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Frequency of Estrogen Receptor-1 (ESR-1) Gene Polymorphism (PvuII and XbaI) in Patients with Coronary Artery Disease

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Abstract Sex hormones might be viewed as biomarkers for cardiovascular health status, as well as protective agents against heart diseases. Coronary artery disease (CAD) is the most common disease in humans and has a complex etiology. In this study, we aimed to investigate the association of CAD with ESR1 PvuII and XbaI gene mutation frequencies and to see if it's important as a genetic risk factor and the susceptibility for CAD in Tanta region, Egypt. By polymerase chain reaction with restriction fragment length polymorphism (PCR- RFLP), we determined the frequency of the ESR1 gene polymorphisms in 110 healthy and 100 CAD sample. Results revealed that there were no significant differences between CAD patients and the control group as regard the frequency AA, AG and GG of XbaI genotype. PvuII genotype frequencies were TT, TC and CC with no significant value. Regarding allele frequencies of PvuII and XbaI polymorphism, they were not statistically important. There was no significant difference among all studied subjects regarding sex, age, menopausal status, cardiac complications and lipid profile, but there was highly significant differences regarding the body mass index. In conclusion, estrogen receptor alpha gene polymorphism Pvu II and XbaI site are not associated with the coronary artery disease.

Keywords: *coronary artery disease, estrogen receptor-1 gene, allele frequency, PCR-RFLP*

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1. Introduction

Coronary artery disease (CAD) is a multifactorial disorder. In developed countries, heart disease is the leading cause of death in both men and women over the age of 60 years [1]. While premenopausal women have a low incidence of CAD as compared to men due to the beneficial effects of estrogen on both the lipid metabolism and on the vasculature, the incidence and mortality among post-menopausal women may even exceed the rates of men [2]. Many new mechanisms are discovered in cardiovascular diseases and lately researches have been focused on the biological role exerted by estrogens, estrogen receptor and androgens. Estrogen receptors exert roles in cardioprotection in both males and females [3]. Sex differences may play an important role in the pathogenesis of CAD [4].

Estrogen has been studied for its beneficial and protective mechanism for the heart in women where the binding of estrogen to its receptors triggers estrogenic effects that are protective against atherosclerotic diseases [5,6].

In humans, there are 2 distinct estrogen receptors; estrogen receptor-α (ESR1) and estrogen receptor-β

(ESR2); both of them are members of a nuclear hormone receptor superfamily. They are encoded by 2 separate genes and both are expressed in endothelial cells and vascular smooth muscle cells [7]. ESR1 was shown to be the main mediator of the atheroprotective effect of estrogen in the vascular system [8,9]. The human estrogen receptor 1 (ESR1) gene is located on the long arm of chromosome 6 (6p25.1) and consists of 8 exons and 7 introns. The ESR1 gene encodes estrogen receptor-α, which is a ligand-activated transcription factor composed of several domains that are important for hormone binding, DNA binding, and activation of transcription [7]. A number of studies have identified that among the polymorphisms in the ESR1 gene, two single nucleotide polymorphisms (SNPs) (PvuII and XbaI) are the most widely investigated. The polymorphisms that were identified include c.454-351 A>G (XbaI, rs9340799) and c.454-397 T>C (PvuII, rs2234693) polymorphic variations. Those are present in the first intron of the ESR1 gene and are associated with different determining factors e.g. onset of menopause, arterial hypertension, bone density and body mass index (BMI) [10,11].

ESR1 is a good candidate gene because estrogen has beneficial effects on cardiovascular health status. Among the polymorphisms identified in the ESR1 gene, two SNPs (c.454-351 A>G and c.454-397 T>C) are widely studied (14, 15). Many studies had declared that the frequencies of the c.454-351 A>G and c.454-397 T>C genotype were not significantly different between CAD patients and controls in various populations [2,12].

On the other hand, some studies point out that homozygosity for the alleles of the c.454-351 G and c.454-397 C polymorphisms was significantly associated with increased CAD severity in males and females in different populations [13,14,15].

These findings suggest that ESR1 may be a proper candidate gene for CAD. In this study, we aimed to investigate the association of CAD with the frequencies of ESR-1 polymorphisms, and to see whether they are important genetic risk factors for CAD development in Tanta population.

