Prevalence of activated protein C resistance among women with recurrent miscarriage

Vytautas Abraitis, Renata Šimoliūnienė1, Aušra Mongirdienė2, Said Makari
Clinic of Obstetrics and Gynecology, Kaunas University of Medicine, 1Department of Physics, Mathematics and Biophysics, 2Institute of Cardiology, Kaunas University of Medicine, Lithuania

Key words: recurrent miscarriage, activated protein C resistance.

Summary. Since 1996 activated protein C resistance is closely associated with various obstetric pathologies. The most widely discussed is that of secondary infertility due to recurrent miscarriage. However, there is still widespread discussion about the role of activated protein C resistance in this and other obstetric pathologies.

Aim. To investigate whether the activated protein C resistance is a cause of early recurrent miscarriage.

Material and methods. A study was designed as a case-control study. Two study groups were formed. Group I included women who have experienced 2 or more miscarriages (61 patients), and Group II included women who have experienced 3 or more miscarriages (33 patients). We investigated the prevalence and compared it in the control and both study groups.

Results. In Group I activated protein C resistance was found for 8 patients (14.7%), in Group II – for 5 patients (16.5%), in the control group – in 4 cases (5%). By comparing different groups the prevalence of activated protein C resistance in Group I was statistically significantly higher than in the control group (p<0.05). The prevalence of activated protein C resistance in Group II and the control group as well as between both study groups was statistically non-significant (p>0.05).

Conclusion. Activated protein C resistance might be a factor behind spontaneous recurrent miscarriage. There was no statistically significant difference between women who had suffered from 2 or 3 spontaneous abortions.

Introduction

Epidemiology

Prevalence: 15–20% of all parous women have experienced at least one miscarriage, 3% of all parous women have experienced 2 miscarriages, and finally 1% of all parous women have experienced three or more miscarriages (1–3). Furthermore, 40 to 70% of all parous women have experienced a miscarriage if we include biochemical pregnancies (pregnancies, which have been diagnosed by establishing an increased level of the human β chorionic gonadotropin without affecting the menstrual cycle, i.e. those pregnancies that have aborted until the beginning of the next menstrual cycle).

Etiology

Only chromosomal abnormalities have been definitely proven to be a reason behind early recurrent spontaneous miscarriages. There is still widespread dispute over the role of immunologic factors, variations in the anatomic structure of the uterus, congenital thrombophillias, infection and hormonal factors as etiologic factors for spontaneous recurrent miscarriages (4–15).

Some data suggest that with increasing age there is an increasing incidence of miscarriage (this may be related to the fact that there is no effective maturation of the ovum in women of increasing age) (16).

Factor V Leiden

Activated Protein C resistance (APC-R) was first mentioned by Dahlback et al in 1993 (17). There is a wide difference between the clotting system factors, which have been explored earlier and which determine a higher risk for venous thromboembolism, and the newly discovered ones. The above-
mentioned factors show a “loss of function”; there is a decrease in the synthesis of normal proteins (Type I) or there is a synthesis of abnormal proteins (Type II). That is why we have weakened anticoagulative effect. The mutation of the newly described factors (APC-R, prothrombin 20210, high levels of factor VIII) reflects the acquisition or strengthening of a certain function (for example APC-R becomes resistant to Protein C which thus becomes more stable). One more difference is that the newly described mutations are more widespread in the population than those earlier known defects of the coagulation system (the incidence of APC-R among European white women can be as high as 15%) Finally, the newly described mutations are remarkably weaker as risk factors for thromboembolism than those described earlier.

The most widespread is APC-R (18, 19). Acquired APC-R is related to the anticardiolipin antibody, lupus anticoagulant and the increased concentration of clotting factor VIII. Inherited APC-R is present due to the mutation in chromosome 1 in 95% of cases (1691 G → A 10 exon). That is why we have the synthesis of an altered Factor V molecule, i.e. APC-R (FV506 Arg-Gln or FV506Q). APC-R is the most common cause of inherited thrombophilias (40–50% of all cases).

**Mechanism of action of APC-R**

Damage of tissue in human leads to the activation of the clotting system – the cascade of serine protease and its cofactor, which later leads to the formation of fibrin (20). Clotting factors V and VIII are the main activating cofactors. Thrombin changes Factors V and VIII into their active forms – FVa ir FVIIIa, in turn these activated forms join on the negatively charged phospholipids, which are on the activated surface of the thrombocytes. Protein C is also a serine protease. Thrombin activates Protein C (PC), by aggregating with transmembrane thrombomodulin receptor on the surface of the undamaged endothelium. This Active Protein C (APC) with cofactor protein S by proteolysis inactivates Factors Va and VIIIa. APC-R is resistant to Protein C and remains active for a relatively long time. This is the reason behind hypercoagulability.

