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Association between factor V Leiden mutation and poor pregnancy outcomes among Palestinian women

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ABSTRACT

Pregnancy is a hypercoagulable state with increased tendency for thrombus formation, a condition that is increased when combined with acquired or inherited risk factors that lead to thrombophilia. Among the inherited risk factors is Factor V Leiden mutation, an autosomal dominant trait with reduced penetrance. The mutation seems to be associated with different poor pregnancy outcomes including recurrent miscarriages. In the present study, we performed a case-control study to investigate the association between the Leiden mutation and poor pregnancy outcome among the Palestinian population in the West bank region of Palestine. The study included 145 subjects with recurrent miscarriages and 205 matched control subjects with successful pregnancies who experienced normal delivery and no apparent complications. Leiden mutation was detected in 41 of the145 study subjects (28.2%), and in 24 of the 205 control subjects (11.7%). Subjects homozygous with the mutant allele were identified only among the test and not the control group. Data analysis indicates a significant association between the mutant allele and recurrent miscarriages (p-value<0.05). Furthermore, this association is significant between the mutant haplotype with miscarriages compared to control group showing time effect where there is no association for miscarriages before week 10. Results here also show strong association of factor V leiden polymorphism among primary aborters compared to secondary aborters or control groups. The odds ratio for the primary aborters was 75 and $p<0.0001$. In conclusion, these results provide evidence for a significant correlation between recurrent miscarriages and Factor V mutation in our population.

1. Introduction

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 [28]. Acquired factors that may lead to this condition include surgery, long distance immobilization, pregnancy, antiphospholipid syndrome, obesity, and the use of oral contraceptive pills [4]. A number of inherited risk factors mutations have been suggested to be associated with thrombophilia including factor V G1691A/R506Q, prothrombin G20210, and C677T/A1298C in methylene tetrahydrofolate reductase [12]; Martinelli *et al.*, [1,17]). Factor V leiden mutation represent the most common genetic risk factor associated with recurrent venous thromoembolism [18,19]) 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 percent while most European studies reporting overall prevalence between 5 and 8 percent (Khan and Dickerman .2006). The prevalence of the mutation seems to be highest among the populations of Greece, Sweden, and Lebanon where it reaches about 15 percent in some regions ([12] and Rees *et al.*, 1995). 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% (Ridker et al., 1997, [25], Cleary-Goldman *et al.* 2003).

Since the turn of the last century, there has been extensive research focusing on both the genetic and acquired causes of thrombophilia, with particular focus on clotting events in venous circulation. In recent years, a number of studies concerning the relationship between recurrent pregnancy loss and thrombophilia have been carried out among several populations around the world with conflicting conclusions about the role of Factor V Leiden mutation in the process [1,25]. They suggested a role of factor V Leiden mutation in cases with unexplained "recurrent pregnancy loss" among some white American women . Similarly, the association between recurrent pregnancy loss and Factor V leiden mutation was established among women with pregnancy loss from Brazil [27], Israel [6], Sweden [29], Austria [15], USA [13], UK [10], Lebanon (Finan et al .2002) and Tunisia [20,31]. On the contrary, other studies failed to demonstrate such association

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between Factor V leiden mutation and recurrent miscarriage ([11,21], Alfirevie et al. 2001, [2,26,32], and Biswas et al. 2008). This may be due to the fact that the incidence of the mutation in different populations is affected by racial and ethnic differences in the gene frequency [7].

In the present study, we investigated the association between Factor V Leiden mutation and adverse pregnancy outcomes among Palestinian women from the West Bank region of Palestine suffering from recurrent miscarriages with unknown etiology compared to control women with uncomplicated pregnancies and deliveries. The results indicate a significant association between this mutation and poor pregnancy outcome in the Palestinian population.

