Research Article

The Association of Activated Protein C Resistance (aPCR) with Recurrent Pregnancy Loss in Syrian Population

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ABSTRACT

Recurrent Pregnancy Loss (RPL) is a multifactorial condition, defined as two or more consecutive pregnancy losses. After chromosome abnormality, thrombophilia is one of the most important genetic factors that could cause RPL. Our Objective is to evaluate the prevalence of aPCR in Syrian women with RPL compared with women who had uneventful pregnancies. This case control study was conducted to evaluates the frequency of aPCR in 100 women with RPL (who have two or more pregnancy losses), compared with 100 women without adverse pregnancy outcome. The presence of aPCR in RPL group was 30% while it was 11%t in the control group. This study has shown that aPCR (regardless of FVL mutation) is significantly associated with RPL compared with controls ($P<0.05$). These data support the hypothesis that thrombophilia accompanied with aPCR may plays a role in the pathophysiology of primary RPL, and suggest that attention should be directed at screening women with recurrent pregnancy loss for aPCR assays.

Keywords: Activated Protein C Resistance, aPCR, Thrombophilia, Recurrent pregnancy loss, RPL.

INTRODUCTION

Recurrent pregnancy loss (RPL), either early or late in the gestational period, is a serious problem and has both psychological and social impacts on the women who suffer from it. In some cases, it may lead to divorce or other social problems[1]. Recurrent Pregnancy Loss (RPL), defined as two or more consecutive pregnancy losses, affecting 1–5 % of reproductive-age woman[2,3]. There is a strong belief that RPL is a multifactorial condition that many factors affect such as chromosomal abnormalities, uterine anatomic malformation, endocrine dysfunction, thrombophilia, immunologic factors, infections, and environmental factors[4-7]. However, the etiology of RPL remains unknown in ~50 % of cases[8-11].

Pregnancy is a hypercoaguable state secondary to an increase in the concentrations of pro-coagulant factors, a reduction in the concentrations of the naturally occurring anticoagulant proteins and a decrease in fibrinolysis[12]. A successful pregnancy requires the development of adequate placental circulation.

Thrombophilia was identified as a major cause of recurrent pregnancy loss (RPL), after chromosomal abnormalities with a rate of up to 40%, especially in the first half of pregnancy[13]. Although numerous studies are available in literature thrombophilia rate seems to vary from study to another due to different selection criteria of patients[14].

Multiple studies have shown that thrombophilia increase the risk of recurrent first and second trimester pregnancy losses through thrombosis of the placental bed[15-18].

Thrombophilia describes an increased tendency to develop thrombosis, either venous or arterial. Thrombophilias may either be inherited or acquired and include protein C deficiency, protein S deficiency, antithrombin deficiency, and the less potent factor V Leiden (FVL) and prothrombin gene mutation (PGM). The combined prevalence of these thrombophilias in the general population exceeds one in ten[19-25]. It is hypothesised that thrombophilias may increase the risk of placental insufficiency because of placental micro- and/or macro-vascular thrombosis, as well as effects on trophoblast growth and differentiation[26-28].

Activated protein C resistance (aPCR) is the most frequent thrombophilic defect associated with venous thrombosis[4,29]. More than 90% of the APC resistance phenotype can be explained by the FVL mutation. This defect is caused by a single point mutation (G-A) at nucleotide position 1691 in the factor V gene resulting in a replacement of Arg by Glu residue[30-33].

The pathophysiology underlying aPCR not caused by the FVL mutation is still not completely understood. In different studies, it has been suggested that acquired factors might be the cause of APCR in the absence of FV Leiden[34,35]. A number of coagulation factors can affect the activated partial thromboplastin time (aPTT). Previous literature suggested a possible positive correlation between levels of factors V, VIII and IX and acquired aPCR. Protein S and protein C, levels can (or may) affect acquired aPCR, but their influence on the resistance seems to be still within the range of normal levels[36-38].

The functional aPCR assay, measures the ratio of APTT clotting times in the presence and absence of a standard amount of exogenous APC. The APC-resistant phenotype is characterized by a minimal prolongation of the APTT in response to activated protein C and a correspondingly low ratio.

The functional assay has a very high sensitivity and specificity for FVL[39,40]. However, it will not identify the rare patient with aPCR not due to factor V abnormalities[41].

Our Objective is to evaluate the prevalence of aPCR and factor V Leiden and its relation in a group of Syrian women with recurrent pregnancy loss.

MATERIALS AND METHODS

In this case-control study the frequency of aPCR were determined in a consecutive series of 100 women referred to Orient Hospital and Maternity Hospital for evaluation of recurrent spontaneous pregnancy loss (case patients) between October 2013 and October 2015.

