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Association Between Angiotensin Converting Enzyme Gene Insertion\Deletion Polymorphism and Coronary Heart Disease in Gaza Strip

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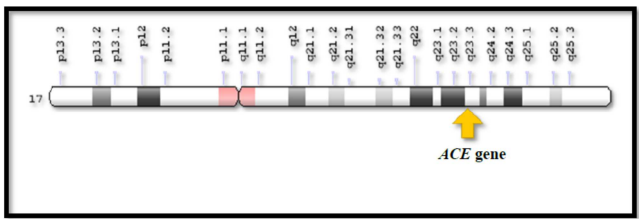
Abstract: The insertion\ deletion (*ID*) polymorphism in intron 16 of the *ACE* gene is a marker for a functional polymorphism, and it is also reported to influence levels of ACE in healthy subjects. *ACE ID* polymorphism is associated with an elevated risk of thrombosis and may be causally associated with coronary heart disease (CHD). To investigate the association between *ACE* gene polymorphism and CHD in the Gaza strip population, we conducted case-control study included 260 samples comprised 160 CHD patients and 100 control subjects. Questionnaire interview was applied. Blood samples were collected in EDTA tube for DNA extraction. Polymerase Chain Reaction (PCR) used to detect *ACE ID* polymorphism. There was significant association between CHD and age, physical activity, education level, occupation, and family history of CHD. No significant association was observed between CHD and gender and smoking. The genotype frequencies were: *ID* (36.9%, 42%), *DD* (53.8%, 54%) and *II* (9.4%, 4%) in case and control groups respectively. The *ACE ID* allele frequencies were: *I* (27%, 25%) and *D* (72.2%, 75%) in case and control group respectively. The *D* allele was the most frequent allele in both groups. No association between *ACE ID* polymorphism and gender. The *DD* genotype was the most frequent in both study groups. There was no statistically significant association between the *ACE ID* polymorphism and CHD in Gaza strip. The results showed that there was no significant association between the *ACE ID* gene polymorphism and CHD in Gaza strip.

Keywords: Angiotensin Converting Enzyme Gene, Coronary Heart Disease, Polymerase Chain Reaction, Polymorphism

1. Introduction

The Angiotensin Converting Enzyme gene (*ACE*) located on chromosome 17q23.3 (figure 1), spans 21 kb, and comprises 26 exons and 25 introns [1]. Exon 26 encodes for the functionally important membrane-anchoring domain of the ACE protein [2].

ACE gene codes for the Angiotensin converting enzyme (ACE) which plays an essential role in two physiological systems, one is the production of angiotensin II which is a vasoconstrictor leading to increase of blood pressure (BP), the other role is the degradation of bradykinin [3].



(Adapted from https://ghr.nlm.nih.gov/gene/ACE/location.png)

Figure 1. *The location of human ACE gene on the long arm of chromosome 17.*

Most studies focused on an *ID* polymorphism in intron 16 of the *ACE* gene as a marker for a functional polymorphism [3]. The polymorphism is due to a 287 bp *Alu* repetitive sequence in intron 16 of the *ACE* gene. The fragment is present in the insertion (*I*) variant and absent in the deletion (*D*) variant, which results in the three genotypes: Homozygotes *II* and *DD* and heterozygotes *ID* [4].

ACE ID polymorphism may play a role in the development of many human disorders including renal complications [5] breast cancer [6], prostate cancer [7], coronary heart disease [8], essential hypertension [9], diabetes and nephropathy [10] and end-stage renal disease [11].

