Association ofACE Gene Polymorphisms with In-Stent Restenosis by Stent Type (Biomime, Supraflex, Xience)

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Abstract

Introduction: Angiotensin Converting Enzyme or ACE is an exopeptidase that causes the conversion of angiotensin I to angiotensin II, vasoconstriction, and aldosterone secretion. ACE gene polymorphism (I/D) causes more enzyme activity and increases the risk of coronary artery disease or CAD.

Aims: To examine the role of ACE (I/D) Gene Polymorphisms by Stent Types (Biomime, Supraflex, Xience) has been investigated in patients who underwent angioplasty in this study.

Material & Methods: Patients in the in-stent restenosis group (ISR^+) (N=53) and patients non-ISR group (ISR^-) (N=68) have been enrolled in this study based on follow-up angiography > 1 year after PCI. Frequencies of allele and genotypes of the ACE (I/D) variant were determined using polymerase chain reaction (PCR).

Results: The genotypes and allele frequencies were not significantly different between the studied populations (p-Values > 0.05). However, there was a significant difference between people with a history of Clopidogrel use in the ISR- and ISR+ groups observed (p-Values > 0.005).

Conclusion: In the present study, there was no statistically significant relationship between ACE (I/D) gene polymorphism and the incidence of restenosis in patients who underwent repeat angiography. However, the ratio of frequency percentage of alleles to each other in terms of frequency shows the highest to lowest alleles I/D, D/D, and I/I in both groups. In the comparison of drugs used among patients, the drug Clopidogrel (Plavix) is discussed in the incidence of restenosis, in this study, the results showed that the number of patients who received Clopidogrel in the ISR+ group was significantly less than the ISR- group. This issue can indicate the inhibitory effect of Clopidogrel in the recurrence of stenosis.

1. Introduction

Atherosclerosis is the leading cause of death and morbidity in developed countries. The disease affects the entire circulatory system and causes specific clinical manifestations depending on the type of vascular bed involved. For example, coronary artery atherosclerosis causes heart attack (MI) and other coronary events. Percutaneous vascular interventions in the coronary arteries (PCI) can treat asymptomatic atherosclerosis (1).

Coronary artery disease and radial infarction (MI) are complex disorder result-disorder the interaction between genetic and environmental factors (2, 3). Percutaneous transluminal coronary angioplasty (PTCA) was introduced in 1979 (3). Restenosis after primary successful percutaneous coronary interventions (PCIs) with balloon angioplasty is one of the principal wound-healing responses to vascular wall trauma, occurring in up to 50% of patients undergoing the procedure without stenting and in about 20% of patients receiving stents. The molecular mechanisms of arterial remodeling are less well understood. Even with extensive efforts using genome-wide linkage studies, the responsible genetic determinants remain largely uncategorized (3-5). Genetic epidemiology might provide insights into the

pathophysiology of coronary restenosis and easily identifiable markers for predicting an increased restenosis risk (3, 5, 6). Despite a lack of good evidence that susceptibility to restenosis is genetically determined, several studies have investigated polymorphisms that might be associated with restenosis (3, 4, 6-10). Among the most studied genes for its association with the pathogenesis of CAD and related outcomes is the angiotensin-converting enzyme (ACE) gene, located on chromosome 17q23 (11).

The growth of vascular endothelial tissue into the stent lumen can cause the stent lumen to narrow again shortly after the stent is implanted. This is called In-Stent Restenosis (12). Accumulation of macrophages and vascular smooth muscle cells are the primary events in native vascular atherosclerosis as well as atherosclerosis after angioplasty balloon and post-stent implantation in the arteries. Several types of growth factors, chemokine's, and cytokines are released from atherosclerotic cells synergistically, they interact to form a network of biological signals that activate and differentiate monocytes, coagulate and proliferate SMCs, and ultimately cause the migration of these agents into the intima and neo-intimal thickening (13).

Neo-intimal thickening is the main pathophysiology in the ISR-causing process. Histopathological examination shows that vascular wall smooth muscle cells are a key element in the formation of neointima, and it is neointima itself that ultimately narrows the coronary arteries by narrowing the lumen (14, 15).

A genetic link to the ISR that has been addressed in recent studies is the association between angiotensin-converting enzyme (ACE) gene polymorphism and ISR, which has been shown in several studies (16).

This enzyme gene (ACE) is involved in regulating blood pressure and electrolyte balanceand catalyzes physiologically active angiotensin I to angiotensin II. Angiotensin II is a large, aldosterone-stimulating peptide that controls blood pressure and fluid and electrolyte balance. ACE also inhibits vasodilator proteins and Bradykinin. Accordingly, the encoded enzyme increases blood pressure and is the drug target of ACE inhibitors, which are often prescribed to lower blood pressure (17).

As studies show, cardiovascular diseases are one of the most important causes of death in Iran and the world, the prevalence of which is increasing widely. To deal with this intervention, several drugs are suggested for treatment, among which Glopidogrel is considered to be one of the most widely used drugs due to its consumption and high therapeutic effects (18).

