INVESTIGATION OF ANGIOTENSIN CONVERTING ENZYME GENE POLYMORPHISM AND BLOOD LIPID PEROXIDATION WITH MI PATIENTS IN TURKISH POPULATION

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ABSTRACT

Myocardial infarction (MI) is the most important reasons of mortality and morbidity in developed countries. It is detected that about half of the deaths, no matter what reason, in the USA is sourced from MI. In recent years, the number of scientific studies have increased on some genetic risk factors responsible from the formation of MI. One of the most popular of these is the one searching for the relations between the I/D polymorphism in the ACE gene and MI. Nowadays there are various studies showing that the free radicals are responsible from the ischemic myocardial wounds. Especially the hydroxy radical is shown to cause some differences in the myocard. In the pathogenesis of ischemic tissue defect, the free radicals are put forward to be effective. malondialdehyde (MDA) which is a subproduct of oxidation, is a value measurable by the TBA method. MDA, one of the compounds formed as a result of peroxidation of membrane phospholipids, is accepted as an indicator of LP. In this study, it is assumed that ACE I/D gene polymorphism is a useful indicator in the detection of the risk of cardiovascular disease, the better control of the people in high risk group and the optimal cure to start at an earlier stage. By the help of these studies the risk factors of genetic origin, using the genetic indicators and new risk factors, and supporting the information by some easily performed biochemical experiments would supply great easiness in diagnosis and cure and save life.The scope of this study is to compare the distribution of gene polymorphism between people suffered from MI and healthy people and to search the relation between ACE gene polymorphism and MDA, the marker of lipid perokxidation (LP) formed as a result of MI. Two groups were covered in our study, one consisting of 104 people of various ages previously suffered from MI and the other consisting of 100 healthy people. Using the blood samples taken from these two groups, data for the following variables was obtained: DNA isolation, PCR, agarose gel electrophoresis and MDA as the marker of blood lipid peroxidation.

Introduction

Myocardial infarction (MI) is the major cause of morbidity and mortality in developed countries (15). MI is a multifactorial disease, influenced by environmental and genetic factors(18). These factors differ in each population. The renin-angiotensin system (RAS) is one of the major regulators of

blood pressure and fluid and electrolyte homeostasis (11). Angiotensin converting enzyme (ACE) is a key component within the RAS and ACE is a dipeptidyl carboxipeptidase that converts angiotensin-I to the potent vasoconstrictor angiotensin-II and inactivites the vasodilator bradykinin (11,14,12). ACE gene is polymorphic (4). The ACE gene has been mapped to chromosome 17q23, and an insertion/deletion (I/D) polymorphism, involving a 287 base-pair alu repeat sequence, has been located to intron 16. (3, 5, 19, 20, 22, 23).

An insertion/deletion (I/D) polymorphism in the ACE gene results in genotypes II, ID, and DD (13,19).

Whereas those people with DD genotype had the highest ACE plasma level, those with II genotype had the lowest. Since people with DD genotype and D allele have a higher level of angiotensin-II due to the higher level of ACE in circulation and tissues compared to other genotypes and alleles, DD genotype and D allele can be considered as a risk factor for a more severe cardiovascular damage (5,12,20,23). ACE D allele is considered as the reason behind a higher ACE activity in both old and young populations (10). According to the method of Lindpainther et.al. mistyping of I/D heterozygotes was controlled using insertion specific primers (12).

In a case control study of four different populations, a homozygous deletion allele in the gene for ACE was associated with an increased risk of MI, especially among individuals with below-average lipid and body mass (5). Lipid peroxidation has been proposed to be a major mechanism of oxygen free radical toxicity (1).

The membrane lipids are vulnerable to peroxidation after death of cells. Thus peroxidation is a mechanism caused by cell death rather than being a cause of it. Malondialdehyde (MDA) formation is subjected to a detailed analysis and it is shown that measurement by thiobarbituric acid (TBA) method is possible (1, 24). The study was approved by the local Ethical Committee.

Materials and Methods

In this study, two blood samples were taken from two groups. The patient group consisted of patients with an MI history (n=94) and the control group consisting of 100 healthy people (n=100). The statistical analysis is conducted with SPSS 9.0 for Windows. The chi-square test procedure and the Mann-Whitney were used. Each observation was marked by numbering only.

