



Anticardiolipin antibodies in pathogenesis of infertility

Antikardiolipinska antitela u patogenezi steriliteta

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Abstract

Background/Aim. Antiphospholipid syndrome (APS) is an autoimmune disorder clinically characterized by arterial or venous thrombosis and/or specific obstetric complications and presence of antiphospholipid antibodies (aPL) in the serum. It occurs in 0.3% of pregnant women, while 1% of them have two spontaneous abortions. The aim of this study was to analyze the frequency of antiphospholipid antibodies in pregnant women with recurrent spontaneous abortions. **Methods.** We analyzed 60 pregnant women who had two or more recurrent miscarriages. The control group included 60 healthy pregnant women. We analyzed titres of anticardiolipin (aCL) IgG and/or IgM with high titres (> 20 U/mL), lupus anticoagulant (LAC) antibodies and anti-beta-2 glycoprotein (b₂-GP1) IgG as well as parameters of coagulation status of pregnant women. **Results.** Analyzing Spearman's rank correlation coefficient in a group of affected patients, we noticed a slightly positive correlation of lupus anticoagulants (LAC) with aCL antibodies of both classes, while the correlation with b₂GP1 IgG was negative. Both classes of aCL antibodies and anti-b₂GP1 IgG were in a discrete positive correlation with the given variables. In the control group, there was a lack of consistency in correlation of the study variables with LAC-aCL IgG, compared to the affected patients, and there was a standard negative coefficient of correlation

with anti-b₂GP1 IgG. The correlation ratio of anti-b₂GP1 IgG was negative for all studied test parameters. Analysis of hemostatic parameters showed a statistically significant difference in the concentration of fibrinogen ($p < 0.01$) and thrombocyte count ($p < 0.05$) between the study and the control group of pregnant women. Lower mean values of fibrinogen (2.90 ± 0.45 g/L) and lower thrombocyte count [$(179.20 \pm 6.00) \times 10^9$] were found in the study group of pregnant women with secondary infertility compared to the mean values of fibrinogen (3.60 ± 0.55 g/L) and thrombocyte count [$(236.05 \pm 5.10) \times 10^9$] in the control group. Activated partial thromboplastin time and prothrombin time were statistically significantly prolonged ($p < 0.05$) in pregnant women with spontaneous abortions compared to controls, suggest anticoagulant activity. **Conclusion.** In pregnant women with spontaneous abortions compared to healthy pregnant women slightly positive correlation of LAC with aCL antibodies of both classes, as well as a positive correlation of aCL antibodies with anti-b₂GP1 IgG exist. On the other hand, hemostatic parameters values suggest an anticoagulant status in the blood of pregnant women with spontaneous abortions.

Key words:

pregnancy complications; abortion, spontaneous; antiphospholipid syndrome; antibodies, antiphospholipid; pregnancy outcome.

Apstrakt

Uvod/Cilj. Antifosfolipidni sindrom (APS) je autoimunski poremećaj koji se klinički odlikuje arterijskom ili venskom trombozom i/ili specifičnim opstetričkim komplikacijama i prisutnošću antifosfolipidnih antitela (aPL) u serumu. Javlja se kod 0,3% trudnih žena, dok 1% njih doživi dva spontana pobačaja. Cilj rada bio je da se ispita učestalost pojave antifosfolipidnih antitela u populaciji trudnica sa višestrukim spontananim pobačajima. **Metode.** U ispitivanje je bilo uključeno 60 trudnica sa dva ili više spontananih pobačaja. Kontrolnu grupu činilo je 60 trudnica bez spontananih pobačaja. Pratili smo titrove antikardiolipinskih antitela (aCL) IgG i/ili IgM, u srednjem ili visokom titru (> 20 IU/mL), lupus antikoagulans (LAC) antitela, kao i anti-beta2-glikoproteina (b₂GP1). Takođe, određivali smo parametre

koagulacijskog statusa ispitivanih trudnica. **Rezultati.** Analizom Spearmanovog koeficijenta korelacije u grupi obolelih uočili smo blago pozitivnu korelaciju lupus antikoagulansa (LAC) sa kardiolipinskim antitelima obe klase, dok je korelacija bila negativna u odnosu na anti-b₂GP1 IgG. Obe klase aCL antitela i anti-b₂GP1 IgG diskretno su pozitivno korelirali sa datim varijablama. U kontrolnoj grupi ispitivane varijable nisu pokazale doslednost korelacije u odnosu LAC – aCL IgG kao kod obolelih, uz standardno negativan koeficijent korelacije prema anti-b₂GP1 IgG. Odnos korelacije anti-b₂GP1 IgG bio je negativan prema svim ispitivanim parametrima testa. Analizirajući hemostatske parametre utvrdili smo da postoji statistički značajna razlika u koncentraciji fibrinogena ($p < 0,01$) i broju trombocita ($p < 0,05$) između ispitivane i kontrolne grupe trudnica. Niže prosečne vrednosti fibrinogena ($2,90 \pm 0,45$

