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Factor V Leiden and Antiphospholipid Antibodies in Pregnancies Complicated by Preeclampsia

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ABSTRACT

Background: Several studies have linked inherited and acquired thrombophilia to adverse pregnancy outcome including preeclampsia. Factor V leiden (FVL) is one of the most frequent thrombophilic mutations. Most of the studies about Factor V Leiden mutation in women were done in developed countries while little is known about the incidence and prevalence in developing countries where preeclampsia is still a major cause of maternal mortality.

Objective: The purpose of our study is to investigate the presence of FVL mutation and antiphospholipid antibodies (APA) in cases with preeclampsia and their relation to the maternal and fetal outcome.

Patients and Methods: We performed a prospective case-control study enrolling 116 preeclamptic and 40 normotensive pregnant women. Complete blood count, urea, creatinine and urine for proteinuria as well as APA IgG & IgM and FVL evaluation were done for cases and controls.

Results: FVL mutation was found in 8.6% of preeclamptic pregnancies and 5% of normotensive pregnancies and APA IgM was 22.4% and 5% in preeclamptic and normotensive pregnancies respectively while APA IgG was 31% and 10% in preeclamptic and normotensive pregnancies respectively. Eight fetuses out of ten (80%) had bad outcome in the heterozygous FVL mothers while 24 out of 106 (23%) had bad outcome in wild FVL mothers and the difference is statistically significant (p=0.001).

Conclusions: The incidence of FVL mutation is higher in preeclampsia than normal pregnancy and this may reflect unfavourable fetal outcome.

Key Words: *Preeclampsia – Factor V leiden mutation – Antiphospholipid antibodies.*

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INTRODUCTION

Preeclampsia (PE) affects 6-8% of all pregnancies and is a major cause of maternal and fetal morbidity and mortality. The pathogenesis of preeclampsia is complex and incompletely understood. Many investigators suggest an association with abnormal trophoblast invasion, coagulopathy, genetic and immunological predisposition, dietary abnormalities and vascular endothelial damage **[1]**.

Prochazka et al., suggest an increased prevalence of obstetric complications in female carriers of hereditary or acquired thrombophilias **[2]**. The G (guanine) to A (adenine) substitution at nucleotide 1691 of factor V gene results in resistance to activation by protein C, causing a pro-thrombotic state in FVL carriers **[3]**. The FVL mutation and other hereditary thrombotic risk factors can moderately increase the risk of preeclampsia, the vasculopathy and secondary thrombosis from hypercoagulopathy may result in inadequate perfusion of intervillous space and preeclampsia **[4]**. Most of the studies about FVL mutation in women were done in developed countries while little is known about the incidence and prevalence in developing countries where preeclampsia is still a major cause of maternal mortality **[15]**.

Several reports suggest that quantification of known APA is not prognostic in the assessment of the risk for PE, others suggest that inherited and/or acquired thrombophilia may play a role in the pathogenesis of PE and/or intrauterine growth retardation (IUGR), as their frequencies are increased in women with histories of the

early-onset PE compared to those with normal pregnancies **[5]**.

The aim of this study was to investigate the frequency of FVL mutation and APA in cases with preeclampsia and to evaluate the maternal and fetal outcome in these cases.

PATIENTS AND METHODS

Beginning in November 2006 and ending in October 2007, women with preeclmpsia who were matched for gestational age to normotensive pregnant controls were approached for participation. One hundred and sixteen women with preeclampsia and 40 normotensive women agreed to participate and provided their signed informed consent. This study was done in the Women's Health Centre, Faculty of Medicine, Assiut University. The APA and FVL were done in Clinical Pathology Department, South Egypt Cancer Institute, Assiut University. The institutional ethics committee at Faculty of Medicine, Assiut University approved this study.