2. Materials and Methods

This study was conducted from April 2012 and March 2014 and included 100 CAD patients that presented to the department of cardiovascular surgery and cardiology, Tanta University, Egypt. The presence and severity of CAD was determined by the Gensini Score [16].

Each patient had given an informed written consent before starting the data collection. 110 healthy control volunteers were collected randomly (who did not have any disease and had no clinical evidence of a family history in first-degree relative of CAD or other disorders).

Exclusion criteria: (acute coronary syndrome; stable angina pectoris with normal coronary arteries or with <50% stenosis in major coronary arteries; cerebrovascular disease; known renal, hepatic, or immunological disorders; obesity secondary to hypothyroidism or Cushing's disease and malignancy).

Demographic data including anthropometric measurements, cardiovascular risk factors, and medication use were recorded for all participants. BMI (kg/m^2) was calculated. The triglycerides (TG) levels, total cholesterol, high-density lipoprotein cholesterol (HDL-c), and low-density lipoprotein cholesterol (LDL-c) were determined using commercially available biochemical assay kits (Randox, UK).

Venous blood samples were collected from all the participants and divided into two aliquots. One was used for measurements of biochemical parameters and the other was placed in EDTA-containing vacutainers for genomic DNA extraction.

The extracted DNA was quantified by measuring its absorbance at 260 nm using GeneQuest (model CE2301, USA) according to manufacturer's instructions. DNA was analyzed using 1% agarose gel electrophoresis stained with ethidium bromide.

ESR1 gene polymorphisms: The c.454-351 A>G (g.34720 A>G, rs9340799) and c.454-397 T>C (g.34650 T>C, rs2234693) within the ESR gene were analyzed using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique described as was previously reported [16].

A PCR product (255 bp) was obtained using the forward primer (5′-CAGGGTTATGTGGCAATGAC-3′) and the reverse primer (5′-TACCTATAAAAATGAC AAAATGAAAT-3′) in 25 ml reaction mixture containing PCR buffer, 1.5mmol/L $MgCl_2$, 0.25mmol/L dNTPs mix, 0.4 mmol/L of each primer, 1.5 U Taq DNA polymerase and ~100ng DNA. PCR conditions included denaturation at 94°C for 4 minutes followed by 35 cycles including denaturation at 94°C for 30 seconds, annealing at 60°C for 30 seconds and extension at 72°C for 30 seconds and ending with extension at 72°C for 10 min using a thermal cycler (Eppendorff`s, Germany).

The PCR product (containing a part of intron1of ESR1 gene) was digested with PvuII and XbaI enzymes at 37 o C for 10 hrs, to produce a 255 bp fragment, which comprises the allele (C) or 97+158 bp fragments, which comprises the allele (T) and of 255 bp for the allele (G) or 142+113 bp for the allele (A), respectively.

Results were described as AA, GG or AG for Xba I and CC, TT or CT for heterozygous allele for PvuII. The products were electrophoresed on 3% agarose gel and stained with ethidium bromide to visualize DNA under ultraviolet illumination.

2.1. Statistical Analysis

The collected data was organized, tabulated and statistically analyzed using Prism 5 software statistical computer package version 5. For quantitative data, the range, mean and standard deviation were calculated. The difference between the two means of the two groups was statistically analyzed using the student t test based on the distribution and a p value of less than 0.05 was considered to indicate statistical significance. The difference between three means of the two groups was statistically analyzed using two way ANOVA where a p value of less than 0.05 was considered to indicate statistical significance. For qualitative data, the number and percent distribution was calculated. Chi square was used as a test of significance and when found inappropriate Fisher exact test was used. Significance was adopted at $P<0.05$ for interpretation of results of tests of significance.

3. Result

A total of 210 subjects were recruited in this study. These subjects comprised 100 CAD group patients and 110 normal volunteers (as the control group). A statistical analysis regarding clinical and biochemical characteristics of the studied groups is described in Table 1 & Figure 1, there was no significant difference between control and CAD subjects regarding sex, age and lipid profile (p-value = 0.73, 0.71 and 0.9). There is significant difference regarding BMI (p value < 0.001), but there is no value with other risk factors as diabetes, smoking, hypertension and family history of CAD (p- value 0.9).

Table 1. Clinical and biochemical characteristics of the studied groups.