Glopidogrel is an anti-platelet agent that selectively prevents the binding of ADP to platelet receptors and the activation of the ADP mediator of the GPIIb/IIIa complex and subsequent platelet aggregation. Glopidogrel acts by irreversibly changing the ADP receptors of platelets so that the platelets affected by Glopidogrel will remain in this form for the rest of their survival (19-21).

In the pharmaceutical market of Iran, Glopidogrel is available under the brand name Plavix and it is administered orally as a platelet aggregation inhibitor. This drug is used as an alternative to aspirin as a prophylaxis in patients with the risk of thromboembolic events such as heart attack, stroke, and peripheral vascular disease. One of the most important uses of this drug is in patients who undergo coronary angioplasty. (22).

Therefore, in this study, we intend to investigate for the first time the association between ACE gene polymorphism in the ISR of patients undergoing angioplasty by stent type (Biomime, Supraflex, and Xience) and their clinical features besides medical treatments including Glopidogrel administration.

2. Materials And Methods

This study is a case-control study in which patients who underwent angioplasty due to coronary artery disease with drug stents (Biomime, Supraflex, and Xience) and within 1 year after angioplasty, for any reason. Underwent re-coronary angiography and were included in the study.

Patients were collected from the cardiac catheterization laboratory of Imam Khomeini Hospital, which is the University Hospital of Tehran University of Medical Sciences. Participants were divided into two groups according to whether or not they had an ISR.

Patients who had significant restenography at the coronary stent site based on Mehran criteria (23) on re-angiography, in the case group, and patients on re-angiography, no restenosis on the coronary stent site, in the control group are located.

Background and baseline characteristics of patients including age, sex, weight, and as well as risk factors for myocardial infarction including diabetes mellitus, hypertension, hyperlipidemia, C/S, positive family history and metabolic syndrome, history of previous diseases, Drug history, clinical scenario referred because of it and Ejection Fraction of patients at the time of referral, using the data in the patients' files, were recorded by the researcher in pre-prepared forms. Information related to angiography of patients including coronary artery involved, stenosis and location, lesion characteristics including type of lesion, its length and presence of calcification, stent-related characteristics including stent diameter, type and number of stents used in angioplasty, and The ISR pattern in each patient was recorded based on Mehran classification (23) as well as the duration of treatment with two anticoagulants and complications during PCI, from the angiography forms of the patients in the pre-prepared forms.

The ISR was defined angiographically as a stenosis of more than 50% in diameter on the stent piece or its edges (5 mm pieces adjacent to the stent) after PCI (24, 25).

Patients who had ISR less than 6 months after stenting, coronary angioplasty with metal stent implantation, coronary angioplasty with drug stent implantation, except for the three mentioned types, age less than 18 years, and dissatisfaction with attending the study was deleted. A comparison was made between the two groups of patients (In- Stent Restenosis Group (ISR+)) (N = 53) and the age and

sex matching of the control group (non-ISR group (ISR-)) (N = 68). After obtaining informed consent, an individual and demographic questionnaire were completed for all patients including risk factors, past medical history, and clinical manifestations.

This study was approved by the Ethics Committee of Tehran University of Medical Sciences (TUMS) and written informed consent was obtained from the study population.

2.1. Genomic DNA analysis

Total genomic DNA was extracted from the blood by the phenol chloroform method. The polymorphism flanking region of the ACE gene for detection of I/D was amplified by polymerase chain reaction (PCR) using the following; Forward: CTGGAGAGCCACTCCCATCCTTTCT and Reverse primers: GATGTGGCCATCACATTCGTCAGAT. PCR was performed with the following program including 35 cycles of initial denaturation; 95 °C for 5 min, denaturation 95 °C for 30 Sec, annealing 62.7 °C for 30 Sec, extension 75 °C for 50 Sec, with a final extension period of 5 min at 75 °C. The amplified product was observed on 2% Agarose gel electrophoresis. The Insertion fragment was 490 bp and the deletion fragment was 190 bp, the genotyping is shown in Figure 1.

2.2. Statistical analysis

Statistical analysis was conducted by SPSS software version 16. The chi-square test was used for comparison of Allele and Genotype frequencies between groups. Odds ratios (OR) and 95% confidence intervals (CI) were calculated using the chi-square test. p-Values <0.05 was considered statistically significant.

3. Results

This study aimed to investigate the relationship between ACE gene polymorphism and In-Stent Restenosis according to the type of drug stent. The results and findings of the research project are presented in this section.

3.1 Results from clinical characteristics and demographic information of patients

The results of this case-control study in which patients referred to Imam Khomeini Hospital due to coronary artery disease underwent drug stent angioplasty (ISR- group) and within one year after angioplasty for any reason under angiography (ISR+ group) were included in this study after complete explanations by the treating physician and completion of a demographic and qualified questionnaire and were included in this study.

In the present study, 68 ISR- patients (non-ISR); control group (67.4%) and 53 ISR+ patients (In- Stent Restenosis); case group (32.2%) were studied.

According to the comparison of the frequency of demographic information of patients, there is a significant difference between age, gender, body mass index (BMI), diabetes mellitus, Hypertension (HTN), Hyperlipidemia (HPL), C/S (Protein C and protein S), dyslipidemia (Positive Familial Hypercholesterolemia), metabolic syndrome and smoking was not observed in both ISR- and ISR+ groups (p-Values <0.05) (Table 1).