2. Methodology

2.1. Study Subjects

A case control study was undertaken to investigate the association between Factor V leiden mutation and recurrent abortion among Palestinian women residing in the West Bank. Study subjects were recruited from four major health centers in the West Bank which operate a comprehensive medical and health program for pregnant women. The case group consisted of 145 women (mean age 31.9 years) who experienced one or more adverse pregnancy outcomes. They included 119 women with three or more consecutive early miscarriages at ≤12 gestation weeks and 26 women with two or more late miscarriages at >12 gestation weeks. Eleven cases had primary recurrent pregnancy loss (RPL) (they had three or more recurrent miscarriages and never delivered a viable fetus), and 134 cases were with secondary RPL (they had at least one successful pregnancy). Subjects were selected in consultation with the medical teams in the indicated centers to include women who are healthy and did not suffer from any medical or nutritional complication that could explain their adverse pregnancy outcome including women who were tested for the indicated mutation. The control group included 205 women who attended the same medical facilities (mean age 32 years) with at least more than 2 normal pregnancies and without any history of adverse pregnancy outcome or recurrent miscarriages. A uniform questionnaire was used to collect relevant information through personal interview with subjects and from official medical records in consultation with the medical staff. The collected information included age, parity, personal and family medical and obstetric history, smoking, residency, consanguinity marriage, and educational level of all participants in the study. Table 1 provides a summary of the collected information of all study subjects.

Table 1
Characteristics of study participants.

Character	Test group		Control group	
	N=145	**(%)**	N=205	(%)
Age (y)	32	-	31.9	-
Marriage age (y)	19	-	18.8	-
Residential distribution				
Camp	93	64.1	141	69.0
City	31	21.4	40	19.4
Village	21	14.5	24	11.6
Smokers				
	19	13.1	19	10.0
Education level				
Low [&]	120	82.8	166	81.3
High[$]	25	17.2	39	18.7
BMI[*]				
Normal	40	27.6	65	31.7
Overweight	60	41.4	66	32.2
Obese	45	31.0	74	36.1

[&] low = Elementary school or less.
[$] High = High school and above.
*BMI = Body Mass Index.

2.2. 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.* [3]. 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'-GGACAAAATACCTGTATTCCCT-3'. A 220 base pair fragment form Exon 10 surrounding nucleotide 1691 was amplified in a 30 µl PCR reaction mixture containing 1X PCR buffer (TaKaRa), 0.2 mM dNTP mixture (TaKaRa), 150 ng each primer (invitrogen), 1U T*aq*™ DNA polymerase (TaKaRa 5U/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).

2.3. 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. Odds Ratio was used for detecting the power of relationship between the determinant and the outcome and 95% confidence interval was calculated. Chi square was used to compare between the different independent groups.

3. Results

Table 1 shows that both the test and control groups possessed basically comparable age, area residential distribution, educational level, smoking habits and body mass index. Live births were significantly lower among the test subjects since about 50% of all pregnancies in this group ended with miscarriages compared to 100% successful pregnancies among the control group. The total number of reported miscarriages in the test group was 499 in the early and late abortion periods compared to 1559 successful pregnancies. The pregnancy losses among the test subjects who were initially referred to us from the indicated medical centers revealed that 85% of cases were recurrent miscarriages while 15% were linked to pregnancy induced hypertension. The latest group was not included in our analysis. In addition, all recurrent miscarriages cases that proved linked to intrauterine fetal death were also excluded. Furthermore, Fig. 1 demonstrates that almost all women with no adverse pregnancy outcomes (controls) had normal vaginal delivery while nearly half the deliveries among the group with adverse pregnancy outcomes ended by abortion or cesarean section. In order to subdivide the miscarriages cases to early and late miscarriages, the term of pregnancy loss (gestation weeks) was drawn with the number of miscarriages cases. Fig. 2 shows that the most adequate threshold for categorizing miscarriages among the cases is at ≤12 gestation weeks.