g/L), odnosno manji prosečni broj trombocita $[(179,20 \pm 6,00) \times 10^9/L]$ bili su zastupljeni u ispitivanoj grupi trudnica sa sekundarnim sterilitetom u odnosu na kontrolnu grupu sa prosečnim vrednostima fibrinogena $(3,60 \pm 0,55 \text{ g/L})$ i brojem trombocita $[(236,05 \pm 5,10) \times 10^9/L]$. Aktivisano parcijalno trombotično vreme i protrombinsko vreme, statistički su značajno bili produženi, ($p < 0,05$) kod ispitivanih trudnica sa spontanim pobačajima u odnosu na kontrolnu grupu trudnica. **Zaključak.** Kod trudnica sa spontanim pobačajima u odnosu na trudnice sa normalnom

trudnoćom postoji blago pozitivna korelacija LAC sa aCL antitelima obe klase kao i pozitivna korelacija aCL antitela i anti-b2GP1 IgG. S druge strane, vrednosti hemostatskih parametara ukazuju na prisustvo antikoagulantnog stanja u krvi trudnica sa spontanim pobačajima.

Ključne reči:

trudnoća, komplikacije; abortus, spontani; antifosfolipidni sindrom; antitela, antikardiolipinska; trudnoća, ishod.

Introduction

Repeated spontaneous abortion is an extremely difficult experience for a couple and, at the same time, a tremendous challenge for their doctor. A habitual abortion is the most common complication associated with pregnancy. It is defined as three or more consecutive spontaneous abortions prior to 20 weeks' gestation¹. It affects 0.3% of pregnant women while 1% of them experience two miscarriages. According to epidemiological data, repeated spontaneous abortions will occur in 24% of pregnant women that had already two miscarriages, 30% of pregnant women that already experienced three miscarriages and in 40% of those that already had four miscarriages².

Antiphospholipid or Hughes syndrome (APS) is an autoimmune disease that is characterized with the presence of the so-called antiphospholipid (aCL) antibodies and the occurrence of arterial and venous thrombosis (blood clots), recurrent spontaneous abortions, preterm delivery and thrombocytopenia (low platelet count). The syndrome occurs due to autoimmune production of antibodies against phospholipids (cell membrane substance), in particular against cardiolipin and beta2-glycoprotein (beta2-GP)³. The disease is also known as sticky blood syndrome or antiphospholipid antibody syndrome, and it was first described by a British rheumatologist Dr. Graham Hughes in the British Medicinal Journal in 1983. The term "primary antiphospholipid syndrome" is used when the disease occurs in the absence of any other related disease. If another autoimmune disorder coexists (like systemic lupus erythematosus – SLE), it is referred to as secondary APS. In rare cases, generalised thrombosis and consequential massive organ failure can occur as a complication, and the disorder is then referred to as the catastrophic antiphospholipid syndrome. The cause of this disorder is not known. Antiphospholipid antibodies decrease the level of annexin V, a protein that binds phospholipids and has a strong anticoagulant effect; therefore, the tendency towards blood clotting and spontaneous abortions, associated with this condition, is increased. In the blood of a patient with anti-APS, different antiphospholipid antibodies can be found. Common tests include anticardiolipin (aCL) antibody test, lupus anticoagulant (LAC) test and VDRL/RPR (test for syphilis that can be false positive in these patients)⁴. Results range from "weakly positive" to "strongly positive". Antiphospholipid antibodies can be found in healthy people in approximately 2% of population. They can also be tempo-

rarily present in blood during an infection caused by bacteria, viruses, or parasites. Some drugs can also cause their presence in blood: antibiotics, cocaine, hydralazine, procainamide and quinine. Treatment of a person with Hughes syndrome must be individualized and according to the present symptoms. Since a great number of manifestations are related to abnormal thrombocyte aggregation, the treatment is directed towards the prevention of coagulation of blood and its dilution. Treatment of this condition includes anticoagulants (drugs that dilute blood), like warfarin, coumarin or low-molecular mass heparin. Aspirin also prevents thrombocyte aggregation. Treatment also includes corticosteroids, e.g. prednisone. It has been noticed that application of the drug hydroxychloroquine in patients with systemic lupus erythematosus and anticardiolipin syndrome protects against coagulation of blood. An intravenous therapy of gamma globulin for a group of patients with a history of pregnancy loss and in patients with low thrombocyte count after pregnancy is also applied⁵. Patients with recurrent miscarriages and family history of deep vein thrombosis, pulmonary embolism, stroke or heart attack require gene testing for Factor V Leiden and prothrombin G20210A mutation.