Study groups:

Pre-eclampsia group:

All the patients were in their late 3rd trimester of pregnancy. Preeclampsia was considered when a pregnant woman developed arterial hypertension after the 20th week of pregnancy associated with proteinuria. Arterial hypertension was defined when the blood pressure was at least 140/90 mm Hg detected twice with a time difference of 6 hours between them. Proteinuria was defined as the presence of 30 mg/dl in the urine analysis. Exclusion criteria for study participation included chronic hypertension, preeclampsia superimposed on chronic hypertension, prior thromboembolism, transient hypertension or multifetal pregnancy.

Control group:

The control group included women who were in their late 3rd trimester and normotensive throughout pregnancy and had no history of thromboembolic event, abnormalities in blood pressure or proteinuria.

Methods:

The following evaluation data were collected: Age, parity, gestational age, blood pressure, proteinuria, maternal outcome, mode of birth, fetal outcome, birth weight of the babies and their Apgar scores.

The following routine laboratory investigations were done for patients and controls: Complete blood count, urea, creatinine and urine for proteinuria. APA IgG & IgM and FVL evaluation were also done.

For specific investigations, FVL and APA, 3ml venous blood were collected in a tube containing EDTA (ethylene diamine tetra acetic acid) for DNA isolation and 5 ml were collected in plain tubes for serum preparation. The samples were centrifuged within 30 minutes at 3000 rpm for 10 minutes and serum collected and stored at -20ºC for APA.

Antiphospholipid antibodies:

The ORGENTEC Anti-Phospholipid Screen IgG/IgM assay is a quantitative enzyme immunoassay (EIA) intended to screen for the presence of IgG and IgM class autoantibodies against Cardiolipin, Phosphatidyl Serine, Phosphatidyl Inositol, Phosphatidic acid and ß2-Glycoprotein, in human serum or plasma.

Principle of the test:

A mixture of highly purified Cardiolipin, Phosphatidyl Serine, Phosphatidyl Inositol, Phosphatidic acid and human ß2-Glycoprotein I is bound to microwells. Antibodies against these antigens if present in serum will bind to the respective antigens. Washing of the microwells removes nonspecific serum and plasma components. The addition of an acid stops the reaction forming a yellow end-product. The intensity of this yellow color is measured photometrically at 450 nm.

The cut off value for APA IgG is <10 GPL/ml and for APAIgM is <10 MPL/ml.

Factor V leiden:

DNA extraction: DNA is extracted from EDTA blood by high pure template isolation kit (Roche diagnostic).

PCR: Using factor V leiden kit (Light Cycler real time PCR, Roche Diagnostics, Mannheim, Germany). The factor V leiden kit allows the detection and genotyping of a single point mutation (G to A at position 1691) of the human factor V gene, from human whole peripheral blood. The test was performed on the Light

Cycler 1.2 instrument. The hybridization probes are used to determine the genotype by performing a melting curve analysis after the amplification cycles are completed and the amplicon is present at increased concentration. The Red 640-labeled hybridization probe hybridizes to a part of the target sequence that is not mutated and functions as an anchor probe. The fluorescein-labeled hybridization probe spans the mutation site (mutation probe).

During the melting curve analysis, increasing temperature causes the fluorescence to decrease because the shorter of the two probes (mutation probe) dissociates first and the two fluorescent dyes are no longer in close proximity. If the FVL mutation is present the mismatch of the mutation probe with the target destabilizes the hybrid so the decrease in fluorescence will occur at a lower temperature. With the wild type genotype mismatch will not occur, and therefore, the heteroduplex DNA has a higher melting temperature (Tm). The heterozygous genotypes exhibits a distinctive combination of properties. The resulting melting peaks allow discrimination between the homozygous (wild type or mutant) as well as the heterozygous genotype (Fig. 1).