Statistical results of the analysis of angiographic reasons at the time of referral of patients, in both case and control groups; no significant correlation, was observed (p-Values <0.05) (Table 2).

In the investigation of the use of bare-metal stents (BMS) and drug-eluting stents (DES) in both ISR- and ISR+ groups, no significant relationship was observed in previous angioplasty and PCI (p-Values <0.05) (Table 3).

The results obtained from the history of Statin and Acetylsalicylic Acid (ASA) use in both groups did not show a significant relationship (p-Values <0.05), however, there was a significant difference between people with a history of Clopidogrel use in the ISR- and ISR+ groups was observed (p-Values < 0.005) (Table 4).

The results of average body mass index and age in both case (ISR+) and control (ISR-) groups (mean ± standard deviation) are summarized in Table 5.

No significant difference was observed in the study of genotypic frequency in ISR- and ISR^+ groups, also in the comparison of genotypic frequency according to patients' clinical information shows that no statistically significant results were reported in any of the demographic parameters (p-Values <0.05) (Table 6, 7). The results of the comparison of the genotypic frequency with the history of taking the drugs Statin, ASA and Clopidogrel did not show any significant difference in the case and control groups (Table 8).

4. Discussion

Coronary artery disease are amongst the diseases that affect the heart and blood vessels and are known as the most common cause of death worldwide. It has been reported that in 2000, about 25% (about 1 billion) of the world's population were affected by high blood pressure, and according to recent findings by Kirini et al., this number is expected to increase to 1.56 billion in 2025 (26).

Many factors can trigger ISRs, but there is growing evidence that genetic factors play an important role in this, and so this has led to new therapeutic strategies for ISRs by targeting these genes (14, 15).

One of the standard treatments for CAD is percutaneous coronary intervention (PCI) with stent placement. However, a portion of patients undergoing PCI suffers from in-stent restenosis, which is the main limitation of coronary stenting. ISR is the formation of scar tissue on the stent and can cause re-narrowing of the opened artery. Although the incidence of ISR has recently decreased with the use of new

therapies, the treatment of ISR remains a difficult clinical issue. Over the past decade, there has been increasing scientific interest in understanding the complex relationship between post-PCI coronary restenosis and the renin-angiotensin system (RAS) (27).

The Renin-Angiotensin System (RAS) is a hormonal system that regulates blood pressure and fluid balance in the body and plays an important role in the pathophysiology of cardiovascular diseases such as congestive heart failure and hypertension (28).

The renin-angiotensin system (RAS) has been implicated in the development and progression of neointimal hyperplasia predominantly through its bioactive peptide, angiotensin II (Ang II). Angiotensin-converting enzyme (ACE) converts angiotensin I (Ang I) to Ang II which promotes the migration and proliferation of VSMCs, causes vasoconstriction, and regulates expression of adhesion molecules via its major cellular receptor, the Ang II type 1 receptor (AT1R) (16).

ACE is the main component of the renin-angiotensin system, which not only controls blood pressure, fluids, and electrical balance, but also changes the function of the kidneys, blood vessels, and heart muscle (29).

The ACE enzyme is non-specific and cuts dipeptide units consisting of two amino acids from the carboxyl end of substrates with different sequences. Substrates are more suitable that have only one free carboxyl group in the amino acid at the end of C and all amino acids except Proline can be present in this position. Excessive activity of ACE leads to increased vasoconstriction and development of high blood pressure, and inhibitory peptides prevent the production of angiotensin II by ACE. A decrease in ACE activity increases the level of Bradykinin, which is a vasodilator (30).

Angiotensin-converting enzyme (ACE) stimulates smooth muscle cell proliferation, and ACE plasma concentration is partially controlled by an insertion/deletion (I/D) polymorphism in the ACE gene on chromosome 17.9. The I/D polymorphism is characterized by the presence (Insertion) or absence (Deletion) of a 288 bp repetitive sequence in intron 16 on chromosome 17 (31). Many studies have linked the presence or absence of a 287 bp Alu repeating element in this gene to the level of circulating enzyme. This polymorphism as well as mutations in this gene are involved in a wide range of diseases including cardiovascular pathophysiology, psoriasis, kidney disease, stroke, and Alzheimer's disease (17).

That is, if this sequence is deleted, D allele (Deletion) and if this sequence is present, allele I (Insertion) is formed. This polymorphism is the determinant of plasma ACE activity. People who are homozygous for allele D have the highest plasma ACE levels, people who are homozygous for allele I have the lowest levels, and people who are heterozygous for I/D show moderate levels. In the presence of this polymorphism, three genotypes DD, II, and ID are created (32).

High levels of angiotensin II develop neointimal hyperplasia after vascular injury. DD genotype of an ACE gene is associated with higher levels of angiotensin II. In addition, treatment with ACE inhibitors (ACEI) is

beneficial after stent placement in coronary arteries. The HOPE and EUROPA trials showed that ACEIs reduce the clinical consequences of atherosclerosis (7).