A 287 bp I/D polymorphism in intron 16 of the ACE gene was examined by PCR in a cross-sectional study of 100 healthy subjects and 95 patients with MI.

Determination of ACE genotypes

Genomic DNA from leucocytes was prepared with standard techniques.(19, 21). PCR was used to detect the insertion(I)/deletion (D) polymorphism of the ACE gene using a previously reported method (5,7).

The presence (allele I) or absence (allele D) of the 287 bp Alu repeat in intron 16 of the ACE gene was determined by evaluating the size of DNA fragments after polymerase chain reaction amplification, using the primers and PCR conditions described by Rigat et. al(19).

Because 4-5 % of samples with the I/D genotype were misclassified as DD with older methods, each sample found to have the DD genotype was subjected to a second PCR amplification with insertion-specific primers (5'TGGGACCACAGCGCCCGCACTAC3' and 5'TCGCCAGCCCTCCCATGCCCATAA3') with 67°C as the annealing temperature to avoid DD mistyping (12).

The ACE genotypes were assessed by PCR using primer sequences and PCR cycling

conditions, as described previously. According to the absence or presence of the 287 base pair insertion in the PCR product, the patients were classified as homozygous DD or II, or heterozygous ID. To prevent mistyping of ID as DD genotypes, a second PCR with an insertion specific primer (5'TTTGAGACGGAGTCTCGCTC3') was performed in all samples classified as homozygous DD in the first PCR.

Amplified DNA was electrophoresed in 2% agarose gels and visualised by ethidium bromide staining. Subjects with one 490 bp band on an agarose gel (2%) classified as II (insertion), subjects with both 490 and 190 bp bands were classified as ID, and subjects with only a 190 bp band were classified as DD (deletion).

Malondialdehyde (MDA) is an end product of lipid peroxidation and MDA content was measured by a modified thiobarbituric acid (TBA) method (1).

The 532 nm. spectrofotometric analysis and the quantity measurement is conducted for the colored complex composed of the condensation of one volume of MDA and 2 volumes of TBA (2,16).

Results and Discussion

Cambien et. al. found that the D allele was a risk factor for acute MI (5). Tiret et. al. showed that an insertion /deletion (I/D) poly-

TABLE 1

Statistical comparison of ACE genotypes of the patient and control groups

ACE GENOTYPE	DD	ID	II
PATIENT GROUP	n=40 %42.5	n=45 %47.8	n=9 %9.7
CONTROL GROUP	n=36 %36	n=46 %46	n=18 %18
	P>0.05*	P>0.05*	P>0.05*

*Statistically insignificant

TABLE 2

MDA mean values of the patient and control groups

MDA	n	Mean value
PATIENT GROUP	60	2.55±2.35 nmol/ml
CONTROL GROUP	70	1.147±0.532 nmol/ml
	130	P=0.000*

* Statistically significant

morphism of the ACE gene located in intron 16 was associated with marked differences of ACE levels; the deletion type (D) allele was associated with higher ACE levels than the I allele (23).

In a study undertaken by Eichner and his colleagues in USA on a patient group of 576 male and 124 females, the relationship between ACE DD genotype and MI was investigated. The gene frequencies were taken as a basis in the study and the most common genotype in the population was detected to be the ID genotype. DD and II genotypes were detected almost in the same frequencies. Among the patients with MI, individuals with an MI history in the family had significantly higher DD genotype(6).

Fatini and his colleagues claimed that ACE DD genotype is risk factor for MI patients based on a study on a sample taken from Italy(8).

In contrast to these studies, Fatini and his colleagues noted that they did not find a statistically significant relation between ACE I/D gene polymorphism and MI based on a study conducted with 304 MI patients in Canarian Islands, Spain (17).

Lindpainther argued that there is no significant relationship between ACE I/D gene polymorphism or DD genotype and MI (12).

Based on a study over the Australian population, Friedl concluded that there is no positive relation between ACE DD genotype and MI(9).

We investigated the relation between ACE gene polymorphism and MDA being the marker of blood lipid peroxidation based on sample of 94 patients with an MI history and 100 healthy people. As a result we found no statistically significant relation between ACE I/D genotype and the occurrence of MI. Comparing the MDA measures of the patient and control group we found that the MDA levels of the patients with an MI history were higher and there existed a statistically significant relationship between the MDA level and MI experience.

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