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Molecular assessment of some cardiovascular genetic risk factors among Iraqi patients with ischemic heart diseases

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ABSTRACT

Objective: The underlying molecular basis of ischemic heart diseases (IHDs) has not yet been studied among Iraqi people. This study determined the frequency and types of some cardiovascular genetic risk factors among Iraqi patients with IHDs.

Methods: This is a cross-sectional study recruiting 56 patients with acute IHD during a 2-month period excluding patients >50 years and patients with documented hyperlipidemia. Their ages ranged between 18 and 50 years; males were 54 and females were only 2. Peripheral blood samples were aspirated from all patients for troponin I and DNA testing. Molecular analysis to detect 12 common cardiovascular genetic risk factors using CVD StripAssay® (ViennaLab Diagnostics GmbH, Austria) was performed.

Results: The genotype frequencies of 12 genetic mutations/polymorphisms were as follows: MTHFR A1298C and C677T were the highest reported mutations (62.5% and 50%, respectively), followed by β-fibrinogen gene mutation, homozygous angiotensin-converting enzyme D/D, heterozygous human platelet antigen-1(a/b) polymorphisms, plasminogen activator inhibitor-1 4G/4G, homozygous E4 allele of apolipoprotein E gene, Leu allele of Factor XIII V34L variant, heterozygous FV R2, Factor V Leiden mutation, prothrombin G20210A mutation, respectively. Genetic risk scores were calculated and a number ranging from 0 to 8 were given to each patient. None (0%) had a risk score >6 or <2; 22 (39.3%) patients had a risk score of 4 and >60% of cases had a risk score of 4 or more.

Conclusion: The obtained results constitute a reference guide where future studies on normal people and older IHD patients can rely on to determine whether these can be used for pre-clinical risk assessment.

Keywords: Angiotensin-converting enzyme I/D, ApoE, genetic risk factors, human platelet antigen 1, Iraq, ischemic heart diseases, MTFHR, plasminogen activator inhibitor-1, β-fibrinogen

Introduction

Ischemic heart diseases (IHDs) are major causes of morbidity and mortality worldwide; their incidence is increasing in developing countries. It is estimated that 17.5 million individuals die from cardiovascular diseases (CVDs) each year, accounting for 31% of all deaths worldwide and more than 75% of these deaths occur in low- to middle-income population.[1] Understanding the pathogenesis of IHD has been modified over the years.[2] Attention is now focused toward understanding the genetic basis of IHD.

The traditional risk factors such as hypertension, diabetes mellitus, hypercholesterolemia, low physical activity, obesity, increased C-reactive protein, elevated plasma homocysteine level, and tobacco use are well known to have their own complex genetic components with individual heritability values.[3]

Enormous efforts have been invested in understanding the genes and specific DNA sequence variations responsible for this heritability because genetic polymorphisms might be risk factors that predispose to IHD.[4] Many new genetic risk factors have been recognized.[2]

Apolipoprotein E (ApoE) has a regulatory role in lipid metabolism through the cellular uptake of ApoE-bearing lipoproteins.[5,6] Three isoforms (Apo E2, E3, and E4) are present in human population.[7] E4 carriers, representing 20% of the population, were reported to have a 40% higher risk of IHD compared with ApoE3/E3 homozygotes, whereas the relationship between the isoform ApoE2 and genetic risk was less obvious.[8]

ApoB (R3500Q) is considered one of risk factors in developing myocardial infarction (MI). ApoB mutations were strongly and positively related to increased risk of MI in men and in women.[9] ApoB 100 mutations cause familial defective, an autosomal dominant disorder.[10]

Angiotensin-converting enzyme (ACE) is the major component of the renin–angiotensin system, which is a triggering factor for the development of coronary artery disease (CAD).[11] The D allele is a risk factor for MI incidence for both Asians and Caucasians,[12] while I allele has a protective effect against elevated serum lipid and lipoprotein levels.[13]

Elevations of plasma homocystein have been associated with genetic defects in enzymes involved in its metabolism.[14] MTHFR C677T and A1298C variant mutations are the most frequent genetic causes of mild hyperhomocysteinemia, which is a risk factor for CVD.[15]

The clotting Factor XIII (FXIII)is essential for maintaining hemostasis by stabilizing the fibrin clot and protecting it from fibrinolytic degradation. FXIII V34L variant conferred a protective effect against MI.[16]

