Genetic polymorphisms in *eNOS, ACE* and *PAI* genes and coronary artery disease

Dr. Basim Mohammad Ayesh

Department of laboratory medical science, Al-Aqsa University, Gaza

ABSTRACT

Background

Cardiovascular diseases (CVD) in general and coronary artery diseases (CAD) in particular are the leading cause of mortality in Gaza strip, as well as in other parts of the globe. As a multifactorial disease, vast genetic and nongenetic factors have been proposed to interplay in susceptibility to CAD. To the best of the author knowledge, genetic risk to CAD has never been evaluated in Gaza strip. Therefore, the main aim of this research is to investigate the role of *ACE, eNOS* and *PAI-1* allelic variants and other risk factors in conferring the individual susceptible to CAD in Gaza strip.

Methods

Patients were recruited upon cardiac catheterization, and subsequently divided into normal and abnormal groups based on their catheterization results. Data and blood samples were collected, and DNA was extracted and genotyped by allele-specific polymerase chain reaction and the data was statistically analyzed.

Results

The allelic frequency the risk alleles in the Palestinian population of Gaza strip is 64.6% for the ACE D-allele, 35.4% for the PAI-1 *4G*-allele and 13.57% for the 4a-allele. The distribution of the different genotypes between normal and abnormal CAD patients was not significant (*P*=0.1981, 0.8922, and 0.5637 respectively). The distribution of CAD cases and normal controls was significant by sex (*P*=0), smoking (*P*=0), abnormal BMI among females (*P* = 0.0490) and unstable angina at presentation (*P*=0.002). However, it was statistically not significant by family income (*P*=0.862), education level (*P*=0.829), presence of diabetes mellitus (*P*=0.37), hypertension (*P*=0.199), BMI (*P*=0.138) and family history (*P*=0.96).

Conclusion

The genetic polymorphism in *ACE*, *eNOS* and *PAI* were not found to be promoting the risk of CAD in the study population. The risk factors for CAD in the study population includes sex, overweight in females, smoking and unstable angina at presentation.

Keywords:

ACE, *eNOS*, *PAI-1*, CAD, GAZA

INTRODUCTION:

Cardiovascular diseases (CVDs) are the leading cause of death worldwide (WHO, 2017). More than 80% of CVD deaths are in low- and middle-income countries (Bowry et al., 2015). Cardiovascular disease was the main leading cause of deaths in Palestine, responsible for 31.5% of deaths in 2018 (MOH, 2019). In Gaza strip, 5,044 deaths were reported in 2016, among which CDV-related deaths were leading (57.1% in 2016 and 44.5% in 2015) (P.H.I.C., 2017). The leading cause of CVD-related deaths in 2013 was ischemic heart disease (IHD), followed by cerebrovascular disease (Nowbar et al., 2019). Coronary artery disease (CAD) is the major source of morbidity and mortality among CVD patients (Benjamin et al., 2017). In CAD, acute occlusion of one or multiple large epicardial coronary arteries for more than 20 to 40 minutes can lead to acute myocardial infarction (Ojha and Dhamoon, 2018).