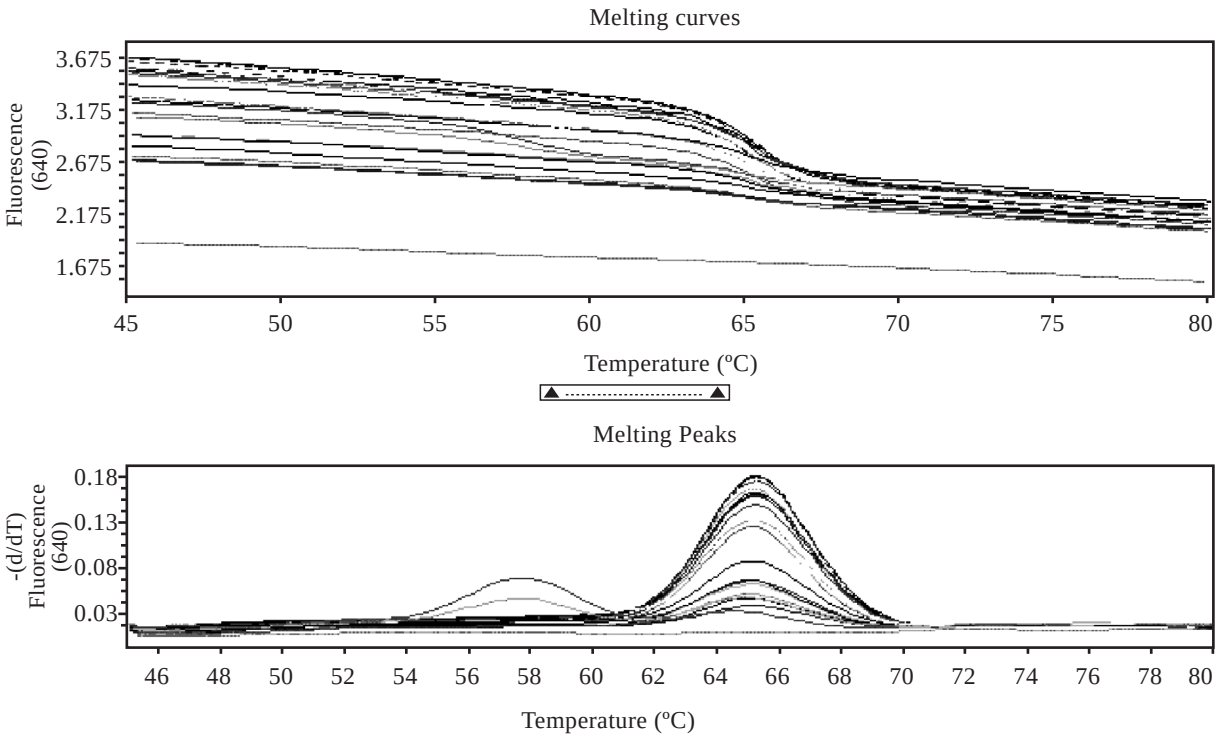

Fig. (1): Melting curve analysis for Factor V Leiden.

This picture reveals 2 peaks at two different melting temperature (one at 58ºC [mutant] and the other at 66ºC [wild]) indicating heterozygous genotype of FVL.

Statistical analysis:

All data were analyzed using SPSS (Statistical Program for Social Sciences) version 11 for windows, 2001, SPSS Inc., Chicago, IL, USA. Comparisons between means for continuous variables were done using independent sample *t*-test. Values are represented as mean ± SD. The relation between values and the outcome was undertaken using the X^2 test. Relationships in variables were assessed by correlation test. A p value <0.05 is considered to be significant. All p values were two-tailed.

RESULTS

One hundred and sixteen preeclamptic women and 40 normotensive pregnant women as a control were enrolled in this study.

Demographic and clinical characteristic of PE and control groups are shown in Table (1).

The laboratory parameters in the PE and control groups are shown in Table (2). Haemoglobin level, leucocytic count, platelets, urea and Creatinine showed comparable values in both groups. The incidence of APA IgG, IgM and FVL (heterozygous) were increased in the PE group than control group but the difference is statistically insignificant.

