Insertion/deletion polymorphism of the angiotensin converting enzyme gene and acute myocardial infarction. A case - control study in Venezuela

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Abstract

Objective: Detection of the ACE I/D polymorphism genotype and the association with essential hypertension and acute myocardial infarction (AMI) in the Venezuelan population. Methods: Samples from 200 patients with AMI and 200 control subjects were analyzed for genotyping ACE I/D polymorphism. A subset of 82 samples underwent the determination of angiotensin II levels (pg/ml). Results: The frequency for the heterozygous ID was 52.50% vs 44.50% and 26.50% vs 36.00% for DD homozygous individuals for AMI and control groups respectively. The D allele frequency was very similar for both populations (0.535 for AMI vs 0.583 for control subjects). The OR for AMI in carriers for the D allele was 0.91 (95% CI: 0.54-1.53, p>0.05). We found a 2.66 fold increased risk between individuals belonging to the AMI group (OR= 2.66, 95% CI: 1.33-5.31, p<0.05) and an 8.46 fold increased risk for AMI in hypertensive individuals (OR=8.46, 95% CI: 5.24-1368; p<0.05). Finally, we detected a statistically significant difference in angiotensin II levels between individuals with DD and II genotypes (6.59±2.93 pg/ml DD genotype vs 4.26±1.40 pg/ml II genotype, p<0.05).Conclusions: We did not find the ACE genotype as a marker for AMI; but we found at statistically significant increased risk between hypertensive individuals carrying the D allele belonging to the AMI group. The lack of direct association between D risk allele and the AMI could be due to the well known multifactorial nature of this pathology.

Key Words: I/D polymorphism, ACE gene, angiotensin II, AMI

Introduction

Hypertension is a major public health concern world-wide; it is a major modifiable risk factor of morbidity and mortality from cardiovascular causes. Is well known that hypertension is a multifactorial and polygenic disorder in which the interaction between several candidate genes and environmental factors play an important role. It has been suggested that the role of hypertension in the pathogenesis of cardiovascular disease is due to the endothelial dysfunction, which is recognized as the initial state in the progress of the pathology[1]. One factor that contributes to development of both hypertension and endothelial dysfunction is the activation of the tissue renin-angiotensin system[2,3], which is an important regulatory mechanism for maintaining normal blood pressure and volume and electrolyte balance. For these reasons, genes coding for components of this system are attractive candidates for the investigation of the genetic basis of essential hypertension. Angiotensin I-converting enzyme (ACE) is a key enzyme in this system, which catalyzes the conversion of angiotensin I to angiotensin II, a potent vasopressor[4]; so, any alteration in ACE activity may cause many pathological conditions including vasoconstriction, coronary thrombosis, heart failure and ventricular remodeling[5].

ACE plasma levels variability has been reported to be associated with the insertion/deletion (I/D) polymorphism of a 287 pair an Alu repeat sequences in intron 16 of the ACE gene, located at chromosome17q23[6,7,8], which results in three genotypes as II, DD and ID[6,9]. Various studies have shown association between the risk allele (D) and several cardiovascular diseases like myocardial infarction,[10,11] left ventricular hypertrophy[12], cardiomyopathy[13] and hypertension[14-19]. In addition to this studies that make

Materials and methods

a positive correlation between the D allele for ACE I/D polymorphism and hypertension in various populations, others have been reported a negative association[20-24]. It has been postulated that the association between the D allele of the polymorphism and hypertension might be related to gender and ethnicity[15,25]. However, to date no study of this type has been conducted in Venezuela, where the prevalence rates of hypertension (25%) are approximated to the worldwide prevalence (26%)[26]. The present study was initiated to determine whether D allele of the ACE I/D polymorphism are associated with essential hypertension and with acute myocardial infarction in the Venezuelan population.

Subjects

The complete sample comprises 400 subjects that were classified in two groups: 200 patients with acute MI (AMI; diagnosed by the presence of increased creatine kinase [CK] with CK-MB >10% and increased troponin I, typical electrocardiographic alteration and evidence of clinical symptoms) and 200 control subjects randomly selected, unrelated and apparently healthy without personal and family history of vascular, arterial or thromboembolic disease. Peripheral blood was collected from all subjects between January 2009 and January 2010, after a signed consent was obtained. A standard pro-forma was filled up with special emphasis on age, gender, smoking (current smokers or non-smokers), presence of hypertension (defined as a use of antihypertensive drugs or by a systolic blood pressure of at least 140 mm Hg and/or a diastolic blood pressure at least 90 mm Hg)[27] and diabetes mellitus (defined by a blood glucose level of at least 6.93 mmol/L)[28] for all subjects. Blood from AMI patients was provided by the "Servicio de Endocrinología y Cardiología del Hospital Militar Dr. Carlos Arvelo" (Caracas, Venezuela).