CAD is multifactorial and its progression is related to an interplay between environmental and genetic factors (Sayols-Baixeras et al., 2014). Various well-known CAD risk factors have been described in the international multi-center case-control study (INTERHEART) (Yusuf et al., 2004). Modifiable CAD risk factors include but are not limited to smoking, abnormal lipid profile, hypertension, diabetes mellitus, abdominal obesity, psychosocial and socioeconomical factors. Non-modifiable risk factors for myocardial infarction include advanced age, male and genetics (heritability of CAD approximately equals 0.42) (Peyser et al., 2002). Association of CAD prevalence and deoxyribonucleic acid (DNA) polymorphism in human genes has been extensively studied (Assimes and Roberts, 2016; Khera and Kathiresan, 2017; McPherson and Tybjaerg-Hansen, 2016). Polymorphisms in a number of genes, including the angiotensin converting enzyme (*ACE*), the Nitric oxide synthase (NOS) and the Plasminogen activator inhibitor-1 (*PAI-1*) were identified as possible genetically predisposing factors in different populations worldwide. An insertion/deletion (*I/D*) polymorphism in *ACE* has been described. Meta-analysis established a significant association between the D allele and an increased risk of HDs (You and Shen, 2016). Association between the D-allele and different pathologies of CAD was reported by several studies (Mani et al., 2017; Prasad et al., 2000; Ruiz et al., 1994). Serum *ACE* level is elevated in association with homozygosity for the D-allele (Suehiro et al., 2004). Polymorphism in a 27 bp variable number tandem repeats in intron 4 within the endothelial nitric oxide synthase (*eNOS*) gene (known as *4a*/*4b*) has been reported as a possible risk factor in CAD pathogenesis (Yang et al., 2014). Nitric oxide (NO) is responsible for the vasodilation and thereby regulation of blood pressure, and has numerous other vaso-protective and anti-atherosclerotic properties (Li and Förstermann, 2000). The activity of *eNOS 4a*/*4a* genotype is lower and thus the basal serum NO level is lower than *eNOS 4b*/*4a* and *eNOS 4b*/*4b* genotype (Dosenko et al., 2006). The plasminogen activator inhibitor-1 (*PAI-1*) is an important regulator counteracting fibrinolysis, therefore increasing the risk of atherothrombotic events and contribute to vascular disease (Vaughan, 2005). *PAI-1* could also have a proinflammatory effect, thereby participating in CAD (Renckens et al., 2005). Plasma *PAI-1* concentrations can be affected by a common single-base polymorphism (4 or 5 guanine) in the promoter region of the gene (Dawson et al., 1991). Homozygotes for the *4G* allele have 25% higher plasma *PAI-1* concentrations than homozygous for the *5G* allele (Eriksson et al., 1995a). Studies could establish a relationship between *PAI-1 4G/5G* polymorphism and risk of CAD (Liang et al., 2015).

As with every polymorphism, prevalence of the *ACE* (*I/D*), *PAI-1* (*4G/5G*) and *eNOS* (*4b/4a*/4c) varies among different populations and ethnic groups. Therefore, this study aimed at determining the relationships between these polymorphisms and coronary artery disease in a cohort of Palestinian CAD patients in Gaza strip.

MATERIAL AND METHODS

Study Population

The study population involved 86 unrelated symptomatic patients presenting with myocardial infarction (MI), stable angina and unstable angina and admitted for coronary artery catheterization. The patients were recruited from the cardiac catheterization departments of the European Gaza Hospital (EGH), Al Hayat center and Al-Shifa hospital. The sample consisted of 57 males and 29 females with ages ranging from 37 to 78 years (mean = 57.69 ± 9.69 years).

Ethical Consideration

The study was approved by the Palestinian Ministry of Health (MOH) ethical committee. All participants gave their consents to donate blood samples for analysis and collection of data, after explaining the aim and objectives of the study.

Blood samples collection

Whole blood samples were collected from the patients in EDTA-anticoagulated tubes. Approximately 3 ml venous blood samples were collected in each tube. The samples were collected at the cardiac catheterization departments of the European Gaza Hospital (EGH), Al Hayat center and Al-Shifa hospital.

Extraction and purification of genomic DNA

DNA was extracted from 300µl well-mixed blood samples using the Wizard Genomic DNA purification Kit (Promega, USA), according to the manufacturer instructions. The recovered DNA was rehydrated with 100µl rehydration solution and stored at -20^{o}C until performing the PCR experiments.

Detection of *ACE*/*eNOS*/*PAI* polymorphism by Allele-specific primer Polymerase chain reaction (ASP-PCR)

Two separate multiplex PCR reactions were carried out for each sample in 20µl reactions containing 1X *GoTaq* Green Master mix (Promega, USA), 50ng DNA and the proper concentration of the primers as indicated in table 1. The primers were reconstituted into two working mixtures prior to the PCR reaction (table 1). The first primer mixture was used for detection of the *ACE I/D* polymorphism and the *4G* allele of the PAI gene. The second primer mixture identified the *eNOS 4a*/*4b*/4c polymorphic alleles in addition to the *5G* allele of the PAI gene.

The PCR cycling conditions consisted a 5-minutes denaturation step at 95˚C, followed by 37 cycles of 95˚C for 40 seconds, 58˚C for 30 seconds and 72˚C for 40 seconds. A final extension step was carried out for 5 minutes at 72˚C.

Table 1. List of primers used for genotyping of *ACE/eNOS/PAI* polymorphisms.

