Research Article

Association of Factor V Leiden G1691a in Women Suffering Repeated Abortions

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Abstract
Purpose: To assess the relationship of factor V leiden G1691A mutation as a risk factor for repeated pregnancy loss. The focus has been on factor V leiden G1691A mutation that may predispose women to microthrombosis during the stages of embryo implantation and placentation. **Methods:** A total of 70 women with recurrent pregnancy loss, mean age 31.1±4.2 years, were involved in the study. As a control group, 70 women [mean age 32.2±3.3 years with at least two live-born child and no history of abortion were included. We used real-time polymerase chain reaction (PCR) to determine the frequencies of factor V leiden G1691A genotype. **Results:** The frequency of heterozygotes for factor V leiden G1691A was significantly higher in women with repeated pregnancy loss compared to women without abortion (p = 0.0001). **Conclusion:** In summary we found an association of factor V leiden G1691A mutation with recurrent pregnancy loss .We recommend for factor V leiden G1691A screening in cases with repeated pregnancy loss so they can start anticoagulant therapy more earlier.
Keywords: G1691A, factor V leiden, pregnancy, abortion

Introduction
Recurrent miscarriage (RM) -defined by ESHRE guideline as ≥2 consecutive pregnancy losses before 20 weeks post menstruation affects approximately 1% of couples trying to conceive[1]. Current diagnostic procedures can identify etiologic factors in approximately 50% of these couples, such as uterine defects, advanced woman age, parental karyotype abnormalities, embryonic aneuploidies, infections and thrombophilia disorders[2,3]. While the role of acquired thrombophilia has been accepted as an etiology of RM, the contribution of specific inherited thrombophilic genes to this disorder has remained controversial[4].

The balance between coagulation and fibrinolysis is an essential part in early pregnancy, and thrombophilia has been postulated to be a contributor to the pathophysiology of recurrent pregnancy loss. Pregnancy is a hypercoagulable state with an increase in procoagulant factors and a decrease in the levels of anticoagulants[5].

Among the causes of these adverse pregnancy outcomes , three in particular are considered as the major factors of recurrent pregnancy loss and other adverse pregnancy out comes including: (i) structural and numerical chromosomal abnormalities, (ii) inflammatory and autoimmune disorders, and (iii) allelic polymorphisms of some pro-thrombophilic genes[6].

Factor V is one of the essential clotting factors in the coagulation cascade. Its active form, factor Va, acts as a cofactor allowing factor X to stimulate the conversion of prothrombin to thrombin. Thrombin is then able to cleave fibrinogen to fibrin and a fibrin clot is formed.

The factor V gene mutation 1691G>A (rs6025) results in an altered variant of factor V, namely Factor V Leiden, which cannot be easily cleaved by activated protein C (aPC). The 1691G>A mutation increases the risk of venous thrombosis (VT) up to 50-100 fold in adult homozygous[7].

The association between the FVL mutation and RPL seems stronger for non-recurrent second-trimester pregnancy loss compared with recurrent early pregnancy loss[8]. Women with factor V Leiden have a substantially increased risk of clotting in pregnancy (and on estrogen-containing birth control pills or hormone replacement) in the form of deep vein thrombosis and pulmonary embolism. They also may have a small increased risk of preeclampsia, may have a small increased risk of low birth weight babies, may have a small increased risk of miscarriage and stillbirth due to either clotting in the placenta, umbilical cord, or the fetus (fetal clotting may depend on whether the baby has inherited the gene) or influences the clotting system may have on placental developmen[9].

Study design

Patients

This study included 70 patients who were selected from the Gynecological and Obstetric Clinic, Faculty of Medicine, Minia University Hospital, Minia, Egypt during the period from December 2018 to June 2019. Patients were further categorized into two Subgroup **Ia**, had the following characters: 23 women with history of two consecutive recurrent pregnancy loss and subgroup **Ib** 47 women with history of more than two times abortions either consecutive or not.

We Included women with two or more recurrent pregnancy loss consecutive or not before 20 weeks gestation and excluded women having Antiphospholipid antibody syndrome, Diabetes mellitus, chronic liver disease, chronic kidney disease, patients with thyroid diseases, dyslipidemia, local uterine abnormalities, patients with policystic ovary syndrome or luteal phase defect and patients with self-induced abortion.

The control group consisted of 70 apparently healthy women with matched age to the patient group attended to the same institutions for regular follow up. They had normal obstetric history with no history of abortion. All subjects volunteer-red to participate in the study. They were subjected to thorough history taking, clinical and radiological examination, Routine laboratory investigations included blood count, Prothrombin concentration (PC) and INR, activated partial thromboplastin time (PTT), Special laboratory investigations involved: Lupus anticoagulant screening test (PTT LA), D-Dimer and qualitative analysis of factor V leiden G1691A mutation.

