Is Factor V Leiden an Important Cause for Recurrent First Trimester Pregnancy Loss

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Abstract

Aim of Work: The aim of this study was to investigate the role of Factor V Leiden (FVL), as a risk factor for first trimester Recurrent Pregnancy Loss (RPL), and to assess the incidence of first trimester RPL in patients with FVL mutation.

Material and Methods: One hundred women were enrolled in this study, fifty with a diagnosis of unexplained first trimester RPL (the case group) and fifty healthy women having at least one child and with no history of RPL (the control group), and Activated Protein C Resistance (APCR) was measured in all subjects of each group, after exclusion of the most common known causes of RPL. We then divided each of the control and case groups into two groups, the first with normal APCR test and the other with abnormal test result, to allow further identification of the incidence of first trimester RPL in patients with FVL mutation.

Results: Nine patients of the case group and four patients of the control group were proved to have abnormal APCR test with no statistically significant differences between both groups. No statistically significant differences in the number of abortions among women with normal and abnormal APCR tests in both groups.

Conclusion: FVL may increase the risk of first-trimester RPL however it is not an important independent cause. Routine FVL screening in first trimester recurrent pregnancy loss should not be the rule.

Key Words: Recurrent pregnancy loss – Inherited thrombophilia – Factor V leiden – Activated protein C resistance.

Introduction

RPL is considered as a major devastating obstetric and gynecological health problem [1]. It is traditionally defined as three or more consecutive miscarriages occurring before 20 weeks post-menstruation [2].

There are many suggested causes and risk factors for RPL including advanced maternal age, Antiphospholipid Antibody Syndrome (APAS), genetic factors, anatomical factors, endocerin factors, immune factors and others [3]; however, only three are widely accepted: Parental chromosomal abnormalities, Antiphospholipid Antibody Syndrome (APAS), and a subset of uterine abnormalities [4]. More than 33% of all cases will remain unexplained [5].

An increased incidence of early and recurrent fetal loss has also been suggested in women with inherited thrombophilia, including FVL, prothrombin G20210A and protein S deficiency [6]. They are one of the most important predisposing factors for Venous Thromboembolism (VTE) in pregnancy and many studies suggested their role in many adverse pregnancy outcomes as RPL [7]. FVL is an inherited disorder of blood clotting. It is the most common cause of inherited thrombophilia and the most common cause of APCR. It is an autosomal dominant genetic condition, which occurs as a result of a single point mutation in the factor V gene. This mutation results in a replacement of Arginine (R) 506 with Glutamine (Q) in one of the factor V cleavage sites for APC (Arg 506) where APC acts. This substitution leads to a factor V species that cannot be degraded by APC [8]. Over the past 3 decades, many studies have suggested the strong association between FVL and many adverse pregnancy outcomes as preeclampsia, Intrauterine Growth Restriction (IUGR), placental abruption and RPL. However many studies have refuted this. Because of the heterogeneity of these results, the American College of Obstetricians and Gynecologists (ACOG) (2013) has concluded that a definitive causal link cannot be made between inherited thrombophilias and adverse pregnancy outcomes [9].
Anatomic uterine defects have also been identified as a cause of RPL which should be excluded in any case of RPL. Anatomic abnormalities can be grouped into congenital (disorders of the müllerian tract) and acquired anomalies (adhesions, cervical incompetence, polyps, and uterine leiomyomas). Although some anomalies may have little to no impact on pregnancy outcome, others may cause RPL, IUGR, preterm labor, malpresentation, and dystocia [10].

Material and Methods

The aim of this case-control study was to investigate the prevalence of FVL, in patients with first trimester RPL and in healthy control women after exclusion of uterine anomalies and APAS. It included 100 women (50 women with history of three or more first trimester RPL and 50 fertile control women with no history of recurrent pregnancy losses), who were recruited from the department of Gynecology and Obstetrics, with the help of the Clinical Pathology Department of Faculty of Medicine, Cairo University during 2014.