In the present study, there was no statistically significant relationship between ACE gene polymorphism and the incidence of restenosis in patients who underwent repeat angiography. In addition, no significant difference was observed between dominant, recessive, and pronounced genetic models with the occurrence of re-stenosis. The present study did not show a significant relationship between the allelic frequency between the two groups with restenosis (ISR+) and the group without restenosis (ISR-).

In addition, comparing the genotypic frequency according to the clinical information of the patients, shows that no statistically significant association with demographic parameters.

The study of Guneri et al reported association between ACE gene polymorphism and stent restenosis. It was observed that the allele D frequency was significantly more frequent in the ISR group than in the Non-ISR group and carriers of allele D were significantly more expected to develop ISR than carriers of the I allele (7).

Genes such as the ACE gene or eNOS gene may play an important role in improving ISR in CAD patients. Endothelin-1, TGF-β, angiotensin II and nitric oxide (NO) are released from damaged endothelial cells participating in hyperplasia. Inner membrane and smooth muscle cell proliferation leading to a subsequent increase in ISR (31).

RAS plays an important role in blood pressure and cardiovascular homeostasis. Recent data suggest that angiotensin II, the main biologically active peptide of the RAS, contributes to the development and progression of coronary restenosis. Angiotensin II production is regulated by ACE, whose serum levels are controlled by genetic variation at the ACE locus. Studies show that genetic polymorphisms are associated with the risk of coronary artery restenosis. ACE gene polymorphisms were considered a risk factor for coronary artery restenosis in several published articles (27).

Studies show that high levels of ACE increase the risk of coronary artery thrombosis. Plasma ACE activity plays a major role in causing restenosis after coronary stent implantation. Also, several of studies have reported the association between ACE I/D polymorphisms and the risk of coronary artery restenosis (27).

The study of the relationship between polymorphisms of different genes and ISR in recent years has been considered by a significant number of researchers. For examplein, a study by Min Zhu et al. in 2017 on the relationship between seven polymorphisms of renin-angiotensin genes with the rate of restenosis after angioplasty, out of 3052 patients who underwent angioplasty using DES stents, 75 patients who underwent angiography-proven stenography underwent genetic testing. The result has indicated that the DD genotype of the ACE enzyme was significantly associated with restenosis, but other polymorphisms were not significantly associated with restenosis. The only other positive finding in this study was the association between angiotensin II polymorphism (A1166C) and restenosis in patients over 60 years of age (33).

The relationship between ACE enzyme DD genotype and extensive ISR in patients undergoing angioplasty was shown in another study with a smaller sample size by Ikuo Taniguchi et al. In Japan. It is also a risk factor for coronary artery disease (17).

The relationship between DD genotype of the ACE enzyme polymorphisms with restenosis after angioplasty seems to be well known. Because Shen Wang and colleagues in a systematic review study by examining evidence from 33 large cohort studies on a total of more than 11,000 patients, showed that the DD genotype of the ACE I / D polymorphism significantly increased the risk of restenosis (18).

A case-control study was conducted between 2019 and 2020 with the aim of investigating the role of VEGF-2549 variants I/D in the formation of ISR. The results showed that the frequency of the I/I allele was higher in the In-Stent Restenosis Group and the D/D allele was higher in the non-ISR Group and a significant relationship between the frequency of the VEGF-2549 genotype and ISR was observed (34).

Genetic changes are the most sensitive factor affecting people's response to drugs. If ISR can be associated with other SNPs, it may be clear why repeat stenting is still needed and why genetic screening is recommended for these patients (31). .Platelet activation and accumulation play an important role in thrombosis, which leads to acute coronary syndrome. Antiplatelet drugs such as Clopidogrel are currently used to prevent ischemic events in patients with acute coronary syndrome (35).

Clopidogrel is a prodrug belonging to the thienopyridine family for antiplatelet effects that require biological conversion (36, 37). It has also been reported in the formation of neointima (38-40).

Thinopyridine derivatives such as Ticlopidine and Glopidogrel (Plavix) are anti-platelet agents and prevent the accumulation of platelets derived from adenosine diphosphate (ADP), which will cause the risk of ischemia (41).

Preventing the activation of platelets and their accumulation using Glopidogrel compounds is one of the most common methods of treating coronary artery diseases (20, 21).

Antiplatelet drugs are used in many cases, including the prevention of vascular ischemia in atherosclerotic patients, acute coronary syndrome, and prevention of thrombosis after stent placement (42, 43). Clinical trials on antiplatelet drugs show the usefulness of these drugs after heart surgery (44).

By binding to Adenosine diphosphate (ADP) to its receptor on platelets, Clopidogrel inhibits platelet aggregation by selective and irreversible inhibition (43, 45).

The process of platelet aggregation begins with damage to the vessel endothelial wall, in which the circulating platelets attach to the vessel wall by interacting with sub-endothelial components such as Collagen and other adhesive proteins, preventing bleeding Becomes (12).

As mentioned, Clopidogrel prevents thrombosis in stented vessels, it is also in heart attacks and unstable angina to prevent the recurrence of ischemic events (46).

Although the relevance of Clopidogrel in the incidence of restenosis is controversial, in this study, the results indicated that the number of patients who received Clopidogrel in the ISR+ group was significantly lower than in the ISR- group. This issue can indicate the inhibitory effect of Clopidogrel on the recurrence of stenosis.

