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Hadeel Abdelelah Abdel Razaaq and Hanadi A. Abdul-Razzaq



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Polymorphism of E-Selection Gene for Coronary Artery Disease in Ramadi / Anbar - Iraq

Hadeel Abdelelah Abdel Razaaq^{1,a)}, Hanadi A. Abdul-Razzaq^{2,b)}

¹ University of Anbar / College of Education for Women / Department of Biology, Anbar, Iraq

² University of Kirkuk / College of Science / Department of Biology, Kirkuk, Iraq

^{a)} Corresponding author: sc.hadeel_aldaraji@uoanbar.edu.iq

^{b)} hanadialdaraji@uokirkuk.edu.iq

Abstract: A lot of factors, such as genetic factors are contributing to coronary atherosclerosis. E- selection gene, which is also referred to as endothelial leukocyte molecule (CD62E or ELAM1), also the role of leukocytes and adhesion to activated endothelial cells is of high importance in the pathogenesis of coronary artery disease (CAD). The major goal of this work associating the mutation related to E-selection gene (Ser 128 Arg) with CAD in Ramadi / Anbar - Iraq. There are 10 control subjects and 50 patients experiencing CAD are enrolled in the presented study. A561C polymorphism in E-selection gene through PCR succeeded via restriction fragment length polymorphism (PCR-RFLP). The frequencies related to CC, AC and AA genotypes have been (10%, 8%, and 82%) in patients experiencing CAD and (10%, 0% and 90%) in control subjects. There have been no considerable differences in genotypes' frequencies in S128R polymorphisms between control and CAD groups, ($P=0.499$). Also, the frequency related to mutant C allele has been high in patients experiencing CAD in comparison to control group (5% vs. 14%). Yet, such differences weren't statistically significant ($P=0.186$). Furthermore, the odds ratio with regard to CAD risks related to C allele is $OR=6.872$; (95% $CI=0.393-120.017$). The results indicated that genetic mutations in the E-selection gene at position 561 (Ser128Arg) do not correlate with the presence of the C allele in Ramadi / Anbar-Iraq.

Keywords: E-selection, polymorphism, Coronary Artery Disease, Restriction enzyme.

INTRODUCTION

One of the major causes of disability and death is CAD, with as many as 40% of all the fatal events [1], also, it might be the cause of diseases spreading all over the world in 2020 [2]. In addition, CAD can be defined as a multifactorial caused by a relation between environmental and genetic risk factors like high-levels of harmful cholesterol, high blood pressure, smoking, obesity and lack of activity. [3]. Atherosclerosis is the main risk factor for CAD, where arteries are found in coronary arteries walls [4]. Also, atherosclerosis is an acute inflammatory process that turns into an acute clinical event through rupture of plaques, that results in thrombosis [4]. Leukocytes' placement in arterial wall's lining was one of the significant stages in creating atherosclerotic plaques [5]. Recruiting leukocytes was multi-chain process which includes leukocyte adhesion then roll-out adhesion and exit from circulatory system to inner layer [6]. An E-selection gene can be defined as a selective family which has been expressed on the endothelial cells throughout their activation. The gene was located in the range of 21-24 on human chromosome-1 which consists of 14 exons exceed approximately 13 kb of DNA [7]. There are many polymorphisms within a gene E-selection which has an effect on the function of the coding protein, as single nucleotide polymorphism (SNP) in gene coding region (A516C) leads to serine (S) substitution with arginine (R) in code 128 [8]. The main function of E-selection is to roll the leukocytes along the vascular walls, which is the first stage in binding white blood cells to the vascular wall, then migrating [7].