	Patients (n=100)	Controls (n=110)	p Value
Sex F/M	45/55	67/43	0.73
Age	56.2±8.5	55.4±8.6	0.71
BMI(kg/m2)	28.2±5.5	26.1± 3.8	<0.001
Triacyl glycerol (mg/dl)	166.2±88.4	165.3±75.0	0.93
HDL-C	39.3 ±18.5	40.1 ±22.5	0.78
LDL-C	130.5 ±38.0	129.2±40.4	0.81
Diabetes (n)	33	10	0.93
Smoking (n)	35	22	0.90
Hypertension (n)	43	20	0.90
Family history of CAD	25	20	0.91

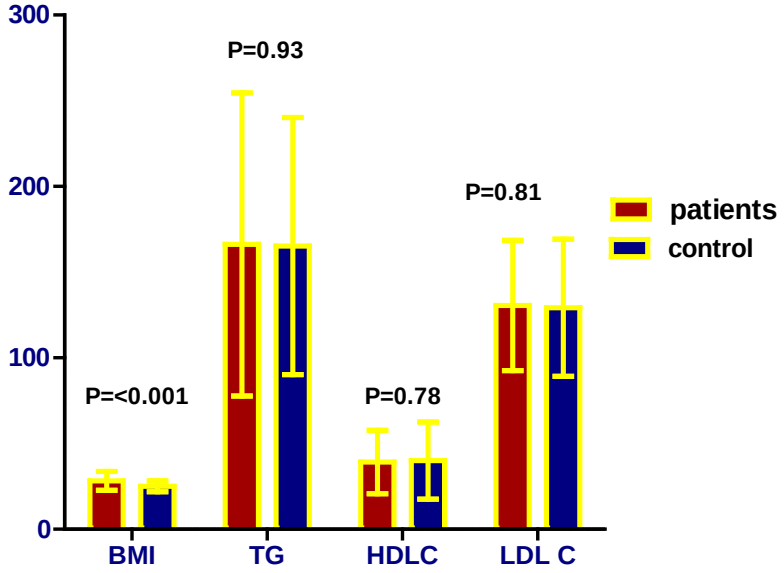

Figure 1. Biochemical characteristics of the studied groups

ESR1 genotypes and allele frequencies: The genotype and allele frequencies of PvuII and XbaI ESR1gene polymorphisms in CAD and control groups are presented in Table-II. There were no significant differences between the CAD patients regarding genotyping and allele frequencies of PvuII and XbaI polymorphism. Demographic, clinical and laboratory characteristics with and without polymorphic genotype for the ESR1 gene in patients with CAD were represented in table III and showed no significant importance. In Table 4, the frequencies of ESR1 gene polymorphisms in relation to sex was represented and showed no significant values. There was no significant difference among all studied subjects regarding sex, age, menopausal status and cardiac complications (data not shown).

Table 2. The genotypes distribution and alleles frequencies.

XbaI genotype frequencies	Patients (n=100)	Controls (n=110)	p value
AA	40(40%)	48 (43.6%)	ref
AG	34(34%)	32(29%)	0.76
GG	26(26%)	30(27%)	0.65
XbaI alleles frequencies			
A	114(%)	128(%)	ref
G	86(%)	92(%)	0.70
PvuII genotypes frequencies			
TT	35(%)	27(%)	ref
TC	52(%)	65(%)	0.92
CC	13(%)	18(%)	0.91
PvuII alleles frequencies			
T	122(%)	119(%)	ref
C	78(%)	101(%)	0.71

Table 3. Demographic, clinical and laboratory characteristics with and without polymorphic genotype for the ESR1 gene in patients with CAD.

	XbaI				PvuII			
	AA	AG	GG	P-	TT	TC	CC	P
Genotype	40	34	26		35	52	13	
Sex (F/M)	15/25	20/14	10/16	0.58	20/15	22/30	3/10	0.12
Age (years)	55.3± 8.2	56.2±7.5	54.5±9.0	0.72	56.2±9.1	55.6±6.9	57.0±7.1	0.82
BMI (kg/m2)	27.4±4.6	28.0±3.5	27.9±4.0	0.85	29.1±2.8	29.0±3.0	28.8±5.0	0.96
TG (mg/dL)	182.4±110.2	165.6±90.2	183.5±104.5	0.73	142.8±100.1	177.0±70.5	152.4±110.2	0.18
HDL-c (mg/dL)	36.6±7.5	34.5±8.5	38.4±6.9	0.15	39.1±3.8	37.4±8.0	40.9±6.8	0.19
LDL-c (mg/dL)	125.0 ± 40.8	128.8 ± 50.0	130.2 ± 46.7	0.88	129.0 ± 48.1	126.2 ± 52.1	130.0 ± 38.9	0.95
Diabetes	23	10	12	0.32	15	20	5	0.14
Smoking	18	12	10	0.75	22	27	6	0.06
Hypertension	16	19	11	0.43	14	25	8	0.07

