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Original Research Article

Estrogen Receptor Gene Polymorphisms and Risk of Myocardial Infarction

N. Sivaranjani[1*], Swaathy Ramamurthy[2], Prathibhabharathi[3], M. Rasheed Khan[4]

[1]Senior Assistant Professor, Dept. of Biochemistry, Government Medical College, Omandurar Government Estate, Chennai, Tamil Nadu.
[2]Senior Assistant Professor, Dept. of Microbiology, Government Medical College, Omandurar Government Estate, Chennai, Tamil Nadu.
[3]Associate professor, Sri Balaji Medical College, Hospital and Research Institute, New Airport Road, Renigunta. Andhra Pradesh.
[4]Associate Professor, Dept. Biochemistry, Srinivasan Medical College and Hospital, Trichy, Tamil Nadu.

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Corresponding author: Dr. N. Sivaranjani,
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Abstract:

Introduction: This study aimed to identify ESR1 gene polymorphisms associated with myocardial infarction susceptibility.

Materials and Methods: The present study was carried out for one year on diagnosed patients of Myocardial infarction admitted to the ICU and Cardiology ward of Narayana Medical College and Hospital, Nellore. 100 subjects were studied, including 50 normal healthy individuals and 50 diagnosed myocardial infarction cases. In all the participants, estrogen receptor -1 gene polymorphism, BMI, blood pressure, fasting blood glucose, serum lipid profile, and creatinine were estimated and the mean values were compared and analyzed.

Results: The mean levels of BMI, BP, serum TC, LDL, TAG, and VLDL were increased in cases but mean HDL decreased in cases was statistically significant ($p< 0.05$). In the present study, TT genotypes of ESR1 gene polymorphism is significantly elevated to 42% in Myocardial infarction patients when compared to control subjects. 26%. CT genotypes are significantly less in patient groups at 28% when compared to control subjects at 34%. CC genotypes are significantly less in patient groups 30% when compared to control subjects 40%. The frequency of the T allele of PvuII was significantly higher in patients (56%) than in controls (44%).

Conclusion: The present study concludes that ESR1 gene polymorphism may be a genetic marker for the development of MI.

Keywords: myocardial infarction, Coronary artery disease, ESR1 gene polymorphisms

Introduction

Myocardial infarction (MI) or Acute myocardial infarction (AMI), commonly known as a heart attack is the interruption of blood supply to a part of the heart, causing heart cells to die. [1] It is one of the most common diagnoses in hospitalized patients in industrialized countries. [2] In India, it has quickly become a major health issue with deaths due to cardiovascular

disease (CVD) expected to double during 1985–2015. [3]

The main causal and treatable risk factors for MI include hypertension, dyslipidemia, obesity, diabetes mellitus, and smoking. In addition to these risk factors, recent studies have shown the importance of genetic factors and of interactions between multiple genes and environmental factors in this condition. [4] some patients who have had a myocardial infarction do not have any conventional risk factors, suggesting the contribution of an uncharacterized genetic component.

One approach to preventing this condition is to identify disease-susceptibility genes. Genetic-linkage studies and candidate-gene analyses have implicated a locus and several candidate genes in the predisposition to myocardial infarction. [5]

Incidence and mortality due to CVD are much higher in men than in women before menopausal age. However, the gender difference diminishes when women go into postmenopause. [6,7] There are many possible mechanisms for the protective effects of estradiol on vasculature, including increased vasodilatation by its positive influence on endothelial nitric oxide (NO) synthase and subsequent production of NO. [8]

The physiological process of estrogen works by binding to estrogen receptors. [9] There are two known estrogen receptors, namely estrogen receptor-α (ESR1) and estrogen receptor-β (ESR2), which are transcription factors and are expressed in a wide range of tissues, including macrophages, adipose cells, vascular smooth muscle and vascular endothelial cells. [10] ESR1 has been identified in most cardiovascular tissues such as the coronary arterial wall in smooth muscle cells, endothelial cells, and myocardial cells. [11]

Two single-nucleotide polymorphisms (SNPs) have been identified in the first intron of the ER α gene: a T-C polymorphism that is recognized by the restriction endonuclease PvuII (T and C alleles correspond to the presence (p allele) and absence (P allele) of the restriction site, respectively) and an A-G polymorphism that is recognized by XbaI (A and G alleles correspond to the presence (x allele) and absence (X allele) of the restriction site, respectively). [12]

Alternation in ER-α expression and function may attenuate the atheroprotective role of oestrogens and increase the chance of myocardial infarction. [13]

The purpose of the present study was to identify ESR1 gene polymorphisms that confer susceptibility to myocardial infarction.