Reaction mixture	Primer	Sequence 5' to 3'	Primer final concentration (µM)
***ACE*/*4G* primers mix**	***ACE*-Out-Sense**	TGGGACCACAGCGCCCGCCACTAC	0.267
	***ACE*-In-Sense**	CTGGAGACCACTCCCATCCTTTCT	0.33
	***ACE*-Out-Antisense**	TCGCCAGCCCTCCCATGCCCATAA	0.53
	PAI-*4G*-Senes	GTCTGGACACGTGGGGA	0.267
	PAI-*4G*-Antisenes	TGCAGCCAGCCACGTGATTGTCTAG	0.53
***eNOS*/*5G* primers mix**	***eNOS*-Sense**	CAGGCCCTATGGTAGTGCCTTG	0.21
	***eNOS*-Antisense**	CTCTTAGTGCTGTGGTCACAGGC	0.21
	PAI-*5G*-Senes	GTCTGGACACGTGGGGG	0.27
	PAI-*5G*-Antisenes	TGCAGCCAGCCACGTGATTGTCTAG	0.43

About 10 µl of the PCR products were analyzed on 2% Agarose gel (Promega, USA), stained with ethidium bromide and visualized by UV transilluminator. The expected size of bands obtained with homozygote or heterozygote for *ACE* gene (*I/D*), *eNOS gene* (*4a*, *4b* or 4c), *and PAI gen*e (*4G/5G*) is shown in table 2. The size of the bands was determined by comparison to 50bp-DNA size marker.

Table 2. The expected fragment size and interpretation.

Polymorphism	Genotype	Amplicon(s) size (bp)
PAI-1	*4G/4G*	139
	4G/5G	138, 139
	5G/5G)	138
ACE	*D/D*	234
	I/D	234, 335 and/or 522
	I/I	355 and/or 522
eNOS	*4a*	393
	4b	420
	4c	447

Statistical analysis

Personal data (name, age, address, education level, family history of heart disease) was collected by interviewing the patients. Current and previous clinical and laboratory data was collected from the medical record of the patient, and included the clinical presentation, result of catheterization, smoking, diabetes, hypertension and others.

The data was summarized, tabulated and analyzed using the IBM statistical package for social sciences software (SPSS) V.22. Differences in proportions were assessed by a *chi*-square test and mean comparisons by the *t*-test, a $P < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Personal and clinical characteristic of the study population

Upon cardiac catheterization 45 patients (52.3%) were found positive (abnormal) and 41 (47.7%) negative (normal). Table 3 shows the distribution of normal and abnormal cases based on different variables. Most of the enrolled patients were from Gaza city (44.2%) and northern area (32.6%) of the Gaza strip. The percentage of abnormal cases was higher in Gaza compared to other areas (P= 0.054).

In total 57 participants (66.3%) were males and 29 were females (33.7%). The results show that 84.4 % of the abnormal cases were males and 15.6% females. The distribution of participating patients by gender was statistically significant (P=0.0). This result is in consistence with previous studies showing that risk of CAD is higher in males than in females (Anand et al., 2008). Gender differences in risk of CAD may be attributed to that endogenous estrogen during the fertile period of life delays the manifestation of CAD in women (Bots et al., 2017). Although they share most of the risk factors for CAD, the relative contribution of each factor differs between males and females (Maas and Appelman, 2010). Studies have shown that both men and women have comparable symptoms of chest pain at presentation, but women tend to have more concomitant symptoms commonly seen in mood disorders, that can mask the chest pain especially at younger ages (Dey et al., 2009; Milner et al., 1999).

As shown in table 3 no statistically significant relationship exists between the outcome of cardiac catheterization and the family income ($P = 0.962$) and the level of education ($P = 0.829$). Previous studies revealed that younger individuals with low income are at higher risk of CAD (Lewis et al., 2015; Schultz et al., 2018). We hypothesized that poverty contributes to stressful lifestyle and ultimately predisposes to a range of chronic diseases including CAD. Lack of contribution of the family income in this study may be due to that almost all of the study population are falling within a low family income classification and are elderly. The majority of the Palestinian people living in Gaza, including cases and controls, are poor, and Palestinian statistics shows that poverty is continually deteriorating. The Palestinian Central Bureau of Statistics (PCBS) reported a significant increase in poverty rates in the Gaza Strip: from 38.8% in 2011 to 53% by the end of 2017, i.e., more than 14% in a period of six years (PCBS, 2018). Similar results were obtained by others who didn't detect any significant difference in the prevalence of CAD between high- and low-income sectors (Schultz et al., 2018).

