study from different geographic region to determine the association of both E-selectin gene A561C and G98T polymorphisms with CAD among Egyptian population.

MATERIALS AND METHODS

Subjects

Two groups of Egyptian individuals were recruited for the present study between August 2014 and March 2015. The pateint group had 152 pateints (105 males and 47 females; mean age 53.7±9.8 years). Patients were recruited from the department of Internal medicine, Mansoura University hospitals and Cardiology departmnet, Dekernis general hospital, Nile Delta region, Egypt. Patients included in the study were diagnosed as acute coronary syndrome including ST segment elevation myocardial infarction (STEMI), non ST segment elevation myocardial infarction (NSTEMI) and unstable angina (UA) according to European Society of

Cardiology (ESC) guidelines. While, any patient with rheumatic heart disease, dilated cardiomyopathy, congenital heart diseases and myocardial infarction due to causes other than atherosclerosis (vasculitis, cocaine abuse, spontaneous dissection) were excluded. The control group included 99 individuals (77 males and 22 females; mean age 52.1±10.1 years). All of them were from the same population, they were apparently healthy with no history of CAD and with negative family history for CAD. The study was approved by the local ethical comittee of Faculty of Medicine, Mansoura University, Egypt.

Relevant clinical history

A detailed clinical history was obtained from all participants including age, sex, smoking, blood pressure; an individual was considered hypertensive if his/her blood pressure is persistently at or above 140/90 mmHg[14], diabeties mellitus, body mass index (BMI); an individual was concidered obese when his/her BMI > 30[15], and lipid profile; an individual was considered hypercholestrolemic when his/her LDL cholesterol ≥ 190 mg/dl in non diabetics and 70-189 mg/dl in diabetics[16] or HDL < 40 mg/dl.[17]

Genomic DNA

Genomic DNA was extracted from EDTA blood obtained from all participating individuals using Whole Blood Genomic DNA Extraction Kit (Fermentas, USA) and stored at -20°C in aliquots until required. Primers were designed as previously described [18,19], are shown in Table (1).

Table 1: E-selectin polymorphisms: primers sequences and corresponding restriction endonuclease.

Gene locus	Amplified product size (bp)	RE	Digested product size (bp)
A561C F: 5´-GCTGATGTCTCTGTTGCACACTG-3´ R: 5´-CCATATGACACCATCTGCACCAG-3´	357	*PstI*	AA (219 & 138) AC (357, 219 & 138) CC (357)
G98T F: 5'-ATGGCACTCTGTAGGACTGCT-3' R: 5'-GTCTCAGCTCACGATCACCAT-3'	332	*HPhI*	GG (194 & 138) GT (332, 194 & 138) TT (332)
F: forward, R: reverse, bp: base pair, RE: restriction endonuclease			

Genotyping of the E-selectin A561C and G98T polymorphisms:

Enzymatic amplification was performed by PCR-RFLP using Master Taq polymerase enzyme and thermal cycler (T personal thermo cycler, Biometra, analytical Jena Company). PCR was performed for each polymorphism in a total volume of 25 µl containing 5.0 µl of genomic DNA, 0.5 µl of each primer (Table 1), 12.5 µl PCR mix (including Taq DNA polymerase, dNTPs [dATP, dCTP, dGTP, dTTP], MgCl2 and PCR buffer) and 6.5 µl sterilized nuclease-free water (negative controls without DNA template). The reaction was performed as follows: an initial denaturation at 94˚C for 5 min, followed by 35 cycles of denaturing at 94˚C for 30 sec, annealing at 59˚C for 30 sec, with extension for 45 sec at 72˚C and a final extension at 72˚C for 10 min. The A561C PCR product (357 bp) was then digested by FastDigest *PstI* restriction endonuclease (Thermo Scientific, USA). The G98T PCR product (332 bp) was digested by *HPhI* restriction endonuclease (Thermo Scientific, USA), and the digested products were detected on a 3% agarose gel and visualised under ultraviolet light after ethidium bromide staining.

Statistical analysis

Statistical analysis was performed using the SPSS software version 16.0 (SPSS Inc., Chicago, IL, USA). Each result was calculated as the mean±SD. The Hardy-Weinberg balance was used to check the sample with group representation. Differences in genotypic and allelic frequency between studied groups were evaluated using the Fisher's exact test or the $\chi 2$ test as appropriate. The odds ratio (OR) was calculated together with its 95% confidence interval (CI). $P<0.05$ was considered to indicate statistically significant differences.