Coronary Heart Disease (CHD) is a collective term for diseases that occur when the walls of the coronary arteries become narrowed by a gradual buildup of fatty material called plaques. Deposition of cholesterol on their walls; this reduces the supply of oxygen and nutrients to the heart musculature, which is essential for proper functioning of the heart. This may eventually result in a portion of the heart being suddenly deprived of its blood supply leading to the death of that area of heart tissue, resulting in heart attack [12]. The variability in the prevalence of heart disease risk factors and their association with stroke or ischemic heart diseases in different populations is certainly due to a complex interaction between environmental and genetic factors. The most genetic variation studied contributed to CHD is the *ACE ID* polymorphism [8]. The presence of *D* allele would increase the serum concentrations of ACE and this would increase the production of angiotensin *II*, potent vasoconstrictor that also affects the vascular smooth muscular cells and myocardiocytes increases the synthesis of the extracellular collagen matrix and inhibits physiological fibrinolysis, all of these being mechanisms related to the pathogeny of CHD [13]. It is thought that because of this mechanism the genotype *DD* of the *ACE* gene *I/D* polymorphism is associated with CHD.

In 2014, the Palestinian health information center of the Ministry of Health reported that the CHD was the main cause of Palestinian deaths by 29.5% of the total reported deaths, according to the report, the number of Palestinian deaths reported 13,297 deaths including 6,601 cases in the Gaza Strip and 6,696 cases in Western Bank. To date, no study has been reported to investigate the association of *ACE* gene polymorphism and CHD in Palestine.

2. Methodology

A retrospective case-control study in which *ACE* genotyping were performed on 260 individuals who were randomly selected. One hundred and sixty subjects (70 female and 90 male) with CHD and 100 normal subject were included in this study (41 female and 59 male). All subjects were asked to fill a questionnaire. EDTA blood sample were collected from all subjects, 160 patients with CHD recruited from the Nasser and Al Shifa hospitals, and 100 healthy individuals as control. DNA was isolated from fresh EDTA whole blood by using Promega kit for human DNA isolation following the manufacturer's instructions. The quality of the isolated DNA was determined by running 5 µl of each sample with 2 µl of DNA loading dye on ethidium bromide stained 1.0% agarose gels and the DNA was visualized on a short wave U.V. transilluminator.

2.1. Data Analysis

The data was enter, stored and analyzed by personal computer using the statistical package for Social Sciences (SPSS) version 20.0. Independent Samples T-test, Chi square test and Odd's Ratio (OR) use to compare between the two group of this study. P value < 0.05 will be consider statistically significant.

2.2. PCR Amplification of ACE Gene

Polymerase chain reaction (PCR) was used to detect *ID* polymorphism of 287 bp *Alu* repetitive sequence near the 3` end of intron 16 of *ACE* gene for both groups (case and control).

PCR was performed using the primers described by Chizynski and Cieplucha [14] from genomic extracted DNA using the following oligonucleotide primers:

Forward: 5'CTGGAGACCACTCCCATCCTTTCT'3'

Reverse: 5'GATGTGGCCATCACATTCGTCAGATTT'3

3µl (~150ng) of prepared DNA template was added to 7 µl master mix (Bioline, UK), and 0.5 µl of each primer (5 pmol) in 0.2 ml thin walled microfuge tube. PCR was performed in a thermal cycler (Biometra, Germany). The cycling conditions were: an initial denaturation for 1 min at 95°C, followed by 35 cycles of 15s at 95°C, 15s at 59°C, 10s at 72°C and an additional 10 min at 72°C for final extension. Upon completion of PCR, the products were analyzed by electrophoresis on 2% ethidium bromide stained agarose gel.

3. Results

3.1. Study Population

The study population consisted of 260 sample (160 case, 100 control). The mean age of subjects was 54.53 ± 11.8. The percentage of males was 57.3% (59% of control group, 56.25% of cases) while that of females was 42.7% (41% of control group, 43.75% of cases). 72.3% of the participants were non- smokers, while about 27.7% were smokers. 62.3% of the study population was non-hypertensive subjects, while 37.7% of them were hypertensive, and about 23.1% of population had a history of heart thrombosis. On the other hand, 74.2% of the study population was non diabetic subjects. All hypertensive and diabetic participants were in case group only. Most of the subjects had no family history of CHD.

