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ACE Gene Polymorphism in Coronary Artery Disease in West Bank, Palestine

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Abstract:

Heart disease is the most hazardous chronic disease that leads to death worldwide. Hypertension is one of the most risk factors that leads to CVD. This study has been conducted to examine the relationship between a genetic polymorphism in the angiotensin gene and coronary heart disease, and the correlation of this polymorphism with environmental and nutritional risk factors. A case-control study has been conducted involving 100 CAD patients and 100 healthy individuals as a control group. DNA was isolated from patient's peripheral blood, and angiotensin-converting enzyme (ACE) I/D gene polymorphism was assayed by polymerase chain reaction (PCR). The results showed that the ACE I/D polymorphism distribution was as follows: Genotype frequencies of DD, II, and DI among cases were 0.42, 0.21, 0.37; respectively. There was a significant association between the studied environmental and nutritional factors and the incidence of CAD I/D especially BMI (≥ 25), cigarette smoking, and family history. The general health level of society can be improved by modifying lifestyle like eating DASH diet (dietary approach to stop hypertension), increasing physical activity and avoiding smoking and processed

food. The study emphasizes the possibility of early detection of heart disease risk by using the ACE gene polymorphism test.

Key words: heart disease, ACE gene Polymorphism, environmental factors, BMI.

الملخص:

تعد أمراض القلب من أخطر الأمراض المزمنة المؤدية إلى الوفاة عالميا، ويعد ارتفاع ضغط الدم أحد أخطر العوامل التي تؤدي إلى أمراض القلب والأوعية (CVD) . وقد أجريت هذه الدراسة لمعرفة العلاقة بين الشكل الوراثي في الجين المحول للانجيوتنسين وأمراض القلب التاجية، ومدى ارتباط هذه الطفرة ببعض عوامل الخطر البيئية والغذائية. شملت هذه الدراسة مجموعتين : تجريبية تألفت من (100) مريض يعانون من مرض الشريان التاجي (CAD)، وعينة الشاهد تكونت من (100) من الأصحاء. وقد تم عزل الحمض النووي (DNA) من خلال أخذ عينة دم من المرضى والأصحاء، ثم تم فحص التباين للجينات المسؤولة عن الإنزيم المحول للأنجيوتنسين (ACE I/D) بواسطة تقنية تفاعل البولميريز المتسلسل (PCR). أظهرت النتائج أن التباين الجيني للإنزيم المحول للأنجيوتنسين ACE كان على النحو الاتي: النسبة المئوية للنمط الجيني لـ DD و II و DI لدى المرضى هي (0.42 و 0.21 و 0.37) على التوالي. وقد تبين وجود ارتباط وثيق بين مرض القلب التاجي (CAD) والعوامل البيئية والغذائية التي تمت دراستها في هذا البحث، وبخاصة مؤشر كتلة الجسم (BMI ≥ 25) والتدخين والتاريخ المرضي في العائلة. وقد اكدت الدراسة إمكانية رفع مستوى الصحة العامة في المجتمع من خلال تعديل نمط الحياة واتباع حمية غذائية صحية وزيادة النشاط البدني وتجنب التدخين والأغذية المصنعة. واظهرت الدراسة ايضا إمكانية الكشف المبكر عن مخاطر الإصابة بأمراض القلب باستخدام اختبار التباين الجيني للإنزيم المحول للأنجيوتنسين (ACE).

الكلمات المفتاحية : أمراض القلب، التباين الجيني للإنزيم المحول للأنجيوتنسين ACE ، العوامل البيئية، مؤشر كتلة الجسم.

Introduction

Coronary artery disease (CAD) is globally known as a major public health concern and is considered as one of the major leading causes of death worldwide. Palestine is experiencing an epidemiological transition, with increasing the burden of chronic diseases as a consequence of rapid modifications in people's

dietary and life style (Abdeen, 2006). Coronary artery diseases (CADs) are highly prevalent in the Palestinian society contributing to a substantial proportion of total mortality. The top two causes that account for the most disease burden in 2010 were heart disease (35.2%), and hypertension (34%) (Mosleh et al., 2016).

