J Thromb Thrombolysis (2012) 34:383–387
DOI 10.1007/s11239-012-0731-9

High prevalence of three prothrombotic polymorphisms among Palestinians: factor V G1691A, factor II G20210A and methylenetetrahydrofolate reductase C677T

Ayman S. Hussein

Published online: 20 April 2012

Abstract Factor V leiden G1691A/R506Q (FVL), prothrombin G20210A (FII) and methylenetetrahydrofolate reductase (MTHFR) C677T are related genetic risk factors for venous thromboembolism. Analysis for those mutations is increasingly being performed on patients exhibiting hypercoagulability. The objective of this study was to determine the prevalence of FVL, FII-G20210A and MTHFR-C677T polymorphisms and their coexistence among apparently healthy Palestinians. After institutional approval, 303 apparently healthy students from An-Najah University representative to North and South regions of West Bank with no previous history of cardiovascular diseases participated in this study. A uniform questionnaire was used to collect relevant information through personal interview with the subjects. The collected information included gender, age, smoking habits, weight and height, diseases such as diabetes, cardiovascular and family history of CVD. The frequencies of allelic distribution of the three prothrombotic polymorphisms factor V G1691A/R506Q), prothrombin G2010A, and MTHFR-C677T were 0.114, 0.050 and 0.071, respectively. The prevalence of the three thrombotic polymorphisms (FVL, FII G20210A and MTHFR-C677T) were 20.1, 9.1 and 13.8 %, respectively. Statistical analysis for factor V leiden showed no significant association between place of residence (P value = 0.953) and gender (P value >0.082). The data presented in this study showed the highest prevalence of FVL among healthy Palestinians compared to other populations and this important finding should be followed in terms of clinical significance.

A. S. Hussein (✉)
Genetics Laboratory, Faculty of Medicine and Health Sciences, An-Najah National University, Nablus, Palestine
e-mail: ashussein@najah.edu

Keywords Thrombophilia · Factor V leiden mutation · Prothrombin · MTHFR · Allelic frequency · Palestine

Introduction

Venous thromboembolism is a major medical problem affecting 1–5 individuals per 1,000 annually [1]. Thrombophilia is a multi-factorial disorder caused by inherited and acquired factors including mutations in genes that code for natural anticoagulants such as anti-thrombin, protein C, and protein S, or clotting factors like prothrombin and factor V [2]. Acquired factors that may lead to this condition include surgery, long distance immobilization, pregnancy, antiphospholipid syndrome, obesity, and the use of oral contraceptive pills [3]. The three most common genetic thrombophilias known to predispose to thrombophilia are factor V G1691A/R506Q, prothrombin G20210, and C677T/A1298C in methylene tetrahydrofolate reductase [4–7]. Factor V leiden mutation represent the most common genetic risk factor associated with recurrent venous thromboembolism [7, 8] accounting for 95 % of cases with activated protein C resistance [9].

The factor V leiden genetic variant is common among Europeans, Israeli Arabs, Canadians and Indian populations, with prevalence that ranges from 1 to 8.5 % while most European studies reporting overall prevalence between 5 and 8 % [10]. The prevalence of the mutation seems to be highest among the populations of Greece, Sweden, and Lebanon where it reaches about 15 % in some regions [4, 11]. On the contrary, the mutation was basically not found or rarely detected among African blacks, east Asian populations including Chinese and Japanese with prevalence ≤1 % [11, 12]. FII G20210A and MTHFR C667T mutations in addition to factor V leiden are

currently the most common known genetic risk factors for venous thrombosis among Caucasians [12].

The West Bank, Palestinian Territories, is undergoing an epidemiological transition characterized by rapid urbanization and changing lifestyles. For Palestinian living in West Bank, circulatory disease was found to be the leading cause of death [13]. In our recent study, we found also a strong association between factor V leiden mutation and recurrent abortion among Palestinian women [14]. But we have little information about the prevalence of the three prothrombotic polymorphisms among Palestinians and thus the aim of this study is to determine the frequency of the important causes of thromboembolism for the first time in West Bank, Palestine.

