Original Research

ASSOCIATION BETWEEN FACTOR V LEIDEN (*FV G1691A*) MUTATION AND CORONARY ARTERY DISEASE DEVELOPMENT IN GAZA STRIP – PALESTINE

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ABSTRACT

Introduction: Coronary Artery Disease(CAD) is multi-factorial and usually results from a combination of acquired environmental and inherited factors with a change in lifestyle. One of these inherited factors is the *G1691A factor V*. Factor V Leiden (FVL) is the most common heritable thrombophilic disorder. It is concomitant with an increased the risk of thrombosis and may lead to CAD. The aim of this study is to investigate the relation of this mutation with CAD in Gaza strip population. **Methods:** it is a retrospective study which includes 180 samples; patients with CAD, n=90 and control group, n=90. An interview with questionnaire was applied. EDTA samples were collected for DNA extraction. The *FVL* mutation was identified by PCR-RFLP. **Results:** The frequency of *FVL* genotypes were: wild type 83.2%, heterozygous16.7% and complete absence of mutant homozygous in control group, whereas in CAD patients were: 70%,26.7% and 3.3%. The *FVL G* and *A* alleles frequencies of the participants were: for case group 0.833 and 0.167 while 0.917 and 0.083 were in control group. Furthermore, the distribution of *FVL* heterozygote and mutant(*AA*) genotypes were not significantly different between the study and control groups. The *GA* and *AA* genotypes increase the risk of developing CAD in patients by 1.9 and 8.3 times respectively. The presence of *FVL A* allele increases the risk of exposure to heart attack among CAD patient (OR =1.7). The frequency of *A* allele among diabetic patients increases the risk of developing CAD 1.5 fold. **Conclusion:** Factor V Leiden *A* allele is associated with the development CAD.

Keywords : Factor V Leiden , Factor V , Coronary Artery Disease , Polymerase Chain Reaction-Restriction Fragment Length Polymorphism

Introduction

Coronary artery disease (CAD) remains one of the leading cause of death in the world (Lozano et al.,2012). Coronary artery disease is a disease where the narrowing occur in the coronary arteries that supplying heart with blood; which is due to deposition of cholesterol, fats, and calcium on the walls of the arteries which lead to atherosclerosis ;that cause hardening in the arteries that supplies the heart with blood (Parmet et al.,2004). There are a lot of predisposing factors considered as risk factors of developing CAD like; old age, sex , hereditary factors , physical inactivity, smoking, high blood pressure, blood fat syndromes, obesity and diabetes (Kesteloot et al.,2006). Abnormalities in specific genes such as factor V, prothrombin, and Methyl tetra hydrofolate reductase causes elevation in the percentage of the risk for myocardial infarction and ischemic stroke, particularly in women and younger patients (Kim et al.,2003). Factor V is a one of coagulation factors which is labile factor called proaccelerin ;also it is a cofactor for the factor Xa which activates prothrombin to thrombin (Rahem and Al-Waeli , 2016).

The *factor V* gene is located on the chromosome 1 (1q23) ,the gene spans 70 kb, consists of 25 exons (Huang and Koerper, 2008). The product of *Factor V* gene is a protein with molecular weight 330-kD ; has an important function in blood coagulation. Naturally; factor V inhibited by a natural anticoagulant protein called activated protein C (APC), thus controlling the amount of thrombin produced (Mannet al.,1990 ,Mann and Kalafatis ,2003 and Camire and Bos ,2009). Factor V Leiden (FVL) is one of the most common causes of thrombophilia and resistance to the action of APC (Ornsteinet al., 2003). In thrombophilia, activated factor V leiden is resistant to the degradation action of APC, that lead to overproduction of thrombin causing thrombosis (Agostini-Vulaj,2016). Factor V Leiden is due to a missense mutation at position 1691, guanine substituted with adenine which replaced arginine (Arg) by glutamine (Gln) at position 506 in the FV protein (Agostini-Vulaj,2016) this substitution, increases the incidence of blood clot ; stay a longer and blockage in a blood vessel ;a condition called thrombophilia (Rahem and Al-Waeli , 2016).The Arginine molecule is a normal cleavage site for APC, so Glutamine substitution slows or prevents cleavage of the FV molecule, and prevents the action of APC on FV and that is called resistance to APC ,while this mutation is called Factor V Leiden ,therefore APC cannot stop FVL from forming extra fibrin. When the coagulation cascade is activated in people have factor V Leiden, it slowly stopped than in persons with regular factor V (Ornsteinet al., 2003). *FVL* mutation is the one of the most important risk factors CAD (Thorelli et al.,1999 , Castoldi et al.,2004 and Taymaz et al.,2007). Heterozygotes of *FVL* increases the risk of venous thromboembolism (VTE) about 4-8 fold, but in homozygous mutations the risk is reached to 80-fold (Agostini-Vulaj,2016) .For that, the aim of this study was to evaluate the association of *FVL* in the developing of CAD.