Plasminogen activator inhibitor (PAI-1) has two common polymorphisms known as 4G and 5G.[17] PAI-1 excess has been identified in survivors of acute MI and plasma PAI-1 performance is higher in MI survivors that go on to develop recurrent MI.[18] PAI-1 is one of the suggested predisposing genetic factors in patients with CAD.[19]

Beta-fibrinogen -455G/A mutation is associated with differences in plasma levels of fibrinogen.[20] β-fibrinogen -455G/A polymorphism contributes to susceptibility to ischemic stroke and IHD,[21] and was related to the progression of CAD.[22]

Human platelet antigen 1 (HPA-1) or called glycoprotein IIIa (GPIIIa) is a membrane receptor for fibrinogen; it plays an important role in platelet aggregation. GPIIIa polymorphism at codon 33 (Leu33Pro), known as HPA-1a and HPA-1b, was reported to be associated with acute MI and is involved in the pathogenesis of coronary syndromes. HPA-1b allele might be an important predisposing factor for acute MI.[23,24]

Factor V Leiden is a variant of human Factor V that may cause hypercoagulability and increases risk of thrombosis.[25] It is one of the most common genetic risk factors for venous thromboembolism.[26]

Factor V R2 (H1299R) was linked to hereditary thrombophilia.[27] Factor V R2 has been shown to influence plasma levels of FV and is related to activated protein C resistance.[28]

Prothrombin (Factor II) plays a role in hemostasis, thrombosis, formation of blood clot, and stimulation of platelet aggregation.[29] Prothrombin gene (G20210A) mutation is an autosomal defect that has been reported as a risk factor for arterial thrombosis with reported increased risks of more than 5-fold for thrombotic cerebrovascular disease.[30] Heterozygous carriers of prothrombin G20210A were reported to have increased risk of deep vein thrombosis.[31]

Materials and Methods

This study was a cross-sectional study that involved 56 patients aged between 18 and 50 years, of which males were 54 (96.4%) and females were only 2 (3.6%). These patients were having a probably higher genetic susceptibility for arterial thrombosis and were chosen from patients admitted to the cardiac care unit (CCU) of Ibn Al-Nafees Teaching Hospital with a clinical diagnosis of acute IHD (MI or unstable angina) during a 2-month period between December 8th, 2015 and February 8th, 2016.

All patients were evaluated and categorized according to the clinical presentation and electrocardiographic finding and cardiac enzyme troponin I (performed by a qualitative rapid strip test).

Inclusion criteria included all cases <50 years old admitted to the CCU with acute MI or angina, while the exclusion criteria included older age (>50 years), and established hyperlipidemia.

History of smoking, hypertension, diabetes mellitus, hyperlipidemia, previous history of IHD, and family history of heart diseases were also reported. Consents from all patients were obtained.

About 2 to 3 ml of peripheral venous blood samples were withdrawn from all patients and were collected in a K_2EDTA tubes and then kept frozen at −20°C for DNA analysis.

For determining the genetic risk factors for CVD, DNA isolation, polymerase chain reaction amplification using biotinylated primers, gel electrophoresis of amplification products, and their hybridization to a test strip containing allele-specific oligoprobes immobilized as an array of parallel lines were performed. The bound biotinylated sequences were detected using streptavidin-alkaline phosphatase and color substrates.

DNA extraction and molecular assessment were performed using Genxtract DNA isolation kit and CVD StripAssay® (ViennaLab Diagnostics GmbH, Austria).

The study was approved by the Ethical Committee at the College of Medicine/Baghdad University and by that of the Training and Development Center/Ministry of Health-Baghdad/Iraq.

Statistical analyses

Statistical analyses were done Microsoft Excel version 2010. Frequency, cumulative relative frequency, and 95% confidence interval (CI) were used for description of the obtained data.

Results

The results of Table 1 of this study revealed that 12 (21.4%) patients were hypertensive, 15 (26.8%) were diabetics, 42 (75%) were smokers, 27 (48.2%) had family history of IHD, and 13 (23.2%) had previous attack of ischemia. Troponin I test was positive in 39 (72.2%) of cases. 41 (73.12%) patients had acute MI while 15 (26.78%) had angina pectoris; 38 (95%) of MI patients and only 1 (7.7%) of angina cases were troponin I positive.

The results of the molecular analysis, as presented in Table 2, showed that MTHFR A1298C and C677T were the highest reported mutations among the study group (62.5% and 50%, respectively), while the least detected mutations were Factor V Leiden and prothrombin G20210A (each reported in 1 case [1.8%]).