Table 2 summarizes the frequency of Leiden mutation distribution between the control and test groups. About 28% of the test subjects possess the mutant allele compared to about 12% of the control group. Moreover, the homozygous mutant allele (AA) was only detected among the case group while the control group possesses only heterozygous haplotype (GA) allele at this location (Table 2). The frequency of the Leiden mutant allele is significantly higher among the

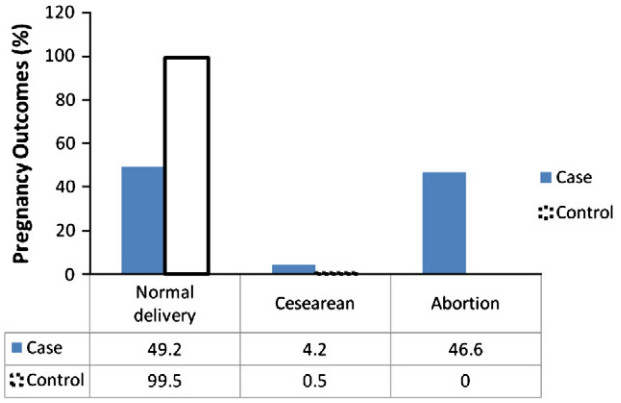

Fig. 1. Distribution of adverse pregnancy outcomes among cases and controls.

test group either in women with early or late miscarriages in comparison to the control group as shown in Table 3. In order to investigate the association of factor V Leiden mutation and the type of abortion, the test subjects were subdivided into two categories as shown in Table 4. The mutant allele was detected in ~90% (10 out of 11 patients) of women with primary RPL (≤12 gestational weeks) which is significantly higher than the mutant allele frequency among the control group ($p<0.0001$; OR = 75 (95% CI = 9.2-616). Furthermore, women with secondary RPL (>12 gestation weeks) had ~23% (31 out of 134 cases) of the mutant allele which is also significantly higher than the mutant allele frequency among control group ($p = <0.006$; OR = 2.25, CI = 1.26-4.08) (Table 4). The timing of the mutant allele frequency was further investigated by dividing test group into three categories (Table 5). Results shows that early miscarriages before 10 weeks are not found to be associated with factor V leiden variant. The association only begins for miscarriages during weeks 10-12, and increase for miscarriages after 12 weeks (Table 5). Table 6 shows the mutant allele frequency among subjects who had successful pregnancies, even if some successful pregnancies happened in patients with RPL (339 out of 350 women) and those women who had three or more recurrent miscarriages and had never delivered a viable fetus (11 out of 350 women). The data shows subjects with miscarriages (childless women) had a significantly higher frequency of factor V leiden than did the women with successful pregnancies ($p<0.001$; OR = 9.24, 95%CI = 1.42-59.92).

4. Discussion

Abortion is a multi-factorial complication that leads to premature loss of fetuses and affects about 20% of all pregnancies in developed and underdeveloped countries with fewer than 50% of cases have definitive and clear causes (Rees *et al* .1996). Thrombophilia, defined as a condition of increased tendency of clot formation, is considered one of the risk factors which contribute to poor pregnancy outcomes including recurrent abortion [8]. More precisely, it may constitute a significant factor that play a major role in 40-60% of unexplained multiple miscarriages [5].

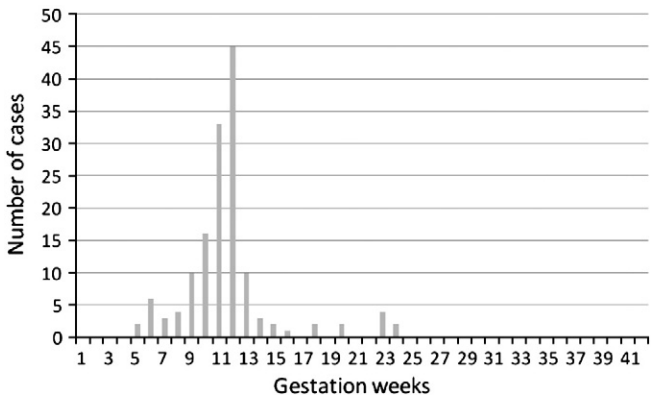

Fig. 2. The frequency of miscarriages among cases according to timing of pregnancy loss.