Investigation of the relationship between the polymorphism of different genes and the rate of ISR has attracted the attention of many researchers in recent years. For example, in a study published in 2017, Zsolt Bagyura et al investigated the relationship between single nucleotide polymorphisms of the VEGF gene (rs2010963) and ISR (rs6999447) in patients who underwent angioplasty with BMS stents. Have paid in this study, 205 patients who underwent angioplasty with BMS were divided into two groups. The patient group included 105 people with disseminated ISR and the control group included 100. This study showed that the rate of ISR in patients with the homozygous genotype of the rs2010963 polymorphism was significantly higher than in other patients, and the homozygous genotype of the rs2010963 polymorphism was significantly higher. It independently increases the rate of ISR in patients. (47).

The relationship between ACE enzyme DD genotype and extensive ISR in patients undergoing angioplasty was shown in another study with a smaller sample size by Ikuo Taniguchi et al. In Japan. It is also a risk factor for coronary artery disease (48).

In a 2021 study by Abdelaziz et al., to investigate the relationship between common single nucleotide polymorphisms (SNPs) in the angiotensin-converting enzyme (ACE) gene and risk of in-stent restenosis (12) and/or ACE inhibitor response in individuals Patients with stable coronary artery disease (49) after stent implantation were performed on 200 Egyptians who were divided into two groups (in-stent restenosis (ISR) group and non-ISR group). This study showed that carriers of the D allele of an ACE gene are significantly more susceptible to ISR. However, I allele carriers was significantly more susceptible to ISR after ACEI administration. There is a negative interaction between the DD genotype of the ACE gene and the administration of ACEI in post-percutaneous ISR intervention (PCI). However, there was a positive interaction between gene II genotype and ACE ID and ACEI administration on ISR after PCI with bare metal stents (BMS).

Also, this study showed a significant increase in the frequency of men, stent length, blood pressure, diabetes, CKD, and obesity in the ISR group compared to the non-ISR group, indicating that these are risk factors for ISR (31).

In this study, the results related to the frequency of men in both the in-stent re-stenosis group and the non-ISR group have the highest number compared to women, which can indicate an increase in the male population in relation to this condition.

Related to this discussion, Ekizler et al found that women were associated with a higher rate of occurrence of ISR after BMS implantation than men (50).

The cardiac muscle, an omnivorous organ, receives energy from the oxidation of fatty acids (51). Factors that reduce the risk of ischemic heart disease are needed to prevent the maintenance of heart function and heart failure, which early diagnosis and also related to gender differences are mandatory for primary or secondary prevention (52).

On the other hand, Solinas study showed that female gender is not a separate indicator of ISR

Wei Miao and Gong conducted a case-control study on the relationship between ACE I/D gene polymorphisms and coronary artery restenosis after percutaneous coronary intervention (PCI). In the present study, performed a meta-analysis to reveal the association of ACE I/D polymorphisms with coronary restenosis. results showed that ACE gene I/D polymorphisms were associated with the susceptibility of coronary restenosis, with the D allele carriers having a 1.92-fold increase in risk for restenosis, compared to the I allele carriers (27).

However, Rebrova et al, found that ACE genetic polymorphism was associated with the risk of coronary artery restenosis (53). Martínez-Ríos et al, found no association between ACE gene insertion/deletion (54).

From the practical aspects of this plan, individuals with specific feature will be identified using a genetic test which helps in prevention and later treatment. Therefore ACE gene polymorphism and factors affecting these changes can be of great help in treating patients.

5. Conclusion

In the present study, there was no statistically significant relationship between ACE gene polymorphism (I/D) and the incidence of restenosis in patients who underwent repeated angiography. However, the percentage ratio of allele frequency to each other in terms of frequency shows the highest to lowest alleles of I/D, D/D and I/I in both groups. In addition, the drug Clopidogrel has been discussed in the incidence of restenosis, in this study; the results showed that the number of patients who received Clopidogrel was significant in the ISR+ group. This issue can indicate the inhibitory effect of Clopidogrel in the recurrence of stenosis.

According to the past studies and the present study, the results are inconsistent or even contradictory. This difference may be due to a small sample size, ethnic difference, false positive results, and/or study design in each study. However, there is still a need for further research and screening of etiological relationships between functional polymorphisms of the ACE gene and susceptibility to coronary artery restenosis.

The reason for the difference in the results in the present study, which did not find a statistically significant relationship between ACE gene polymorphism and the incidence of re-stenosis in patients who

underwent re-angiography, can be the duration of the study and the number of patients. In addition, in future studies, more accurate results will be obtained by increasing the time period and the number of people.

Also, if possible and with the discretion of surgeons and cardiologists, the use of new drugs and their effectiveness should be added to the study with desired intervals. In addition, further population studies can help to confirm and generalize the findings. Therefore, inconsistent findings among individual studies may be due to differences in research approaches.