METHODS AND MATERIAL

Subjects

Fifty adults with sex and age matched subjects, CAD patients have been selected from Ramadi Cardiology Department. CAD was counted existing in the case when: (a) minimum of one suffers is present in any of 15 segments of coronary arteries shrink the lumen via facultative diagnostic angiography. Documented suffering to the heart muscle even without positive results via angiography: positive ECG results as well as high cardiac signs. Twenty subjects are presented with age and gender matched as a normal control group, and all subjects underwent a complete history with a special concentrate on the coronary risk factors involving family history, smoking of coronary artery disease, hyperlipidemia, laboratory tests, hypertension and lipid profile

Methods and Sampling

After fasting for 12hrs, samples of blood without/with EDTA have been taken from the subjects. HDL cholesterol (HDL-C), triglyceride (TG) and total cholesterol concentrations are evaluated via standard low density lipoprotein cholesterol (LDL-C) estimated with Friedewald formula. Also, the DNA has been extracted from EDTA blood with the use of standard primers developed with Fast PCR software and specified as ESR: 5'-CCATATGACACCATCTGCACCAG-3' and ESF: 5'-GCTGATGTCTCTGTTGCACACTG-3'. In addition, the region related to E-selection gene which contains A561C site have been amplified from the genomic DNA through PCR, which is done via reaction mixture that is subjected to denaturation for 5mins at a temperature of 95°C, succeeded via 30 cycles for 45 seconds at a temperature of 95°C, 45 seconds at 59°C, 45 seconds at 72°C, after that via final extension for 5mins at 72°C in MY Cyclor system (Bio Rad Co, USA). Furthermore, the PCR product has been verified via electrophoresis in 1.5% agarose gel pre-stain with EtBr. Subsequently, 10µL PCR product (323 bp) has been digested via 10 unites Pst I and the subsequent fragments are separated through gel electrophoresis in 2.5% agarose gel.

Statistical Analyses

The statistical analysis of the levels of the serum lipid and the demographic properties with the use of SPSS program v16.0 (SPSS Inc., U.S.). Every one of the results has been computed as mean \pm standard deviation. The frequencies of the genotypes in the control and patient groups have been compared with the Chi-Square test. The binary logistic regression has been utilized for the calculation of the ORs and 95% of the CIs. P value has been taken under consideration < 0.05 has been considered to have statistical significance

RESULTS

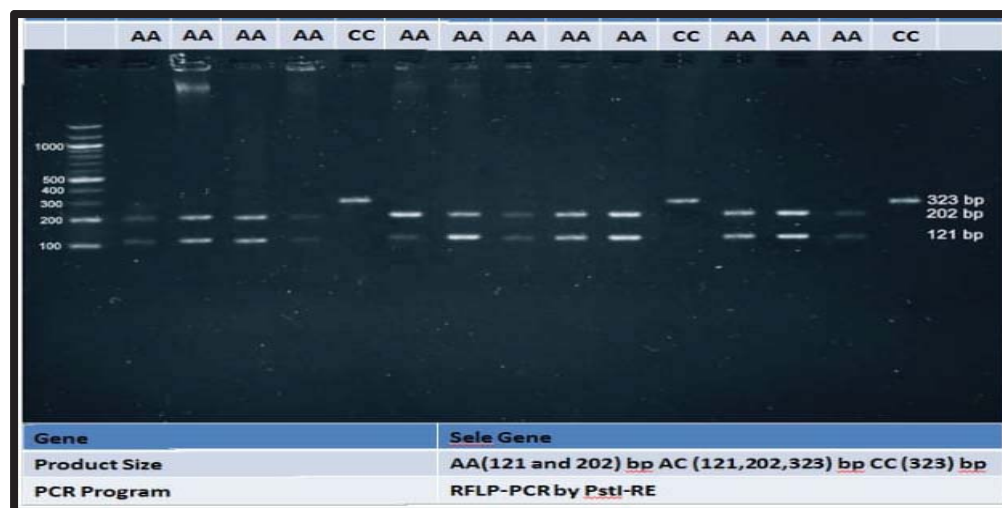
The present study included 60 subjects that have been categorized to fifty adult patients with the CAD and 10 healthy controls. TABLE1 lists the patient and control groups' demographic properties. There have not been any significant differences between the two patient groups, as sex, age, hypertension, smoking, and having a family history of CAD (TABLE1). Had statistical significance with higher levels of the total cholesterol, LDL cholesterol and triglyceride in comparison with the controls, whereas the concentration of the HDL cholesterol had statistically significant lower levels in the patients in comparison with the controls

TABLE 1. Properties of the Patients and Controls (Mean \pm SD)