Table 3 also shows the relationship between diabetes mellitus and the outcome of Catheterization. It reveals that 51.1% of the abnormal cases are diabetic compared to 41.5% of the normal cases. However, this difference was not statistically significant ($P = 0.370$). All diabetic cases were from type 2 diabetes mellitus (T2DM). The role of diabetes in increasing the morbidity and mortality in CAD patients is well established, and is particularly important in the management of CAD (Einarson et al., 2018). The lack of significance in our study may be explained by the fact that both cases and controls are elderly, which is a risk factor for diabetes as well for CAD.

No statistically significant relationship was seen between hypertension and the result of cardiac catheterization ($P = 0.199$), although 57.8% of the abnormal patients were hypertensive compared to 43.9% of the normal. Different studies demonstrated that the effect of hypertension on CAD onset may be modulated by various environmental and genetic factors. It is widely accepted that strategies adapted to lower blood pressure play a protective role against CAD by delaying atherosclerotic lesion formation (Hajar, 2017; Milane et al., 2014).

A history of CAD in the family of patient had no statistically significant role in increasing the risk of CAD in our study population (44.4% of abnormal and 43.9% of normal had a family history; $P = 0.960$). It may be worth mentioning that the family history was significantly higher in the abnormal (85.7%) compared to normal (40.9%) female subjects ($P = 0.0490$) but not in males. The prevalence of CAD is particularly high in the Palestinian males, which may mask any role of the family history in increasing the risk. Family history is most prominent in CAD patients with the lowest overall cardiovascular risk profile, such as females, suggesting the possibility that in this group there is familial aggregation of a lower threshold for developing clinically apparent disease due to absence of other risk factors (Shea et al., 1984). Others suggested that family history for ischemic heart disease is a significant and independent risk factor for coronary artery disease (Hajar, 2017).

Smoking is more common among the abnormal cases 66.7% compared to 26.8% of normal. The relationship between smoking and CAD was statistically significant ($P = 0.000$), and these results concord with other studies showing that cigarette smoking can result in a two-fold increase in the risk of coronary artery disease and other CVDs (Banks et al., 2019). Smoking contributes to atherogenic vessel wall changes characteristic of CAD, by direct physical damage to endothelial cells, tissue remodeling, and prothrombotic processes together with activation of systemic inflammatory signals (Messner and Bernhard, 2014).

The mean BMI for the abnormal group was 29.2 ± 6.3 Kg/m2 and for the normal group was 31.0 ± 4.4 Kg/m^2. The mean difference was not significantly different ($P = 0.138$). It is noticeable that 89% of abnormal and normal patients were having a BMI of more than 25 Kg/m^2, who are overweight and/or obese according to the WHO classification. This fact may be responsible for the lack of significant relationship between the BMI and the occurrence of CAD. The BMI is generally higher in females than in males (Banks et al., 2019). In this study, about 53.8% of controls are females, who have a higher BMI than men (32.1 compared to 29.1 Kg/m^2; $P = 0.018$). Similar results were reported by (Jamee Shahwan et al., 2019). However, many researches have established that increasing BMI is tightly associated with increasing the risk for developing cardiac events (Gregory et al., 2017).

Regarding the clinical presentation, the present study indicates that there is a statistically significant relationship between the result of cardiac catheterization and the clinical presentation of patient (P=0.002). The presence of unstable angina among abnormal cases was 64.4%, while it was 26.8%. Many studies have shown that patients with a history of dyspnea (unstable angina) had an annualized mortality rate of 6.4% compared with 2.4% among those with typical angina without dyspnea (stable angina) in CAD patients (Abidov et al., 2005).

Table 3. the relationship between cardiac catheterization result and different variables.