This work is conducted according to the ethical rules of the Faculty of Medicine, Minia University , Egypt , and approved by the faculty board .The study conforms to the ethical guidelines of the 1975 Declaration of Helsinki. Informed consent was obtained from all patients.

Methods

All participants were screened for anti-phospholipid syndrome by sensitive APTT reagent (KACZOR D.A., BICK FORD N.M 1992), The kit was supplied by DIAGNOSTICA STAGO SAS, France. The reading was obtained via (CoaDATA 2001, Germany). The principle of the PTT-LA test is based on the measurement of plasma recalcification time in the presence of cephalin and activator .The presence in the test plasma of lupus anticoagulant prolongs the clotting time. Sensitization of the reagent specially enhances the prolongation of the clotting time due to the LA in the plasma.

All participants were screened for any thromphophilic manifestation or presence of microthrombus through D. Dimer .It was measured by enzyme immunoassay method (EIA) by Humareader plus 3700-1272 Germany. The kit was supplied by Wuhan EIAab Science Co., Ltd , **China.**

Molecular study

DNA extraction was performed using PROBA-NA DNA mini kit from DNA-Technology company. One ml in ethylene diamine tetra acetic acid (EDTA) containning tube for genotyping technique. DNA extraction was done from fresh whole blood samples then DNA was stored at -20 ºC till amplification by real time PCR.

Real time PCR was performed using Taq Man Gene Expression Assays followed by

melting curve analysis, The Kit were supplied by DNA-TECHNOLOGY (catalog no.334-1), Russia.

Procedure: The following were left to thaw, completely re-suspended by doing gentle vortex, then were briefly centrifuged to bring liquid to the bottom of the tube: Taq-AT-polymerase, DNA samples, Probes, PCR buffer. The required number of 0.2 mL PCR-tubes were marked for each mutation to be tested. The PCR master mix was prepared as following:

20 µL of corresponding PCR-mix were added into the marked tubes. Vortex of the tubes with PCR-buffer and Taq-AT-polymerase were performed for 3-5 seconds, then spinning for 1-3 seconds was done to collect the drops.10 µL of PCR-buffer and Taq-AT-polymerase mixture was added into each PCR-tube. Then 20 µL of mineral oil were added in each PCR-tube. The tubes were closed tightly .Finally, each tube was opened and 5.0 µL of DNA sample were added into corresponding PCR-tubes, then the tube was closed again before proceeding to the next DNA sample. The tubes were spun for 1–3 seconds to collect the drops.
The tubes were set to Real-time PCR instrument (DNA-TECHNOLOGY, Russia)

Statistical analysis

The collected data were tabulated and analyzed by Statistical Package for Social Sciences program (SPSS) software version 20. Descriptive statistics were done for numerical variables by mean, standard deviation, median and interquartile range, while they were done for qualitative variables by number and percentage.

Mann-Whitney test was used to determine the statistical difference between the two groups for normally distributed quantitative variables and independent samples **t-test** for not normally distributed quantitative variables. **Fisher's exact** were used to determine the statistical difference between the two groups for **factor V leiden G1691A** variable.

Pearson's and **Spearman's** correlation were used to correlate between different variables. According to (r) ranged from (0±1), the degree of correlation was determined (0-0.24 weak, 0.25-0.49 fair, 0.5-0.74 moderate and ≥0.75 strong). The level of significance was taken at **p value** less than or equal to 0.05 as significant.

Results

All groups in our study showed similar age There was no statistical significant difference between the studied groups as regard age (p=0.08). Frequency of abortion among cases ranged from 2-10 times with mean ± SD 3.6±1.8, while the control group (group II) had no abortion. There was highly statistical significant difference between the studied groups as regarding frequency of abortion (p=0.0001*).

Comparison between studied groups regarding HB, WBCs and platelets.

	Group I N=70	Group II N=70	P value
HB :(g/dl)			
Mean ± SD	12.1±0.9	12.4±1.1	0.1
Median	12	12.5	
Range	10-14.8	10-15	
WBCs **:($\times 10^3$/µl)**			
Mean ± SD	6.8±2.1	6.5±1.7	0.4
Median	6.7	6.2	
Range	4.-12	4-10.8	
PLT **:($\times 10^3$/µl)**			
Mean ± SD	282.5±60	281.5±62	0.8
Median	279.5	283	
Range	167-400	153-400	

Regarding HB, WBC's and platelet count, there was no statistical significant differences were found between the studied groups (p= 0.1,0.4,0.8 respectively).

Comparison between studied groups as regarding PC, INR and aPTT.

