Factor v leiden mutation (fvlm) compared to placental histopathology in recurrent unexplained second and third trimester fetal loss

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ABSTRACT

Objective: This study was performed to detect FVL mutation in women with unexplained second and third trimester fetal loss and to compare the presence of FVL mutation with placental histopathology in these women.

Patients & Methods: The present study included two groups: group (I) represented sixty pregnant patients with history of recurrent unexplained fetal loss. The group is subdivided ***into group (Ia):*** 30 patients in their 2nd trimester and group (Ib): 30 patients in their 3third trimester. Group (II): 20 healthy multiparous patients with no previous history of fetal loss as a control group. Ultrasound scanning of the placenta and blood sampling for detection of FVLm for all women were done during pregnancy. Histopathologic examination of the placenta was performed after the pregnancy has ended.

Results: There was no significant difference in the presence of FVL mutation between patients with recurrent unexplained fetal losses (5/60 = 8.3%) and the control group. Normal factor V genotype was detected in 55/60 of the patient with fetal loss (28/30) of the 2nd trimester abortion and 27/30 of the 3rd trimester fetal death; these results were not statistically significant. Ultrasonography of the placenta reflected pathological findings (calcification and degeneration) in second trimester abortion and third trimester intrauterine fetal death which was highly statistically significant than the control group ($P<0.01$). Histopathological examination of the placenta (infarction and thrombosis) was statistically of higher significance in cases with history of fetal loss than the control group ($P< 0.01$).

Conclusion: Screening for FVL mutation is not recommended as routine screening test in cases with recurrent fetal loss although still highly indicated in cases with past history of thrombosis.

Key words: Thrombophilia, Recurrent pregnancy loss, Facto V Leiden mutation.

INTRODUCTION

Recurrent fetal loss is a significant public health problem with two or more losses affecting up to 3-5% women. Identified causes of recurrent fetal loss include chromosomal abnormalities, anatomic alterations of the uterus, autoimmune and endocrinal abnormalities. However, a significant fraction of poor pregnancy outcomes remains unexplained by these factors and many researches have focused on identifying further risk factors. Given that a successful pregnancy outcome is highly dependent on the establishment and maintenance of an adequate placental circulation1 (Martinelli et al., 2000).

It is possible that abnormalities of placental vasculature leading to inadequate feto-maternal circulation may be responsible for at least some poor pregnancy outcomes. This has led to an interest in the thrombophilia as risk factors for fetal loss. Factor V Leiden mutation is the most common form of inherited thrombophilia. A point mutation in the factor V gene at nucleotide position 506 resulting in an arginine to glutamine substitution, reduces the sensitivity of the factor V protein to inactivation by activated protein C (activated protein C resistance) resulting in pro-coagulant state and an inserted risk of thrombosis 2(Tracy and John, 2004).

Factor V Leiden mutation is present in 4–10% of people of Caucasian origin. The mutation induces a hypercoagulable state which increases the risk of venous thrombosis 7-fold among heterozygous carriers and 80-fold among homozygous carriers compared to non carriers. It has been suggested that factor V Leiden mutation may be associated with negative outcomes of reproduction such as recurrent abortion, preeclampsia, prematurity and small for gestational-age neonates3 (Pauer et al., 2003).

The aim of this study is to detect the prevalence of factor V Leiden mutation in pregnant women with history of unexplained recurrent 2nd and 3rd trimester fetal loss and comparing it with placental histopathology in these women.

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MATERIAL & METHODS

This study was conducted in the Obstetrics Department in Al Zahraa University Hospital between December (2006) and November (2009). Eighty pregnant women were included in this study and divided into two groups. Group (1) (Study group) included 60 pregnant women with history of recurrent two or more unexplained fetal loss. This group was further subdivided into; group (Ia) of 30 patients presented with second trimester abortions and group (Ib) of 30 patients in third trimester of pregnancy with intrauterine fetal deaths. Group (II) (control group) consisted of 20 healthy women with uncomplicated pregnancies and with no history of fetal loss, both groups were matched for age, parity and blood group.