APC-R can be found in 40–80% of all pregnant women suffering from venous thrombosis. The most common cause of congenital AACP is Factor V Leiden (95%); the Cambridge factor is less common etiologic factor of inherited APC-R (this factor is further divided into subtypes, however they are usually found in very closed populations, for example in the Chinese population living in Hong Kong (21). Furthermore, this factor is an etiologic factor in only 5% of all cases of inherited APC-R. This is why in medical literature inherited APC-R is interchangeably used only with the Leiden factor (21–25). Other thrombophilias are very rare.

The German physiologist Virchow’s theory of the triad (1848) can explain the negative effect of the APC-R on the fetus:

1. Decreased blood supply.
2. Hypercoagulability.
3. Damage to the blood vessels.

During pregnancy there is a mechanical disturbance of the blood supply:

1. There is a decreased blood flow to the lower venous reservoirs due to compression by the enlarging uterus.
2. The likelihood for venous stasis is great in the deep veins (DV) of the left leg; this is because the left iliac vein is compressed by the right iliac artery.
3. Due to the hormonal changes and increasing blood volume that occur during pregnancy valves located in DV of the lower veins become insufficient.

Pregnancy itself is a hypercoagulable state. There is constant change of the clotting and fibrinolytic systems, especially during pregnancy; there is an increase in the concentration of clotting factors (I, II, VII, VIII, IX, XII), the concentration of Protein S decreases, the activity of protein C changes, (acquired APC-R occurs) (7, 26–28). All these changes tend to increase coagulability. Lindquist et al stated that these changes were the result of the evolution of a biological mechanism that was aimed at protecting the gravid woman from a life threatening hemorrhage during labor. However, this mechanism increases the risk for thromboembolic complications (29).

When the blood vessels of the growing chorion invade the capillaries of the endometrium, there is an activation of the extrinsic pathway of the coagulation cascade. This explains the appearance of thrombi that are found when histologically examining the chorionic material either after miscarriage or by examining the placenta after delivery.

The role of the Leiden factor in obstetrical and gynecologic pathologies is being widely investigated.
Obstetric pathology is classified according to the time of its onset: from conception to the 14th week – first trimester, 14–29 weeks – second trimester, 28–42 weeks – third trimester. There is a reason to believe that the APC-R influences pregnancy in all trimesters.

Material and methods
We evaluated 66 women who have had 2 or more miscarriages in the period from October 2001 to February of 2003. The study was carried out in the Clinic of Obstetrics and Gynecology of Kaunas University of Medicine. The sample size was determined according to the criteria of Chi Square (or Pearson). We evaluated the qualitative size. The sample size was determined according to published data and calculated using Epi-Info program.

The Ethics Committee at Kaunas University of Medicine endorsed the methods of the study (protocol no. 71/2002).

The study was designed as a prospective case-control study. The study group and control groups included respectively 61 women who have suffered 2 or more spontaneous miscarriages with no evident cause and 80 women who have successfully conceived and delivered. Spontaneous miscarriage was diagnosed in woman who had a documentation of their pregnancy (gestational age until 13 weeks) by ultrasound examination and by rising levels of beta human chorionic gonadotropin hormone. Women, whose miscarriage was associated with traumas, infectious states, karyotypic anomalies, and anatomic anomalies of the internal genitalia, were excluded from the study.

Women in both the control and study groups underwent the below-mentioned tests:
APC-R, anticardiolipin antibody (ACLA), Lupus anticoagulant (LA), INR (international normalized ratio), activated partial thromboplastin time (APTT), fibrinogen, and complete blood count (looking for thrombocytopenia, and possible hemolytic anemia).

Those women, who tested positive for ACLA and LA, were evaluated 6 to 8 weeks later.

Women in the study group additionally underwent the following tests:
1. Vaginal ultrasound examination to evaluate for possible anatomic variations of the inner genitalia. Anatomic anomalies were found in 3 cases: 1 - uterus bicornus, 1 - saddle-form uterus, and 1 - hypoplasia of the uterus.

2. Cytogenetic tests (Department of Biology of Kaunas University of Medicine and the Human Genetics Center) for both partners. The leucocyte culture from venous blood was evaluated. A karyotype anomaly was found in one case, consisting of a balanced Robertson translocation.