Methods

A retrospective study in which *FVL* genotyping were done on 180 Palestinian subjects who were selected randomly. Ninety subjects with CAD and 90 normal subject were included in this study (in total :78 females and 102 males). All participants were fill a questionnaire as we requested from all subjects and blood sample were collected in EDTA tube from them, 90 patients with CAD collected from Al Shifa and Nasser hospital complexes , and 90 healthy individuals as control group. Informed consent gave to the all volunteers for donating a blood sample for genetic investigation. The ethical agreement was agreed by the Palestinian Ministry of Health -Ethics Committee. Controls with hypertension, diabetes, and endocrine or metabolic disorders were excluded from the study. Cases with CAD and age ranging from 25 to 80 years were included. These cases were admitted into the Intensive Care Unit of Al Shifa and Nasser hospital complexes.

DNA extraction and amplification of FVL gene

DNA was extracted from peripheral blood leukocytes with DNA extraction kit (Bioline, UK) according to the manufacturer's protocol. The quality of isolated DNA detected by running 5 µl of each sample with 2 µl of loading dye on 1.0% agarose gels stained with ethidium bromide and the DNA was visualized using a U.V. transilluminator.

To detect *G1691A* polymorphism in exon 10 of factor V for all subjects ;PCR was done using the primers described by Koksal et al . A169 bp fragment was amplified from the extracted DNA using the following primers (hy.labs):

Forward: 5'ACATCGCCTCTGGGCTAATA-3′

Reverse, 5′-TTGAAGGAAATGCCCCATTA-3′

The reaction mixture in 0.2 ml microfuge tube consisted of :3µl (~150ng) of isolated DNA , 20 µl master mix (Bioline, UK), and 1.0 µl of each primer forward and reverse (5 pmol). PCR was performed in a thermal cycler (BOECO, Germany). The reaction conditions were: first step with denaturation for 1 min at 95°C, followed by 35 cycles of 15s at 95°C, 15s at 57°C, 10s at 72°C and an final extension for 10 min at 72°C. The PCR product was identified in 2% agarose gel stained by ethidium bromide under UV transilluminator (Bio-Rad,USA).

RFLP Analysis

Genotyping for *FVL G1691A* was accomplished by the digesting of the 169bp fragment with MnlI restriction enzyme (New England Biolabs). The mixtures for RFLP analysis in 0.2 ml microfuge tube consisted of : 10 µL PCR product, 34 µL of nuclease-free water, 5 µL of 10X Buffer and 1µL of MnlI (500 U/ml) added. The final volume were 50 µL , mixed and centrifuged for a few seconds; then incubated at 37°C for 15 mins.

Digestion of the 169 bp fragment with MnlI generated 17 bp, 37 bp and 115 bp fragments ; the17-bp was a result of MnlI cutting site. The homozygote factor V Leiden(*AA*) gave a 17 bp and 152 bp fragments after digestion. Digestion fragments were separated by electrophoresis on 4% agarose gels and were visualized by ethidium bromide.

Results

The case sample consisted of 90 subjects (52 males and 38 females), the mean age was 60.9±14.9 years and 90 of healthy subjects as control group with mean age 49.5±8.3. The general characteristics of the study populations are: (1) The percentage of previous smokers among patients was 31.11% and 68.89% had never smoked (the corresponding data for controls were 27.78% and 72.22%) ; (2) The frequency of patients with hypertension was 56.67% and no one of controls had hypertension ; (3) 33.33% of patients and no one of controls had diabetes; (4) The percentage of cases and control had family members with heart disease were 37.78% and 8.9% respectively.

Table 1 showed the demographic data. There is no any significant difference between the groups of this study in terms of gender and smoking. The percentage of family history of CAD was significantly higher in patients in comparison to the control group (*P*=0.000).

Table 1: Demographic characteristics of the study groups

Characteristic	Control N=90 (%)	Case N=103 (%)	OR(CI 95%)	P-value
Age(mean ±SD)	49.5±8.3	60.9±14. 9	7.8 - 14.8	**<0.001**
Gender				
Male	50(55.56)	52(57.78)	0.93(0.53-1.6)	0.88
female	40(44.44)	38(42.22)		
Smoking				
Yes	25(27.78)	28(31.11)	1.2(0.6-2.2)	0.74
No	65(72.22)	62(68.89)		
Family history of CHD				
Yes	8(8.9)	34(37.78)	6.2 (2.7-14.4)	**<0.001**
No	82(91.1)	56(62.22)		

Table 2 demonstrate the *FV* (*G1691A*) genotypes frequencies among the study population; both groups were in Hardy-Weinberg equilibrium. The frequencies of the heterozygote *FVL* genotype and *AA* genotype were not significantly different between the both groups : P= 0.08, and 0.16 , respectively .