3.2. CHD, Education, Occupation and Smoking

In the overall sample, there was a statistical differences

in the education level between case and control groups (p-value:0.000). Similarly, there was a significant differences between gender and education level among the subjects (p-value:0.023) with low levels of education among women. (Table 1). Our findings showed that 30.6% of patients in case group were employees, while 69.4% of them were not. Moreover, 51% and 49% of controls were employee and non-employee respectively (p-value: 0.001). There was no statistically significant correlation between smoking and CHD (p-value: 0.630). 71.3% of case group were smokers while 28.7% of them were not. Moreover 74% and 26% of controls were smokers and non-smokers respectively.

Table 1. *Relationship between gender, study groups and education.*

Education	Study group		Gender	
	Case No. (%)	Control No. (%)	Male No. (%)	Female No. (%)
Illiterate	17 (10.6%)	2 (2%)	8 (5.4)	11 (9.9)
Primary	61 (38.1%)	56 (56%)	59 (39.6%)	58 (52.3)
Preparatory	45 (28.1%)	15 (15%)	36 (24.2%)	24 (21.6)
High school	8 (5%)	0 (0%)	3 (2%)	5 (4.5)
High education	29 (18.2%)	27 (27%)	43 (28.8%)	13 (11.7)
Total	160 (100%)	100 (100%)	149 (100%)	111 (100%)
P-value	0.000		0.023	

3.3. Family History of CHD Among Study Groups

There was a strong positive relationship between family history and status of participants in study groups (p-value = 0.000). Of cases, 91.7% had at least one family member with CHD while only 8.3% of controls had a family history of CHD. The chance of having CHD was increasing eleven times in people with family history more than those without a family history of CHD (OR:11.00, CI: 4.55-26.61). There is a statistical significance between family history of CHD and gender among case group (p-value:0.009). Female have about 2.4 times of having family history of CHD compared to males (OR: 2.4, CI: 1.2 – 4.5). (Table 2)

Table 2. *Relationship between gender and family history of CHD.*

Family history of CHD	Male No. (%)	Female No. (%)	Total No. (%)
Yes	29 (32.2%)	37 (52.9%)	94 (58.8%)
No	61 (67.8%)	33 (47.1%)	66 (41.2%)
Total	90 (100%)	70 (100%)	160 (100%)
P- value	0.009		

3.4. History of Heart Thrombosis, Gender, Age, Hypertension (HTN) and Diabetes

The results of this study showed that there was no statistical significance between the mean of age and previous exposure to heart thrombosis among case group (p-value: 0.190). Also the results revealed that there was a statistically significant difference between history of heart thrombosis and hypertension in case group (p-value:0.002) and the relative of developing heart thrombosis is increasing about three times in hypertensive patients more than non-hypertensive ones (OR: 3.033, CI: 1.48-6.20). While we didn't found any statistical significance between diabetes and history of heart thrombosis (P-value: 0.34) and with smoking (P-value: 0.787) as shown in table 3.

Table 3. *Relationship between history of heart thrombosis and some of demographical variables among case group.*

History of heart thrombosis	HTN No. (%)	Non-HTN No. (%)	Diabetic No. (%)	Non Diabetic No. (%)	Smoker No. (%)	Non-smoker No. (%)
Yes	46 (46.9%)	14 (22.6%)	28 (41.8%)	32 (34.4%)	18 (39.1%)	42 (36.8%)
No	52 (53.1%)	48 (48.4%)	39 (58.2%)	61 (65.6%)	28 (60.9%)	72 (63.2%)
Total	98 (100%)	62 (100%)	67 (100%)	93 (100%)	46 (100%)	114 (100%)
P- value	0.002		0.341		0.787	

3.5. PCR Results

The amplicon (PCR product) generated from *ACE* gene should yield a 490 bp or 190 bp long ds. DNA fragment for *I* and *D* allele respectively, as giving rise to three genotypes: the homozygotes *II* and *DD*, and the heterozygote *ID* [15]. A negative control (with water instead of the DNA template) was included in each reaction. The size of the amplicon was estimated by comparing it with a DNA molecular size marker (50 bp ladder DNA) run on the same gel. A represented photograph of ACE PCR amplification product is illustrated in figure 2 below. Lane 1 in the figure shows the 50bp DNA marker, 490 bp product in the presence of insertion of *Alu* repeat (*I* allele), and 190 bp fragment in the absence of the *Alu* repeat (*D* allele).