All cases had no previous history of thrombosis and were subjected to detailed history and clinical examination. Also, they had routine investigations as blood group and Rh, CBC, urine analysis and culture and sensitivity, random blood sugar, HBs Ag screening. Pelvic U/S was done then 2ml of blood was withdrawn in sterile EDTA containing tubes and stored at 4 degrees for detection of factor V gene by PCR. Postpartum placental sample was taken from feto-maternal side for histopathology examination (taken in natural buffered formalin and stored at room temperature).

Exclusion criteria included patients age above 40 years, systemic lupus disease, RH negative patients, elevated TORCH antibodies, medical disease with pregnancy e.g. diabetes mellitus, hypertension, renal disease, fetal malformations, positive APS or cases with hypothyroidism.

Material assay:

Two ml of venous blood withdrawn from each patient and control by venepuncture under complete sterile conditions, and put the sample on sterile EDTA continuing tubes, collected and stored in 4°C until the factor V assay. Determination of factor V Leiden was done by genomic DNA extraction followed by PCR amplification. Placental biopsies taken from fetomaternal surface were formalin fixed paraffin embedded and five micron section were prepared for routine hematoxylin and eosin stain. Sections were examined microscopically for infarctions and vascular thrombosis. Data was statistically analyzed using SPSS (statistical package for social science) program version 13 for windows and Epi info program version for all the analysis.

RESULTS

Eighty pregnant women were included in this study and divided into two groups. Table (1) shows that both groups were matched as regard maternal age blood groups and marital periods. Table (2) for the relation between FVL (wild and positive) and placental findings by US and histopathology in the study group shows that all women that were positive FVL showed placental pathological changes. There was highly significant difference between FVL and placental pathology detected by US and histoapthological examination ($P < 0.01$). There was no statistically significant difference in the presence of FVLm in the studied groups ($P < 0.05$). Table (3) for Logistic regression analysis of predictors of FVL in aborted cases shows that Parity, placental histopathology, maternal age, marital period, number of recurrent fetal loss, gestational age are dependent predictors of FVL positive.

Table III : Correlation coefficient between inhibin B and FSH & E2 in PID patients and control group

Studied variables	Group I (Cases) GIA (no. 30)		Group I (Cases) GIB (no. 30)		(Group II) Controls (n. 20)	
✓ Age (years)						
Range	22 - 39		22 - 40		22 - 32	
Mean	29.7		31.07		27.85	
± SD	±3.9		±5.8		± 2.89	
✓ Parity	No	%	No	%		
- P0	5	16.7	0	0.0	0	0.0
- P1	15	50.0	0	0.0	0	0.0
- P2	10	33.3	10	33.3	7	35.0
- ≥ P3	0	0.0	20	66.7	13	65.0
Gestational age						
Range	13 – 20		25-36		18-36	
Mean	16.5		28.9		28.7	
± SD	± 1.91		± 2.56		± 5.5	
✓ No of abortion						
Range	3 – 8		---		---	
Mean	4.2					
± SD	± 1.27					
✓ IUFD						
Mean± SD	---		3.87± 0.89		---	
✓ Marital period (years)						
Range	5 – 19		3 – 18		4 – 15	
Mean	9.73		9.03		8.75	
± SD	±3.43		±4.29		± 3.11	
✓ Blood group						
A	13	43.3	13	43.3	10	50.0
B	6	20.0	6	20.0	5	25.0
AB	5	16.7	6	20.0	5	25.0
O	6	20.0	5	16.7	0	0.0

Table (2) Relation between FVL (wild and positive) and placental findings by US and histopathology in the study group