Patients with preeclampsia were more likely to undergo caesarean section and have intrauterine growth restriction than the control group. Newborn admission to neonatal intensive unit was more in PE patients than the control group and the differences are statistically significant (Table 3).

Eight fetuses out of ten (80%) had bad outcome in the heterozygous FVL mothers while 24 out of 106 (23%) had bad outcome in wild FVL mothers and the difference is statistically significant (p=0.001).

The incidence of bad maternal outcome was more (36%) when APA IgM was positive than when it was negative (9%). The difference is statistically significant (p=0.01).

Table (1): Demographic and clinical characteristics of preeclampsia patients and control group.

Clinical features	PE Patients N=116	Control group N=40	*p* value
Age (years)	16-19 y = 16 (13.8%) 20-35 y = 84 (72.4%) >35 y = 16 (13.8%)	16-19 = 4 (10%) 20-35 = 32 (80%) >35 = 4 (10%)	NS
Parity	Primigravida: 66 (56.9%) Multigravida: 50 (43.1%)	Primigravid: 18 (45%) Multigravid: 22 (55%)	NS
Gestational age (Week)	<37 = 62 (53.4%) >37 = 54 (46.6%)	<37 = 12 (30%) >37 = 28 (70%)	NS
Systolic bp (mm Hg)	156.3	118.2	<0.001
Diastolic bp (mm Hg)	94.6	74.4	<0.001

PE: Preeclampsia. bp: Blood pressur. y: Year. N: Number. NS: Non significant. 0.001: Highly significant.

Table (2): Laboratory parameters in preeclampsia patients and control group.

	PE Patients N=116	Control group N=40	*p* value
Hb. (g/dl)	9.9±1.5	10.1±1.1	NS
WBC (109/L)	7.8±2.5	7.6±2.2	NS
Plt (109/L)	214±77	206±78	NS
Urea (umol/L)	4.7±2.2	4.1±1.4	NS
Creatinine (umol/L)	74±1.3.3	72.1±12.7	NS
APA (IgG)	Positive: 36 (31%) Negative: 80 (69%)	Positive: 4 (10%) Negative: 36 (90%)	NS
APA (IgM)	Positive: 26 (22.4%) Negative: 90 (77.6%)	Positive: 2 (5%) Negative: 38 (95%)	NS
FVL	Wild: 06 (91.4%) Heterozygous: 10 (8.6%)	Wild: 38 (95%) Heterozygous: 2 (5%)	NS

PE : Preeclampsia. WBC : White blood cells. IgG : Immunoglobulin. M, FVL : Factor Vleiden.
N : Number. Plt : Platelet count. G, IgM : Immunoglobulin. NS : Non significant.
Hb : Hemoglobin. APA : Antiphospholipid antibodies.

Table (3): Maternal and fetal outcome in preeclampsia patients and control group.

	PE Patients N=116	Control group N=40	*p* value
Maternal outcome	Good: 98 (84.5%) Bad*: 18 (15.5%)	Good: 34 (85%) Bad: 6 (15%)	NS
Mode of birth	VD: 28 (24.1%) C/S: 88 (75.9%)	VD: 28 (70%) C/S: 12 (30%)	*p*=.001
Fetal outcome	Good: 84 (72.4%) Bad#: 32 (2736%)	Good: 40 (100%)	*p*=0.009
Birth wight (gm)	<2500: 44 (37.9%) >2500: 72 (62.1%)	>2500: 40 (100%)	*p*=0.001
Apgar score 5	Mean: 9.1±1.3	Mean: 10	*p*=0.001
Apgar score 10	Mean: 8.1±1.9	Mean: 10	*p*=0.001

PE : Preeclampsia. VD : Vaginal delivery. * : Intensive care unit admission.
N : Number. C/S : Cesarean section. # : Neonatal intensive care unit admission.

DISCUSSION

Our results showed that the frequency of FVL was (8.6%) in PE group which is more than the control group (5%) but the difference is statistically insignificant.