Materials and methods

Study subjects

A cross-sectional study was undertaken to investigate the prevalence of three prothrombotic polymorphisms among Palestinian (factor V G1691A, factor II G20210A and methylenetetrahydrofolate reductase (C677T). Study subjects were recruited from An-Najah National University students that represent all governorates of West Bank (Fig. 1). West Bank can be divided into North and South with Nablus and Jerusalem as the main cities of those regions, respectively (see Fig. 1). After institutional review board approval, three hundred and three healthy students were selected randomly. A uniform questionnaire was used to collect relevant information through personal interview with the subjects. The collected information included gender, age, smoking habits, weight and height, diseases such as diabetes, cardiovascular and family history of CVD. Table 1 provides a summary of the basic collected information of all study subjects.

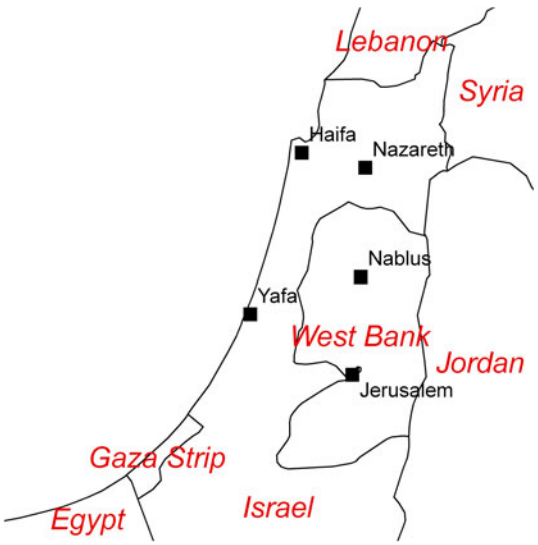

Fig. 1 Map of West Bank showing two cities Nablus in the north and Jerusalem in the south

DNA extraction and haplotype analysis

Total genomic DNA was isolated from whole blood samples using leukocyte-rich buffy coat interphase using the commercially available master pure DNA purification kit for blood (Epicenter Biotechnologies, Wisconsin, USA). Purified DNA was stored in nuclease free water at −20 °C until further use. To detect the leiden point mutation, amplification refractory mutation system (ARMS) was used as described by Bathelier et al. [15]. According to this protocol, PCR primers were designed based on Exon 10 sequence of the human factor V gene. The primers used in the PCR amplification reactions include a common primer 5′-ACATC TTAG A GTTTGATGA-3′, a normal allele-specific primer 5′-GGACAAAATACCTGTATTCCGC-3′ and a mutation-specific primer 5′-GGACAAAATACCT GTATTCCCT-3′. A 220 base pair fragment form Exon 10 surrounding nucleotide 1691 was amplified in a 30 µl PCR reaction mixture containing 1× PCR buffer (TaKaRa), 0.2 mM dNTP mixture (TaKaRa), 150 ng each primer (invitrogen), 1U *Taq*™ DNA polymerase (TaKaRa 5 U/µl), and 200 ng genomic DNA (0.2 µg/µl). Amplification was done for 30 cycles of 94 °C for 30 s, 57 °C for 1 min, 72 °C for 1 min followed by extension at 72 °C for 5 min. The PCR product (220 nucleotides) was resolved on 1.4 % agarose gel. Accordingly, all subjects were categorized as homozygous normal (GG), heterozygous (GA) or homozygous with the mutant allele (AA).

For the detection of prothrombin G20210A, a 345 bp fragment from the exon 14 of the 5′-untranslated region of

Table 1 General characteristics of the participants

Characteristics	Number (%) (total, $n = 303$)
Age (years)	20.2 ± 1.7
Male gender	173 (57.1)
Current smokers	130 (42.9)
BMI > 30	15 (4.9)
Diabetes type 1 or 2	0 (0)
Cardiovascular diseases[a]	0 (0)
Family history of cardiovascular diseases[b]	180 (59.4)

[a] Cardiovascular diseases: We asked about thromboembolism, hypertension, myocardia infarction