RESULTS

Clinical parameters in the patient and control groups

The studied participants were divided into two groups: apparently healthy control group (controls) consisted of 99 subjects: 77 males (77.8%) and 22 females (22.2%), with mean age 52.1±10.1 years. The CAD group (cases) included 152 subjects: 105 males (69.1%), 47 females (30.9%), with mean age 53.7±9.8 years. Demographic and clinical characteristics of patients and control subjects are shown in Table (2). Smoking, hypertension, diabetes mellitus, obesity and hypercholesterolemia were significantly higher in the CAD group when compared to controls ($p=0.02$, $p<0.0001$, $p<0.0001$, $p<0.0001$, and $p<0.0005$, respectively).

Table 2: Demographic and clinical characters of the studied groups.

Parameter	Cases (No= 152)	Controls (No= 99)	P	OR	95% CI
Age (years) (mean±SD)	53.7±9.8	52.1±10.1	0.1		
	No. (%)	No. (%)			
Male	105 (69.1)	77 (77.8)	0.13	0.6	0.35-1.14
Female	47 (30.9)	22 (22.2)			
Smoking	54 (35.5)	22 (22.2)	**0.02**	1.9	1.06-3.39
Hypertension	98 (64.4)	35 (35.4)	**<0.0001**	3.2	1.92 -5.55
Diabetes Mellitus	101 (66.4)	23 (23.2)	**<0.0001**	6.4	3.6-11.4
Obesity	87 (57.2)	30 (30.3)	**<0.0001**	3.03	1.77-5.18
Hypercholesterolemia	98 (64.4)	41 (41.4)	**<0.0005**	2.5	1.49-4.28
P: P-value, OR: odds ratio, CI: confidance interval, SD: standerd deviation					

E-selectin gene polymorphisms

E-selectin gene A561C and G98T polymorphisms were studied by Hardy-Weinberg equilibrium both in CAD group and controls. The association between the A561C polymorphism was not statistically significant in CAD patients when compared to controls (p=0.53). The genotypes frequency and distribution were in the CAD group: AA 75 (49.3%), AC 69 (45.4%) and CC 8 (5.3%) while in control subjects were: AA 50 (50.5%), AC 48 (48.5%) and CC 1 (1.0%) as shown in Table (3).

Table 3: Frequency of E-selectin A561C genotypes and alleles in patients and controls.

	Cases (No=152) No (%)	Controls (No= 99) No (%)	P	OR	95% CI
Genotypes					
AA (r)	75 (49.3)	50 (50.5)	--	--	--
AC	69 (45.4)	48 (48.5)	0.8	0.95	0.57-1.60
CC	8 (5.3)	1 (1.0)	0.11	5.3	0.64-43.9
Alleles					
A (r)	219 (72.1)	148 (74.7)	--	--	--
C	85 (27.9)	50 (25.3)	0.53	1.14	0.76-1.72
P: P-value, OR: odds ratio, CI: confidance interval, r: reference					

The association between the G98T polymorphism was not statistically significant in CAD patients as compared to control subjects (p=0.36). The genotypes frequency and distribution were in the CAD group: GG 77 (50.6%), GT 70 (46.1%) and TT 5 (3.3%) while in control subjects were: GG 48 (54.5%), GT 40 (45.5%) and CC 0 (0%) as shown in Table (4).

Table (4): Frequency of E-selectin G98T geneotypes and alleles in patients and controls.

	Cases (No=152) No (%)	Controls (No= 88) No (%)	P	OR	95% CI
Genotypes					
GG (r)	77 (50.6)	48 (54.5)	--	--	--
GT	70 (46.1)	40 (45.5)	0.7	0.9	0.53-.53
TT	5 (3.3)	0 (0)	0.19	0.14	0.007-2.6
Alleles					
G (r)	224 (73.7)	136 (77.3)	--	--	--
T	80 (26.3)	40 (22.7)	0.36	1.2	0.79-1.89
P: P-value, OR: odds ratio, CI: confidance interval, r: reference					

DISSCUSION

CAD is a major problem in Egypt. According to the latest WHO data published in April, 2011; coronary heart disease deaths in Egypt reached 78,897 or 21.73% of total deaths.[20] The rise of CAD occurrence in Egypt has been attributed to the major changes in the life-style.