Following the approval of the ethical committee of Gynecology and Obstetrics Department of Cairo University and after obtaining a written informed consent from each patient, 100 non-pregnant female patients less than 40 years old with history of one or more prior pregnancies, who were fulfilling the inclusion and exclusion criteria for the study which included:

**Inclusion criteria:**
- Females less than 40 years old for both groups.
- Patients with history of three or more first trimester RPL for the case group.
- Fertile women and had at least one successful pregnancy with no history of previous abortion for the control group.

**Exclusion criteria:**
- APAS.
- Anatomical Intrauterine abnormalities.
- Other identifiable causes of RPL (tests done only if clinically indicated) e.g., diabetes, thyroid disease.

A written informed consent was taken from all women prior to enrollment. Case group patients were subjected to detailed history with special focus on maternal age, medical and surgical histories, family history of RPL or recurrent thrombosis, in addition to detailed obstetric history. Complete physical examination was done also for case group patients including general, abdominal and pelvic examination. Also, investigations were done for case group patients to exclude the most common causes of RPL, which included, 1) Liver functions, renal functions, urine analysis, serum Thyroid Stimulation Hormone (TSH), fasting and 2 hour postprandial blood sugar etc..., done if abnormalities were suspected by history and general examination of the patients of the case group, to exclude general causes of pregnancy loss. 2) Screening for APAS was also done by detailed medical and obstetric history and by measuring LAC IgG and/or IgM antibody titre. 3) A 3-Dimensional Transvaginal Ultrasound (3-D TVS) was done for assessment of uterine size, endometrial thickness, ovaries and uterine cavity for exclusion of uterine anomalies, using the transvaginal probe of the Accuvix XQ 3-D system transducer with frequency range of 5-8 MHz. 4) Office hysteroscopy for assessment of the uterine cavity. If an anatomical anomaly was identified the patient was excluded from the study. 5) FVL screening: this was done for both case and control groups women. The screening was done by functional APCR testing. This test identifies patients who have APCR, depending on the fact that more than 95% of cases of APCR are due to FVL mutation [8]. This test depends on that in cases of APCR; there is a reduced anticoagulant response of patient plasma after adding a standard amount of APC. So in this assay, the activated Partial Thromboplastin Time (aPTT) clotting test fails to prolong significantly after the addition of APC. The APC test was performed on anti-coagulated blood. At first a blood sample was withdrawn, collected in a suitable tube containing a standard amount of an anticoagulant (aPTT siliconized glass tube), the sample was then centrifuged for 15 minutes so as to remove the platelets, and then divided into two samples, the first was used to measure the aPTT of the patient without adding the APC, and the second was used to measure the aPTT after adding a carefully standardized amount of APC reagent, and results are expressed as the quotient (APC ratio) of the latter clotting time divided by the former.

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\text{APC resistance ratio} = \frac{[\text{aPTT with APC (seconds)}]}{[\text{aPTT without APC (seconds)}]}
\]

Patients with APCR usually have a ratio of <2.0 compared with a ratio of >2.0 for patients without APCR. The test was done using purified APC reagent which was obtained from purified human protein C activated with human thrombin which was subsequently completely removed before vialing. Protein C was purified from human plasma. The reagent was provided in a vial containing...
approximately 10 microgram of APC, freeze dried. The APC reagent was reconstituted with 1ml of distilled water, then 0.025 M CaCl2 was used to prepare a 1:10 dilution of the reconstituted reagent to obtain a final APC concentration of approximately 1 microgram/ml. A carefully standardized amount of APC was used for testing. The APC ratio indicates the severity of the APC-R and also detects thrombosis prone APC-R cases without the FV gene mutation. The high sensitivity and specificity of the APC ratio (approaching 100%), together with its reliability and ease with which it is performed, make it an adequate screening method.

**Statistical analysis:**

Data were statistically described in terms of mean ± Standard Deviation (±SD), median and range, or frequencies (number of cases) and percentages when appropriate. For comparing categorical data, Chi square ($\chi^2$) test was performed. Exact test was used instead when the expected frequency is less than 5. $p$-values less than 0.05 was considered statistically significant. All statistical calculations were done using computer program SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, United States of America (USA) release 15 for Microsoft Windows (2006).

**Results**

We compared both groups as regards many variables including demographic data, obstetric history and prevalence of APCR (FVL screening test).