Parameter	CAD (Number= 50)	Control (Number= 10))	p-value
Age (Years)	52.8 \pm 9.6	46.8 \pm 7.42	0.34
	No. (%)	No. (%)	
Male	(56) 28	(80) 6	0.14
Female	(44) 22	(20) 4	
Smoking	(70) 35	(55) 11	0.43
Hypertension	(80) 40	(50) 10	0.91
Family History	(76) 38	(60) 12	0.95
Cholesterol Level (mg/dl)	204.5 \pm 40.01	167 \pm 25.1	<0.01**
Triglyceride Level (mg/dl)	212.2 \pm 45.8	111.03 \pm 30.4	<0.01**
LDL cholesterol (mg/dl)	131.4 \pm 44.9	103.2 \pm 25.1	<0.01**
HDL cholesterol (mg/dl)	40.5 \pm 5.1	50.6 \pm 6.50	<0.01**

(** High significant, $p < 0.01$)

The product of the PCR (323 bp) after the digestion resulted in bands of 121 & 202bp in the AA homozygote, and 323bp, 202bp, and 121bp in the AC heterozygote. PCR product in the CC homozygote stayed intact (FIGURE 1). The CC genotype frequency has been statistically different between the CAD patient and control groups.

**FIGURE 1.** A-561C Polymorphism Electrophoresis Pattern of the E-selection Gene with the use of the PCR-RFLP

In this area, the serine mutation into arginine results from nucleotide A-561 transversion (9). Through restricting the mapping of the enzyme it has been discovered that this transversion abolished a PstI site of recognition. The fragment which has been amplified by the PCR is 323bp long, transversing the nucleotide 1,843 in the 3rd intron to the nucleotide 2,200 in the 4th intron (10, GenBank accession: M58017). In the Homozygous Wild-type AA genotype where there are PstI sites of recognition, the products of the PCR have been entirely digested in 2 small portions: 121bp and 202bp, respectively (FIGURE 1). In the heterozygous CA genotype where the PstI site of recognition of 1 allele has been abolished and there is PstI site of recognition of a different allele, 1 allele has not been digested and a different allele has been entirely digested, therefore, all 3 potential fragments appear: 121, 202 and 323bp respectively (FIGURE 1). The frequency of the C allele in CAD patients has been 14% whereas normal controls 5% (TABLE2). There has not been any significant difference in genotype frequencies in S-128R polymorphism between CAD patients and controls ($P = 0.186$). The Odds ratio for CAD risk which is related to C allele has been OR= 6.872; (95% CI=0.393 -120.017). The mutant C allele frequency has been higher in CAD group (14%) compared to it in the control group (5%) (TABLE2), however, those differences have not been statistically significant.

TABLE 2. Distribution of the genotype and allele frequency of E-selection gene polymorphism of study group.

Genotype	Patients No. (50)		Control No. (10)		P value	OR	(95% CI)
	No.	%	No.	%			
AA	41	82	10	100	0.10	1 Ref.	-
AC	4	8	0	0	0.590	2.277	0.1135 - 45.701
CC	5	10	0	0	0.499	2.783	0.142 - 54.424
Alleles	No.	%	No.	%	P value		
A	86	86	20	95		1 Ref.	-
C	14	14	0	5	0.186	6.872	0.393 -120.017

The variety of the clinical and biochemical properties of patient and control group (n = 60) have been analyzed based on the E-selection genotype types, in other words, as AA, AC & CC (TABLE3). The numbers of the men have been considerably higher (p = 0.023) in AA genotype (35 males / 10 females), AC genotype (5 males / 2 females) and CC genotype (8 males / 0 females). The smoking status has been considerably greater (p = 0.05) in AA genotype (16 nonsmokers / 29 smokers), AC genotype (1 nonsmokers / 6 smokers) and CC genotype (1 nonsmokers / 7 smokers). The Age, hypertension, family history, mean serum HDL and LDL have not shown any significant differences between AA, AC and CC genotype (p = 0.047, p = 0.04, p = 0.05, p = 0.0374 and p = 0.0787), respectively (TABLE 3). The mean serum HDL and mean serum LDL have not shown any significant differences between AA, AC & CC genotype (p = 0.0374 and p = 0.0787), respectively (TABLE 3). Also, the average serum HDL level has been lower in AC (34.25±2.217) than in the AA and CC genotype (35.048±2.312, 36.4±3.130), and the mean serum LDL level was lower in the CC genotype (173.8±2.167) than in AA and AC genotype (177.634±12.887 and 176.25±4.787) respectively (TABLE 3). However, the average value of the serum cholesterol level and the average serum Triglyceride has shown significant difference between AA, AC & CC genotype (p = 0.02, p= 0.02), respectively (TABLE 3). Also, the mean serum Cholesterol and mean serum Triglyceride significantly lower in the CC genotype (267.4±7.5, 290.8±9.679) than in the AA (279.122±23.346, 316.122±28.641) and AC group (306.25±7.5, 342.75±9.5) (TABLE 3).