Single patient’s spouse did not agree to undergo testing for cytogenetic abnormalities and thus her results were not included in the statistic analysis of the data.

Determination of resistance to activated protein C. The analysis was carried out at the Institute of Cardiology at Kaunas University of Medicine. Inherited APC-R was established from the women’s plasma. Venous blood was taken and placed in a container with trisodium citrate (4.5 ml). The citrated blood was centrifuged at 2500 rpm for 30 minutes. The plasma was collected and frozen at −80°C using liquid nitrogen for a period not longer than 6 months. Before examining the blood sample, the plasma was thawed in a water basin for 15 min at a temperature of 37°C. The APC-R study was done using 2nd generation Diagnostica Stago method based APTT with an automated STACOMPACT coagulometer or semi automatic BIOS-4 coagulometer. The principle of this test is disproportionate prolongation of the clotting time, in the presence of APC and calcium. Before the test plasma is mixed with a factor V deficient plasma, to ensure the normal starting concentration of the other factors. Clotting is initiated with Croalus viridis helleri poison, which activates factor X. The sensitivity of the method for the mutation the Leiden V factor is 99.6%, specificity is 99.7%, positive prognostic value 99.2%, negative prognostic value 99.9%. The results and reliability of the method were not influenced by treatment using indirect acting anticoagulants, heparin and other preparations from the same group, lupus anticoagulant, other acquired or inherited defects of the coagulation system.

The plasma, whose clotting time was 120 sec or more, was APC-R negative, whereas plasma, whose clotting time was less than 120 sec, was APC-R positive.

Evaluation of the statistical data. The statistical analysis of the data was carried out using an IBM personal computer, with SPSS (Statistical Package for Social Sciences) 8.0 for Windows (Chicago, Illinois, USA) software. The data was stored using “MS Excel”. The significant difference in the qualitative size was calculated using Pearson
(Chi Square) criteria. The statistical analysis was done in two stages: the first time, when the first study group comprising women (61 cases), who had experienced 2 or more miscarriages, was formed, and the second time, when the second study group comprising women (33 cases), who had suffered three or more miscarriages, was formed.

Results

We investigated 61 patients in the Clinic of Obstetrics and Gynecology of Kaunas University of Medicine, who have been diagnosed with two or more spontaneous miscarriages (study group) and 80 healthy women who have successfully delivered and have not experienced a spontaneous miscarriage. The study group was further divided into 2 groups. Group I was comprised of women, who have had 2 or more spontaneous miscarriages (61 cases). The Group II was comprised of women, who have had 3 or more spontaneous miscarriages (33 cases).

Statistic analysis of the data was done separately in each group. The results of both study groups were compared with those of the control group (Table 1).

In women from study Group I the prevalence of APC-R was statistically significantly higher than in women of the control group. In study Group I it was 14.7%, in the control group 5%, p=0.0439 (p<0.05). Dependency grade (contingency coefficient) was 0.17 (Table 2). Between Group II and the control group there was no statistically significant difference, this can be due to the small number of cases included in the study. The difference of prevalence of APC-R in both study groups was statistically non-significant.

Discussion

According to the results of our study APC-R incidence is higher in women of study Group I compared with women in the control group. This result is statistically significant: in the control group – 5%, I study group – 14.75% (p<0.05). Between Group II and the control group there was no statistically significant difference (p>0.05), this can be due to the small number of cases included in the study. The prevalence of APC-R in the control group is similar to the prevalence of APC-R in the only study carried out in 1999 in Lithuania: 7.1% are APC-R positive in the healthy population. This somehow contradicts to what is commonly accepted in the publications of obstetricians, gynecologists, immunologists and clotting specialists, where the prevalence of APC-R is higher in late pregnancy miscarriage (14–21 gestational week). There is a no statistically significant relation between the prevalence of APC-R and those women who had suffered from 2, 3 or more spontaneous miscarriages (30). However, it is possible that certain racial or ethnic factors play a role here.