CAD is a multifactorial disease in which environmental, nutritional, and genetic factors interact. Many risk factors have been known to be associated with CAD including hypertension, dyslipemia, smoking, diet, physical activity and many others. It is also well known that family history of heart disease is a strong CAD risk factor (Hunt et al., 1986). Interactions between genetic and environmental factors influence the pathological progression of the disease and the susceptibility to treatment (ZAK et al., 2003). Genetic factors help in explaining the molecular basis of the disorder and in designing prevention and treatment of the disease. Several polymorphisms in different genes in the renin-angiotensin-aldosterone system (RAAS) have been linked to heart disease. The RAAS system is essential for vascular homeostasis and plays an important role in the development of cardiovascular diseases (Fuster, 1994). Angiotensin I-converting enzyme (ACE) is a key component of the RAAS. Angiotensin-converting enzyme (ACE) is a zinc metallopeptidase that cleaves the C-terminal dipeptide (His-Leu) from angiotensin 1 and generates a vasoconstrictor (angiotensin II) (Davis et al., 1997). The ACE gene maps to chromosome 17q23, and consists of 26 exons and 25 introns (Villard et al., 1996).

Insertion/Deletion (I/D) polymorphism of the gene that codes for ACE has been proposed as a genetic marker of risk of CAD. It is based on the presence or absence of 287 base pairs in intron 16 of the gene (Rigat et al., 1990), which results in three genotypes insertion homozygote (I/I), insertion/deletion heterozygote (I/D) and deletion homozygote (D/D) (Lindpaintner et al., 1995). The D allele is accompanied by the highest plasma and tissue concentrations of ACE activity and the I allele is the lowest which could provide a pathophysiological explanation for the higher incidence of myocardial infarction

and CAD in individuals with the DD genotype (Cambien et al., 1992). Some studies on the association between ACE genotypes and the risk of CAD have provided controversial results (Lindpaintner et al., 1995). Especially when combined with bad food choices that contains high fat and salt methods.

To the best of our knowledge, the study is the first to report on possible association of ACE polymorphisms as predisposing factors in the development of the CAD in the West Bank, Palestine.

2. Materials and Methods

2.1 Study cases

One hundred clinically diagnosed cases with CADs were randomly chosen from the Cardiology Department of Al-Ahli and Rafedia hospitals in West Bank. One hundred age- and sex-matched healthy controls from these hospitals were included in the study. All the cases were diagnosed by biochemical tests and other medical tests like ECG and Echo. Information regarding their clinical, lifestyle habits, and socioeconomic conditions was collected using an approved questionnaire. All subjects were asked to sign the consent statement before involving them in the study.

2.2 ACE polymorphism study

Two-three ml blood was collected from each individual in an EDTA vial. Genomic DNA was extracted from leukocytes by a salting-out method (Suguna et al., 2014). The D and I alleles were identified on the basis of Polymerase chain reaction (PCR) of the fragment in intron 16 of ACE gene and visualized by gel electrophoresis. Detection of the I/D polymorphism was done by specific primer sequence: sense oligo: 5′ CTGGAGACCACTCCCATCCTTTCT 3′ and anti-sense oligo: 5′ GATGTGGCCATCACATTCGTCAGAT 3′. The DNA was amplified for 30 cycles with denaturation at 95 ºC for 1 min, annealing at 58ºC for 1 min, and extension at 72 ºC for 1 min. Genotypes of all individuals were

noted as DD: 190 bp; ID: 490 bp, 190 bp; II: 490 bp (Fig. 1). All the results were double checked.

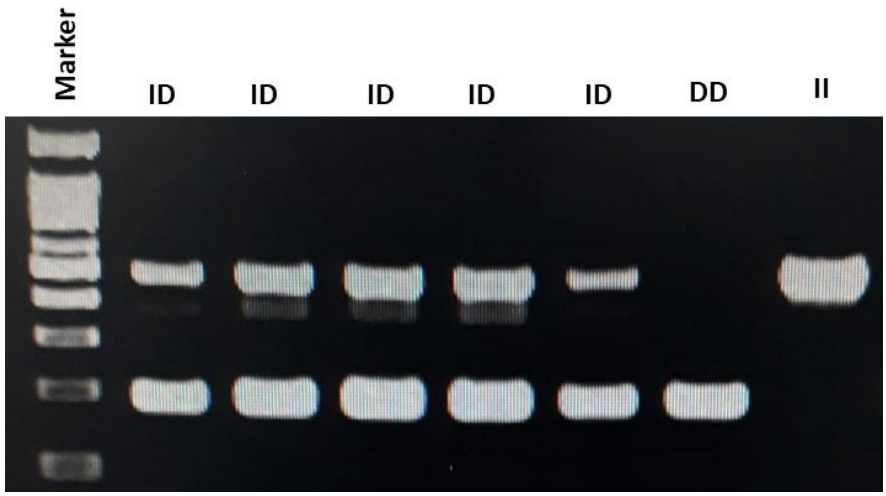

Figure 1: Patterns of ACE polymorphisms. Lane 1: 100 bp molecular weight marker (Genei, /USA). Lane 2-6: ID genotype (490 bp, 190 bp). Lane 7: DD genotype (190 bp). Lane 8: Lane 8: II genotype (490bp).