Materials and Methods

The present study was carried out for one year on diagnosed patients of Myocardial infarction admitted in the ICU and Cardiology ward of Narayana Medical College and Hospital, Nellore.

The study includes 50 myocardial infarction patients age group of 35-65 years. Both the sexes were included. Fifty healthy individuals working in Narayana Medical College & Hospital in the age group 35-65 were included in the control group.

Patients with diagnosed MI, and having family H/O of MI, H/O hypertension, diabetes, smoking, alcoholism, and MI attack at the past were included in the study.

Patients diagnosed with other cardiac abnormalities, renal, liver, and nutritional disorders, pregnant women, and children were excluded from the study.

The data on history regarding the onset of the disease, family history and personal history were collected through a standard questionnaire. A standard written informed consent, reviewed and approved by the Institutional ethical committee of Narayana Medical College and Hospital, Nellore Andhra Pradesh, India was obtained from all participating subjects

who were given a full explanation of the study

Sample Collection:

Blood samples were collected in a fasting condition. 6ml of venous blood was drawn from the patients and controls under aseptic precautions. 2ml of blood was taken in EDTA coated vacutainer tube for DNA isolation. 2 ml in Plain vacutainer for estimation of Lipid profile and Creatinine. 2ml in Fluoride coated vacutainer for estimation of Fasting Blood Glucose. The samples were separated by centrifugation and analysed or stored at 2-8˚c.

History was collected from both cases and controls like the onset of the disease, family history of cardiac disease, smoking & alcohol history, hypertension and diabetes mellitus history with treatment, in case of female-post-menopausal history.

Body Mass Index (BMI) was calculated as weight in kilograms divided by the square of the height in meters.

Blood pressure was measured using a mercury sphygmomanometer with the patients in the sitting position. After 5 minutes of rest in the sitting position. BP was measured on both the arms and higher value was taken into consideration.

Pulse pressure was calculated by subtracting SBP and DBP.

Plasma glucose was determined by the glucose oxidase method using a commercially available kit Human (gmbh Germany) using Humastar 300 chemistry analyzer (Human gmbh Germany).

Total cholesterol, triglycerides and HDL-cholesterol were measured using reagent kits from Human (gmbh Germany) using Humastar 300 chemistry analyser (Human gmbh Germany).

Serum creatinine was determined by the modified Jaffe's method using a commercially available kit Human (GmbH Germany) using Humastar 300 chemistry analyzer (Human GmbH Germany).

Estrogen receptor 1 T/C Gene Polymorphism (Genotypes) Analysis was done by

- Genomic DNA extraction
- PCR amplification
- Digestion by RFLP
- Electrophoresis separation.
- DNA bands analyzed by UV transilluminator.

The mean and standard deviation was calculated for all the Biochemical parameters. The significance between the groups was determined using Student **t-** test for Equality of means. p-value of < 0.05 was considered significant.

Results

A total of 100 individuals, aged between 35-65 yrs were recruited for this study including 50 healthy controls and 50 Myocardial infarction patients. 100 samples was analyzed to study the levels of Fasting Blood glucose, lipid profile, creatinine and ESR1 genotype.

The overall mean age of controls and case are 50.34 and 53.3 (range 35 to 65 yrs). The overall female/male ratio of controls and cases was (8/ 42) 0.19 and (41/9) 0.21, with male gender predominant in two groups.

BMI, SBP, DBP, PP, serum FBS, serum TC, serum TAG, serum LDL Cholesterol, serum VLDL Cholesterol and serum creatinine were increased in cases than controls and was statistically significant. There was decrease in the serum HDL levels in cases than controls and was statistically significant as shown in table 1.