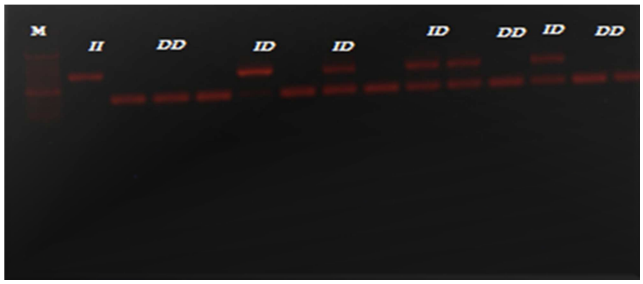


Figure 2. *A photograph of ACE gene amplification product:490 bp forII,490 bp and 190 bp for ID,190 bp for DD genotype.*

3.6. ACE Alleles and Genotypes Frequencies

The *ACE* allele frequencies in the control subjects were 25% for the *I* allele and 75% for the *D* allele. The frequencies in the CHD group were 27.8% and 72.2% for the *I* and *D* alleles respectively. The distribution of the subjects according to *ACE* genotypes were: *DD*: 53.85%, *ID*: 38.85%, and *II*: 7.30% (Table 4). The *ACE* genotype frequencies among study population in the control subjects were 42% for the *ID,* 54% for the *DD*, and 4.0% for the *II* genotypes, and in case subject were 36.9%, 53.8% and 9.4% for *ID*, *DD* and *II* genotype respectively. As shown in table 4 there was no statistically significant relation between study groups and *ACE* gene polymorphism (p-value: 0.241).

Table 4. *Distribution of the ACE alleles and genotypes frequency among the study groups.*

Alleles	**Case No. (%)**	**Control No. (%)**	**Total**
I	89 (27.8%)	50 (25%)	139 (26.73%)
D	231 (72.2%)	150 (75%)	381 (73.27%)
Total	320 (100%)	200 (100%)	520 (100%)
P-value	0.48		

***ACE* genotype**	**Case group No. (%)**	**Control No. (%)**	**Total**
ID	59 (36.9%)	42 (42%)	101 (38.85%)
DD	86 (53.8%)	54 (54%)	140 (53.85%)
II	15 (9.4%)	4 (4%)	19 (7.30%)
Total	160 (100%)	100 (100%)	260 (100%)
P-value	0.241		

3.7. ACE Genotype and Gender

The results of the study showed that there was no statistically significant difference between the *ACE* genotype and the gender among study groups (P-value: 0.266), and for case group was (P-value: 0.162). The most frequent *ACE* genotype among male and female in the study population where *DD*: 49.7% and 59.5% respectively (OR:1.6, CI: 0.91-2.4).

3.8. Relation Between ACE Genotype, Family History of CHD, Hypertension and Diabetes

The results showed that there was no risk of developing CHD in the *DD* genotype group and having family history of CHD when compared with *ID* and *II* genotype group (OR:1.5, CI: 0.87-2.62) (P-value: 0.146). Also results of our study indicated that the most *ACE* genotype among hypertensive patient in case group was *DD* genotype (59.2%), and showed that there was no statistically significant relationship between hypertension and *ACE* genotype in case group (P-value:0.194), (OR: 1.7, CI: 0.926-3.347). There was no statistically significant relation between diabetes and *ACE* gene polymorphism in case group (p-value: 0.748), (OR: 0.52, CI: 0.43-1.53). (Table 5)

