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Polymorphism studying of the Angiotensin-converting gene for a group of patients with myocardial infarction in Saladin Governorate

Wasan Nazhan Al-Assie*[1], Hadeel Abdulhadi Omear[2], Faik Ibrahime Ali[1], Adnan Fadhel Al-azawey[2]

1- College of pure science,Tikrit university, Tikrit, Iraq

2- College of science, Tikrit University ,Tikrit ,Iraq

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***Corresponding author :**
Email :
drwasannzhan@tu.edu.iq

Contact To Journal
E-mail tjops@tu.edu.iq

Abstract :

Blood samples were collected from 120 people, including 70 people affected by Myocardial Infarction (MI) and 50 healthy people aged between 40 - 70 years. Patients were diagnosed by basis of clinical symptoms and after made laboratory tests in addition to electricity cardio Gram (ECG) by specialists. Also it is measured The concentration of homocysteine for both of groups, where its concentration Patient groups was (45.26 ± 0.71) µmol / L compared to the healthy group (7.86 ± 1) µmol / L. There was a significant difference obviously at level (0.01 and 0.05) between the two groups beside, a mutation Ins / Del was found. Using PCR technique, where appeared three genotypes (II, ID and DD), there was a clear difference in the value of the Allele frequency for Allele D in patients group compared to healthy, where was in patients group(0.664) while in healthy group (0.37). On the contrary, value of the Allele frequency has decreased for Allele I to patients where was(0.336) compared with healthy(0.63) This indicates the association of the deletion mutation (D) with MI and heights level of concentration Homocysteine in plasma. After analysis of Chi square χ^2, the resent studying proved that society of patients applies Hardy-Weinberg law for balance on it at a significant level (0.01) and Hardy-Weinberg law ($p^2 + 2pq + q^2 = 1$) where value of Chi square (7.61)which was less than its tabular value at a significant level (0.01) (9.21). As for the healthy society, it was a balance and applied Hardy-Weinberg law on it at a significant level (0.01) and (0.05) where value of Chi square (5.867) which was smaller than its tabular value at tow levels (9.21) and (5.99).

المستخلص

جمعت عينات الدم من 120 شخص ، منها 70 شخص يعاني من احتشاء العضلة و 50 شخص سليم تتراوح اعمارهم بين (40-70) عام تم تشخيص المرضى على اساس الاعراض السريرية وبعد أجراء الفحوصات المختبرية وتخطيط القلب الكهربائي Electromyo Cardio Gram (ECG) من قبل الأخصائيين، كما تم قياس تركيز الهوموسستين للمجموعتين حيث بلغ تركيزه في مجموعة المرضى (45.26 ± 0.71) µmol/L مقارنة بمجموعة الاشخاص الاصحاء (7.86 ±1) µmol/L فقد كان هناك فرق معنوي واضح وعلى مستوى معنوية (0.01 و 0.05) بين المجموعتين، كما تم ايجاد طفرة Ins/Del وباستخدام تقنية الـPCR حيث ظهرت ثلاث طرز وراثية (II ، ID و DD) ، كان هناك فرق واضح في قيمة التكرار الاليلي للاليل D في مجموعة المرضى مقارنة بمجموعة الاصحاء حيث بلغ في المرضى (0.664) اما في الاصحاء (0.37) على العكس من ذلك انخفضت قيمة التكرار الاليلي للاليل I لدى المرضى حيث بلغت (0.336) مقارنة بالاصحاء (0.63) يدل هذا على ارتباط طفرة الحذف D

بمرض احتشاء العضلة القلبية وبارتفاع مستوى تركيز الهوموسستين في بلازما الدم .كما اثبتت الدراسة الحالية وبعد اجراء تحليل مربع كاي χ^2 ان مجتمع المرضى ينطبق عليه قانون هاردي –واينبرك للتوازن عند مستوى معنوية (0.01) وينطبق عليه قانون هاردي واينبرك Hardy-Weinberg Law ($p^2 +2pq +q^2 =1$) حيث كانت قيمة مربع كاي المحسوبة (7.61) وهي اقل من قيمتها الجدولية عند مستوى معنوية (0.01) (9.21) . اما بالنسبة لمجتمع الاصحاء فقد كان متوازناً وينطبق عليه قانون هاردي- واينبرك عند مستوى معنوية (0.01) و (0.05) حيث بلغت قيمة مربع كاي المحسوبة (5.867) وهي اصغر من قمتها الجدولية عند المستويين (9.21) و (5.99) .