Table 2
Distribution of Factor V leiden haplotypes among the test and control groups.

Group[a]	G1691A haplotypes					
	G/G		G/A		A/A	
	N	%	N	%	N	%
Control	181	88.3	24	11.7	-	-
Test	104	71.7	36	24.8	5	3.4

[a] A total of 145 cases and 205 controls were analyzed.

In the Palestinian community, the incidence of abortion is relatively high, estimated at 4 -8% among women attending antenatal care clinics. Therefore, in order to reveal the possible risk factors that contribute to this serious complication among young women in our society, this investigation was initiated to study the possible association between Factor V Leiden mutation and recurrent abortion. Ultimately, this will provide an opportunity to minimize the chance of fetal loss and provide bases for an appropriate medical intervention for women who prove to be at high risk of abortion due to this genetic risk factor. Study subjects comprised of test and control groups of women who regularly attend antenatal care clinics in various locations in the country. Apparently, the test and control groups were closely matched for a number of parameters including age, smoking habits, and body mass index. The results showed that test subjects with recurrent miscarriages had a significant higher incidence of factor V leiden mutation compared to controls. The percentage of abortions among the case group was relatively high, reaching about 43% of all deliveries in this group, while about 95% of first pregnancy outcome among the control group ended by normal vaginal deliveries compared to only about 55% of deliveries in the case group. These results further reflect the high incidence of abortion among the Palestinian population (reaching about 20% of all pregnancies) in comparison to other developed and underdeveloped countries (Rees et al. 1996).

The present study demonstrates that Factor V Leiden mutant allele is correlated with recurrent abortion in our population. The mutant haplotype was detected with significantly higher frequency among subjects who experienced fetal loss (~28%) in comparison to the control group (~12%), with the homozygous mutant haplotype only detected among the test group. Moreover, our results provide evidence that the mutant haplotype allele is significantly correlated with pregnancy poor outcome during early and late miscarriages. Although, the Leiden mutation was found to be significantly associated with subjects with either early or late miscarriages, the data show that there is a time effect on this association. It starts eventually during weeks 10-12 and increased after 12 weeks. This association is found to be stronger among primary aberters group compared to secondary aborters indicating that early foetal losses (before week 10) are not due to factor V Leiden mutation. These results are in agreement with earlier reports which showed that the prevalence of factor V Leiden mutation among cases was higher with second trimester than with first trimester abortions ([23]; Souza *et al*. 1997; [6,10,12–14,15,20,30,31],). On the contrary, other studies failed to show significant association between the actor V leiden mutation and pregnancy adverse outcomes [2,11,16,22,29]. This could be due to limited number of subjects, bias in subject selection or heterogeneity of ethnicity among patients [11,16,22,29];). A recent study conducted by Rashmi and colleagues [24] on fetal gene defects and pregnancy failure among mothers with factor V Leiden mutation established a cause–effect relationship in the observed epidemiologic association between maternal factor V leiden mutation and fetal loss [24]. These

Table 3
Comparison of factor V leiden mutation prevalence among women with various pregnancy outcomes compared to controls.

Group[a]	N	%	Genotype				Comparison with control		
			Normal		Mutant		*P = Value*	*OR*	*95% CI*
			N	%	N	%			
Control	205	100	181	88.3	24	11.7	-	-	-
Early[#] miscarriages	119	82.1	90	75.6	29	24.36	0.005[*]	2.377	1.310-4.315)
Late miscarriages[&]	26	17.9	14	53.8	12	46.2	<0.001[*]	6.464	2.670-15.597)

[a]A total number of 145 cases and 205 control subjects were analyzed.
[*]Pearson's chi-square test.
[#] Women with three or more consecutive early miscarriages at ≤ 12 gestation weeks.
[&] Women with two or more late miscarriages at > 12 gestation weeks.