Genotyping of the ACE gene Alu I/D polymorphism

Genomic DNA was extracted from total peripheral blood as described by Bowen and Keenney[29]. ACE genotyping for the Alu I/D polymorphism was performed by Polymerase Chain Reaction (PCR) amplification of the respective fragments from intron 16 of the ACE gene using the primers: ACE-Fwd1 5'-CTGGAGAGCCACTCCCATCCTTTCT-3' and ACE-Rev1 5'-GACGTGGCCATCACATTCGTCAGAT-3' (modified from Acartürk et al.[11]). The insertion allele (I) was detected as a 490 bp DNA fragment, and the deletion allele (D) was detected as a 190 bp DNA fragment. Because the D allele in heterozygous samples is preferentially amplified and to prevent underestimation of heterozygous and overestimation of DD genotype, each DD type was subjected to a second, independent PCR amplification with a primer pair that recognizes an insertion-specific sequence: ACE-Fwd2 5'-TGGGACCACAGCGCCCGCCACTAC-3' and ACE-Rev2 5'-TCGCCAGCCCTCCCATGCCCATAA-3', that yields a 335 bp fragment only in the presence of an I allele, and no product in samples homozygous for DD[30]. The first PCR was performed using 60 ng of genomic DNA in a 20 µL PCR reaction containing 0.025 U/µL of Taq DNA polymerase, 1.0 pmol/µL of each primer, 0.2 mM deoxynucleotide triphosphates (dNTPs), 2 mM $MgCl_2$, and 1X Taq polymerase buffer (10 mM Tris-HCl pH 8.3 and 50 mM KCl). Thirty five cycles were performed following denaturation step at 95ºC for 10 min. Each cycle consisted of incubations at 95°C for 1 minute, 58°C for 1 minute and 72°C for 1 minute. A final extension step was carried out at 72°C for 5 min. The second PCR was performed with identical PCR conditions except for an annealing temperature of 67°C. PCR products were analyzed by 2.5% agarose gel electrophoresis containing SYBR Safe (1X). Gel images were documented by using a digital camera (Fotodyne) equipped with ultraviolet filters, and the intensities of electrophoretic signals were estimated by Foto Analyst PC Image program.

Biochemical Measurements

Blood samples are collected in tubes kept at 4°C containing EDTA. The samples are centrifuged at 2000 g for 15 minutes. The plasma extracted should be stored at -20°C until biochemical determination. Angiotensin II levels were measured with enzyme immunoassay (EIA) according to kit SPIbio.

Statistical Analysis

Statistical analysis was performed with the Statistical Package for the Social Sciences (SPSS), version 9.0. Values of continuous variables were expressed as means ± standard deviations (SD). The frequencies of the alleles and genotypes among the case patients and controls were counted and were compared by the chi-square test with the values ($\chi^2_{HW}=\sum(O-E)^2/E$, one degree of freedom). Odds Ratios (OR) was calculated as a measure of the association of the ACE genotype with the phenotypes of AMI and Hypertension. Multivariable logistic curve regression analyses were used to evaluate the risk to develop AMI under various conditions: genotype, age, gender, smoking and presence of hypertension and diabetes mellitus. The regression coefficients that were obtained represent the probability to suffer the disease as a consequence of the presence of the D allele and the others variables studied. Statistically significance was set up at a p value ≤ 0.05.

Results

General characteristics

The general characteristics of the AMI patients and the control groups are shown in Table 1. The AMI group has the highest mean age (57.87± 11.85) and the highest percentages of males, smokers, subjects with hypertension and diabetes mellitus (75, 68, 74 and 32%, respectively).

Determination of the ACE gene I/D polymorphism

First we determined the principle of Hardy-Weinberg equilibrium in the control group, in order to determine which frequencies should be observed in the population for each genotype as a function of allele frequencies. In this sense, the χ^2 calculated was 1.45, so there is a probability between 20 and 25% of the differences between observed and expected are randomly, so it is accepted that this population is consistent with the Hardy-Weinberg equilibrium.