TABLE 3. The clinical and Biochemical values of the patients and controls in relation to the different E-selection genotypes.

Parameter	AA (No. 45) Mean ± SD	AC (No. 7) Mean ± SD	CC (No. 8) Mean ± SD	P value
Age	42.8±6.03	42.4±10.02	44.1±8.01	0.47
Sex (M/F)	35:10	5:2	8:0	0.023*
Smoking status (No/ Yes)	16:29	1:6	1:7	0.05*
Family History (No/ Yes)	34:11	5:2	6:2	0.4
Hypertension (No/Yes)	33:12	6:1	6:2	0.5
Cholesterol	279.122±23.346	306.25±7.5	267.4±7.5	0.02 *
Triglyceride	316.122±28.641	342.75±9.5	290.8±9.679	0.02 *
HDL	35.048±2.312	34.25±2.217	36.4±3.130	0.374
LDL	177.634±12.887	176.25±4.787	173.8±2.167	0.787

(*Significant, p<0.05), Color indicates the lowest level

DISCUSSION

The variety of the genetic factors, which include the genetic types (single nucleotide polymorphisms) can have an impact on the atherosclerosis pathogenesis, SNPs substitute a nucleotide by a different nucleotide in the structure of the gene. When the SNPs arise in a gene or a regulatory are near it, they can have a direct impact on the disease through having an impact of the function of the gene (11, 12). In the case where the SNPs happen in the gene coding locations, they cause replacing an amino acid in the structure of the protein with a different amino acid, resulting in altering the activity of the protein (13). The study results have shown that there has not been any significant differences between the controls and the CAD patients, not in the genotype (p = 0.499) nor in the frequencies of the allele. In this work, it has been discovered that mutant C allele has been greater in CAD group (14%) compared to it in the controls

(5%), however, this difference has not been of a statistical significance ($p = 0.186$) OR = 6.872 (95% CI= 0.393 - 120.017), which indicates that C allele has not been a factor of risk for the CAD in the population of Al-Anbar governorate. Our results have been coinciding with Ghilaridi et al. results, where the differences in the genotype and the allele distributions between the patients and the controls has not been of a statistical significance (14). Which has been coinciding with the results of the present study, Tripathi et al. have stated that S-128R polymorphism in the gene of the E-selection in the Indian people was not related to CAD (15). In addition to that, in a different research, it has been shown that in the subjects that suffer from T2D, E-selection S-128R polymorphism has not been related to the CAD (16). In addition to that, the results of the present study have been coinciding with (Soliman *et al.*) results, which have stated that there aren't any associations of E-selection gene mutations (Ser-128Arg) with the CAD in Egyptian populations (17). The results have shown that the mutant C allele prevalence has been 5% in the controls and it has been similar to a work by (Zheng *et al.*) that have found an identical percentage in the control group (18). On the other hand, Li et al. Have stated that the existence of the C allele in the A-561C polymorphism has been related to the CAD in the Chinese populations (8). There has been a significant correlation found between C allele and CAD in the Arab patients. The CAD odds ratio that has been related with the 128-R allele has been 1.760 (95% CI 1.14-2.72; $p = 0.007$) thereby, showing a significant correlation of this allele with the CAD in Saudi populations (19). None-the-less, it has been seen in the Japanese populations that substituting Ser by Arg in the EGF domain of the E-selection can be a factor of risk for the severe atherosclerosis (20). Substituting Ser by Arg at the 128 positions in either one of E-selection EGF domains may result in increasing binding activity of those selections to the leukocyte and ligand surfaces (21). In addition to that, the present study does not agree with a study that has been reported by Alireza et al. that discovered that the existence of the C allele at the position 561 E-selection gene has been related to an increase in the risks of the atherosclerosis in south-eastern Iran populations. Such polymorphism can have the ability of affecting the interactions of leukocyte-endothelia that can be responsible for the atherosclerosis pathogenesis (22). In this research, the family history, hypertension have been analyzed based on the genotype of the E-selection, no significant associations between genotypes and earlier risk factors of CAD have been discovered. The results of the present study have been in agreement with Li et al. and Zheng et al. results, who have stated equivalent results (19) (23). In the present research, triglycerides and serum total cholesterol, have been a significant correlation between genotypes (AA, AC & CC) and risk factors of CAD. Which has not been in agreement with Li et al. results, who have stated that even though the frequency values of numerous conventional risk factors of CAD, which include total cholesterol, smoking, tri-glycerids and LDL-cholesterol, no statistical significance correlation between genotype and the risk factors of CAD has been discovered (23). In this research there has not been any significant correlation of genotypes (AA, AC & CC) with the levels of the serum HDL-cholesterol and LDL-cholesterol ($p = 0.374$ and $p = 0.787$) respectively. Which has been in agreement with results that have been stated by a study by (Zheng *et al.*) that have stated equivalent results (19).