[b] Relatives including father, mother, grandfather, grandmother, sisters, brothers, uncles and ants who had been diagnosed with cardiovascular problems such as hypertension, myocardial infarction and thromboembolism

the prothrombin gene was amplified using the primers 5′-TCT AGA AAC AGT TGC CTG GC-3′ and 5′-ATA GCA CTG GGA GCA TTG AAG C-3′ as described by Poort et al. [16]. A total of 10–15 µl amplified PCR product was digested with ten units *Hin*dIII restriction enzyme and electrophoresed on 2 % agarose gel. The presence of a mutated allele produces a fragment of 322 bp. PCR amplification of a 198 bp DNA fragment followed by *Hin*f I digestion as described by Frosst et al. [17] for the region containing the MTHFR mutation was follows: 40 pmol of both sense primer 5′-TGA AGG AGA AGG TGT CTG CGG GA-3′ and antisense primer 5′-AGG ACG GTG CGG TGA GAG AGT G-3′ were used in 25 µl containing 200 µmol/l dNTP's, 1.5 mmol/l MgCl2, 50 mmol/l Tris–HCl (pH 9), 50 mmol/l KCl, 2 U Tag polymerase (promega), 200 ng of genomic DNA, and 1 % Triton X-100. The PCR cycling conditions were 94 °C for 1 min, 63 °C for 1 min, and 72 °C for 1 min for 40 cycles. PCR products were electrophoresed on 2 % agarose gel, stained with ethidium bromide, and visualized under UV light. Each PCR test for the three prothrombotic polymorphisms has been repeated and cohen kappa has been calculated using SPPS statistical program.

Statistical analysis

Collected data was analyzed using the statistical package for social sciences program (SPSS) version 16. Descriptive results were expressed as frequencies and percentages. *P* value <0.05 was accepted as statistically significant. Chi square was used to compare between the different independent groups. Cohen kappa was used to test reliability of PCR methodology.

Results

Table 1 shows the general characteristics of the study participants. The mean age of our sample is 20.2 years. About 95 % of participants are not obese and none of them has diseases like diabetes or cardiovascular diseases (CVD). Surprisingly, about 60 % of them have family history with CVD. The frequencies of allelic distribution of the three prothrombotic polymorphisms factor V G1691A/R506Q, prothrombin G20210, and MTHFR-C677T are presented in Table 2; 20.1 % of the study participants have factor V leiden mutation. The prevalence of FII-G20210 and MTHFR-C677T among Palestinians living in West Bank is 9.1 and 13.8 %, respectively (Table 2). The prevalence of homozygous mutations for the three polymorphisms FVL, FII-G1691A and MTHFR-C677T is 2.6, 0.7 and 0.3 %, respectively. Cohen kabba for the three polymorphisms FV leiden, FII and MTHFR was found to be 0.92, 0.98 and 0.93, respectively. Statistical analysis for factor V leiden showed no significant association between place of residence (*P* value = 0.943) (Table 3) and the distribution of factor V leiden mutation. Although no significant association between gender and the distribution of factor V alleles (*P* value >0.082) (Table 3), males have significant difference in homozygous alleles than females among the study participants (*P* value = 0.035) (data not shown). The association of the mutation in FII or MTHFR and either gender or place of residence was not significant (*P* value >0.05) (data not shown).

Discussion

Death attributed to cardiovascular diseases among Palestinian living in West Bank become alarming. A survey in

Table 2 Genotype and allele frequencies of FV, FII and MTHFR polymorphisms among Palestinians living in West Bank

Polymorphism	Genotype	Number	%	Frequency of A allele	Frequency of T allele
Factor V	GG	242	79.9	0.114	–
	GA	53	17.5		
	AA	8	2.6		
	Total	303			
Prothrombin	GG	275	90.7	0.050	–
	GA	26	8.6		
	AA	2	0.7		
	Total	303			
MTHFR	CC	261	87.1	–	0.071
	CT	41	13.5		
	TT	1	0.3		
	Total	303			

Table 3 Association of gender and residence with FV leiden polymorphism among Palestinians living in West Bank

FV Genotype					*P* value[a]
Variable	GG (%)	GA (%)	AA (%)	Total number (%)	
Gender					
Male	140 (79.1)	35 (19.8)	6 (2.6)	177 (58.4)	0.082
Female	102 (81.0)	18 (14.3)	2 (1.1)	126 (41.6)	
Residence					
North	88 (80.7)	18 (16.5)	5 (2.6)	109 (36)	0.943
South	154 (79.4)	35 (18.0)	3 (2.8)	194 (64)	