The aim of this paper was to investigate the frequency of antiphospholipid antibodies in population of pregnant women with recurrent spontaneous abortions.

Methods

We analyzed 60 pregnant women with the average age of 29.09 ± 3.2 years, hospitalized in the Department of Fertility Control, Clinic of Gynecology and Obstetrics, Clinical Center Kragujevac, in the period from 2004 to 2008. The patients had two or more recurrent miscarriages, and they were included into the study after other causes of spontaneous abortions had been excluded. The control group consisted of 60 healthy pregnant women with the average age of 27.1 ± 2.95 years. Lupus anticoagulant was detected by kaolin clotting time (KCT), while the ratio values of the preliminary: final titre ≥ 1.3 were considered positive. Normal values for this method are 25–35. Analytic aCL ELISA tests by INEP were used for detection of aCL. A detection limit, precision and linearity were determined. Normal values for aCL IgM are $< 13 \text{ U/mL}$, while for aCL IgG they are $< 20 \text{ U/mL}$. Values of b2GP1 IgG were determined using the ELISA Zy-mutest test anti-b2GP1 IgG by the Aniera Corporation. Lupus anticoagulant was investigated after the viability of the

fetus had been confirmed by an ultrasound examination prior to 12 weeks' gestation. In addition to detection of LAC, prothrombin time (PT), activated partial thromboplastin time (PTT), fibrinogen and thrombocyte count were also investigated. Prothrombin time, activated PTT and fibrinogen were determined by the commercial tests IL (Instrumentation Laboratory). Prothrombin time was expressed as percentage, while PTT in seconds. Thrombocytes were determined on a standard hematological counter Coulter ONYX. Statistical analysis was performed by using of the χ^2 test and Mann-Whitney test. The laboratory data were presented as a mean value \pm standard deviation (SD). The test values of $p < 0.05$ were considered statistically significant.

Results

The results of the coagulation status, LAC, aCL antibodies and anti-b2GP1 IgG and IgM are presented in the Tables 1 and 2.

relation of lupus anticoagulants (LAC) with aCL antibodies of both classes, while the correlation with b2GP1 IgG was negative. Both classes of aCL antibodies and anti-b2GP1 IgG were in a discrete positive correlation with the given variables. In the control group, there was a lack of consistency in correlation of the study variables with LAC-aCL IgG, compared to the affected patients, and there was a standard negative coefficient of correlation with anti-b2GP1 IgG. The correlation ratio of anti-b2GP1 IgG was negative for all studied test parameters.

Discussion

Antiphospholipid syndrome is an autoimmune disorder clinically characterized by arterial or venous thrombosis and/or specific obstetric complications and the presence of aPL antibodies in the serum. Antiphospholipid antibodies involve a heterogeneous group of antibodies of different reactivity mainly directed against phospholipid-binding proteins, alone or in complex with phospholipids like cardiolipin, phos-

Table 1
Coagulation status in the group of patients with recurrent spontaneous abortions (the study group) and in health pregnant women (the control group)

Parameters	Study group (n = 60)	Control group (n = 60)	p
Fibrinogen (g/L)	2.90 \pm 0.45	3.60 \pm 0.55	< 0.01
Prothrombin time (%)	88.10 \pm 2.64	78.60 \pm 2.35	< 0.05*
Activated partial thromboplastin time (s)	36.10 \pm 3.90	26.95 \pm 2.60	< 0.05
Thrombocyte count ($\times 10^9/L$)	179.20 \pm 6	236.05 \pm 5.1	< 0.05

Note: results are presented as $\bar{x} \pm SD$; Mann Whitney test

Table 2
Spearman's rank correlation of studied parameters in the group of patients with recurrent spontaneous abortions (the study group) and in health pregnant women (the control group)

Parameters	Patients	Parameters			
		LAC	aCL IgG	aCL IgM	anti-b ₂ GP1
LAC	Study group	1	0.053	0.162	-0.062
	Control group	1	-0.034	0.324	-0.177
aCL IgG	Study group	-0.054	1	0.491	0.337
	Control group	-0.034	1	0.026	-0.020
aCL IgM	Study group	0.162	0.491	1	0.222
	Control group	0.324	0.026	1	-0.334
Anti-b ₂ GP1 IgG	Study group	-0.062	0.337	0.222	1
	Control group	-0.177	-0.020	-0.334	1

LAC – lupus anticoagulant; aCL – anticardiolipin IgG antibodies; aCL/IgM – anticardiolipin IgM antibodies; anti-b₂GP1 – anti-beta2 glycoprotein 1 IgG

Analysis of hemostatic parameters showed a statistically significant difference in the concentration of fibrinogen ($p < 0.01$) and thrombocyte count ($p < 0.05$) between the study and the control group of pregnant women. Lower mean values of fibrinogen (2.90 \pm 0.45 g/L) and lower thrombocyte count [(179.20 \pm 6.00) $\times 10^9$] were found in the study group of pregnant women with secondary infertility compared to the mean values of fibrinogen (3.60 \pm 0.55 g/L) and thrombocyte count [(236.05 \pm 5.10) $\times 10^9$] of the control group. Activated partial thromboplastin time and prothrombin time were statistically significantly prolonged, ($p < 0.05$) in pregnant women with spontaneous abortions compared to controls, suggesting an anticoagulant activity.