Statistical studies between both groups showed no significant differences as regards maternal age with $p$-value >0.05.

Regarding APCR test results, abnormal APCR test values were found in a total of 13 cases in our study, 4 cases in the control group and 9 in the case group. No statistically significant differences were found between both groups as regards APCR test values (with $p$-values >0.05).

We then divided each of the control and case groups into two groups, the first with normal APCR test and the other with abnormal test result, to allow further studying of the impact of abnormal test on the pregnancy outcome.

**Control group:**

Analysis of the data obtained from the control group revealed that a total of 46 patients (92%) had a normal APCR test value (above 2) (Group A), while only 4 patients (8%) showed abnormal values (below 2) (Group B).

Demographic data showed no statistically significant differences regarding the age, number of abortions and number of successful pregnancies, while, significant difference was found as regards APCR test values, as shown in the (Table 1).

**Table (1): Comparison between both groups regarding age, number of abortions, number of successful pregnancies and APCR test value.**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Group A (n=46)</th>
<th>Group B (n=4)</th>
<th>$p$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>29.96 (±5.538) (20-40)</td>
<td>27.5 (±3.697) (24-32)</td>
<td>0.38 (NS)</td>
</tr>
<tr>
<td>Number of abortions</td>
<td>0.33 (±0.598) (min: 0, max: 1)</td>
<td>0.75 (±0.5) (min: 0, max: 1)</td>
<td>0.067 (NS)</td>
</tr>
<tr>
<td>Number of successful pregnancies</td>
<td>2.24 (±0.9)</td>
<td>1.5 (±0.58)</td>
<td>0.1 (NS)</td>
</tr>
<tr>
<td>APCR test value</td>
<td>3.1 (±0.8)</td>
<td>1.62 (±0.13)</td>
<td>&lt;0.00001 ($S$)</td>
</tr>
</tbody>
</table>

Data are presented as mean (±SD). NS: Not Significant. N: Number. S: Significant.

**Case group:**

Analysis of the data obtained from the case group revealed that a total of 41 patients (82%) had a normal APC resistance test value (above 2) (Group C), while only 9 patients (18%) showed abnormal values (below 2) (Group D).

Demographic data showed no statistically significant differences regarding the age, number of abortions, gestational weeks at abortion and number of successful pregnancies, while significant difference was found as regards APC resistance test values, as shown in the (Table 2).

**Table (2): Comparison between both groups regarding age, number of abortions, average gestational weeks at abortion, number of successful pregnancies and APC resistance test value.**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Group C (n=41)</th>
<th>Group D (n=9)</th>
<th>$p$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>30.46 (±5.9) (21-40)</td>
<td>29.56 (±5) (22-38)</td>
<td>0.64 (NS)</td>
</tr>
<tr>
<td>Number of abortions</td>
<td>3.71 (±1.6)</td>
<td>5 (±4.1)</td>
<td>0.16 (NS)</td>
</tr>
<tr>
<td>Average gestational week at abortion</td>
<td>8.3 (±1.8)</td>
<td>8.2 (±2.3)</td>
<td>0.94 (NS)</td>
</tr>
<tr>
<td>Number of successful pregnancies</td>
<td>1.14 (±0.36)</td>
<td>1.25 (±0.5)</td>
<td>0.6 (NS)</td>
</tr>
<tr>
<td>APC resistance test value</td>
<td>3.1 (±0.9)</td>
<td>1.6 (±0.27)</td>
<td>&lt;0.00001 ($S$)</td>
</tr>
</tbody>
</table>

Data are presented as mean (±SD). NS: Not Significant. N: Number. S: Significant.
In the case group, 21 patients with normal APC values experienced successful pregnancies, 18 only once and 3 twice. However, only 4 patients with abnormal APC values had successful pregnancies, 3 for only once and 1 has twice.

Discussion

In fact, the relationship between inherited thrombophilias including, FVL and RPL has been extensively studied since the 1990’s, however unfortunately, the results are highly conflicting. Most studies have been retrospective studies comparing the prevalence of FVL among RPL women and a control population. Some studies have espoused a link between the two, while others have refuted any association. The discrepancies concerning the study design, the type of control, the definition of the obstetrical event studied and the frequently small number of cases and controls enrolled, have contributed to the state of uncertainty. This was one of the important causes that motivated us to perform this study.