CONCLUSIONS

In the present study, it can be concluded that there has not been a correlation between E-selection gene mutation (Ser128 Arg) and the CAD in the population of Al Ramadi. The contributions of one new polymorphism to the general risks of those multi-factorial disorders can be obscured with the existence of an additional dominant factor of risk or through the linkage of other mutation types (24). The variety of the properties of chosen group of the patients, can be a result of the ethnic differences. The small size of the group of the subjects under study can be a reason that affected those results.

REFERENCES

1. Franchini M, Peyvandi F, Mammucci PM. (2008) . The genetic basis of Coronary Artery Disease: from candidate genes to whole genome analysis. *Trends Cardiovasc Med* 18: 157-162.
2. Murray CJ, Lopez AD. (1997) . Alternative projections of mortality and disability by cause 1990-2020: Global Burden of Disease Study. *Lancet* 349: 1498-1504.
3. Ho, S., Fang, X.,....& Asia Pacific Cohort Studies Collaborations.(2016). The impact of body mass index on the associations of lipids with the risk of Coronary Heart Disease in the Asia Pacific region. *Preventive Medicine Reports* 3: 79-82.
4. Lüscher, Thomas F. (2015) . Atherosclerosis and CAD. *European heart journal* 36.8 : 457-459.

5. Jousilahti, P., Vartiainen, E., Tuomilehto, J., & Puska, P. (1999) . Sex, age, Cardiovascular risk factors, and Coronary Heart Disease A prospective follow-up study of 14 786 middle-aged men and women in Finland. [Circulation](#) 99,9: 1165-1172.
6. Clemmons, David R. (2015) . Role of Dysglycemia in Atherosclerosis. *Atherosclerosis: Risks, Mechanisms and Therapies* : 15-26.
7. Hartiala, J., Li, D., Conti, DV., Vikman, S., Patel, Y., et al. (2011) . Genetic contribution of the leukotriene pathway to coronary artery disease. [Hum Genet.](#) 129: 617-627.
8. Tripathi, R., Singh, PK., Tewari, S., Tamhankar, PM., Ramesh, V., Agarwal, S. (2009) . Genetic predisposition of E-selection gene (S128R) polymorphism in patients with coronary herat disease (CAD). *Indian J Med Res.*; 130 (4): 423-7.
9. Collins, T., Williams, A., and Johnston, GI. (1991). Structure and Chromosomal location of the gene for endothelial – leukocyte adhesion molecule 1. [J Biol Chem](#); 266 (4) : 2466-73.
10. Wenzel, K., Hanke, R., and Speer, A. (1994). Polymorphism in the human E-selection gene detected by PCR-SSCP. [Hum Genet](#); 94(4) : 425-3.
11. Hashemi M, Moazeni-Roodi AK, Fazaeli A, Sandoughi M, Bardestani GR, Kordi-Tamandani DM, et al. (2010). Lack of association between paraoxonase-1 Q192R polymorphism and rheumatoid arthritis in southeast Iran. [Genet Mol Res.](#) 9(1):333-9.
12. Wang Z, and Moul J. (2001). SNPs, protein structure, and disease. [Hum Mutat.](#) 17(4):263-70.
13. Ng PC, and Henikoff S. (2003). SIFT: Predicting amino acid changes that affect protein function. [Nucleic Acids Res.](#) 31(13):3812-4.