Table 1. Characteristics of the women participating in the study

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Study group I (2&lt;s. m.)</th>
<th>Study group II (3&lt;s. m.)</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average age</td>
<td>28.1</td>
<td>29.5</td>
<td>30.6</td>
</tr>
<tr>
<td>Age (years)</td>
<td>min. 18, max. 42</td>
<td>min. 20, max. 42</td>
<td>min. 22, max. 47</td>
</tr>
<tr>
<td>Average number of miscarriages</td>
<td>3.45</td>
<td>4.07</td>
<td>–</td>
</tr>
<tr>
<td>Miscarriages</td>
<td>min. 2, max. 9</td>
<td>min. 3, max. 9</td>
<td>–</td>
</tr>
<tr>
<td>Average number of deliveries</td>
<td>–</td>
<td>–</td>
<td>1.43</td>
</tr>
<tr>
<td>Number of deliveries</td>
<td>–</td>
<td>–</td>
<td>min. 1, max. 3</td>
</tr>
</tbody>
</table>

s. m. – spontaneous miscarriage.

Table 2. The comparison of the prevalence of APC-R in Study Groups I and II in addition to the control group

<table>
<thead>
<tr>
<th>Group</th>
<th>Study group I n=61</th>
<th>Control group n=80</th>
<th>p</th>
<th>Study group II n=33</th>
<th>Control group n=80</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>APC-R</td>
<td>9</td>
<td>4</td>
<td>&lt;0.05</td>
<td>5</td>
<td>4</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>
However, whether APC-R might be an etiologic factor behind spontaneous miscarriage is still controversial (31, 32).

It is evident that the results for the markers of thrombophilias are identical in the study Group I and the control group, as well as in the control group and the study Group II. Even though recurrent miscarriages are diagnosed when a woman experiences three or more miscarriages, the problem should be addressed after the second miscarriage, since woman react very sensitively to this problem of reproductive health. An article published in 1995 compared two groups of women: in one group women, who had conceived after two spontaneous abortions, were included, and in the second group - women after three spontaneous abortions. No preventive measures were applied to them, even though prophylactic anticoagulants have already been described in literature. In the first group 32% of women carried to term in comparison with only 11% of women in the second group.

**Conclusions**

The incidence of APC-R is statistically significantly higher in women in study Group I (2 or more recurrent miscarriages) – 14.7% than in women in the control group – 5% (p<0.05), however the dependency is small (contingency coefficient 0.17). There was no statistically significant difference in the incidence of APC-R between women who had 2 early recurrent miscarriages or those who had 3 early recurrent miscarriages.

**Prevalence of activated protein C resistance among women**

Vytautas Abraitis, Renata Šimoliūnienė, Aušra Mongirdienė, Said Makari

Kauno medicinos universiteto klinikų Akušerijos ir ginekologijos klinika, Kauno medicinos universiteto 1Fizikos, matematikos ir biofizikos katedra, 2Kardiologijos institutas

Raktažodžiai: trys ir daugiau iš eilės savaiminių persileidimų persileidimas


Medžiaga ir metodas. Atliktas atvejo-kontrolės tyrimas. Sudarytos dvi tiriamųjų grupės (pirma grupė: du ir daugiau persileidimų patyriusios moterys – 61 pacientė; antra grupė: tris ir daugiau savaiminių persileidimų patyriusios moterys – 33). Kontrolinė grupė – 80 moterų. Ištirtas ir palygintas atsparumo aktyvuotam C proteinui paplitimas tiriamosiame ir kontrolinėje grupėse. Rezultatai. Atsparumas aktyvuotam C proteinui nustatytas: I grupėje – 8 tiriamosios (14,7 proc.), II grupėje – 5 (16,5 proc.) kontrolinėje grupėje – 4 tiriamosios (5 proc.). Lyginant tiriamųjų grupes, atsparumo aktyvuotam C proteinui paplitimas statistiškai patikimai didesnis pirmoje tiriamųjų grupėje (p<0,05). Lyginant kontrolinę ir antrąją tiriamųjų grupes nenustatyta statistiškai patikimo skirtumo (p>0,05), manome, dėl per mažo tiriamųjų skaičiaus šioje grupėje. Atsparumo aktyvuotam C proteinui paplitimas, palyginus pirmą ir antrą tiriamųjų grupes, statistiškai patikimai nesiskyrė (p>0,05). Išvados. Atsparumo aktyvuotam C proteinui paplitimas pirmoje tiriamųjų grupėje statistiškai patikimai didesnis negu kontrolinės grupės (p<0,05). Atsparumo aktyvuotam C proteinui paplitimas, palyginus pirmą ir antrą tiriamųjų grupes, statistiškai patikimai nesiskiria (p>0,05).

Adresas susirašinėjimui: V. Abraitis, KMUK Akušerijos ir ginekologijos klinika, Eivenių 2, 3007 Kaunas El. paštas: v.abraitis@delfi.lt

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