The control group included 100 women from the same ethnic background and with at least one successful pregnancies and no history of pregnancy loss, which matched by age with patients.

All women with known independent risk factor for pregnancy complication, such as uterine malformation, systemic disease (Diabetes mellitus, Lupus erythematosus), endocrine abnormality (Prolactin, Thyroid Stimulating Hormone, Follicular Stimulating Hormone and Luteal Hormone during the early follicular phase), and women who received induced abortion upon their request were excluded, in addition to the women with other thrombophilic defects, such as antiphospholipid antibodies syndrome (Lupus anticoagulant, Anticardiolpin), or deficiency of activities of antithrombin III, protein C and protein S were also excluded.

72 patients were with primary RPL (they had never delivered a viable fetus), and 28 patients were with secondary RPL (they had delivered a viable fetus and then experienced recurrent pregnancy loss).

18 patients had two miscarriages, 38 had three, and 44 had more than three.

A full and thorough clinical history was taken from patients, including their demographic details. Past and present history associated with any infection, medical disease, and any gynaecological problem, previous or present history of thrombophilia. Ethnically, women of only Syrian origin from paternal and maternal sides were included.

Venous blood samples were collected with minimal stasis using a 19-gauge butterfly needle into 0.109 mol/l *Vacuette* trisodium citrated blood tube (*Grenier* Bio-One International AG, Kremsmünster), STA-clot Test was used in order to Identify APCR Samples. Blood samples were centrifuged at 4,000 rpm for 5 min, and aliquots of platelet-poor plasma were frozen at −80 °C until the assay took place.

An activated protein C resistance (aPCR) assay was performed using an activated partial thromboplastin time (aPTT)–based assay.

The aPTT was measured in the presence and absence of activated protein C and in a calcified medium using an STA-Staclot aPCR kit (Diagnostica Stago, France).

The test was performed with undiluted patient plasma. Plasmas whose clotting times obtained with the STA-Staclot aPCR kit, measured using STA COMPACT fully automated analyzer (Diagnostica Stago, France), are equal to or greater than 120 seconds are regarded as aPCR Negative, on the other hand, plasmas whose clotting times are less than 120 seconds are regarded as aPCR Positive.

According to our experience (unpublished data) the Leiden mutation was never found if the APC–SR was higher than 120 seconds.

In all subjects, prothrombin time (PT) and activated partial thromboplastin time (aPTT) were determined using commercial reagents.

A full blood count was carried out on the EDTA anticoagulated sample using the sysmex KX 21 N Hematology analyzer.

Cellulose acetate hemoglobin electrophoresis at alkaline pH was used to determine hemoglobin phenotypes while ABO and Rhesus blood types were determined using the tube method.

All Participants gave informed consent, according to the protocol approved by local Ethics Committee at Damascus University and health ministry.

Data was analyzed using SPSS V23. Continuous variables like age of patients were expressed as mean ± standard deviation (SD), whereas categorical data was expressed in the form of frequency and percentage.

Any association was analyzed by Chi- square test. $P<0.05$ was considered statistically significant. Odd ratio and 95% confidence intervals (CI) were calculated.

Convenience sampling was employed and sample size was calculated using matched case-control situation.

RESULTS

In cases 100 women with RPL were evaluated, which their mean age was 28.2±6.7 years (range 16-41). In controls mean age was 26.6±6.4 years (range 17-39).

Differences between ages of cases and controls was not significant ($p>0.05$).

The aPCR was found in 30 out 100 RPL patients and in 11 out of 100 controls as mentioned in table (1). Compared

with parous controls, acquired aPCR was significantly more common among patients with RPL (OR:3.47). The aPCR was revealed in 27 out 72 primary RPL patients (37.5%) and in 4 out 28 secondary RPL patients (14.3%).

Table 1: Illustrated the distribution of aPCR in recurrent pregnancy loss.

Variable	Number	aPCR n (%)	Odd ratio (CI 95%)*	P-Value**
Controls	100	11 (11%)		
RPL patients	100	30 (30%)	3.47 (CI:1.62-7.40)	0.001411
Primary RPL patients	72	27 (37.5%)	4.86 (CI:2.21-10.67)	0.000064
Secondary RPL patients	28	4 (14.3%)	1.35 (0.45-3.77)	0.74

*Odd ratio (Confidence Interval: 95%); **Fisher's two-sided exact P

DISCUSSION

The fate of fetus is highly affected by the placental development and function, which, in turn depend upon the development of an adequate maternal-fetal circulation[42].