		Normal N (%)	Abnormal N (%)	Total N (%)	*P-value*
Address	**North Gaza**	18 (43.9)	10 (22.2)	28 (32.6)	0.054
	Gaza City	16 (39.0)	22 (48.9)	38 (44.2)	
	Mid-Zone	2 (4.9)	9 (20.0)	11 (12.8)	
	South Gaza	5 (12.2)	4 (8.9)	9 (10.5)	
Sex	**Male**	19 (46.3)	38 (84.4)	57 (66.3)	0
	Female	22 (53.7)	7 (15.6)	29 (33.7)	
Family Income	**<500**	11 (26.8)	10 (22.2)	21 (24.4)	0.862
	500-1500	23 (56.1)	26 (57.8)	49 (57.0)	
	>1500	7 (17.1)	9 (20.0)	16 (18.6)	
Education level	**School**	17 (43.6)	17 (37.8)	34 (40.5)	0.829
	Graduate	7 (17.9)	10 (22.2)	17 (20.2)	
	Post-graduate	15 (38.5)	18 (40.0)	33 (39.3)	
Diabetes Mellitus	**Yes**	17 (41.5)	23 (51.1)	40 (46.5)	0.37
	No	24 (58.5)	22 (48.9)	46 (53.5)	
Hypertension	**Yes**	18 (43.9)	26 (57.8)	44 (51.2)	0.199
	No	23 (56.)	19 (42.2)	42 (48.8)	
Family History (CAD)	**Yes**	18 (43.9)	20 (44.4)	38 (44.2)	0.96
	No	23 (56.1)	25 (55.6)	48 (55.8)	
Smoking	**Smoker**	11 (26.8)	30 (66.7)	41 (47.7)	0
	Nonsmoker	30 (73.2)	15 (33.3)	45 (52.3)	
Clinical Presentation	**Unstable Angina**	11 (26.8)	29 (64.4)	40 (64.5)	0.002
	Stable Angina	28 (68.3)	15 (33.3)	43 (50.0)	
	Acute MI	2 (4.9)	1 (2.2)	3 (3.5)	

Genotyping results:

Blood samples from 72 patients, only, were successfully amplified by PCR for (*ACE, PAI* and *eNOS*) genes by the two multiplex reactions. Figure 1A and B show representative gel electrophoresis results for *PAI-4G/ACE* and *PAI-5G/eNOS* multiplexes respectively.

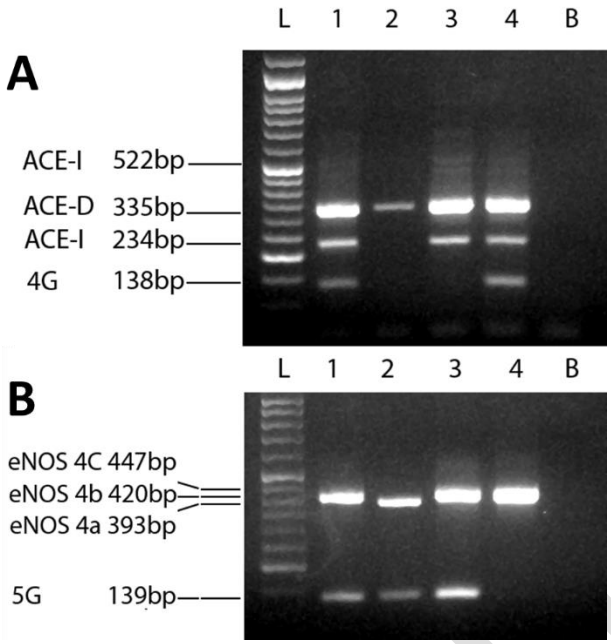

Figure 1. A representative gel electrophoresis.

The genotyping results of 4 samples are depicted: The gel for *ACE-I/D* and *PAI-4G* polymorphism (A), and for *eNOS-4a/4b/4c* and *PAI-5G* polymorphism (B). Sample 1 is *4G/5G, 4b/4b, I/D*; Sample 2 is *5G/5G, 4a/4a, D/D*; Sample 3 is *5G/5G, 4b/4b, I/D*; and Sample 4 is *4G/4G, 4b/4b, I/D*. L is a 50bp-DNA size marker and B is a blank contamination control. The sizes of expected amplicons are indicated.