In Table 3, to assess the combined effect of the determined mutations in this study, genetic risk scores were calculated for eight risk factors in each patient including all determined mutations/polymorphisms except those related to venous thrombosis Factor V G1691A (Leiden), Factor V R2 (H1299R), and prothrombin G20210A plus FXIII that has a protective effect against arterial thrombosis. A number ranging from 0 to 8 were given and were considered as the risk score for each patient.

None (0%) had a risk score >6 or <2; 22 (39.3%) of patients had a risk score of 4 (the most commonly encountered risk score) and >60% of cases had a risk score of 4 or more.

In Table 4, genetic risk scores for patients were evaluated along with a history of traditional risk factors (namely, hypertension, DM, and smoking); the patients were classified into four groups.

The patients with genetic risk score of 3 or more who had one or more traditional risk factors had higher cumulative frequency than those of free of the traditional risk factors (93.7%, 92.9%, and 100% for those who had 1, 2, and 3 traditional factors, respectively, as compared to 71.4% found in those free of traditional risk factors).

Discussion

Molecular basis of IHD has not been investigated earlier among Iraqi people.

Patients rather than healthy Iraqi individuals were included in this study as these patients may have higher genetic susceptibility that is related, both etiologically and pathologically, to CVD. The recruited patients have angina or MI (the most common CVD).

In this study, younger patients were selectively chosen to minimize the effect of increasing age on the incidence of

Table 1: The relative frequency of clinical and laboratory variables in patients with IHD

Clinical/laboratory variables	*n* (%)		
	Negative	Positive	Total
Hypertension	44 (78.6)	12 (21.4)	56 (100)
Diabetes mellitus Type II	41 (73.2)	15 (26.8)	56 (100)
Smoking	14 (25)	42 (75)	56 (100)
Previous history of ischemia	43 (76.8)	13 (23.2)	56 (100)
Family history of IHD	29 (51.8)	27 (48.2)	56 (100)
Troponin I test	15 (27.8)	39 (72.2)	54* (100)
Total			56 (100)

*Troponin I test results were not available in 2 cases. IHD: Ischemic heart diseases

Table 2: The relative frequency of 12 cardiovascular genetic risk factors (mutations/polymorphisms) and zygosity status among young Iraqi IHD patients

Mutation/polymorphism	*n* (%)	95% CI*
MTHFR A1298C		
Normal (AA)	21 (37.5)	
Heterozygous (AC)	25 (44.6)	(6.4–26.2)
Homozygous (CC)	10 (17.9)	8.9–30.4)
MTHFR C677T		
Normal (CC)	28 (50.0)	
Heterozygous (CT)	20 (35.7)	(23.3–49.6)
Homozygous (TT)	8 (14.3)	
β-Fibrinogen -455G/A		
Normal (GG)	30	
Heterozygous (GA)	24 (42.6)	(29.8–56.8)
Homozygous (AA)	2 (3.6)	(0.4–12.4)
PAI-1		
Homozygous (5G/5G)	5 (8.9)	
Heterozygous (4G/5G)	42 (75)	(61.6–85.6)
Homozygous (4G/4G)	9 (16.1)	(7.6–28.4)
ACE		
(I/I)	10 (17.9)	
(I/D)	26 (46.4)	
(D/D)	20 (35.7)	(23.3–49.6)
HPA1 (a/b)		
Homozygous (a/a)	40 (71.4)	
Heterozygous (a/b)	16 (28.6)	(17.3–42.2)
Homozygous (b/b)	0 (0)	-
ApoE (E2/E3/E4)		
Homozygous (E3/E3)	44 (78.6)	
Heterozygous (E2/E3+E2/E4)	8 (14.3)	
Homozygous (E4/E4)	4 (7.1)	(2.0–17.2)
Factor XIII V34L		
Normal	42 (75.0)	
Heterozygous	12 (21.4)	(11.6–34.4)
Homozygous	2 (3.6)	0.4–12.4)

(Contd...)