Introduction

Myocardial infarction (MI), occurs when blood flow decreases or stops to a part of the heart for a short time, causing the death of some cardiac cells as a result of coronary artery occlusion (1,2). This disease considers one of the leading causes of death in developing countries and it is a complex disease and multi reasons, it is caused by the environmental factors that differ from one society to another and there are genetic factors, include occurrence of mutations and polymorphism of some genes, in addition to the interference which happens between genes and other genes on the one hand and between genes and environmental factors on the other hand(3,4). The most important risk factors for myocardial infarction are cardiovascular disease, age, smoking, high blood pressure, high blood lipids (triglycerides, high density lipids, low density lipids), diabetes, obesity and others. Beside to those known factors, some studies have shown the important of genetic factors, where The genetic basis of myocardial infarction was studied extensively to find a clear correlation between some genetic markers that increase occurring of disease(5). Homocysteine is one of an amino acid containing sulfur (6) and has referred to it in many recent researches as accredited factors affecting on the heart and blood vessels (7). Level of peripheral homocysteine is directly related to thickness wall of carotid artery (8) and is inversely related with wall thickness And coronary blood flow (9). The use of modern molecular biology techniques in the genetic diagnosis of diseases present the possibility of studying the mechanism of preparedness and how to achieve disease for the individual and family(10). Where multiform of the gene are indicators of biodiversity and some differences in genetics of genes associated with some diseases that affect humans(11). polymorphism of gene can contribute to the genetic predisposition of the disease at different rates and thus some of the genetic structures of those genes may associate with disease or be considered indicators of these diseases(12). Genetic indicators related to the Renin-angiotensin system, have taken a great attention, especially in coronary artery disease, because of their effect on the internal balance of blood vessels(10). Some studies were indicated, that there is a link between the polymorphisms of the angiotensin converting enzyme and coronary heart disease, where is observed that the genetic pattern DD of the mention gene can be an independent risk factor not only for myocardial infarction but for chronic heart failure, sudden cardiac death and others(13). Several studies were conducted in recent years on some of the dangerous genetic factors which responsible for myocardial infarction, the most important of those genes was the encoded gene of the angiotensin-converting enzyme (4). The changes in blood pressure play an important role in cardiovascular disease, therefore there is a necessary to analyze the correlation between those disorders and the allelic diversity of the angiotensin-converting gene, which converts the inactive angiotensin 1 to active angiotensin 2, where The inhibitors of those enzymes are used widely in the treatment of blood pressure, heart failure And myocardial infarction (14). The angiotensin-converting enzyme works as initial stimulating for the production of angiotensin-II. Therefore, any factor affects on the effectiveness and level of the angiotensin-converting enzyme will affect on the Ring-angiotensin system. Thus, high or low level of the angiotensin-converting enzyme leads to

the high or low conversion process of angiotensin 1 to angiotensin 2. Subsequently, high or low blood pressure (15,16). Rigat and his companions discovered the functional effect of the multiform of the angiotensin-converting enzyme gene, which includes insertion or deletion of the base pair 287 at the Introetn 16 of that gene(17). The angiotensin-converting enzyme gene is located on chromosome 17, long arm, package No23. The length of this gene is 23 kilo a base pair and consists of 26 Exon and contains two types of promoters, therefore gives two types of symmetric enzymes, the first is somatic, largely encoded from the first Exon to the twenty-sixth except Exon thirteen, the second testicular encodes from the promoter thirteen to twenty-six, which has important in fertility of male (17,18), the level of the angiotensin-converting enzyme varies from one person to another and this may be due to genetic influence at the level of the enzyme(19). So, there are three genotypes of that gene, genotype DD which leads to increase the concentration of the enzyme and its effectiveness in the blood and tissues and thus lead to increase blood pressure by increasing the production of angiotensin 2, genotype II which leads to a decrease in the effectiveness of the enzyme and its concentration and lead to increased muscle tolerance and increase the ratio of free fiber, while type ID gives an medium effectiveness of the enzyme (17,20). Aim of this studying was to define multiform of the angiotensin-converting enzyme (Insertion / deletion)mutation of a patients group with MI in Saladin governorate.

Materials and methods of work:

1- Sampling: Ninety blood samples were collected from 90 patients (52 males and 38 females) whose were infected with myocardial infarction from the intensive care unit at Tikrit Teaching Hospital, aged between 40 to 70 years. It was confirmed to be symptoms them after laboratory tests and ECG Electromyo Cardio Gram (ECG) by specialists in the hospital and based on the clinical symptoms in addition to the information which collected from patients according to the questionnaire prepared for this and (50) healthy people from both sexes with almost identical ages, Collected 5 ml of blood from each An individual of the healthy and patients then it divided into two parts the first (2 ml) kept in a plastic tube with a lid, contained EDTA as an anticoagulant, those tubes carried to laboratory by cooling box and were stored at -20 ^{0} C for genomic DNA extraction. The second (3 ml) Blood plasma was separated from it to measure the total homocysteine ratio in the plasma.