Table 4
Comparison of factor V leiden mutation prevalence among women with primary and secondary aborters compared to controls.

Group[a]	N	%	Genotype				Comparison with control		
			Normal		Mutant		*P = Value*	*OR*	*95% CI*
			No	%	No	%			
Control	205	100	181	88.3	24	11.7	-	-	-
Primary aborters[$]	11	7.1	1	9.1	10	89.9	<0.0001[*]	75	9.20-616.00
Secondary aborters[#]	134	92.9	103	76.8	31	23.2	<0.0060[*]	2.25	1.26-4.08

[a]A total number of 145 cases and 205 control subjects were analyzed.
[*]Pearson's chi-square test.
[$]Primary aborter: Women with no successful pregnancy (Childless).
[#]Secondary Aborters: Women with at least one successful pregnancy.

authors identified fetal gene defects as risk modifiers of pregnancy failure in prothrombotic mothers and showed that pregnancy failure is mediated by Par 4-dependent activation of maternal platelets at the feto-maternal interface that likely involves a pathogenic pathway independent of occlusive thrombosis. They also demonstrated that the interaction between two given thrombosis risk factors produces markedly disparate consequences on disease manifestation (i.e., thrombosis or pregnancy loss), depending on the vascular bed in which this interaction occurs [24].

In conclusion, the present study provides evidence for the significant association between factor V leiden mutation and poor pregnancy outcome among the Palestinian population. The association of the mutation has an effect on miscarriages that occur after week 10 and becomes stronger after 12 week of gestation. Some early foetal losses seen in this study seem to have no association with factor V variant. Evidently, the incidence of the leiden mutation does not represent the only factor contributing to this adverse situation as expected. Other additional factors definitely play a major role in the

Table 5
Comparison of factor V leiden mutation prevalence among women with various pregnancy outcomes compared to controls.

Group[a]	N	%	Genotype				Comparison with control		
			Normal		Mutant		*P = Value*	*OR*	*95% CI*
			No	%	No	%			
Control	205	100	181	88.3	24	11.7	-	-	-
<10 wks[$]	26	17.9	23	88.4	3	11.6	0.98[*]	0.98	0.28-3.52
10-12 wks[$]	93	64.1	72	10.7	26	28	0.0007[*]	2.94	1.57-5.45
>12wks[#]	26	17.9	14	53.8	12	46.2	<0.0001[*]	6.46	2.68-15.60

[a]A total number of 145 cases and 205 control subjects were analyzed.
[*]Pearson's chi-square test.
[$] Women with no successful pregnancy (Childless).
[#] Women with at least one successful pregnancy.

Table 6
Comparison of factor V leiden mutation prevalence among women with successful pregnancies compared to cases with at least three miscarriages and no viable fetus.

Group[a]	N	%	Genotype				Comparison with control		
			Normal		Mutant		*P = Value*	*OR*	*95% CI*
			No	%	No	%			
Successful pregnancy[&]	339	100	285	84.1	54	15.9	-	-	-
Abortion[#]	11	100	1	9.1	10	89.9	<0.001[*]	9.24	1.42-59.92

[a]A total number of 11 cases with miscarriages (childes women) and 339 subjects with at least one successful pregnancy were analyzed.
[*]Pearson's chi-square test.
[&]Women with at least one successful pregnancy (included 205 controls plus 134 cases).
[#]Women with no successful pregnancy (Childless).

process and are involved in the overall pathogenesis in recurrent abortion. These factors primarily involve other players involved in the blood coagulation event including thrombin, prothrombin, thrombin receptor, Methylene tetrahydrofolate reductase, and enzymes of the Vitamin K metabolism. The potential involvement of these and other factors in this adverse pathological condition is currently under investigation by our group.

Conflict of interest statement

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

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