The genotype distribution of *ACE* is presented in table 4. Ten patients (13.9%) were homozygotes for the insertion allele (*I/I*), while the remaining 62 were equally distributed between heterozygous (*I/D*) and homozygous for the deletion (*D/D*) allele. The allelic frequency of the *I*-allele is 35.4% and of the *D*-allele is 64.6% (Table 5). Both alleles are in Hardy-Weinberg equilibrium ($P = 0.6177$). Our data is similar to data reported in the Palestinian population of Gaza strip, with similarly high frequency of the *D* allele (Al Sallout and Sharif, 2010; Saqer and apos, 2016). Our results are also comparable to other Middle Eastern populations, like Omani (*D* = 71%), Emirati (*D* = 66%), Somali (*D* = 73%) and Sudan population (*D* = 64%) (Bayoumi et al., 2006). In contrast, other populations have different frequencies of the *D*- allele, like the Japanese (34%) and Chinese (29%) who have lower frequencies (Kario et al., 1997; Lee, 1994). As depicted in table 4, the genotype of 16.7% of the patients was homozygous (*4G/4G)* for the *PAI-1* gene. The remaining patients were either heterozygotes (37.5%) or homozygotes for the *5G/5G* genotype (45.8%). The allelic frequency of the *4G*- allele is 35.4% and of the *5G*-allele is 64.6% (table 5). Both alleles are in Hardy-Weinberg equilibrium ($P = 0.1261$). Our allele frequency is similar to that previously reported in a cohort of Palestinian women with recurrent abortion (Al Sallout and Sharif, 2010). According to The Single Nucleotide Polymorphism Database (dbSNP), the worldwide *4G* allele frequency ranges from 39.3% in Asian populations to as high as 73.1% in African Americans (NCBI, 2021).

The genotype distribution of the *eNOS* gene polymorphism is presented in table 4. Only 2.9% of the patients were homozygous for the *4a* allele, while 21.4% were heterozygous *4a/4b*; 74.3% were homozygous for the *4b* allele and 1.4% of patients were heterozygous for *4b/4c*. The allelic frequency is 13.57% for the *4a*-allele, 85.71%

the *4b*-allele and 0.71% for the *4c*-allele (table 5). The alleles are in Hardy-Weinberg equilibrium (*P*=0.8819). Our data of allele frequency is similar to that reported among female Palestinian women with recurrent abortion (Al Sallout and Sharif, 2010) in whom the allele frequencies of *4a ,4b* and *4c* were 19% , 80.5% and 0.5% respectively.

Table 4: The genotype frequency of *ACE, PAI-1* and *eNOS gene*

		Normal N (%)	Abnormal N (%)	Total N (%)	*P-value*
ACE	***I/I***	2 (7.4)	8 (17.8)	10 (13.9)	0.1981
	I/D	15 (55.6)	16 (35.6)	31 (43.1)	
	D/D	10 (37.0)	21 (46.7)	31 (43.1)	
PAI-1	***5G/5G***	12 (44.4)	21 (46.7)	33 (45.8)	0.8922
	4G/5G	11 (40.7)	16 (35.6)	27 (37.5)	
	4G/4G	4 (14.8)	8 (17.8)	12 (16.7)	
eNOS	***4b/4b***	18 (72.0)	34 (75.6)	52 (74.3)	0.5637
	4b/4a	5 (20.0)	10 (22.2)	15 (21.4)	
	4a/4a	1 (4.0)	1 (2.2)	2 (2.9)	
	4b/4c	1 (4.0)	0 (0.0)	1 (1.4)	

Table 5: The allele frequency for *ACE, eNOS and PAI-1* polymorphic alleles.

Gene	Allele	Normal		Abnormal		Total	
		Frequency	*P**-value	Frequency	*P**-value	Frequency	*P**-value
ACE	I	35.2%	0.2572	35.6%	0.1327	35.4%	0.6177
	D	64.8%		64.4%		64.6%	
PAI	*5G*	64.8%	0.5791	64.4%	0.1327	64.6%	0.1261
	4G	35.2%		35.6%		35.4%	
eNOS	*4a*	14.0%	0.9200	13.3%	0.7964	13.6%	0.8819
	4b	84.0%		86.7%		85.7%	
	4c	2.0%		0.0%		0.7%	

* *The P-value is calculated from comparison between expected and obtained allelic frequencies based on the Hardy-Weinberg equation.*