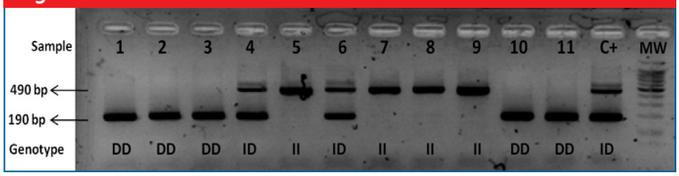
Table 1. Some basal characteristics for the study population

Characteristic	AMI Patients (n= 200)	Controls (n= 200)
Mean age ± SD	57.87 ± 11.85	38.34 ± 15.06
Male sex (%)	75.43	38.81
Female sex (%)	24.57	61.19
Smokers (%)	67.53	21.30
Hypertension (%)	74.07	16.41
Diabetes mellitus (%)	31.54	1.82

Abbreviations: SD: Standard Deviation; AMI: Acute Myocardial Infarction

The I/D genotypes were determined by PCR as described in materials and methods. The products were separated by agarose gel electrophoresis and the presence of the insertion allele (I) was detected as a 490 bp DNA fragment, and the deletion allele (D) as a 190 pb DNA fragment (Figure 1). In heterozygous genotypes (ID) both products were present (Figure 1: Samples 4,6), while the product corresponding to the mutant homozygous genotype[24] was detected and confirmed by the unique presence of the band of 190 bp (Figure 1: Samples 1-3, 10-11).

Figure 1



Agarose gel electrophoresis for detection of ACE I/D polymorphism by PCR. Samples: 7-9, insertion homozygous genotype (II); samples 1-3 and 10-11, deletion homozygous genotype[24]; samples 4 and 6 heterozygous genotype (ID); C+, positive heterozygous control and 100 bp molecular weight marker (MW).

The distribution of genotypes and allelic frequencies of the ACE I/D polymorphism in both groups are shown in Table 2. It was determined that the frequency for the heterozygous (ID) was 52.50% (AMI group) vs 44.50% (control group) and 26.50% vs 36.00% for homozygous individuals for the deletion (DD)[24], respectively. Furthermore, the deletion allelic frequency was very similar for both populations with values of 0.535 for the AMI patients compared to 0.583 for the controls individuals (Table 2).

Table 2. Some basal characteristics for the study population

Characteristic	AMI Patients (n= 200)	Controls (n= 200)
Mean age ± SD	57.87 ± 11.85	38.34 ± 15.06
Male sex (%)	75.43	38.81
Female sex (%)	24.57	61.19
Smokers (%)	67.53	21.30
Hypertension (%)	74.07	16.41
Diabetes mellitus (%)	31.54	1.82

Abbreviations: SD: Standard Deviation; AMI: Acute Myocardial Infarction

The Odds Radio (OR) for AMI in carriers for the deletion or risk allele D (ID and DD genotypes) was 0.91 (95% CI: 0.54-1.53, p>0.05) (Table 2). Thus, the risk allele (D) presence was not significantly associated with an increased risk to this pathology when comparing the two populations (AMI vs controls). In contrast, when we subdivided the population between hypertensive and non-hypertensive individuals in each group (AMI and controls), we found a 1.06 fold increased risk for hypertension in control subjects carrying the D allele although not statistically significant (OR=1.06, 95% CI: 0.43-2.67, p>0.05), and a 2.66 fold increased risk statistically significant between individuals belonging to the AMI group (OR= 2.66, 95% CI: 1.33-5.31, p<0.05) (Table 2).

ACE gene I/D polymorphism and angiotensin II

A subset of 82 samples underwent the determination of angiotensin II levels (pg/ml) in order to determine the existence of any possible association of the D allele and increased levels of this biochemical parameter. In this regard, we observed that carriers of the D allele showed higher levels of angiotensin II compared with the insertion homozygous individuals II (6.59±2.93 pg/ml DD genotype; 5.68±1.72 pg/ml ID genotype vs 4.26±1.40 pg/ml II genotype), corresponding to a statistically significant difference in angiotensin II levels between individuals with DD and II genotypes (p<0.05).