Table 5. *ACE genotype and allele frequencies among the hypertensive and diabetic patients.*

***ACE* genotype**	**With HTN No. (%)**	**Without HTN No. (%)**	**Diabetic No. (%)**	**Non Diabetic No. (%)**
ID	31 (31.6%)	28 (45.2%)	27 (40.3%)	32 (34.4%)
DD	58 (59.2%)	28 (45.2%)	34 (50.7%)	52 (55.9%)
II	9 (9.2%)	6 (9.6%)	6 (9%)	9 (9.7%)
Total	98 (100%)	62 (100%)	67 (100%)	93 (100%)
P- value	0.194		0.748	
Allele Frequency	With HTN (%)	Without HTN (%)	Diabetic (%)	Non Diabetic (%)
I	25%	32.3%	29%	27%
D	75%	67.7%	71%	73%
P- value	0.16		0.66	

4. Discussion

4.1. CHD and Demographical Variables

Over the past decade CHD, also known as ischemic heart disease or atherosclerotic heart disease, has been the leading cause of death worldwide, as well as Palestinian territories, with persistently rising incidence [16]. Approximately 80% of all CHD related deaths occur in low- and middle-income countries [17]. For our knowledge, this study is the first one to investigate the association between *ACE* gene polymorphism and CHD and to assess the frequencies of the *ACE ID* genotypes and alleles in Gaza strip.

There is a marked difference in CHD risk between sexes [18]. CHD was approximately 3 times higher in men than women [19]. CHD incidence was ≈3-fold and mortality ≈5-fold among men greater than in women [20]. Sex-specific differences in the prevalence of CHD are known and attributable to biological factors, differences in health behavior, as well as to aspects of medical care [21]. Although studies have shown that hormonal dysfunction in premenopausal women is associated with an increased risk of atherosclerosis and CHD events [22].

One theory on why men suffer from heart disease earlier than women is stress. men still endure more stress from heavy physical activities or actions than women [23].

Genetic variation within the male-specific region of the Y chromosome [24] and dominance of androgens over estrogens [25] have been proposed as the major factors contributing to the male predisposition to CHD [26]. This

suggests that certain sex hormones may be important risk factors of heart disease. Reduction of estrogen concentrations in menopause leads to changes in female lipid profile by reducing HDL, and elevating apolipoprotein levels, thus increasing the risk for cardiovascular disease [27]. Estrogen may have cardio-protective effects [28] through glucose metabolism and the hemostatic system, and it may also have a direct effect on endothelial cell function [29]. Our findings showed that the percentage of females in CHD group was higher than in control may be due to the advanced age of the women (mean age was 60.03 years).

The findings of the present study showed that 48.8% of cases were physically low-active. We noted that there was a good agreement with evidence that physical inactivity is one of the major modifiable risk factors for CHD. Physical activity appears to slow the initiation and progression of CHD through salutary effects [30]. Sedentary people have about twice the risk of developing or dying from CHD and 37% of deaths from CHD can be attributed to physical inactivity [31]. Physical activity prevents the blood vessels from narrowing further (anti-atherosclerotic), prevents blood clotting (anti-thrombotic), helps deliver blood to the heart (anti-ischemic), and helps to maintain a normal heart rhythm (anti-arrhythmic). These changes reduce the load on the heart at rest and during exercise, which helps to lessen some of the symptoms as well as decrease the risk of death from CHD [32].

One major contribution to the increased risk of CHD among smokers is tobacco's effect on increasing overall blood cholesterol levels. This occurs as a result of the chemical acrolein, which affects the way the body processes cholesterol, allowing greater amounts to remain in the blood system [33]. This compound, also decreases the ratio of high HDL,(the "good" cholesterol) to low-density lipoprotein (LDL), (the "bad" Cholesterol) [34]. The risk of heart thrombosis is also raised due to tobacco's effect on fibrinogen levels (a protein which causes blood to clot) and its effects on increased platelet aggregation which makes the blood more sticky [35]. Finally, it has been shown that smoking causes the body's blood vessels to constrict (vasoconstriction) by decreasing nitric oxide which dilates blood vessels and increasing endothelin-1 which causes constriction of blood vessels [36]. The net result is raised blood pressure and a transient reduction in blood supply. Our study showed that the majority of CHD patients were smokers (71.3%) but we didn't fount any significant association between smoking and CHD.