2.3 Statistical analysis

Collected data were analyzed using the statistical package for social sciences program (SPSS) version 16. Chi-square test was used to test the significance of the observation and to compare between cases and controls. A value of $p < 0.05$ was considered statistically significant. Odds Ratio was used to determine the power of relationship between the determinant and the outcome, and 95% confidence interval was calculated.

3. Results

3.1 Characteristics of the study population

The mean ages of the case patients and controls in the study sample were 52.6 and 50.1 years, respectively. The distribution of several other risk factors among the cases and controls is shown in Table 1, which indicates the differences in some of the known risk factors. 49.6% of the cases were found to be smokers, which is almost the same as in controls (45.3%). There was a positive relationship between BMI > 25 and hypertension with regard to genotype. Family history showed a strong correlation with the development of the disease. Diabetes, obesity and hypertension also elevate the risk of the disease. No differences in educational and socioeconomic levels were observed between the cases and controls.

Table 1: Characteristics of the study population

	Cases (N=100)	**Controls (N=100)**	**P-value***
Age (Yrs)	52.6	50.1	0.
Cigarette smoking (%)	49.6	45.3	0.
Diabetes (%)	40.2	19.7	<0.01
Hypertension (%)	80.1	13.4	<0.01
BMI (Kg/m^2) < 25%	25.2	62.8	0.
BMI (Kg/m^2) > 25%	74.8	37.2	<0.01
Family history (%)	43.9	18.2	<0.01

* p< 0.05 is considered significant

3.2 Genotypes of the study population

Among the controls, the I and D alleles had frequencies of 0.45 and 0.55, respectively. The frequencies of the genotypes DD, II, and DI in controls were 0.28, 0.18, and 0.54, respectively. These values were virtually identical to those predicted by the Hardy–Weinberg equilibrium. Genotype frequencies of DD, II, and DI among cases were 0.42, 0.21, 0.37, respectively.

Table 2 shows the distribution of different ACE genotypes and the corresponding allele frequencies among the subjects. The frequencies were compatible with Hardy-Weinberg equilibrium. It was found that both the DD and ID genotypes impart statistically significant effect on the development of CAD. The number of D allele carrying subjects was significantly higher in CADs.

Table 2: Distribution of ACE genotype and allele frequencies

	Genotype frequencies			**Allele frequencies, Chi-squared (chi2)**	
	DD	II	ID	D allele	I allele
Cases	0.42	0.21	0.37	0.60	0.39 (0.051)
Controls	0.28	0.18	0.54	0.55	0.45 (0.0083)

4. Discussion

The results of the study illustrated that the existence of DD genotype was more common than ID genotype among CAD patients of the study population although both genotypes showed obvious correlation in the development of CAD. This result is consistent with a study by Dhar et al., 2012 who investigated the relationship between CAD and ACE polymorphism among an Indian population who reported that both DD & ID genotypes are correlated significantly with the incidence of CAD. The results of this study can be considered as a reference for the whole population in the West Bank. The study depicted that the frequencies of ACE alleles in CAD were: 0.60 for D, and 0.39 for I, while in control group the frequencies were 0.55 for D and 0.45 for I.

However, there was no statistically significant relation between smoking and ACE gene polymorphism among the study groups. This result is inconsistent with the results of a study by Sayed-Tabatabaei et al. (2005), who reported that

smoking is a correlated factor to ACE gene polymorphism. However, the study revealed that there was a clear relationship between diabetes and hypertension with ACE gen polymorphism in the case group which is consistent with Sayed-Tabatabaei et al. (2005).

In general, BMI over 25 shows a significant correlation with the incidence of CAD; however, it is more hazardous for patients with ACE gene polymorphism. Overweight or obese people have higher chances of having CAD more than people with BMI less than 25.

The study illustrates considerable correlation between family history and ACE gene polymorphism among the study population. The probability of having CAD was accreting in people with positive family history more than in those with negative family history of CHD.

Conclusion:

Based on the results obtained from this study, there is a positive interrelationship between ACE gen polymorphism and the incidence of CAD among the Palestinian people. Other studied risk factors play a key role in increasing or decreasing the influence of the gene polymorphism, especially hypertension, diabetes, weight (BMI) and family history. For those with ACE gene polymorphism, the incidence of CAD can be reduced by adopting lifestyle changes that manage and control the risk factors.

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