	Group I N=70	Group II N=70	P value
PC: %			
Mean ± SD	93.5±8.4	87.1±9	0.0001*
Median	99	88	
Range	72-100	70-100	
INR			
Mean ± SD	1.04±0.05	1.09±0.07	0.0001*
Median	1	1.08	
Range	1-1.17	1-1.25	
aPTT :(Sec)			
Mean ± SD	30.1±5.9	27.3±3.6	0.008*
Median	29.4	27.8	
Range	18.6-41	17.9-38.6	
PTT.LA: (Sec)			
Mean ± SD	32.9±5.1	30.9±3.5	0.01*
Median	33.1	30.9	
Range	21.3-39.5	22.3-37.9	
D.dimer:(ng/ml)			
Mean ± SD	40.5±83.9	24±24.8	0.03*
Median	9.7	13.5	
Range	5.3-400	5-106	

Comparison between both groups as regarding ProthrombinA20210G expression .

	Group I N=70	Group II N=70	p value
F5 1691G>A(FVL)			
Wild G/G	46 (65.7%)	67 (95.7%)	0.0001*
Heteromutant G/A	24 (34.3%)	3 (4.3%)	

On comparing the expression of F5 1691 G>A (factor V Leiden) among the studied groups there was statistically significant increase in the expression of F5 1691G>A in group I when compared with group II (p=0.0001) as 24 cases of group I (34.3 %) were heteromutant (G/A) while in group II 3 subjects only (4.3%) expressed heterogeneous mutation

Comparison between group I subgroups (Ia and Ib) as regarding ProthrombinA20210G expression .

	Group Ia N = 23	Group Ib N = 47	P value
F5 1691G>A(FVL)			
Wild G/G	22 (95.7%)	24 (51.1%)	0.0001*
Heteromutant G/A	1 (4.3%)	23 (48.9%)	

Regarding the expression of F5 1691G>A (factor V Leiden) among cases there was statistical significant increase in the expression of F5 1691G>A in group Ib when compared with group Ia (p=0.0001) as 23 cases (48.9%) of group Ib were heteromutant (G/A) while in group Ia only one case (4.3%) expressed heterogeneous mutation

Correlation studies between frequency of abortions and the studied coagulation tests among cases .

Frequency of abortions		
Parameters	**Pearson's rho**	**P value**
D. Dimer	0.28	0.01*
PTT.LA	0.13	0.2
PTT	0.19	0.1
INR	0.13	0.2
PC	-0.05	0.6

The present study showed that there was a statistically significant fair positive correlation between frequency of abortions and level of D. Dimer among cases (r= 0.28 & p=0.01). Also, there were statistically insignificant weak positive correlations between frequency of abortions and PTT LA. among cases (r=0.13 & p=0.2), PTT showed statistically insignificant weak positive correlations with frequency of abortions among cases (r=0.19 & p=0.1).

There was statistically insignificant weak negative correlation between frequency of abortions and prothrombin concentration among cases (r= -0.05& p=0.6).Also there was statistically insignificant weak positive correlations with frequency of abortions and INR among cases (r=0.13 & p=0.2).

Correlation studies between frequency of abortions and genetic mutations among cases

Frequency of abortions		
Parameters	**Spearman's rho**	**P value**
factor V A1691G	0.38	0.001*

Our study showed that there was statistically significant fair positive correlations between frequency of abortions and factor V Leiden A1691G expression among cases (r=0.38 & p=0.001).

Discussion

We examined the relationship between unexplained RPL and thrombophilia gene mutations. In the present study, in terms of age no meaningful difference was determined between the studied groups (p>0.05) The causes of RPL are still unexplained .Successful pregnancy requires an even balance of coagulation and fibrinolysis, in order to continue adequate placental perfusion. Spiral artery thrombosis and infarction occurs, and as a result of these, uteroplacental insufficiency may be the final common pathophysiologic pathway in RM and later pregnancy complications associated with inherited thrombophilia become possible[10].

In our study we found that the prevalence of F5 1691G>A(factor V Leiden) mutation was higher in the studied cases in comparing with the control group, and it was statistically significant in cases when compared with the control group (p= 0.0001). Also Factor V Leiden is reported as a common inherited risk factor for RPL, with the incidence range of 8-32% in patients and 4-10% in controls[11]**.** Estimated frequencies of FVL allele and genotypes in our research significantly differ between RPL patients and controls.

Mahmutbegovic E., found that the presence of A allele of FVL was associated with a nearly four times higher risk of recurrent miscarriage in RPL patients[12]. On the other hand other studies[13] did not find strong association between FVL and RPL.

Conclusion

Incidence of F5 1691G>A mutation was significantly increased in patients having recurrent miscarriages.

Recommendation

It is recommended that women with a family or personal history of thrombosis should be screened for F5 1691G>A mutation before their conceptions or at the beginning of their pregnancies. A history of venous thromboembolism related to pregnancy or oral contraceptive use and early unexplained pregnancy loss are also considered to be indications for testing.

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