[a] Pearson's Chi-square test

2003 showed that circulatory disease is the leading cause of death among Palestinians [13]. We have noticed that incidence of myocardial infarction (MI) is also increasing among younger Palestinians (Hussein et al., unpublished data). Few data are available with regard to the prevalence and incidence of cardiovascular diseases; for example Husseini et al. [13] showed that the acute MI among Palestinians reside in West Bank is 78.5 per 100,000. A previous report from Kark et al. [18] showed that a high rate of coronary events (344 per 100,000) among Palestinians living in Jerusalem. Thrombotic polymorphisms analysis has become an integral part of diagnostic evaluation of patients with signs and symptoms of venous thrombosis [19]. The prevalence of thrombotic polymorphisms, which is one of the frequently observed and important risk factors for genetic thrombophilia, varies in different populations and the difference in distribution can be explained by ethnicity and geographical differences [9, 20]. Although the incidence of FVL is reported as almost absent in non-European whites, native populations of Africa, America, Asia and Australia [1, 6, 13, 25], and around 3–5 % (occasionally up to 15 % in certain areas) in European countries, there is a high prevalence of the FVL in the Middle East [21–23, 25]. We found that the prevalence of FVL in healthy individuals in our region was 20.1 %, which is higher than the threshold value reported elsewhere (for example 15–17.7 % among healthy Jordanian individuals [24, 25]. To exclude the possibility of errors from PCR technique, reliability of PCR test was performed and results showed that the test is reproducible (Kabba = 0.92). In this regard, our results showed that family history of our participants with regard to CVD is found to be surprisingly high (see Table 1). FVL prevalence was found also high from other studies on Palestinians and Egyptians with MI [26, 27]. Our results showed also no significant association with gender or place of residence and thus may indicate common ancestor of all Palestinians living in West Bank.

FII prothrombin G2010A mutation, a risk factor for thrombophilia, was found in 9.3 % of our healthy students. This result is slightly higher than those values (6–8 %) reported for other countries (6–8 %) [28]. The prevalence of MTHFR-C677T was 13.8 % which is lower than values reported for the same mutation among healthy Jordanians (24 %) [24] and Lebanese (39.7 %) [29].

This study is the first report about the frequency of the three thrombotic polymorphisms among Palestinians living in West Bank. Compared to other populations reported so far, the Palestinians harbor the highest prevalence of FVL polymorphism. This is an important finding to be followed in terms of clinical significance. In light of this data, we may advise families of affected subjects to be genotyped and counseled for proper prophylaxis.

Acknowledgment We would like to thank Ms Alaa Darwish for her help in this study.

Conflict of interest There is no conflict of interest of this work with anyone.

References

1. Dahlbäck B (2008) Advances in understanding pathogenic mechanisms of thrombophilic disorders. Blood 112(1):19–27
2. Walker JD (2000) Thrombophilia in pregnancy. J Clin Pathol 53(8):570–573
3. Bertina RM, Koeleman BP, Koster T et al (1994) Mutation in blood coagulation factor V associated with resistance to activated protein C. Nature 369:64–67
4. Finan R, Tamim H, Ameen G et al (2002) Prevalence of factor VG1691A (factor V-leiden) and prothrombin gene mutations in a recurrent miscarriage population. Am J Hematol 71:300–305
5. Martinelli I, Taioli E, Cetin I, Marinoni A, Gerosa S, Villa MV, Bozzo M, Mannucci PM (2000) Mutations in coagulation factors in women with unexplained late fetal loss. N Engl J Med 343(14):1015–1018
6. Isotalo PA, Wells GA, Donnelly JG (2000) Neonatal and fetal methylenetetrahydrofolate reductase genetic polymorphisms: an examination of C677T and A1298C mutations. Am J Hum Genet 67(4):986–990
7. Lensen R, Rosendaal F, Vandenbroucke J, Bertina R (2000) Factor V leiden: the venous thrombotic risk in thrombophilic families. Br J Haematol 110(4):939–945
8. Kist WJ, Janssen NG, Kalk JJ, Hague WM, Dekker GA, de Vries JL (2008) Thrombophilias and adverse pregnancy outcome—a confounded problem. Thromb Haemostat 99(1):77–85