14. Ghilardi G., Biondi ML., Turri O., et al. (2004). Ser 128 Arg gene polymorphism for E-selection and severity of atherosclerosis arterial disease. *J Cardiovascular Surg (Torino)*, 45(2):143-7.
15. Tripathi R, Singh PK, Tewari S, Tamhankar PM, Ramesh V, Agarwal S. (2009). Genetic predisposition of E-selectin gene (S128R) polymorphism in patients with coronary artery disease (CAD). *Indian J Med Res.* 130(4):423-7.
16. Endler G, Exner M, Raith M, Marculescu R, Mannhalter C, Endler L, et al. (2003). The E-selectin S128R polymorphism is not a risk factor for coronary artery disease in patients with diabetes mellitus type 2. [Thromb Res.](#) 112(1-2):47-50.
17. Soliman M. A. A., Kassem H. H., Hamed M. A., Ibrahim S. (2007). E-Selection Gene Polymorphism and Coronary Artery Disease: A Genetic Association Study. *Heart Mirror J.* Vol. 1, No. 2, ISSN: 1687-6652.
18. Abu-Amero KK, Al-Boudari OM, Mohamed GH, Dzimir N. (2006). E-selectin S128R polymorphism and severe coronary artery disease in Arabs. *BMC Med Genet.* 7(52):1-5.
19. Zheng F., Chevalier J. A., Zhang L. Q., et al. (2001). An HphI polymorphism in the E-selection gene is associated with premature coronary artery disease. [Clin Genet](#); 59 (1): 58-64.
20. Yoshida M, Takano Y, Sasaoka T, Izumi T, Kimura A. (2003). E-selectin polymorphism associated with myocardial infarction causes enhanced leukocyte-endothelial interactions under flow conditions. [Arterioscler Thromb Vasc Biol.](#) 23(5):783-8.
21. Hashemi M, Moazeni-Roodi AK, Fazaeli A, Sandoughi M, Bardestani GR, Kordi-Tamandani DM, et al. (2010). Lack of association between paraoxonase-1 Q192R polymorphism and rheumatoid arthritis in southeast Iran. [Genet Mol Res.](#) 9(1):333-9.
22. Alireza N. , Masoumeh A. , Seyed P. T. , Kouroush T. F. , Mohammad H. (2013). Association Between A561C Polymorphism of E-Selectin Gene and Coronary Arterial Disease in Southeastern Iranian Population. [Health Scope International Quarterly J.](#) 2(1) : 47-51.
23. Li Y., Wei Y. S., Wang M., et al. (2005). Association between the Ser128Arg variant of the E-selection and risk of coronary artery disease In the central china. [Int J Cardiol](#) ; 103 (1) : 33-6.
24. Miller M. A., Kerry S. M., Dong Y. et al. (2005). Circulating soluble E-selection levels and the Ser 128 Arg polymorphism in individuals from different ethnic groups. [Nutr Metab Cardiovasc Dis](#); 15(1) : 65-70.
25. Li Y., Wei Y. S., Wang M., et al. (2005). Association between the Ser128Arg variant of the E-selection and risk of coronary artery disease In the central china. [Int J Cardiol](#) ; 103 (1) : 33-6.
26. Miller M. A., Kerry S. M., Dong Y. et al. (2005). Circulating soluble E-selection levels and the Ser 128 Arg polymorphism in individuals from different ethnic groups. [Nutr Metab Cardiovasc Dis](#); 15(1) : 65-70.