Abbreviations

ACE: Angiotensin-Converting Enzyme

ISR: In-Stent Restenosis

MI: Myocardial Infarction

PCI: Percutaneous Coronary Intervention

CAD: Coronary Artery Disease

PTCA: Percutaneous Transluminal Coronary Angioplasty

PCIs: Percutaneous Coronary Interventions

BMS: Bare-Metal Stent

DES: Drug-Eluting Stent

SMC: Smooth Muscle Cell

VEGF: Vascular Endothelial Growth Factor

ADP: Adenosine Diphosphate

ISR^+: In-Stent Restenosis Group

ISR^-: non-ISR Group

RAS: Renin-Angiotensin System

Declarations

Authors' contributions

Zeynab Nickhah Klashami: Perform laboratory tests, Data Collection and/or Processing, Analysis and/or Interpretation, Writing and Critical Review.

Mahsa M Amoli: Design, Supervision, Fundings, Analysis and/or Interpretation Literature Review and Critical Review.

All authors have read and agreed to the published version of the manuscript.

Author contributions

All authors contributed to the conception and design of the study.

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Data availability

There is no data and material to be shared.

Conflict of interest statement

The authors declare no conflict of interest.

Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethics protocol of the institutional and/or national Research Committee and the 1964 Helsinki declaration and its later amendments or comparable ethical standards. This study was approved by Endocrine and Metabolic Research Institute (EMRI) Ethics Committee (IR.TUMS.MEDICINE.REC.1398.693).

Consent to participate

Informed consent was obtained from all individual participants included in the study.

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Tables

Table 1. Association between Clinical Characteristics of the Study Groups with In- Stent Restenosis Group (ISR+) to non-ISR Group (ISR-).

Parameters	ISR+ Groups (N=53)	ISR- Groups (N=68)	Total	p-Values
Gender				
Male	39 (50.0%)	39 (50.0%)	78	0.06*
Female	14 (32.6%)	29 (67.4%)	43	
Diabetes				
Negative	32 (43.2%)	42 (56.8%)	47	0.8
Positive	21 (44.7%)	26 (55.3%)	74	
HTN				
Negative	24 (46.2%)	28 (53.8%)	52	0.6
Positive	29 (42.0%)	40 (58.0%)	69	
HPL				
Negative	37 (43.0%)	49 (57.0%)	86	0.7
Positive	16 (45.7%)	19 (54.3%)	35	
C/S				
Negative	33 (43.4%)	43 (56.6%)	76	0.9
Positive	20 (44.4%)	25 (55.6%)	45	
Positive FH				
Negative	48 (42.5%)	65 (57.5%)	113	0.2
Positive	5 (62.5%)	3 (37.5%)	8	
Metabolic syndrome				
Negative	43 (45.7%)	51 (54.3%)	94	0.4

Positive	10 (37.0%)	17 (63.0%)	27	
Smoker				
Negative	11 (35.5%)	20 (64.5%)	31	0.9
Positive	9 (36.0%)	16 (64.0%)	25	

- **ISR:** In- Stent Restenosis
- **HTN:** Hypertension
- **HPL:** Hyperlipidemia
- **C/S:** Protein C and protein S (Protein C and protein S are proteins in the blood that work together to prevent your blood from clotting too much. Tests of proteins C and S use a sample of blood to measure how much of these proteins you have and how well they're working.)
- **Positive FH:** Positive Familial Hypercholesterolemia (Dyslipidemia)
- p-Values <0.05 has been considered significant

Table 2. Clinical Scenario at Presentation (Reason for Angiography) with In- Stent Restenosis Group (ISR+) to non-ISR Group (ISR-).

Parameters	ISR+ Groups (N=53)	ISR- Groups (N=68)	Total	p-Values
Reason for Angiography				
Positive Noninvasive Test	8 (47.1%)	9 (52.9%)	17	
STEMI	3 (75.0%)	1 (25.0%)	4	0.1
Unstable Angina or NSTEMI	15 (33.3%)	30 (66.7%)	45	
Chronic Stable Angina (including recent MI)	27 (50.9%)	26 (49.1%)	53	
• p-Values <0.05 has been considered significant				

Table 3. Type of Stent with In- Stent Restenosis Group (ISR+) to non-ISR Group (ISR-).

Parameters	ISR+ Groups (n=53)	ISR- Groups (n=68)	Total	p-Values
BMS	1 (50.0%)	1 (50.0%)	2	0.6
DES	48 (43.6%)	62 (56.4%)	110	
DES & BMS	0 (0.0%)	1 (100.0%)	1	

- **DES:** Drug Eluting Stents
- **BMS:** Bare Metal Stents
- p-Values <0.05 has been considered significant

Table 4. Drug History with In- Stent Restenosis Group (ISR+) to Non-ISR Group (ISR-).

Parameters	ISR+ Groups (N=53)	ISR- Groups (N=68)	Total	p-Values
ASA				
Negative	21 (45.7%)	25 (54.3%)	46	0.7
Positive	32 (43.2%)	42 (56.8%)	74	
Clopidogrel (Plavix)				
Negative	41 (53.9%)	35 (46.1%)	76	0.005*
Positive	12 (27.3%)	32 (72.7%)	44	
Statin				
Negative	29 (50.0%)	29 (50.0%)	58	0.1
Positive	23 (37.7%)	38 (62.3%)	61	

- **ASA** (Aspirin): Acetylsalicylic Acid
- **Statin**: HMG-CoA reductase inhibitors
- p-Values <0.05 has been considered significant

Table 5. The Results of the Difference in Mean BMI and Age with In-Stent Restenosis Group (ISR+) to non-ISR Group (ISR-).