Table 1: Comparison of baseline characteristics between the groups

Variable	Groups		p-value
	Controls(n=50) Mean ± SD	Cases(n=50) Mean ± SD	
BMI (kg/m^2)	21.61 ± 1.10	24.17 ± 2.55	<0.0001
SBP (mmHg)	116.2 ± 5.1	128.92 ± 11.3	<0.0001
DBP (mmHg)	79.84 ± 4.23	83.16 ± 6.6	<0.001
PP (mmHg)	36.36 ± 6.9	45.76 ± 15.51	<0.001
FBS (mg/dl)	100.74 ± 8.3	126.52 ± 15.3	< 0.0001
TC (mg/dl)	183.72 ± 40.50	223.94 ± 39.99	< 0.0001
TAG(mg/dl)	115.88 ± 36.97	182.8 ± 56.27	< 0.0001
HDL(mg/dl)	51.86 ± 10.35	41.68 ±15.22	<0.001
LDL(mg/dl)	155.03 ± 38.05	218.83 ± 44.52	< 0.0001
VLDL(mg/dl)	23.17 ± 7.39	36.57 ± 11.25	<0.0001
CREATININE (mg/dl)	0.83 ± 0.09	1.28 ± 0.44	< 0.001

Percentage of ESR1 genotype in patients and controls was first calculated. Allele frequencies are then calculated for each genotype by allele counting. The distribution of genotype among cases and controls studied using chi-square test. Odds ratio was calculated in 95% confidence interval (CI) and all p values was 2-tailed, with statistical significance defined by $p \leq 0.05$. The distribution of PvuII ER1 variants in controls and cases are as follows : Controls: TT = 26 %, CT = 34 %, CC = 40 %.

Cases: TT = 42 %, CT = 28 %, CC = 30 % as shown in table 2.

Table 2: Percentage of Estrogen receptor 1 genotype in controls and cases

Genotype	Controls (%)	Cases (%)
TT	13 (26 %)	21 (42 %)
CT	17 (34 %)	14 (28 %)
CC	20 (40 %)	15 (30 %)

On the basis of these finding the incidence of T allele and C allele in cases and controls are : Controls T allele was 43 % and C allele was 57%. Cases T allele was 56 % and C allele was 44 % as shown in table 3.

Table 3 : Allelic frequency of T and C in controls and cases

Study group	Allelic – frequency		Total
	C	T	
Controls (%)	57 (0.57)	43 (0.43)	100
Cases (%)	44 (0.44)	56 (0.56)	100

TT genotype of MI cases vs. CC genotype of controls was statistically significant ($p<0.005$) among the two groups. TT vs. CT was statistically significant ($p<0.005$) among the two groups. TT vs. CC+ CT was statistically significant ($p<0.005$) among the two groups as shown in table 4**.**

Table 4: Distribution of ESR1 genotypes between MI patients and controls

Patients vs. Controls	Chi-square (χ^2)	Odds ratio	CI 95%			p value
			L.limit		U.limit	
TT vs. CC	30.07	3.9	2.4		6.1	<0.005
TT vs. CT	6.04	1.3	0.9		2.2	<0.005
TT vs. CC+ CT	18.5	2.5	1.6		3.9	<0.005

Discussion:

Myocardial infarction (MI) is an important clinical problem because of its large contribution to mortality. The main causal and treatable risk factors for MI include hypertension, hypercholesterolemia or dyslipidemia, diabetes mellitus, and smoking.

Estrogen receptor -1 gene polymorphism

We found significant differences in allele frequencies of PvuII between cases and controls . The frequency of the T allele of PvuII was significantly higher in patients (56 %) than in controls (44 %). Our results were consistent with those of previous studies, which suggested that sequence variants in the ESR-1 gene polymorphism might modify the effects of estrogens on the cardiovascular system, and be associated with cardiovascular disease susceptibility. The work of Herrington et al (2002), [14] Ferrero et al (2003), [15] Demissie et al (2006), [16] Hayashi et al (2007), [17] Lamon-Fava et al (2009), [18] Teng Zhao, et al (2010), [19] are some of the notable references.