Studied variables	FVL Wild (no. 55) No.	%	FVL Positive (no. 5) No.	%	X^2	P-value
Placenta in US						
Normal placenta	0	0	0.0	0.0	16.8	<0.05*
Placental calcification	9	16.4	0.0	0.0		
Placental degeneration	46	83.6	5	100		
Placental histopathology						
No infarction	8	14.5	0	0.0	15.46	<0.01**
Focal infarction	5	9.2	0	0.0		
Severe infarction	2	3.6	2	40.0		
Wide infarction	39	70.9	2	40.0		
Thrombosis	1	1.8	1	20.0		

Table (3): Logistic regression analysis of predictors of FVL in aborted cases

	Positive FVL R square = 0.04 β	SE	Odds ratio (95% CI)
Constant	-10.17		
No parity	-1.4	1.17	0.25 (0.02-2.43)
Placental histopathology	0.45	0.62	1.57 (0.47-5.29)
Maternal age	0.1	0.2	1.11 (0.75-1.64)
Marital period	0.18	0.21	1.19(0.79-1.79)
Recurrent fetal loss	0.21	0.39	1.23 (0.57-2.69)
Gestational age	0.04	0.08	1.05 (0.89-1.23)

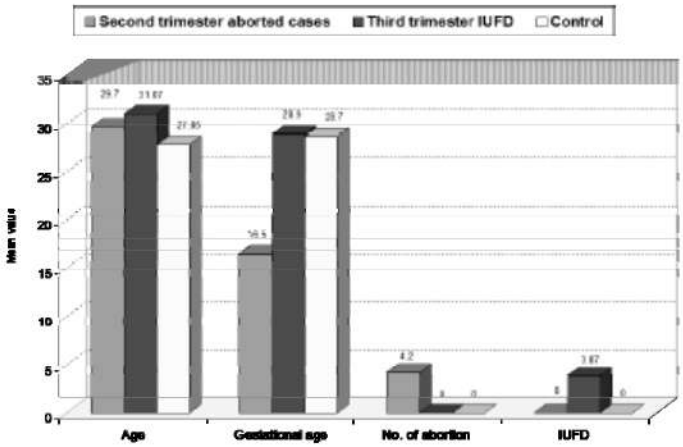


Figure (1): Shows the distribution of age, gestational age, no of abortion and IUFD distribution in studied groups.

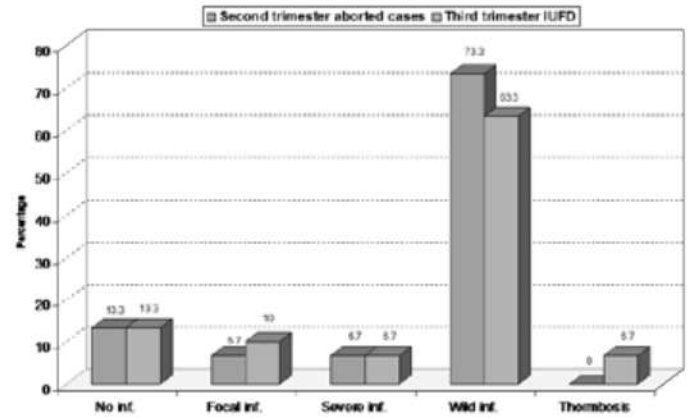


Figure (2): Shows the placental histopathology in the studied groups.

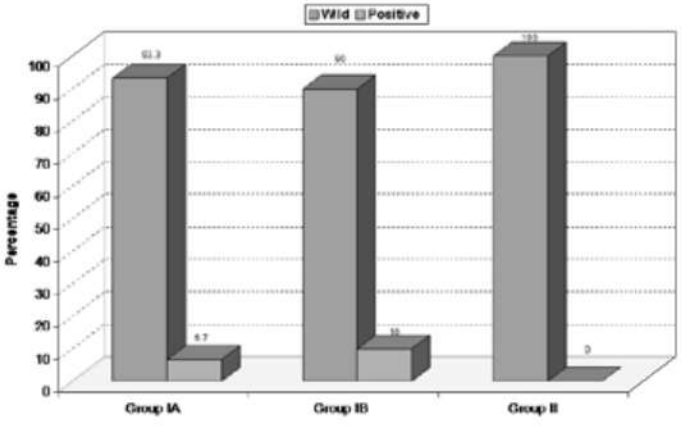


Figure (3): Shows the distribution of FVL mutation in the studied groups.