Education is a measure of socioeconomic state (SES) that takes into account the person's nonmaterial resources (e.g. knowledge and problem-solving skills). Education can be measured by years of education or the highest educational degree completed, but it is, nevertheless, easy to categorize, obtain and can be measured whether employed or unemployed. In addition, education is usually fixed after early adulthood, and therefore unlikely to be affected by possible poor health of adulthood. [37]. More education are likely to have greater knowledge of health conditions and treatment regimens and have better self-management skills than those with less education [38]. Individuals with low educational levels are less likely to be knowledgeable about the health effects of smoking, particularly the effects of smoking during pregnancy [39].

The educational level of parents can influence child and family health related behaviors .Studies have shown that the education level of mothers is likely to have a greater impact than that of fathers [40]. An association has been found between higher parental education level and increased likelihood of consuming a healthy diet [41]. Adolescents in families with low maternal education may also be more likely to use illegal drugs [42].

Our study, showed that there was a statistical differences in the education level between cases and controls. Similarly, there was a significant differences between gender and education level with low levels of education among women.

There was a strong positive relationship between family history and status of participants in study groups. The chance of having CHD was increasing eleven times in people with family history more than those without any family history of CHD. A study was done in USA reported that the persons with a positive family history of CHD were almost 4 times as likely than those with a negative family history to believe that they were very likely to develop heart disease or stroke in the future. A positive family history of CHD was associated with increase odds of having high blood pressure, compared with persons those having a negative family history [43].

The results of our study showed that the percentage of patients who had previous exposure to heart thrombosis was 37.5% with mean age of 59.35 years, and 60% of them was males. We found that 76.7% of them were hypertensive and 46.7% were diabetic, while 43.3% of them were suffering from hypertension and diabetes together. More than half of patient whose had previous heart thrombosis were physically low-active (65%). These findings showed agreement with the fact that age, hypertension and diabetes and physical activity are strong risk factors for heart thrombosis. The National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) had published that having diabetes increases the risk for heart attack at least twice as likely as someone who does not have diabetes. Over time, high blood glucose levels damage nerves and blood vessels, leading to complications such as heart disease and stroke. If someone has high blood pressure his heart must work harder to pump blood. High blood pressure can strain the heart, damage blood vessels, and increase risk of heart attack, stroke, eye problems, and kidney problems (NIDDK, 2013).

4.2. Genotype Frequencies of ACE Gene in Gaza Strip

Most studies focused on *ID* polymorphism in intron 16 of the *ACE* gene [3]. The polymorphism is due to a 287 bp *Alu* repetitive sequence in intron 16 of the *ACE* gene in chromosome 17. The fragment is present in the insertion (*I*) variant and absent in the deletion (*D*) variant, which results in the three genotypes: Homozygotes *II* and *DD* and

heterozygotes *ID* [4].

The observed genotype frequencies were in Hardy-Weinberg equilibrium for both groups. According to the results obtained from PCR the *ACE ID* polymorphism genotype frequencies in Gaza Strip were: *II*: 7.3%, *ID*: 38.8, *DD*: 53.8%. Table 6 illustrates the distribution of *ACE ID* polymorphism among some population.