9. Dahlbäck B (1997) Resistance to activated protein C as risk factor for thrombosis: molecular mechanisms, laboratory investigation, and clinical management. Semin Hematol 34(3): 217–234
10. Khan S, Dickerman JD (2006) Hereditary thrombophilia Thromb J 4:15
11. Rees DC, Cox M, Clegg JB (1995) World distribution of factor V leiden. Lancet 346(8983):1133–1134
12. Koksal V, Baris I, Etlik O (2007) Primer-engineered multiplex PCR-RFLP for detection of MTHFR C677T, prothrombin G20210A and factor V Leiden mutations. Exp Mol Pathol 83(1):1–3
13. Husseini A, Abu-Rmeileh NM, Mikki N, Ramahi TM, Ghosh HA, Barghuthi N, Khalili M, Bjertness E, Holmboe-Ottesen G, Jervell J (2009) Cardiovascular diseases, diabetes mellitus, and cancer in the occupied Palestinian territory. Lancet 373(9668): 1041–1049
14. Hussein AS, Darwish H, Shelbayeh K (2010) Association between factor V leiden mutation and poor pregnancy outcomes among Palestinian women. Thromb Res 126(2):e78–82
15. Bathelier C, Champenois T, Lucotte G (1998) ARMS test for diagnosis of factor V leiden mutation and allele frequencies in France. Mol Cell Probes 12:121–123
16. Poort SR, Rosendaal FR, Reitsma PH, Bertina RM (1996) A common genetic variation in the 3′-untranslated region of the prothrombin gene is associated with elevated plasma prothrombin levels and an increase in venous thrombosis. Blood 88(10): 3698–3703
17. Frosst P, Blom HJ, Milos R, Goyette P, Sheppard CA, Matthews RG, Boers GJ, den Heijer M, Kluijtmans LA, van den Heuvel LP et al (1995) A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. Nat Genet 10(1):111–113
18. Kark JD, Fink R, Adler B, Goldberger N, Goldman S (2006) The incidence of coronary heart disease among Palestinians and Israelis in Jerusalem. Int J Epidemiol 35(2):448–457
19. Cleary-Goldman J, Nakhuda GS, Zimmermann RC, Sauer MV (2003) The role of factor V leiden mutation in recurrent pregnancy loss. J Am Med Womens Assoc 58(3):165–172
20. Ozmen F, Ozmen MM, Ozalp N, Akar N (2009) The prevalence of factor V (G1691A), MTHFR (C677T) and PT (G20210A) gene mutations in arterial thrombosis. Ulus Travma Acil Cerrahi Derg 15(2):113–119
21. Dashti AA, Jadaon MM, Abdulsamad AM, Dashti MH, Lewis HL (2011) Thrombosis risk in carriers of the factor V leiden mutation: is it associated with a defined skin color? Pathol Res Pract 207(11):671–673
22. Almawi WY, Keleshian SH, Borgi L, Fawaz NA, Abboud N, Mtiraoui N, Mahjoub T (2005) Varied prevalence of factor V G1691A (Leiden) and prothrombin G20210A single nucleotide polymorphisms among Arabs. J Thromb Thrombolysis 20(3): 163–168
23. Dashti AA, Jadaon MM (2011) Race differences in the prevalence of the factor V leiden mutation in Kuwaiti nationals. Mol Biol Rep 38(6):3623–3628
24. Eid SS, Rihani G (2004) Prevalence of factor V leiden, prothrombin G20210A, and MTHFR C677T mutations in 200 healthy Jordanians. Clin Lab Sci 17(4):200–202
25. Nusier MK, Radaideh AM, Ababneh NA, Qaqish BM, Alzoubi R, Khader Y, Mersa JY, Irshaid NM, El-Khateeb M (2007) Prevalence of factor V G1691A (Leiden) and prothrombin G20210A polymorphisms among apparently healthy Jordanians. Neuro Endocrinol Lett 28(5):699–703
26. Rosen E, Renbaum P, Heyd J, Levy-Lahad E (1999) High frequency of factor V leiden in a population of Israeli Arabs. Thromb Haemostat 82(6):1768
27. Settin A, Dowaidar M, El-Baz R, Abd-Al-Samad A, El-Sayed I, Nasr M (2008) Frequency of factor V leiden mutation in Egyptian cases with myocardial infarction. Hematology 13(3):170–174
28. Akar N, Misirlioğlu M, Akar E, Avcu F, Yalçin A, Sözüöz A (1998) Prothrombin gene 20210 G-A mutation in the Turkish population. Am J Hematol 58(3):249
29. Almawi WY, Finan RR, Tamim H, Daccache JL, Irani-Hakime N (2004) Differences in the frequency of the C677T mutation in the methylenetetrahydrofolate reductase (MTHFR) gene among the Lebanese population. Am J Hematol 76(1):85–87