Table 2: (*Contined*)

Mutation/polymorphism	*n* (%)	95% CI*
FV R2 (H1299R)		
Normal	49 (87.5)	
Heterozygous	7 (12.5)	(5.2–24.1)
Homozygous	0 (0)	-
FV Leiden (G1691A; R506Q)		
Normal (GG)	55 (98.2)	
Heterozygous (GA)	1 (1.8)	(0.05–9.6)
Homozygous (AA)	0 (0)	-
Prothrombin (G20210A)		
Normal (GG)	55 (98.2)	
Heterozygous (GA)	1 (1.8)	(0.05–9.6)
Homozygous (AA)	0 (0)	-
ApoB R3500Q		
Normal	56 (100)	
Heterozygous	0 (0)	-
Homozygous	0 (0)	

CI: Confidence interval; MTHFR: Methylenetetrahydrofolate reductase; PAI: Plasminogen activator inhibitor; ACE: Angiotensin-converting enzyme; HPA: Human platelet antigen; Apo: Apolipoprotein; FV: Factor five, IHD: Ischemic heart diseases

Table 3: The risk score of 8 selected cardiovascular genetic risk factors in young Iraqi IHD patients

Risk score (0–8)	Number of patients (%)	CF*
8	0 (0)	0
7	0 (0)	0
6	2 (3.6)	3.6
5	10 (17.9)	21.5
4	22 (39.3)	60.8
3	17 (30.3)	91.1
2	5 (8.9)	100
1	0 (0)	100
Total	56 (100)	

* CF: Cumulative relative frequency. IHD: Ischemic heart diseases

IHD, since the prevalence and severity of IHD these diseases increases with age.[32,33]

Patients having hyperlipidemia were excluded as they probably had other genetic mutations and monogenic diseases with clear Mendelian inheritance.

Male patients were predominant and consisted of 54 (96.4%) patients, while females were only 2 (3.6%). The two females were in their premenopausal period; this lower incidence of IHD among premenopausal women and the subsequent age-related rise of IHD in women postmenopausally compared with age-matched men is related to endogenous sex hormones including estrogen.[34]

In this study, known traditional risk factors of CVD such as hypertension, diabetes, and smoking were encountered in our recruited IHD patients. There were 21.4% hypertensive patients; 26.8% diabetics, 75% smokers, 48.2% had positive family history of IHD, and 23.2% patients had previous attack of ischemia. These findings were similar to other studies.[35-37]

One previous Iraqi study reported MTFHR mutations among Iraqi Kurds in 2009; their findings were slightly different than ours as MTHFR C677T heterozygous (CT) and MTHFR C677T homozygous (TT) states were found in 44% and 8%, respectively, among 150 healthy blood donors in Duhok. While in this study, they were 35.7% (CT) and 14.3% (TT). This MTHFR C677T (TT) genotype was a significant risk factor for ischemic stroke among Iraqi population.[38]

Although those studies and our study were conducted on Iraqi people, this variation may be attributed to different geographic locations (Duhok vs. Baghdad) and ethnic groups of enrolled individuals (Kurds vs. Arabs), variation in type of sample (normal healthy people in their study vs. IHD patients in this study), and difference in sample size (56 in ours vs. 150 cases in theirs). This finding encourage future research plans to assess the role of these factors in incidence of this mutation and its pathoetiologic role in CVD.

The current study found that β-fibrinogen was present in 53.6% cases. Heterozygous β-fibrinogen -455G/A (GA) allele was detected in 42.9% of patients, while homozygous (AA) allele was detected in 3.6% cases with 95% CI (33.0–60.3). An association between smoking and elevated fibrinogen levels was reported earlier.[39] In this study, β-fibrinogen (GA and AA) alleles were studied in association with smoking among IHD patients; a higher incidence of these alleles was found among smokers than non-smokers (50% for smokers vs. 35.71% for non-smokers); these results are in concordance with previous reports.[39]

In the current study, 91.1% of patients were carriers for PAI - 1 4G allele; 75% of them had heterozygous 4G/5G polymorphism and 16.1% had 4G/4G polymorphism, while only 8.9% had homozygous 5G/5G. This polymorphism was reported to be significantly associated with early-onset CAD risk.[40,41]

Since 1992, when the association between the ACE (D/D) genotype and IHD was published, several articles reported the prevalence of the ACE (I/D) polymorphism in different populations and ethnicities, indicating the positive effect of the D allele on IHD development.[42] This study showed that the frequency of homozygous ACE (I/I) polymorphism was 17.9%; heterozygous ACE (I/D) was detected in 46.4%, and the homozygous (D/D) polymorphism was detected in 35.7%. Collectively, the D allele was present in 82.1% in our cases.