Figure (4): Chorionic tissues showing viable chorionic villi (on the right) while the left side shows ischemic necrosis due to infarction (H&Ex100) in group IB.

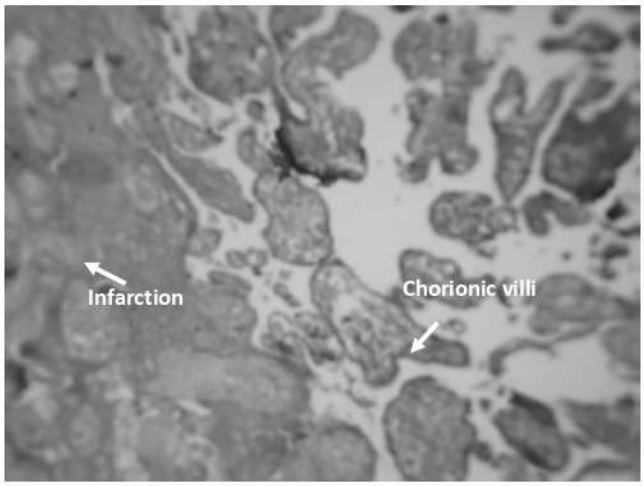


Figure (5): Infarcted placental tissue with evidence of intravascular thrombosis formation (H&E x 100) in case of group IB.

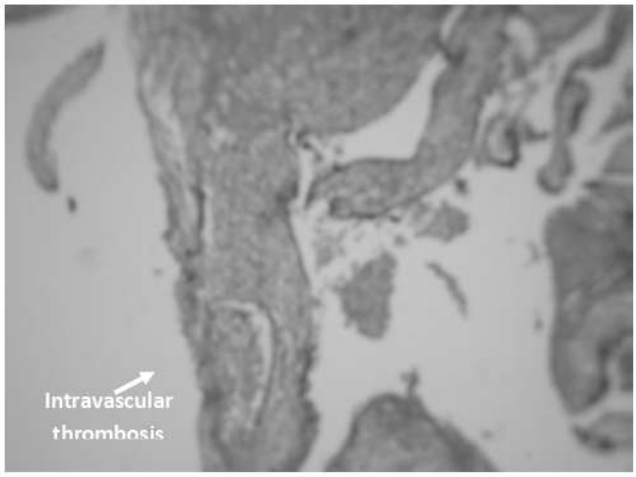


Figure (6): A normal fundal anterior placenta in a 26 weeks pregnancy.

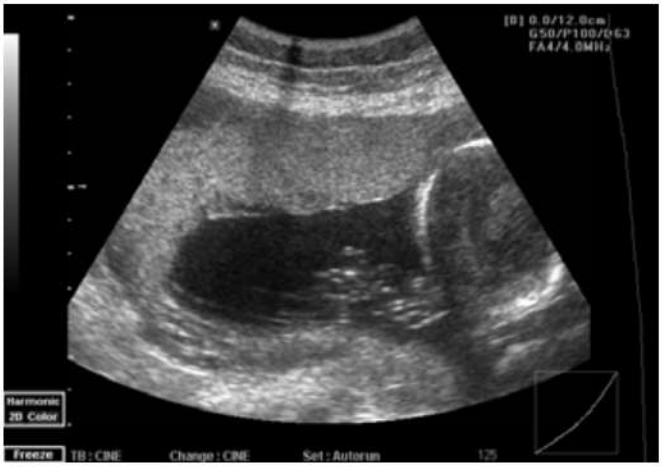


Figure (7): An anterior placenta of a 27 weeks pregnancy showing multiple placental cysts surrounded by calcificaltion. Doppler study of umbilical vessels showed compromized utreoplacental circulation.