Table2: *FVL* frequencies (Genotypes and Alleles) in CAD case and Control groups

FV Leiden	Control N=90 (%)	Case N=90 (%)	OR(CI 95%)	P-value
GG (wild type)	75(83.3)	63(70)	**1	0.11
GA	15(16. 7)	24(26.7)	1.9(0.92-3.9)	0.08
AA	0(0)	3(3.3)	8.3(0.42-164.2)	0.16
GA+ AA	15(16.67)	27(30)	2.14(1.05-4.38)	**0.034***
G	165(91.7)	150(83.33)	**1	**0.019***
A	15(8.3)	30(16.67)	2.2(1.14-4.25)	

**** Reference , *Significant level at P value <0.05**

However, the *GA* and *AA* genotype increases the risk of developing CAD in patients by 1.9 and 8.3 times respectively (OR=1.9,OR=8.3 respectively) . The presence of *FVL A* allele (*GA* and *AA* genotype) increases the risk of exposure to heart attack among CAD patients (OR =1.7, 95% CI =0.79-3.8).There was no any significant association between frequency of *A* allele and the incidence of hypertension (OR :1.04 ,95% CI=0.44-2.5) but the *A* allele frequency increases the risk of developing CAD among diabetic patients 1.5 fold (OR: 1.5,95% CI 0.62-4.07) (Table 3).

Table 3: *FVL* Genotypes in CAD Patients

FV Leiden	***GG (wild type)***	***GA+AA***	**OR(CI 95%)**	**P-value**
Gender				
Male	34	18	1.7(0.67-4.3)	0.26
Female	29	9		
Heart Attack				
No	**39	21	1.7(0.79-3.8)	0.14
Yes	24	6		
HTN				
No	**36	12	1.04(0.44-2.5)	0.93
Yes	40	15		
DM				
No	**44	16	1.5(0.62-4.07)	0.33
Yes	19	11		
Family history of CHD				
No	**43	14	2.3(0.92-5.8)	0.14
Yes	20	13		

** Reference

Discussion

This is the first study carried out for studying the association between *FV G1691A* Polymorphism and CAD in Gaza Strip. Coronary artery disease is one of the most complex and multifactorial diseases, results from several genetic alternations in combination with many environmental and lifestyle factors (Liu et al.,2008). Our results showed that the *A* allele frequency was 0.083 in healthy individuals and 0.1667 in CAD cases.

The observation of this study approved a relationship between *FVG1691A* mutation and the risk of CAD occurrence in an Palestinian population. In comparison to *GG* genotype; this study showed that the *AA+GA* and *GA* genotypes increase the risk of developing CAD with 2.14 fold; while the *AA* genotype showed 8.3-fold higher risk for CAD but with no statistically significance which may be because we find only three patients with the *AA* genotype (n = 3) and complete absence in control group. The prevalence among the study population of *FVL* mutation was 23.33% (homozygote and heterozygote) in total: 30% in patients with CAD and 16.67% in control group. The *FVL* mutation frequencies were different according to sample size, geographic area and ethnic differences (Gurlertop et al.,2007 and Dajani et al.,2013).

In Europe, the *FVL* mutation was detected in high prevalence (Bauduer and Lacombe ,2005) , but the prevalence of this mutation diverges between 2 and 15% in Caucasian populations (Juul et al.,2002) and with no existence in America, Asia ,Australia and Africa. Many researches showed no relation between the *FVL* and risk of CAD (Ridker et al.,1995, Gowda et al.,2000, Baykan et al.,2001, Caner et al.,2008, and Himabindu 2012). Other studies have approved that the frequency of *FVL* increased the risk of coronary atherosclerosis or myocardial infarction in different ethnic groups (Rosendaal et al.,1997, Makris et al.,2000,Ye et al.,2006 and Gurlertop et al.,2007) .A current study done on 180 participants in Gaza Strip revealed an

increased prevalence of *G1691A* variants(*GA+AA*) in CAD patients who had diabetes mellitus but this prevalence was not statistically significant.

The incidence of the *FVL* polymorphism was not related to increase risk of myocardial infarction or stroke in a study comprised of 14,916 healthy men; (Caner et al.,2008) .Likewise, a study done on 850 CAD patients failed to confirm any relationship between *FVL* heterozygote and ischemic disease or myocardial infarction (Juul et at.,2002). Other study found that was an elevation in *FVL* frequency among patients with CAD more than in control; this elevation increases the risk of CAD by 2.4 times (Marz et al.,1995). The *FVL* frequency in a study conducted in Turkey was reported to be 10% in case group but this polymorphism was absent in control group (Gurlertop et al.,2007) and the authors proposed that *FVL* polymorphism may be a major risk factor in progressing CAD in northeast Turkey. Our results are in agreement with these studies donating that *FVL* influence is associated with the risk of CAD and the presence of high prevalence of *FVL* mutation among our case group (OR:2.14 ,95% CI 1.05-4.38,P=.034). The differences in the results of previous studies maybe due to rare *AA* homozygous genotype frequencies , number of cases , deviation in *A* allele (G1691A mutation) frequency in different population and alteration in inclusion and exclusion criteria used ;so the associated risk for CAD is hardly to be determined. The small size of study groups and low *AA* genotypes frequency were consider as a limitations in our study.

Conclusion

Our findings in this case control study reveals the association of FVL mutation in genetic predisposition to CADs in Palestinian population .The present study approves the suggestion that *FVL* homozygous is one of CAD risk factors . Additional research is needed to detect other risk factors with mutated factor V that predispose patient to CAD.

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Conflicts of Interest

The author declare no conflicts of interest.

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