2- HPLC measurement: High performance liquid chromatography (HPLC) using UV detector at wavelength 338mn depending on the method adopted (21) .

3- Genetic analysis: Genomic DNA isolates from the total blood according to method (22), then estimates concentration DNA using the Nanodrop device. The multi- form of the angiotensin-converting gene were identified using PCR technique by specialized Primers (Table 1) as mention (23) using Kit(GoTaq Master Green) provided by American Promega Corporation and according to the instructions attached.

Table (1): Demonstrate the Primers which used in PCR technique for the ACE gene and its specific conditions.

Name of Primer	Sequence	Heat degree of correlation *Tm*
Forward primer	5'- CTG GAG AGC CAC TCC CAT CCT TTC T-3'	56 °C
Reverse primer	5' GAC GTC GCC ATC ACA TTC GTC AGA T 3'	56 °C

Some initial experiments were conducted to reach the optimum concentration of the used primates and DNA template which gives the best result of the doubling. put (12.5 µl) of the essential reaction mixture in each tube, then add the DNA template to it at concentration (100 ng) and the mention parameters at concentration (10 bl / 10 ml) , then complete the reaction amount with distilled water to 25 µl. So, the components of the reaction mix well and then put the tubes in Thermo- cycler carefully to achieve the doubling reaction after programming it, according to the program: one cycle for 5 minutes at 95°c for the initial scan of the DNA streak followed by 30 doubling cycles, embody each cycle 45 seconds at 94°c into mold scan and 45 seconds at 56°c to connect the primers with DNA tape follow it 30 doubling each cycle includes 45seconds at 72°c for elongation with a final cycle for 10 minutes at 72 °c for finality elongation. The amplification results on agarose gel at concentration 2% with the DNA Ladder were migrated for 90 minutes at amount (5 volts / cm). finally, The gel was examined after dyeing it with ethidium bromide dye for 30-45 minutes under (UV-light) and images using Gel Documentation System (24).

Statistical analysis

Statistical analysis was performed using SPSS (version 11.). results of the biochemical tests were analyzed by (Student's t-test) of the patients and healthy group at a significant level of 0.01, 0.05. then assumed allelic frequency of the angiotensin conversion gene and the number and percentages of genotypes which are wild type, also assumed heterozygous and mutant of the patients and healthy group(25). Apply the analysis of Chi square χ^2 to find matching studied population into law of Hardy- Weinberg

Results and discussion

Table 2 show level of homocysteine amino acid concentration in people with myocardial infarction and healthy subjects. The arithmetic mean for patients with myocardial infarction (45.26 ± 0.71) µmol / L compared to the healthy group (7.86 ± 1) µmol / L.

Table (2): Homocysteine concentration in group with MI and healthy group

Group	Concentration of homocysteine Mean± S.E(µmol/L)	Value of t and p at significant level 0.05,0.01
Affected group with MI	45.26 ± 0.71	t = 28.635 ,0.00001≥ p
Healthy group	7.86 ± 1	at significant level 0.01,0.05

Homocysteine scratch the lining of the blood vessels by stimulating the production of

hydrogen peroxide and super-ions (26) through reducing the antioxidants of sugary proteins (27). Therefore, high level of homocysteine is related with myocardial infarction, there is a significant difference in the mean level (0.01 and 0.05) between the healthy and patients. scratching the internal lining of the blood vessels caused by the height of the homocysteine, which will lead to narrowing of the blood vessels then that will be easy to embolism and forming Thrombosis. After makes of Insertion / Deletion technique using specialized primers,

Results of the electrophoresis showed the appearance of three genotype, the homozygous allele II, which alleles were inserted a DNA piece leading to reach the molecular size of the PCR product at 490 pb, heterozygous allele ID (490, 190) bp, which one of the alleles occurred inserted on it while other allele removed a piece of DNA piece from it, which led to become the molecular size of the PCR product 190 bp, homozygous allele (DD) (190) bp and it is a type which Both of alleles suffered from the deletion process.