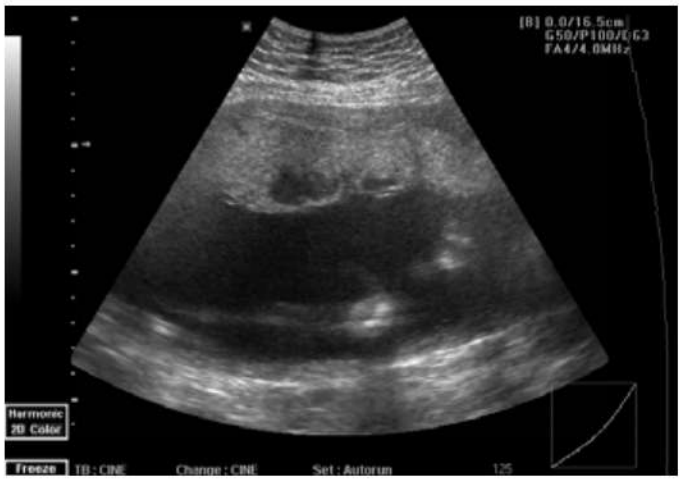


Figure (8): A third trimester fundal placenta showing calcifications and fibrin deposition early in 31 weeks gestation. The fetus who was growth restricted was delivered at 34 weeks gestation

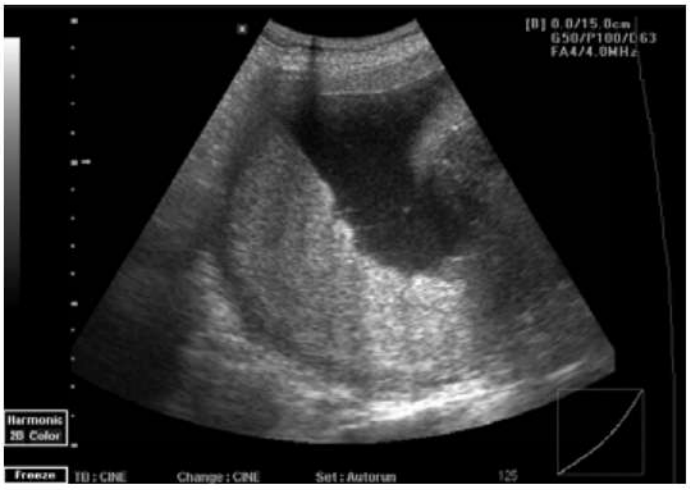
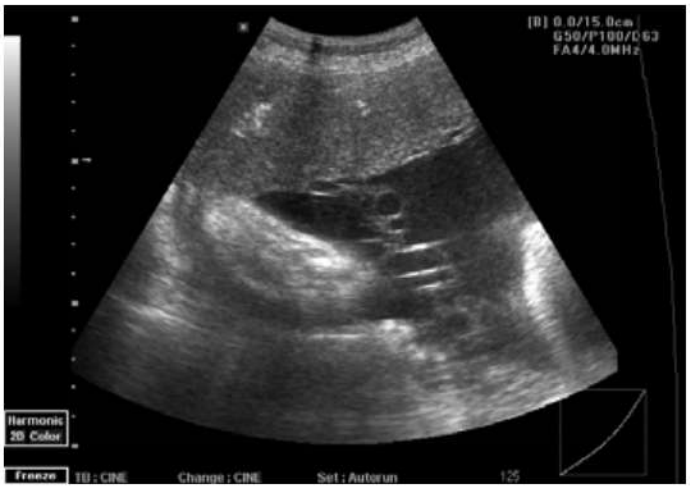


Figure (9): A fundal anterior placenta in 33 weeks pregnancy showing calcification and exaggerated coiling of the umbilical cord.



DISCUSSION

The current study was done to detect FVL mutation in women with unexplained second and third trimester fetal loss and to compare the presence of FVL mutation with placental histopathology in these women.