Resistance to APC is the commonest genetic defect known to confer a predisposition to thrombosis, occurring at least ten times more frequently than other hereditary defects[43].

In 1993, a Swedish research team led by B. Dahlbäck recognized an unusual phenomenon affecting the coagulation system. They were studying the effect of addition of external APC to plasma of patients with VTE. Normally, APC should inactivate clotting Factor V (FV) and therefore slow down the coagulation process. However, in certain patients studied by Dahlbäck and his team, this slowing down did not occur. They called this phenomenon "aPCR", and they originally though this could be due a deficiency in a yet unknown protein that co-helps aPC in inactivating FV[44]. One year later, another group of researchers from Holland, led by R. M. Bertina, discovered a missense point mutation in the FV gene, where adenine (A) replaced guanine (G) at nucleotide position 1691 of exon 10 of the FV gene, only eleven nucleotides upstream of the beginning of intron 10. They called this mutation as FV Leiden mutation (FVL) after the Dutch city where they made their discovery in[30]. This nucleotide replacement happened to be in the codon for the amino acid residue arginine 506 (CGA) normally present in the factor V molecule, creating a new codon (CAA) which is translated as glutamine.

In order to inactivate FV, APC needs to recognize arginine at position 506 of the FV molecule. Because of the amino acid change in FVL, aPC can no longer inactivate FV efficiently, but FV retains its coagulation capabilities and therefore carriers of FVL develop hypercoagulability[45,46].

RPL occurs due to multiple etiologies; genetic factor is considered one of those etiologies. Advances in molecular genetics technology provide an accurate and reliable tool for precise study of the genetic abnormalities associated with RPL. The role of some thrombophilia in foetal loss has been well studied in different populations[47-49]. On the other hand, the role of aPCR is still under debate, and geographical differences also account for the variation in the reported allele frequency of aPCR and factor V Leiden[50-52]. Therefore, it is of great importance to explore the association between aPCR and RPL.

The frequency of aPCR has been reported as approximately 5% in the general Caucasian population[53]. This varies from 1% to 15% in different countries with a frequency of 3% reported in Italy and Spain and a frequency of 15% reported in Northern Sweden[54]. aPCR in the general population and during pregnancy is reported to be most frequently caused by FVL mutation[55,56] inherited aPCR.

The prevalence of aPCR was tested and calculated in both case and control groups. The presence of aPCR in RPL group was 30% while it was 11%t in the control group. This study has shown that aPCR (regardless of FVL mutation) is significantly associated with RPL compared with controls (P=0.0014).

It is of interest to note that the aPCR constitutes a major risk factor if Primary RPL is considered in comparison with control (OR:4.86), while the aPCR constitute a minor risk factor if secondary RPL is considered in comparison with control (OR: 1.35).

Our results documented a clear association between aPCR and RPL (OR: 3.47). Such findings are consistent with those of Rai[57] who reported on APC resistance in 1111 recurrent losses, and found that 15.5 % of their cases were APC resistant, compared to 11.3 % of the controls, and resistance was due to FVL mutation in 6.7 % of the patients and 8 % of the controls.

However, failure of documentation of the association between aPCR, FVL and RPL has also been reported by several other investigators[58-60]. Kujovich reported that FVL is associated with a 2–3-fold increased relative risk of pregnancy loss and other obstetric complications[61]. Some evidence suggests that FVL is more responsible for late pregnancy loss than for early first trimester loss[62,63].

However, it appears logical to assume that an acquired aPCR would certainly be amplified by pregnancy, which also induces an aPCR state[64]. This may lead to placental thrombosis and infarction and consequent pregnancy loss.

Although 95% of cases of aPCR reflect the presence of the factor V Leiden mutation, 5% of individuals have repeatedly abnormal aPCR tests in the absence of the factor V Leiden allele. Depending on the screening assay used, some cases may represent acquired aPCR caused by high factor VIII levels, pregnancy, Use of hormonal contraceptives or a lupus anticoagulant effect[65, 66]. Another reports have shown, however, that between 5 and 10% of APCR in Caucasians does not involve the FVL, and the cause of positive APCR in these cases is not known[67-69].

CONCLUSION

According to our knowledge, this is the first report highlighted the relation of aPCR with RPL in Syrian population. In conclusion, our data support the hypothesis that thrombophilia accompanied with aPCR may plays a role in the pathophysiology of RPL[70,71], and suggest that attention should be directed at screening women with recurrent pregnancy loss associated with placental thrombosis for aPCR assays which are advantageous as they are easily automated and cost effective.

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