Excessive intake of fast foods, obesity, diabetes, excessive smoking, stress and hypertension, all of which are considered CAD risk factors. The early detection of genes susceptible to occurrence of CAD can lead to early disease prevention and avoidance of risk factors. Single nucleotide gene polymorphism (SNP) may affect disease pathogenesis. It is a substitution or deletion of a single nucleotide of a gene resulting in an allelic variant that exists stably in a population. SNP may lead to an amino acid substitution resulting in functionally altered protein.[21] If occurs in regulatory region of the gene may alter binding affinity of transcription factors changing the rate of gene transcription resulting in higher or lower protein levels.[22,23] E-selectin (SELE), a glycoprotein molecule, is expressed on the surface of cytokines-activated endothelial cells.[24] It helps leukocytes rolling on the activated endothelial cells and maintains circulating monocytes and lymphocytes adhesion to endothelial cells.[25] It has been observered that SELE expression increases in the arterial endothelium interacting with lymphocytes and macrophages in human atherosclerotic lesions.[26] Several polymorphisms have been discribed in SELE gene. Among them, the A561C polymorphism, that leads to substitution of the amino acid serine to arginine at position 128 (Ser128Arg), is proven to increase the ligand-binding function of the protein[27], and the G98T polymorphism, a mutation of G toT in the untranslated region of SELE [28], that might affect the SELE expression.[29]

Here, we studied the association of the A561C and G98T polymorphisms in E-selectin gene in Egyptian CAD patients and matched controls. We found that both A and C alleles in the A561C polymorphism were higher in patients than controls but without statistical significance between both alleles when compared to control group P=0.53 ($p<0.05$). Although, this polymorphism may be related to increased endothelial responses to injury during atherosclerotic plaque formation, thereby potentially serving as a risk factor.[30] Regarding different genotypes of E-selectin polymorphism (A561C), the distribution of AA, AC and CC were higher in patient when compared to controls but without any statistical significance confirming that CAD is not influenced by different genotypes of this polymorphism. The relationship between A561C polymorphism of E-selectin gene and CAD have been studied in various populations worldwide. Considering Caucasians, our results are in concordance with the results of Ghilardi et al. [31] in which the difference in genotype and allele distribution between Italian patients and control was not statistically significant and with Sakowicz et al.[8] who found no significant association between A561C polymorphism of E-selectin gene in Polish pateints with myocardial infarction. Thus, we could confirm the previous findings of Hamid et al.[12] who found the insignificatnt associaition between E-selectin A561C polymorphism and Egyptian CAD patients. On the other hand, our work disagrees with the findings of Motawi et al.[13] who found that the frequency of the mutant AC genotype and C allele of E-selectin A561C polymorphism in peripheral, cerebral and cardiovascular atherosclerotic Egyptian patients was significantly higher than in control subjects suggesting a possible role of the mutant allele in predisposition to atherosclerosis, and with the work of Wenzel et al.[28] who found a significant difference between German CAD patients and normal controls in frequency of C allele. In the same line, the Iranians reported a significant association between A561C polymorphism and CAD pateints[19], as well as the Saudi Arabs who showed a significant association of A561C polymorphism with angiographic severe CAD that lost when adjusted to the risk factors of CAD.[10] While, in Americans the C allele was significantly associated with early onset CAD.[32]

In the G98T polymorphism in E-selectin gene, we found that both G and T alleles were higher in patients than controls but without any statistical significance between both alleles when compared to control group P=0.36 ($p<0.05$). Regarding different genotypes of this polymorphism, the distribution of GG, GT and TT were higher in patients than controls but with no statistical significance confirming that CAD is not influenced by different genotypes of this polymorphism. This polymorphism had been studied in different Caucasian populations and was found to be significantly associated with premature CAD in Americans[11] and significantly correlated with the A561C polymorphism assuming synergistic interactions between both SNPs of E-selectin gene and hypercholesterolemia which cause a significant increase in the susceptibility to CAD among Poles. [33]

CONCLUSION

Although there were no significant associations between the E-selectin gene A561C and G98T polymorphisms and CAD in Egyptian patients, the important role of E-selectin in the pathophysiology of coronary artery disease cannot be ruled out. Thus, our results confirm that the same genetic variant has variable role in different ethnic groups.

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CONFLICT OF INTEREST: The authors have nothing to declare.

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