There were highly statistical significant differences between control group and second trimester cases and also the third trimester cases regarding US findings of placental pathology (p- value < 0.01). As the control cases showed no placental abnormalities. However there was no significant change in the placental pathology, detected by ultrasound between cases of second trimester abortion and intrauterine fetal death (P> 0.05). Suggesting that the importance of ultrasound in reflecting placental pathology in cases of second trimester abortion and third trimester intrauterine fetal death.

As regard placental histopathology the present study showed highly statistical significant differences between control and both second trimester cases and third trimester fetal death groups (p- value < 0.01). On the other hand the present study showed that there was high incidence of placental infarcts and thrombosis in both women with and without thrombophilia. These findings were in agreement of the results of 4(Mousa and Alfirevic, 2000).

On the contrary, Many et al., 2001, have compared the placental findings in women with severe pregnancy complications with and without thrombophilias5. The number of women with villous and multiple infracts was significantly higher in women with thrombophilias. The number of placentas with fibrinoid necrosis of the decidual vessels was also significantly higher in women with thrombophilias. The results of the present study shows that there was no significant difference in the presence of FVL mutation between patients with recurrent unexplained fetal losses (5/60=8.3%) shown as 6.7% of the 2nd trimester abortion cases (2/30) and 10% of 3rd trimester fetal loss (3/30) and the control group. These results supported that FVL mutation may play a role in only some cases of unexplained recurrent fetal loss.

Grandone et al., 1997, looked at 43 Italian women-with 2 or more unexplained losses. They found that the mutation was found in 7 of 43 patients (16.28%) and in 5 of 118 controls (4.24%). Among the 16 women with stillbirth, 5 (31.25%) tested positive for the mutation. Two of the 27 women who suffered first-trimester losses (7.41%) had the mutation. They concluded that the frequency of the factor V Leiden mutation is significantly higher among women with recurrent pregnancy loss and that the mutation is particu¬larly associated with late events6.

Meinardi et al., 1999, evaluated fetal wastage in 228 women with the factor V Leiden mutation and in 121 non affected controls. Approximately 32% of carriers and 22% of non-carriers experienced fetal loss. Carriers had a 29.4% rate of miscarriage, and non-carriers had a 17.4% rate. Stillbirth rates were similar for both groups, 5.7 % and 5.0% respectively, Carriers had a 10.1% risk for recurrence, and non-carriers had a 4.1% risk. These investigators concluded that stillbirth and miscarriage are increased in women with the factor V Leiden mutation7.

Although the above investigators found an association between the factor V Leiden mutation and recurrent preg¬nancy loss, other studies have suggested that there is no such association. Rai et al., 2001, found that the prevalence of FVL was not high than in controls for both first and second trimester abortions8. This discrepancy may stem for the different study design. Carp et al., 2002, reported that thrombophilia was not found to be associated with recurrent pregnancy loss9. Mukhopadhyay et al., 2009, study did not come up with a significant association between FVL and RPL among a north Indian patients, but a trend toward probable association between FVL and women with more than two habitual recurrent abortion was found in our study population10. Another two studies on Indian population did not find any association

between FVL and RPL cases Biswas et al., 2008. Similarly, some other studies conducted on different world populations suggested that FVL is not a predisposing factor for RPL (Aksoy and Karabulut, 2005)11, 12.

We concluded that FVL mutation may play a role in only some cases of recurrent unexplained fetal loss in the second and third trimester of pregnancy. Neverthless, study of FVLm is not recommended in all cases of recurrent unexplained fetal loss. But in those cases with inherited thrombophilias, although playing a small contributary role in recurrent abortions, it may be the straw that breaks the camel's back regarding the thrombotic risk faced by these patients during pregnancy and more important after delivery . Therefore, it remains highly indicated to be searched for in these cases. Ultrasonographic study of the placenta is essential for detection of the placental calcification and thrombosis in cases of unexplained recurrent fetal loss This must be confirmed by postpartum histopathological examination of the placenta.

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