Parameters	N	Mean	Std.Deviation	p-Values
Age				
ISR+ Groups	51	61.43	± 8.91	0.6
ISR- Groups	62	62.04	± 7.89	
BMI				
ISR+ Groups	34	26.42	± 3.85	0.2
ISR- Groups	46	27.49	± 3.67	

- **BMI**: Body Mass Index
- p-Values <0.05 has been considered significant

Table 6. Analyzing the Genotypic Frequency of ACE (I/D) Gene Polymorphisms with In-Stent Restenosis Group (ISR+) to non-ISR Group (ISR-).

	Valid		Missing		Total
Diagnose ACE Gene	114 (69.5%)		50 (30.5%)		164
Parameters	Deletion (D/D)	Indel (I/D)	Insertion (I/I)	Total	p-Values
ISR+ Groups	15 (30.0%)	24 (48.0%)	11 (22.0%)	50	0.73
ISR- Groups	22 (34.4%)	26 (40.6%)	16 (25.0%)	64	

- p-Values <0.05 has been considered significant

Table 7. Comparison of Genotype Frequencies According to Clinical Symptoms in Two Groups of In-Stent Restenosis (ISR+) and non-ISR (ISR-).

Parameters	Deletion (D/D)	Indel (I/D)	Insertion (I/I)	Total	p-Values
Gender					
Male	10 (33.3%)	19 (63.3%)	1 (3.3%)	30	0.2
Female	3 (50.0%)	1 (16.7%)	2 (33.3%)	6	
ISR+ Groups					
Male	11 (30.6%)	18 (50.0%)	7 (19.4%)	36	0.7
Female	4 (28.6%)	6 (42.9%)	4 (28.6%)	14	
ISR- Groups					
Male	11 (30.6%)	15 (41.7%)	10 (27.8%)	36	0.7
Female	11 (39.3%)	11 (39.3%)	6 (21.4%)	28	
ISR+ Groups DM					
Negative	8 (26.7%)	15 (50.0%)	7 (23.3%)	30	0.8
Positive	7 (35.0%)	9 (45.0%)	4 (20.0%)	20	
ISR- Groups DM					
Negative	13 (31.0%)	18 (42.9%)	11 (26.2%)	42	0.7
Positive	9 (40.9%)	8 (36.4%)	5 (22.7%)	22	
ISR+ Groups HTN					
Negative	6 (26.1%)	13 (56.5%)	4 (17.4%)	23	0.5
Positive	9 (33.3%)	11 (40.7%)	7 (25.9%)	27	
ISR- Groups HTN					
Negative	13 (46.4%)	9 (32.1%)	6 (21.4%)	28	0.1
Positive	9 (25.0%)	17 (47.2%)	10 (27.8%)	36	

ISR+ Groups C/S					
Negative	10 (32.3%)	12 (38.7%)	9 (29.0%)	31	0.1
Positive	5 (26.3%)	12 (63.2%)	2 (10.5%)	19	
ISR- Groups C/S					
Negative	15 (37.5%)	17 (42.5%)	8 (20.0%)	40	0.4
Positive	7 (29.2%)	9 (37.5%)	8 (33.3%)	24	

- **DM:** Diabetes
- **HTN:** Hypertension
- **HPL:** Hyperlipidemia
- **C/S:** Protein C and protein S (Protein C and protein S are proteins in the blood that work together to prevent your blood from clotting too much. Tests of proteins C and S use a sample of blood to measure how much of these proteins you have and how well they are working.)
- p-Values <0.05 has been considered significant

Table 7. (Continued), Comparison of Genotype Frequencies According to Clinical Symptoms in Two Groups of In-Stent Restenosis (ISR+) and non-ISR (ISR-).

Parameters	Deletion (D//D)	Indel (I/D)	Insertion (I/I)	Total	p-Values
ISR+ Groups Positive FH					
Negative	12 (26.7%)	23 (51.1%)	10 (22.0%)	45	0.2
Positive	3 (60.0%)	1 (20.0%)	1 (20.0%)	5	
ISR- Groups Positive FH					
Negative	21 (34.4%)	25 (41.0%)	15 (24.6%)	61	0.9
Positive	1 (33.3%)	1 (33.3%)	1 (33.3%)	3	
ISR+ Groups Metabolic syndrome					
Negative	10 (25.0%)	22 (55.0%)	8 (20.0%)	40	0.1
Positive	5 (50.0%)	2 (20.0%)	3 (30.0%)	10	
ISR- Groups Metabolic syndrome					
Negative	17 (35.4%)	18 (37.5%)	13 (27.1%)	48	0.6
Positive	5 (31.3%)	8 (50.0%)	3 (18.8%)	16	
ISR+ Groups Smoker					
Negative	4 (36.4%)	3 (27.3%)	4 (36.4%)	11	0.1
Positive	4 (44.4%)	5 (55.6%)	0 (0.0%)	9	
ISR- Groups Smoker					
Negative	8 (40.0%)	10 (50.0%)	2 (10.0%)	20	0.2
Positive	5 (31.3%)	6 (37.5%)	5 (31.3%)	16	