Table 4. Frequencies of ESR1 gene polymorphisms in relation to sex

SNP	Patients (n=100)	Controls (n=110)	P-value
women	45	67	
XbaI Genotypes			
AA	16	26	ref
AG	20	29	0.06
GG	11	12	0.53
Alleles			
A	52	81	ref
G	42	55	0.27
Men	55	43	
AA	25	21	
AG	14	20	0.54
GG	16	20	1.0
Alleles			
A	64	44	ref
G	64	42	0.32
PvuII Genotypes			
Women	45	67	
TT	20	15	ref
TC	22	39	0.66
CC	3	13	0.79
Alleles			
T	62	69	ref
C	28	65	0.33
Men	55	43	
Genotypes			
TT	15	12	Ref
TC	28	26	0.81
CC	12	5	0.32
Alleles			
T	58	50	ref
C	52	36	0.25

4. Discussion

When estrogen receptor (ER) is combined with estrogen, it forms a dimer, then it stimulates transcription of target genes, by regulating the growth, reproduction, differentiation and function of many target organs, which included breast, uterus, ovaries, bone, liver, cardiovascular system and nervous system [17].

Estrogens are known to regulate the cardiovascular system via their effect on cholesterol, triglycerides and the blood vessel wall (e.g. vascular cell proliferation) [18]. The role of estrogens in ischemic heart disease (IHD) is uncertain.

Polymorphisms of ER gene might affect the role of estrogen on atherosclerosis. Evidence suggests that genetic variations in the estrogen receptor-α (ESR1) gene may influence ischemic heart disease (IHD) risk, but the role of common sequence variations in the ESR1 gene is unclear. However, the genetic factors underlying this form of cardiovascular disease are complex and not clearly established [17].

In this study we aimed to investigate the association of CAD with the frequencies of ESR1 PvuII and XbaI gene polymorphisms, and to see whether they are important as genetic risk factors for CAD or not. We did not find an association of ESR1 c.454-351 A>G and c.454-39 T>C genotypes and alleles with CAD.

Similarly, we did not observe an association between CAD and the frequencies of the ESR1: c.454-397 CC genotype and C allele (P >0.9). Our results agree with Abdussemet hazar and his colleges [19] who examined 100 healthy individuals and 80 CAD patients and they found that ESR1 variants were not associated with CAD and the two variants can be considered as an independent risk factor or a predictor for CAD. These two variants of the gene studied in different populations as in a Chinese population [20], Iranian population [21], and Spanish population [22] where the results showed no difference between control and patients.

On the contrary, c.454-351 A>G and c.454-397 T>C genotype distributions were significantly different between the CAD and non-CAD groups in a Greek population, in postmenopausal women and in men in a Japanese population [23].

In addition, subgroup analysis by sex did not demonstrate any significant relation in the two variant genes ($p > 0.05$). These findings for sex are in agreement with Karadag et al [5]. We found also no significant association with other risk factors as high lipid profile, diabetes and hypertension except BMI. These contradictory results might be partially caused by increasing age among normal controls where these age group populations always complain of these disorders. This agrees with Abdussemet hazar [19] who had found no statistically significant differences in TG, HDL-C, and the prevalence of current cigarette smoking between the CAD group and healthy subjects and attributed these result to limited number of studied groups. There was no significant difference among all studied subjects regarding sex, age, menopausal status and cardiac complications.

In conclusion, we found that the ESR1 gene c.454-351 A>G and c.454-397 T>C polymorphisms are not a risk factors for CAD in an Egyptian population in Tanta region. However, studies of larger numbers of patients and controls in various populations may be needed in order to determine the effect of these genes on the risk of CAD.

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