The genotype frequency of GPIIIa HPA-1(a/b) polymorphisms was as follows: 71.4% for HPA-1 (a/a); 28.6% for HPA-1 (a/b); and 0% for HPA-1 (b/b) (95% CI 17.3–42.2). An association between HPA-1b and premature MI was reported earlier.[24]

Table 4: Correlation of the calculated genetic risk score of 8 risky genes with the number of traditional risk factors among young Iraqi IHD patients

Risk score (0–8)	Free of traditional risk factors		Had only 1 traditional risk factor		Had any 2 traditional risk factors		Had all 3 traditional risk factors	
Risk score	*n* (%)	CF*	*n* (%)	CF*	*n* (%)	CF*	*n* (%)	CF*
6	0 (0)	0	2 (6.3)	6.3	0 (0)	0	0 (0)	0
5	0 (0)	0	4 (12.4)	18.7	4 (28.6)	28.6	2 (66.7)	66.7
4	3 (42.8)	42.8	15 (46.9)	65.6	4 (28.6)	57.2	0 (0)	66.7
3	2 (28.6)	71.4	9 (28.1)	93.7	5 (35.7)	92.9	1 (33.3)	100
2	2 (28.6)	100	2 (6.3)	100	1 (7.1)	100	0 (0)	100
Total	7 (100)		32 (100)		14 (100)		3 (100)	

*CF: Cumulative relative frequency. IHD: Ischemic heart diseases

ApoE4 allele is considered as an independent risk factor for premature MI.[43,44] In this study, E3/E3 polymorphism was detected in 78.6% of patients; E2/E3 was detected in 14.3%; E2/E4 polymorphism was detected in 1.8%; E3/E4 polymorphism was detected in 5.3% of patients, while E4/E4 was not detected in any patient (0%). Collectively, the risky E4 allele was present in 7.1% of the studied cases (95% CI 2–17.2).

FXIII Val34Leu polymorphism has a significant ethnic heterogeneity. The incidence of Leu allele is quite high in Western countries (43%-51%), and this polymorphism is rare in Asian populations (as low as 0–2%).[45] The current study showed that heterozygous FXIII V34L variant was detected in 21.4% patients, while homozygous allele was detected in 3.6% patients, i.e., ~25% of selected cases had Leu allele (95% CI 14.4–38.4).

This study found that the frequency of the heterozygous FV R2 state was 12.5% while homozygous FV R2 was not reported in any case (0%) (95% CI 5.2–24.1). The mutation frequency ranged between 5.8% and 10.4% in different populations around the world.[46-51]

Results of the current study demonstrated that the frequency of Factor V Leiden mutation was 1.8% (95% CI 0.05–9.6) of enrolled patients. A previous Iraqi study showed that the prevalence of Factor V Leiden was 3% which was lower than the prevalence rates reported in regional countries (5.5%–14.2%),[52] but similar to the prevalence of 2.5% reported in Saudi Arabia.[53]

The current study demonstrated that the frequency of prothrombin G20210A mutation in IHD patients was (1.8%) (95% CI 0.05–9.6). This wide range of CI might reflect the frequency of this allele in our population as no previous figure was reported for this mutation and a larger scale study is needed to define the exact frequency in our population. The incidence of this variant in our people seems to lie in the middle of Asian, African, and American figures (95% CI 0.5–4%).[54,55]

IHD is an example of a complex disease, in which the effect of a single gene on the risk is suspected to be weak. A larger number of abnormal alleles may confer a significant risk of developing IHD.[56,57] Using the polygenic approach, this study estimated the cumulative effect of the risky gene variants, and showed that all enrolled cases carry at least two mutant alleles of IHD risk genes and none carry more than six mutant alleles, and that 60.8% of enrolled cases carry 3–6 mutant alleles. The patients with risk score 6 had the least frequency (3.6%).

From the results of genetic risk scores, it is observed that increasing number of mutant alleles may have an increasing risk of developing acute coronary artery disease. However, proper assessment of genetic risk score and its role with environmental and other risk factors on CVD need to be analyzed and compared with figures in normal controls.

It became clear that the presence of multiple risk factors did increase the risk of coronary heart disease and individual risk factors could be combined into a multivariate function to give an assessment (or the probability of or the risk) of developing a CVD event over a specific period (e.g., 10 years).[58] This study showed that the risk of IHD increases when associated traditional risk factors in those who carry three or more genetic risk factors.

Conclusions

In this work, although several cardiovascular genetic risk factors were studied, the role of each of them as an individual risk of IHD need to be compared and analyzed with figures from normal controls volunteers and with other group of patients with arterial thrombosis (stroke and older IHD more than 50 years) as well as their correlation with traditional/environmental risk factors. The same applies on genetic risk scores observed in this study. Actually, the obtained figures of this study may represent a baseline and documented review for other future studies in Iraq.

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