Analyzing Spearman's rank correlation coefficient in a group of affected patients, we noticed a slightly positive cor-

relation of lupus anticoagulants (LAC) with aCL antibodies of both classes, while the correlation with b2GP1 IgG was negative. Both classes of aCL antibodies and anti-b2GP1 IgG were in a discrete positive correlation with the given variables. In the control group, there was a lack of consistency in correlation of the study variables with LAC-aCL IgG, compared to the affected patients, and there was a standard negative coefficient of correlation with anti-b2GP1 IgG. The correlation ratio of anti-b2GP1 IgG was negative for all studied test parameters.

C, protein S, annexin V, thrombomodulin or kininogen has not yet been fully understood⁷.

The theory that aPL antibodies play a vital role in pathogenesis of APS is founded in the results of investigations on animal models. Namely, it has been shown that a passively transferred aPL antibodies into a normal mouse cause clinical manifestations of human APS. The most probable mechanisms by which aPL antibodies induce thrombosis can be divided into those in which these antibodies interfere with maintenance of coagulation homeostasis and those in which they induce the activation of endothelial cells, monocytes and thrombocytes. Since many people with high levels of aPL antibodies never develop thrombosis, it is assumed that an additional risk factor is needed, such as trauma, long immobilization, oral contraceptive, pregnancy (hypercoagulable state) or an infection that leads to activation of endothelial cells. The most probable mechanism by which aPL antibodies induce a spontaneous abortion is through interaction with placental annexin V and complement activation. Recent researches show that the presence of aPL antibodies presents an independent risk factor for arteriosclerosis, most probably through binding to b2GP1 which is found in many lipoprotein fractions including oxidised LDL^{7,8}.

Analyzing Spearman's rank correlation coefficient in a group of affected patients, we noticed a slightly positive correlation of LAC with cardiolipin antibodies of both classes, while the correlation with b2GP1 IgG was negative. Both classes of aCL antibodies and anti-b2GP1 IgG were in a discrete positive correlation with the given variables. In the control group, there was a lack of consistency in correlation of the study variables with LAC-aCL IgG, compared to the affected patients, and there was a standard negative coefficient of correlation with anti-b2GP1 IgG. The correlation ratio of anti b2GP1 IgG was negative for all studied test parameters.

The presented results were expected because two contradictory options were compared in the different groups of patients⁸. The literature gives a high positive rank correlation in cases when variables of patients with primary and secondary APS are compared, which is, again, expected because these are different modalities of two similar pathological conditions.

A number of researches have shown that aCL IgA antibodies and their ratio with other parameters in APS are not of importance due to their clinical unreliability; therefore, they were not taken into consideration in this study.

Along with obstetric pathology and thromboses, thrombocytopenia remains one of the important characteristics of this disorder⁹. The frequency of thrombocytopenia in patients with APS varies from 5 to 10%, and it is even more frequent in 293 patients described in the Italian Registry of Antiphospholipid Antibodies, where it reaches 26%. Some authors associate thrombocytopenia in APS patients with a high risk of development of SEL in a further follow-up period¹⁰⁻¹². Follow-ups of patients with APS have shown that thrombocytopenia rarely stays the only symptom of APS within a few years. Treatment of thrombocytopenia alone in pregnant women with APS is not usually needed; however, a significant decrease in thrombocyte count sometimes requires high doses of glucocorticosteroids, immunosuppressors, or intravenous immunoglobulin⁹.

Many questions concerning antiphospholipid syndrome in pregnancy are left unanswered. Pathogenesis of pregnancy complications is not truly known, although it is certain that pregnant women with previously unfavourable obstetric anamnesis (miscarriages, intrauterine fetal death) belong to a high-risk group for development of APS syndrome. It is possible that the interaction between antiphospholipid antibodies and other comorbid states (congenital thrombophilia) plays an important role in the development of complications or unsuccessful pregnancy¹².

Conclusion

In pregnant women with spontaneous abortions compared to healthy pregnant women a slightly positive correlation of LAC with aCL antibodies of both classes, as well as a positive correlation of aCL antibodies with anti-b2GP1 IgG exist. On the other hand, hemostatic parameters values suggest an anticoagulant status in the blood of pregnant women with spontaneous abortions.

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