These results agree with the results of Dona et al. **[6]**, who reported that 8.9% of severe PE were heterozygous for FVL compared to 4.2% in the control group but their results were statistically significant and with the results of Rigo et al. **[7]** who reported 18.33% heterozygous carriers of Factor V Leiden mutation among preeclamptic women and 2.97% heterozygous carriers among healthy controls with the differences between the two groups found to be statistically significant. Dudding et al. **[8]**, reported that maternal FVL is significantly associated with preeclampsia, and Tempelhoff et al. **[9]**, also reported that FVL was 20% in their patients compared to 5% in control.

Our results showed that homozygosity for FVL is not found in either group which agrees with the results of Dona et al. **[6]**, but contrasts with the results of Tempelhoff et al. **[9]**, who found one of their patients and one of the controls to be homozygous for FVL.

Nurk et al. **[13]** found that FVL mutation conferred increased risk of preeclampsia, the risk was highest for preeclampsia at less than 37 weeks. So this latter study supports that FVL mutation may be a risk factor for preeclampsia.

Many studies found no associations between FVL variant allele and preeclampsia **[10-12]**. A meta analysis by Kosmas et al. **[10]** including 2742 hypertensive women and 2403 controls suggested that the associations observed in early and small studies may be due to time-lag bias and publications bias.

The association was evident with FVL mutation (heterozygosity), when the presence of moderate and severe forms of preeclampsia is analyzed separately, the existence of heterogeneity in the moderate form is shown which is not found in the studies related to the severe form **[14]**.

In patients with negative histories for thromboembolism the heterozygous FVL mutation is associated with a lower risk of thromboembolism in pregnancy and therefore neither the screening of all pregnant women nor the treatment of the low risk carriers is recommended **[15]**.

The current study also showed that FVL was not associated with adverse maternal outcome which agrees with the results of Van Pumpus et al. **[15]** who reported that FVL are important genetic risk factor associated with thrombotic risks but this mutation is apparently not related to perinatal outcome in women with preeclampsia. It also agrees with the results of Rigo et al. **[7]** who reported that no statistically significant different perinatal outcomes were found between

Factor V Leiden positive and negative preeclamptic women.

On the other hand, FVL may affect the fetal outcome in which adverse fetal outcome is more in the heterozygous FVL than the wild type in PE group. This result agrees with that of Calderwood **[16]**, who observed an association between maternal FVL and fetal or neonatal stroke and with Dena et al. **[17]**, who concluded that women who are carriers of FVL are faced with increased risk of stillbirth, early onset preeclampsia, severe abruption and possibly fetal growth restriction. Our results are contrast with these of Dudding et al. **[8]**, who observed that there was no association between maternal FVL and fetal growth restriction.

In our study we found positive APA IgG in 31% in PE compared to 10% in controls and positive APA IgM in 22.4% in PE compared to 5% in controls but these differences are statistically insignificant. Studies of women with preeclampsia have confirmed the high incidence of antiphospholipid antibody **[18]**. A number of groups have described increased rate of APA ranging between 10% and 20% among women with preeclampsia **[19]**, severe preeclampsia **[20]** or eclampsia **[21]**. However, at least 2 groups of investigators have found no increased rate of APA among women with preeclampsia **[22,23]**.

Katano et al. **[24]** who did prospective study of 800 unselected obstetric patients have found that the rate of preeclampsia is significantly higher among those women with positive results for APA than among those with negative results. In this study 2% to 7% of the tested women had APA with 0.7% to 7% having anticardiolipin antibodies, and preeclampsia developed in 22% to 50% of these women.

The maternal outcome was affected in our series by APA IgM in which adverse maternal outcome was higher when APA IgM is positive than when it is negative.

We conclude from this study that FVL is more evident in preeclamptic pregnancies than normal pregnancies but these differences are statistically insignificant, FVL is not associated with perinatal outcome but is associated with bad fetal outcome and APA IgM is associated with bad perinatal outcome.

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