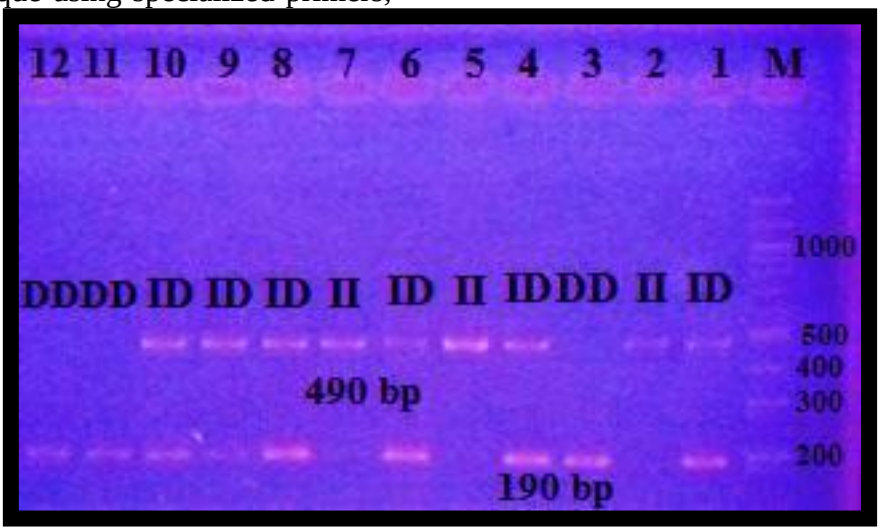

Fig.(1): Shows electrophoresis of the Agarose gel to product of PCR after apply of the Ins / Del technique on the DNA samples

Enzyme gene of angiotensin-converting ACE is located on chromosome 17q23 and consists of 26 Exon and 25 Intron. Intron of No. 16 is suffered from polymorphism, which characterized with an insertion (I) or Deletion (D) for recurrence of successive from DNA *Alu* pieces (28). This difference leads to get three genetic types (DD,ID and II). (29) . As shown in Table (3) and (4),shows the results discovered height of percentage of the genotype (DD) in the patients group compared with the other genotype, where was 51.43%. As for the healthy group, the percentage of this type was 22%, also it is noticed that frequency value of allele (D) in patients group, was (0.664) comparing to the healthy group which was (0.37). That results indicate a connection the mutation of Deletion in the ACE gene with myocardial infarction. That results conform researches which were made (30) and were Proved that individuals with genotype DD had a high concentration in plasma and tissue from An angiotensin-converting enzyme that is associated with coronary artery disease (29) .

Table (3):Represents the genetic types, their viewing and predicted frequency, in addition to percentages of the observed numbers of patients

Genetic type Frequency	expected number frequency	Observed number frequency	Percentage of Observed number %
II p^2	8 (0.113)	13 (0.186)	18.57 %
ID **2pq**	31 (0.446)	21 (0.3)	30 %
DD q^2	31 (0.441)	36 (0.514)	51.43 %
Total number	70	70	100 %
frequency of allele I (p) = 0.336 frequency of allele D (q) =0.664 1=(p+q)			
value of calculated χ^2 = 7.16≤ of tabular value at significant level 0.01 and ≥ tabular value at significant level (5.99,9.21)0.05free dgree=2.f.d			

Table (4):Represents the genetic types, their viewing and predicted frequency, in addition to percentages of the observed numbers of healthy group.

Genetic type Frequency	expected number frequency	Observed number frequency	Percentage of Observed number %
II p^2	20 (0.397)	24 (0.48)	48 %
ID **2pq**	23 (0.466)	15 (0.3)	30 %
DD q^2	7 (0.137)	11 (0.22)	22 %
Total number	50 %	50 %	50 %
frequency of allele I (p)=0.63 frequency of allele D(q)=0.37 1=(p+q)			
Calculated value of χ^2 = 5.867≤ of tabular value at significant level 0.01 and 0.05(9.21,5,99) and free dgree = 2.f.d			

The result indicate that the ratio of genotype(II) is low in patients (18.57%) compared with healthy (48%) as shown in table (3) and (4). In the case of the heterozygous genotype (ID) which carries allele from each mutation, its ratio was equal in healthy and patients group (30%). When was applied the analysis of the Chi square (χ^2)on the patients and healthy group, it was found that the population of the patients was balanced at a significant level (0.01) and The Hardy-Weinberg Law agree it (p2 + 2pq + q2 = 1) (31) where value of the calculated Chi square (7.61) and it is less than its tabular value at a significant level (0.01) (9.21). As for the healthy population, it was balanced and the Hardy-Weinberg law applied on it at a significant level (0.01) and (0.05), where value of Chi square (5.867) and it is less than its tabular value at two level (9.21) and (5.99) .

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