ISR+ Groups					
Reason for Angiography					
Positive Noninvasive Test	2 (28.6%)	2 (28.6%)	3 (42.9%)	7	0.2
STEMI	0 (0.0%)	1 (33.3%)	2 (66.7%)	3	
Unstable Angina or NSTEMI	6 (42.9%)	7 (50.0%)	1 (7.1%)	14	
Chronic Stable Angina (including recent MI)	7 (26.9%)	14 (53.8%)	5 (19.2%)	26	
ISR- Groups					
Reason for Angiography					
Positive Noninvasive Test	1 (12.5%)	3 (37.5%)	4 (50.0%)	8	0.1
STEMI	0 (0.0%)	0 (0.0%)	1 (100.0%)	1	
Unstable Angina or NSTEMI	11 (39.3%)	9 (32.1%)	8 (28.6%)	28	
Chronic Stable Angina (including recent MI)	10 (40.0%)	12 (48.0%)	3 (12.0%)	25	

• **Positive FH:** Positive Familial Hypercholesterolemia (Dyslipidemia)

- p-Values <0.05 has been considered significant

Table 8. Comparison of Genotype Frequencies According to Drug History in Two Groups of In-Stent Restenosis (ISR+) and non-ISR (ISR-).

Parameters	Deletion (D/D)	Indel (I/D)	Insertion (I/I)	Total	p-Values
ISR+ Groups					
Drug History Statin					
Negative	10 (38.5%)	9 (34.6%)	7 (26.9%)	26	0.1
Positive	5 (21.7%)	14 (60.9%)	4 (17.4%)	23	
ISR- Groups					
Drug History Statin					
Negative	11 (37.9%)	12 (41.4%)	6 (20.7%)	29	0.8
Positive	11 (32.4%)	14 (41.2%)	9 (26.5%)	34	
ISR+ Groups					
Drug History ASA					
Negative	6 (30.0%)	9 (45.0%)	5 (25.0%)	20	0.9
Positive	9 (30.0%)	15 (50.0%)	6 (20.0%)	30	
ISR- Groups					
Drug History ASA					
Negative	8 (32.0%)	11 (44.0%)	6 (24.0%)	25	0.9
Positive	14 (36.8%)	15 (39.5%)	9 (23.7%)	38	
ISR+ Groups					
Drug History Clopidogrel					
Negative	11 (28.2%)	19 (48.7%)	9 (23.1%)	39	0.8
Positive	4 (36.4%)	5 (45.5%)	2 (18.2%)	11	
ISR- Groups					
Drug History Clopidogrel					

Negative	12 (35.3%)	14 (41.2%)	8 (23.5%)	34	0.9
Positive	10 (34.5%)	12 (41.4%)	7 (24.1%)	29	

• **ASA** (Aspirin)**:** Acetylsalicylic Acid

• **Statin:** HMG-CoA reductase inhibitors

- p-Values <0.05 has been considered significant

Figures

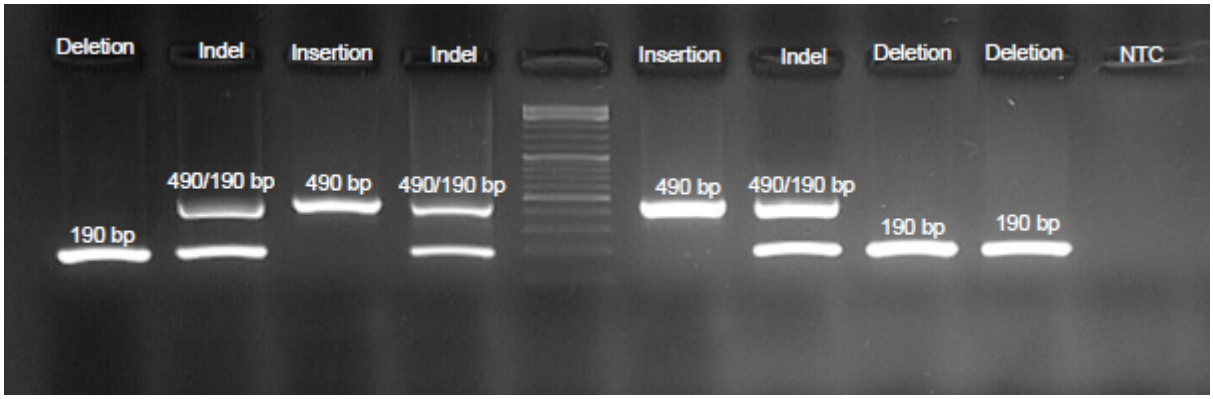

Figure 1

The Image Depicts the ACE (I/D) Genotyping. The Genotypes were Visualized on 2% Gel Electrophoresis. The 490 bp Represents (I allele), the 190 bp Band Indicates the Deletion Allele (D allele), and the Insertion/Deletion (I/D) Allele has 490 bp and 190 bp Bands. NTC, Non-Template Control.