The relation between gene polymorphism and risk of CAD

Distribution of the different genotypes among the normal and abnormal patients was not statistically significant (*P*s for *ACE* = 0.198, for *PAI-1* = 0.892, and for *eNOS* = 0.564; tables 4). Moreover, the allele frequency for the three genes did not differ significantly between the normal and abnormal groups (table 5). Previous studies have shown that the *D*- allele of *ACE* is associated with significantly increasing the risk for CAD events and severe coronary stenosis (Kryczka et al., 2020; Vladeanu et al., 2020). This effect has been reported in different populations worldwide. The *DD* genotype was suggested to be associated with increased risk of CAD in Asian Indian population (Bhatti et al., 2017). An association between the *DD* polymorphism and CAD was also

reported in Saudi patients with T2DM (Al-Jafari et al., 2017). Studies also suggested that *ACE I/D* polymorphism is an important predictor of coronary artery disease in different Turkish populations (Nacak et al., 2004; Temel et al., 2019). Homozygosity for the *D* allele might be associated with higher plasma fibrinogen levels in women but not men with premature CAD (Kryczka et al., 2020). On the other hand, a number of studies failed to establish any correlation between the *DD* genotype and CAD (Bayramoglu et al., 2020; Heidari et al., 2019; Zhang et al., 2019). *ACE I/D* polymorphism and *ACE* activity were both unable to predict ST-Elevation Myocardial Infarction (STEMI) or in-hospital mortality after STEMI in Indians (Moorthy et al., 2021). No association was also observed in African-Brazilians and Caucasian-Brazilians (Bonfim-Silva et al., 2016). *ACE I/D* polymorphism was excluded from being an independent risk factor in the development of atherosclerosis in Iranian population (Nouryazdan et al., 2019). The lack of significance in the relationship may result from the high prevalence of the risk allele among the Palestinian population.

Contrary to our results, the *PAI-1 4G allele* is considered a risk allele for CAD (Bayramoglu et al., 2020; Liang et al., 2015). *4G/4G* homozygotes have higher plasma levels of *PAI-1* than *5G/5G* homozygotes (Yousef et al., 2020). The expression of *4G* allele is increased over that of the *5G* allele as a result of transcription activation (Eriksson et al., 1995b). The effect of *4G* allele in conferring CAD risk is more profound in younger individuals (Yousef et al., 2020). On the other hand, similar to our results *PAI-1 4G/5G* was found not significantly associated with Myocardial infarction in a cohort of Iraqi patients (Mohammad et al., 2020). Lack of association was also reported in Egyptian, young Italian, Finnish patients and other populations (Al-Wakeel et al., 2018; Gazi et al., 2014; Viitanen et al., 2001).

Meta-analysis revealed that *eNOS 4b/4a* polymorphisms could confer susceptibility for developing CAD in population-based subgroups, particularly in African and Middle Eastern populations (Rai et al., 2014; Yang et al., 2014). In our study however, the *eNOS 4a*/*4b* polymorphism was not associated with CAD. In agreement, other studies show no association with premature CAD, but the clustering of classical risk factors play more important role in premature CAD in Caucasian women (Letonja, 2004). The *4a*/*4b* variants were not associated with risk for CAD and occurrence of angiography-assessed st*enos*is in Northern Iranian population (Joshaghani et al., 2018). *eNOS 4a*/*4b* polymorphism was not associated with CAD under any of the genetic models tested in Tunisians (Ben Ali et al., 2015). There was no positive association with premature CAD in an Australian Caucasian population (Granath et al., 2001). Furthermore, a study revealed that the *4a* allele could be associated with reduced CAD incidence in a Korean population, when present in a haplotype with other *eNOS* alleles such as the T allele of -786T>C (Bae et al., 2010; Sung et al., 2015). Such modulating effect has not been studied in our study.

CONCLUSION

The risk factors for CAD in the study population includes sex, increased BMI in females, smoking and unstable angina at presentation. Other recognized risk factors were found not significantly associated with CAD risk in the study population of Gaza strip. The genetic polymorphism in *ACE*, *eNOS* and *PAI* were not found to be influencing the risk of CAD. The results of the study may be limited by the small number of cases and controls.

ACKNOWLEDGMENTS

The author is indebted to departments of cardiac catheterization of the European Gaza Hospital (EGH), Al Hayat center and Al-Shifa hospital for provision of patients and data. The author thanks Dr. Amal Shahwan who kindly facilitated access to the patients and data. The author would like also to thank Mr. Nedal Qaddoura, Ms. Nedaa Al-Majayda and Ms. Doaa' Al-Majayda for their valuable assistance in sample collection and laboratory work.

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