Although the number of patients with abnormal APC resistance test results (<2) was higher in the case group (9 out of 50 with 18% prevalence) than in the control group (4 out of 50 with 8% prevalence), while the mean test values were higher with the control group (3.03) than with the case group (2.86), however, none of these parameters showed any statistically significant difference. However, In spite of this, the relatively higher incidence of abnormal APCR test results in the case group, suggests that it may have a role in first trimesteric RPL.

These results agree with many studies who could not demonstrate any clear association between RPL and hereditary APCR [11-25].

In contrast, many studies did not agree with our study [26-31]. However, the accuracy of these studies may be limited by the variation between studies in many items including the definition of RPL, the number of women involved, the inclusion and exclusion criteria and the thrombophilias to be examined. For example, regarding the definition of RPL, unlike our study, some studies defined it as 2 or more pregnancy losses [27,28,30], which can be a cause of the different results. Also the main drawback of some studies was that they included pregnancy loss in general so did not differentiate between first trimesteric and late recurrent pregnancy losses which may affect the results also [28, 29,31].

As shown in (Table 1), in the control group, only 4 out of 50 (8%) had APCR as shown in (Table 2). Again number of abortions was higher in the group with abnormal results than those with normal results but also, no statistical significant difference was found. No statistically significant differences were found between both groups as regards the age, number of abortions and number of livebirths.

Of the 50 women in the case group who were included in our study, all had a clinically recognized pregnancy at least once. 25 out of 50 (50%) gave a history of at least one livebirth. Only 9 of them (18%) showed abnormal APCR test, while 41 (82%) had normal results as seen in (Table 2). By comparing both groups, the number of abortions was higher among the group with abnormal APCR test (5 vs. 3.7); however this didn’t show the statistical significance as shown in (Table 2). No statistically significant differences were found between both groups as regards the age, number of abortions, gestational weeks at abortion and number of livebirths as shown in (Table 2). It is to be noted that one of patients who had abnormal results gave a history of 16 abortions; all were first trimesteric, in addition to 2 episodes of Deep Venous Thrombosis (DVT).

As many studies, including those that found a significant association between FVL mutation and RPL, have noted that some women who carry the FVL allele have uncomplicated pregnancies, and that maternal carriage of this thrombophilic mutation does not, therefore, preclude a successful, uncomplicated live birth at term, they concluded that not all women who carry a thrombophilic mutation suffer a pregnancy loss and perhaps it is those who carry multiple thrombophilic defects who are at greatest risk [32]. Also, Dana Mierla et al., 2012, concluded that the incidence of FVL and Prothrombin gene mutation in female patients with fertility problems and RPL, is not significantly higher than that in the control group, but there was a significant correlation in case of combination between the two polymorphisms (p-value <0.05) [25].

Also, it should be noted that multiple studies and four meta-analyses suggest that FVL heterozygotes have a higher risk for late pregnancy loss than early first-trimester loss [33-35]. Also, a meta-analysis found that a heterozygous FVL mutation is associated with a twofold increased risk for a late unexplained fetal loss and a fourfold higher risk for loss in the second trimester compared to the first trimester [36].
From all the previously stated studies, it is obvious now that there is a great discrepancy in their results, which may partly be explained by selection bias, the small numbers of women that have been included in some studies, by genetic polymorphism, bias in patient selection, or ethnic heterogeneity among the patients studied. In spite of the negative association reported in some studies, possible association between FVL and Prothrombin G20210A mutations and RPL could not be completely ruled out, suggesting that the difference may become statistically significant if more patients and controls were recruited.

As a result of this great controversy, the ACOG (2013) has concluded that a definitive causal link cannot be made between inherited thrombophilias and adverse pregnancy outcomes. Similarly, the American College of Chest Physicians (ACCP) recently concluded that it was unclear whether screening for inherited thrombophilias is prudent in women with pregnancy complications [37].

Finally, in our study, we concluded that isolated FVL may increase the risk of RPL; however it is unlikely to be an important cause as no statistically significant difference in the incidence of RPL was found between the case and control groups.

References
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