We also studied the other cardiac risk factors like lipid profile, ECG, diabetes mellitus, hypertension, smoking among myocardial infarction patients. The classical cardiovascular risk factors considered in the study were as follows :

Blood pressure: The incidence of atherosclerosis increases as blood pressure rises and this excess risk is related to both systolic and diastolic blood pressure as well as pulse pressure. [**20]** Arterial hypertension is a well-known risk factor for the development of ischaemic heart disease and approximately one-third of patients admitted to hospital with an acute myocardial infarction has a history of hypertension. Left ventricular hypertrophy may lead to impaired coronary reserve and myocardial ischaemia even in the presence of normal coronary arteries and left ventricular hypertrophy is a well established independent predictor of mortality. [**21]** There is an increase in mean values of BP in MI cases when compared to controls and is statistically highly significant ($p<0.0001$).

Fasting Blood Sugar: Diabetes Mellitus is a potent risk factor for all forms of atherosclerosis and is often associated with diffuse disease that is difficult to treat. Insulin resistance is associated with obesity and physical inactivity, and is also a potent risk factor for coronary heart disease.[20] The presence of diabetes mellitus in the setting of an acute myocardial infarction is a marker of a poor prognosis. [**22]**

In this study the mean levels of FBS in controls were 100.74 and in cases were 126.52 . There is decrease mean level of FBS in controls than in cases and it is statistically highly significant ($p < 0.0001$).

Dyslipidemia: Dyslipidemia is a major modifiable risk factor for CAD. Dyslipidemic patients are more prone to myocardial infarction due to increased free radical generation and ischemia. Low high density lipoprotein cholesterol (HDL-C) levels are common in MI patients. The HDL-C level is the most important independent protective factor against arteriosclerosis which underlies coronary heart disease. HDL-C associated paraoxonase-1 enzyme (PON1) is protective against lipid peroxidation. Numerous cohort studies and clinical trials have confirmed the association between a low HDL-C and increased risk of CAD. LDL-C is considered as the most important risk factor for CAD. Its oxidized form promotes foam cell formation which initiates the process of atherosclerosis by accumulating in sub-endothelium cells leading to fatty streaks and complex fibro-fatty or atheromatous plaque formation. [**23]**

In this study the mean levels of serum TC**,** LDL**,** TAG**,** VLDL in controls were decreased (table 2) than in cases and it is statistically highly significant ($p < 0.0001$). But there is an increase in mean level of

serum HDL in controls than MI cases and it is statistically extremely significant ($p < 0.0001$). These findings were in accordance with work of Patil et al **(**2007). [**24]**

BMI : Obesity increases mortality in adult and elderly patients with cardiovascular diseases. This relation could be due to the higher prevalence of diabetes, hypertension, and hypercholesterolemia observed in obese patients. Overweight is also associated with increased plasminogen activator inhibitor activity and, therefore, with altered fibrinolytic activity. Finally, hypertriglyceridemia is frequent in patients with a high BMI and is an independent risk factor for CAD. [**25]** In our study the mean levels of BMI in cases were higher than controls and was statistically extremely significant ($p < 0.0001$). [26]

Creatinine: Renal dysfunction is a strong independent predictor of cardiovascular outcomes and mortality in the general population after myocardial infarction, heart failure. Baseline renal function is a potent independent risk factor for adverse events after AMI. This increased mortality risk continued until 6 months after myocardial infarction. These findings suggest that close monitoring of renal function during the first few weeks after acute MI.**[26]** In this study the mean levels of creatinine in cases were higher than controls and was statistically extremely significant ($p < 0.0001$).

Conclusion:

The mean levels of BMI, BP, serum TC, LDL, TAG, VLDL, and creatinine were increased in cases but mean levels of HDL decreased in cases. In the present study, TT genotypes of ESR1 gene polymorphism is significantly elevated in Myocardial infarction patients 42% when compared to control subjects 26%. CT genotypes are significantly less in patient groups 28 % when compared to control subjects 34 %. CC genotypes are significantly less in patient groups 30 % when compared to control subjects 40 %. In our study, T allele of PvuII was strongly associated with Myocardial infarction patients (56 %). Screening for ESR1gene PvuII polymorphism genotypes may help to identify patients who are at risk of developing MI. The present study concludes that ESR1 gene polymorphism may be a genetic marker for the development of MI**.**

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