Interaction of other risk factors and AMI

Multivariable logistic regression analysis was performed to determine the effect of conventional risk factors and the I/D genotype on AMI (Table 3). We found a positive correlation between AMI and the following variables: age, male gender, presence of hypertension and smoking (p<0.001) (Table 3). Particularly we found an 8.46 fold increased risk for AMI in hypertensive individuals (OR= 8.46, 95% CI: 5.24-1368; p<0.05). However, in our study the presence of the D allele did not increase the risk of AMI.

Table 3. Multivariable logistic regression analyses of AMI risk factors

AMI				
Variable	B	SE	z value	p value
(Intercept)	-6.04119	0.98846	-6.112	9.85e-10 ***
Age	0.06869	0.01879	3.655	0.000257 ***
Male sex	1.73522	0.45171	3.841	0.000122 ***
Hypertension	1.92749	0.49835	3.868	0.000110 ***
Smoking habit	1.99067	0.45473	4.378	1.20e-05 ***

Significance codes: *p≤0.05; **p≤0.01; ***p≤0.001

Abbreviations: B: indicates estimated coefficient; SE: Standard Error

Discussion

Coronary artery disease (CAD) is the main cause of death in industrialized countries. In Venezuela specifically these diseases cause the 20.18% of all deaths and the AMI represent the most common cause (12.87%)[31]. The AMI, a clinical manifestation of CAD, is caused by atherosclerosis, a degenerative disease condition affecting the arterial vessel walls. Has been suggested that AMI have a multifactorial genetic basis involving a number of genes and environmental factors that interact to determine whether a person will develop the disease. Among these, ACE gene polymorphism (DD genotype) has been proposed as an AMI genetic risk

factor. The first report in this regard was made by Cambien et al.,[32] in a retrospective, multicenter, case-control study, which found that the frequency of the DD genotype was increased in subjects with myocardial infarction recruited between 3 and 9 months after the event. Since then, studies both supporting the finding as well as those questioning the veracity of the association have been published[8,10,30,33-41], leading to an uncertain picture at present about the importance of the polymorphism.

In the current study we assessed the relation between ACE gene I/D polymorphism and the development of AMI, and indeed found not association between the risk allele (D) presence with an increased risk to this pathology. However, we found a positive correlation between hypertension and AMI (Table 3) and 8.46 fold increased risk for AMI in hypertensive individuals (OR= 8.46, 95% CI: 5.24-1368; p<0.05). This association is stronger than those found in INHEART study[42,43]. The data obtained only from Latin America countries, showed the hypertension as the risk factor most strongly associated with AMI (OR= 2.81, 99% CI: 2.39-2.68)[42]. In the same way, the data obtained from 52 countries (representing every inhabited continent) also showed an association between hypertension and AMI (OR= 2.48, 99% CI: 2.30-2.68)[43]. Because of the association with AMI, hypertension is an important public-health challenge worldwide. In fact, an interesting study published by Kearney et al.,[44], reported that more than a quarter of the world's adult population totaling nearly one billion had hypertension in 2000, and that this proportion will increase to 29% (1.56 billion) by 2025.

Multiple genetic causes had been associated with hypertension, such as the presence of D allele for the ACE gene I/D polymorphism. Several studies have indicated a positive association between the risk allele (D) and hypertension[14-19]. These findings are in agreements with our results, particularly between hypertensive individuals belonging to the AMI group, where we found an increased risk of hypertension in presence of D allele (OR: 2.66; p<0.05).

The polymorphism ACE /ID is strongly associated with the level of circulating ACE. The DD genotype is associated with higher levels of circulating enzyme than the ID and II genotypes[7,32,45]. In our study, we found that homozygous individuals of the D allele showed higher levels of angiotensin II compared with the insertion homozygous individuals II (6,59±2,93 pg/ml DD vs 4,26±1,40 pg/ml II genotype; p<0,05) respectively; which could be associated with an increased activity of ACE.

In conclusion, our results do not support the postulated role of the ACE genotype as a marker for AMI; however we found that hypertension is strongly associated with this pathology and showed at increased risk statistically significant between hypertensive individuals carrying the D allele belonging to the AMI group (Table 2). The presence of the D risk allele for the ACE gene I/D polymorphism could be associated indirectly with the development of AMI. The lack of association between D risk allele and the AMI could be due to the well known multifactorial nature of this pathology. The finding in this study that the D allele risk presence was associated with hypertension, support the importance of detecting this polymorphism in the molecular diagnostic tests for genetic risk estimation associated with hypertension and indirectly with AMI.

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