Table 6. *Distribution of ACE ID polymorphism for many populations.*

Country	Allele frequency		*ACE* genotypes			Reference
	I	***D***	***II%***	***ID%***	***DD%***	
Egypt	0.567	0.433	39	35.5	25.5	Rashed, et al., 2015 [44]
Algeria	0.384	0.616	11.3	54.2	34.5	Semmame, et al., 2015 [45]
Kuwait	0.379	0.621	17.4	41.3	41.3	Al-Serri, et al., 2015 [46]
Lebanon	0.328	0.672	9.8	46	44.2	AlBacha, et al., 2015 [47]
Soudi Arabia	0.398	0.602	23.45	32.8	43.75	Alharbi, et al., 2013 [48]
Turkey	0.405	0.623	14.7	47.48	38.45	Inanir, et al., 2016 [49]
South Indian	0.36	0.64	26.7	46.7	26.6	Shanmuganathan et al., 2015 [50]
Romania	0.391	0.609	11.5	55.2	33.3	Carmen, et al., 2011 [51]
China	0.362	0.638	22.6	31.3	46.1	Zhou, et al., 2012 [52]
Iraq	0.358	0.652	19.4	29.1	51.5	Al-Jebouri and Al-Alwani, 2015 [53]
Palestine\Gaza Strip	0.267	0.733	7.3	38.8	53.8	Present study

4.3. Relationship Between CHD and ACE Gene Polymorphism

Several studies have been conducted to analyze the relationship between the *ID* polymorphism of the *ACE* gene and the development of CHD (Table 7). These studies revealed that the presence of the *ACE* gene *DD* variant is associated with an increased risk of developing atherosclerotic and vascular disease [54]. The presence of the *D* allele homozygotes corresponds to an increased risk of CHD by an average in 1.3 times [55]. The *DD* genotype and *D* allele may serve as the criteria for identifying patient groups which are at high risk for MI and CHD [56]. A studies conducted in Iraq [53], Algeria [45] and Lebanon [47] showed that there was statistically significant between *ACE* genotype among the study groups which differ from our findings, while there was good agreement between our results and the results that revealed from Turkish one [49].

No significant differences were detected in allele or genotype frequencies between patients and controls (p = 0.48). Table 7 showed distribution of *ACE ID* polymorphism for many populations among CHD group and control.

Table 7. *Distribution of ACE gene ID polymorphism for cases and controls among different studies.*

Country	Case			Control			P-value	Reference
	II%	***ID%***	***DD%***	***II%***	***ID%***	***DD%***		
Algerian	14.46	46.55	38.99	8.13	61.87	30	0.02	Semmame et al., 2015 [45]
Turkey	12.7	47.1	40.2	16.19	48.09	35.72	0.40	Inanir et al., 2016 [49]
Iraq	13.4	28.9	56.7	35.15	29.7	35.15	<0.05	Al-Jebouri and Al-Alwani, 2015 [53]

5. Conclusion

The results of this study can be summarized as follows:

- In Gaza Strip, the *DD* genotype was the most common genotype among the control and the CHD groups. *ID* was the next most common genotypes.
- The frequencies of *ACE* alleles in the CHD subjects were: 27.8% for the *I* and 72.2% for the *D*. These frequencies are comparable to those found in the control group where: 25% for the *I*, and 75% for the *D*.
- No statistically significant differences in *ACE* genotypes were found between the patients and the control groups and between male and female in terms of the *ACE* genotypes.
- There is statistical significance in physical activity among study groups, and significant relationship between education level and risk of CHD, and this association was stronger in women participated in this study.
- There was a strong positive relationship between family history and status of participants in study groups. The chance of having CHD was increasing eleven times in people with family history more than those having negative a family history of CHD.
- There was no statistically significant relation between diabetes and hypertension with *ACE* gene polymorphism in case group, also there was no statistically significant relation between smoking and *ACE* gene polymorphism among study groups.

Based on the data presented by this study changes in lifestyle and CHD prevention strategies should be followed to reduce risk factor for CHD developing. These strategies include the management of high blood pressure, high blood cholesterol, unhealthy diet and physical inactivity. People should be encouraged to increase their daily physical activities to achieve additional health benefits, by increasing the intensity, duration, or frequency of physical activity. Further studies recommended to establish the role and

relative contribution of CHD and candidate genes.

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