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Original scientific paper

Antibody Profile of Pregnant Women with Antiphospholipid Syndrome and Pregnancy Outcome After Treatment with Low Dose Aspirin and Low-Weight-Molecular Heparin

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A B S T R A C T

*The aim of the research was to show our diagnostic and therapeutic experience with antiphospholipid syndrome (APS) in pregnant women. 36 pregnant women suspect on APS were included in the study: 32 with primary antiphospholipd syndrome (PAPS) and 4 with secondary antiphospholipid syndrome (SAPS). All pregnant women received low-molecular-weight-heparin (LMWH) and low dose aspirin (LDA) therapy. Control group represented 26 women with SAPS and previous bad reproductive anamnesis. Average pregnancy lasted 37.06±0.707 weeks. LMWH and LDA therapy was successful in 97.22%. Lupus anticoagulant (LA) was found to be more frequent in PAPS group (71,87%). Anticardiolipin antibodies (aCL) were found to be more frequent in SAPS (26,66%). For three patients (3.37%), PAPS was diagnosed due to a fact that they had positive antibeta2-glycoprotein1 (anti*β*-GP1). To make APS diagnosis, it is of great importance to search for all antiphospholipid antibodies. LMWH and low dose of acetylsalicylic acid should be the first choice therapy.*

***Key words**: antiphospholipid syndrome, pregnancy, antibodies*

Introduction

The antiphospholipid syndrome (APS) or Hughes syndrome is characterized by venous and/or arterial thrombosis, recurrent pregnancy loss and presence of antiphospholipid antibodies[1]. Primary antiphospholipid syndrome (PAPS) occurs in patients without clinical evidence of another disease, whereas secondary antiphospholipid syndrome (SAPS) occurs in association with autoimmune or other disease[2]. Anticardiolipin antibodies (aCL) and lupus anticoagulant (LA) are the most frequently found antibodies related to repeated pregnancy losses and thrombosis episodes[3]. Obstetric manifestations of APS, according to current criteria, also include early delivery due to pre-eclampsia, intrauterine growth retardation (IUGR) or fetal distress[4]. The aim of our research was to determine antibody profile of pregnant women with APS, as well as the outcome of their pregnancies after treatment with low dose aspirin (LDA) and low-molecular-weight heparin (LWMH), which represent the standard treatment of APS[5]. LWMH is adequate for prevention and treatment of venous thrombosis and recurrent pregnancy loss because it does not cross over placental barrier, it can be applied subcutaneously once a day, and it does not require laboratory tests. Compared with unfractioned heparin (UFH), there are no side effects such as haemorrhage, thrombocytopenia and osteoporosis[6]. LDA prevents thrombosis development and initiates production of interleukin 3 (IL-3) that regulates placental growth[7].

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Subjects and Methods

Sixty-two female patients fulfilling classification criteria for APS were included in the research. Thirty-two of them had PAPS, and thirty of them had SAPS. The most frequent diagnosis in SAPS group was systemic lupus erithematosus (SLE). Out of sixty-two women included in the research, 36 were pregnant, 32 from PAPS group and 4 from SAPS group. All patients had antiphospholipid antibody level determined. All pregnant patients received LDA and LMWH therapy and were prospectively observed throughout their pregnancies and for six weeks after the delivery. Women who were not pregnant were used for antibody comparison between two groups of women with ASP.

Lupus anticoagulant was determined with test of Dade Behring Marburg firm using LA 1 screening reagent and LA 2 confirming reagent, which is simplified reagent Dilute Russells Viper Venom Test (DRVVT) for lupus anticoagulant verification.

Anticardiolipin antibodies were determined with Autostat^{TM}II ACA test of Hycor firm, for every isoclass, based on enzyme-linked immunosorbent assay (ELISA), which is used for detection of specific antibodies against cardiolipin in human serum.

Antiβ_2 -glycoprotein-1 (antiβ_2-GP1) antibodies were identified with ETI-Beta-Glycoprotein I IgG kit for quantitative analysis of IgG antibodies against β_2-glycoprotein 1 in human serum.

Preliminary tests, prothrombin time, activated partial thromboplastin time, fibrinogen and thrombin time (DADE Behring) were used for coagulogram screening, and platelet number was determined with Cell-Dyn-4000.

Protein C (APC resistance) was determined with ProC Global test (DADE Behring).

Factor V von Leiden mutation was determined by method Bertina et al. (1994), prothrombin mutation was determined according to Poort et al (1996) and C677T mutation in methylentatrahydrofolat reductase (MTHFR) was determined according to Frosst et al. (1995).

Data input and processing was done in a computer table calculator Microsoft Excel 2003, whereas software packages SPSS 13.0 for Windows and Statistica 6.0 were used for statistical analysis.

Results

Out of sixty-two female patients fulfilling clinical criteria for APS and included in the research, thirty-six (58%) were pregnant by the time they were recalled to our department. Thirty-two of them had PAPS and just four of them reported with SAPS. Other patients had at least one clinical and one laboratory criterion for APS in previous pregnancies. All patients had clinical manifestation in form of obstetric complication. Average age in PAPS group was 31.31±4.65 years, whereas average age in SAPS group was 44±12.30 years. Antiphospholipid antibody analysis showed that 71.87% patients from PAPS group, and only 33.33% patients from SAPS group had positive LA antibody. In SAPS group 26.66% patients had positive had aCL, which is more frequent than in PAPS group where only 9.37% of patients were aCL positive. Other antiphospholipid antibodies were also represented (Tables 1, 2 and 3).

On the significance level, the possible presumption is that there is statistically great difference in average results of IgM class of anticardiolipin antibodies titre between PAPS and SAPS patients ($p<0.05$, Table 4).

TABLE 1
MAIN CARACTERISTICS OF THE PATIENT WITH ANTIPHOSPHOLIPID SYNDROME

	PAPS	SAPS
Age(yeras)	31.31±4.65	44±12.30
Number of pregnant women	32 (100%)	4 (13.33%)
LA	23 (71.87%)	10 (33.33%)
aCL	3 (9.37%)	8 (26.66%)
antiβ_2GP1	3 (9.37%)	8 (26.66%)
LA+aCL	3 (9.37%)	2 (6.66%)
LA+aCL+ antiβ_2GP1	N=32 (100%)	1 (3.33%)
LA+ antiβ_2GP1		1 (3.33%)
aCL+antiβ_2GP1		N=30 (100%)

N – sample size, PAPS – primary antiphospholipid syndrome, SAPS – secundary antiphospholipid syndrome, LA – lupus anticoagulant, aCL –anticardiolipn antibody, antiβ_2GP1 – antibeta2-glycoprotein1

TABLE 2
STATISTICAL CARACTERISTICS OF ANTIPHOSPHOLIPID ANTIBODIES IN PATIENT WITH PRIMARY ANTIPOSPHOLIPD SYNDROME

	LA	IgGaCL	IgMaCL	IgAaCL
Arithmetic mean	1.461	16.905	8.113	4.228
Median	1.475	8.400	5.525	3.750
Interkvartil	0.325	2.720	3.780	2.840
Standard deviation	0.227	31.494	8.548	5.035
Variation coefficient	15.537	186.230	105.362	119.087
Skewness	–0.417	4.117	2.567	3.429
Kurtosis	–0.669	18.227	6.451	14.218

LA – lupus anticoagulant, aCL –anticardiolipin antibody, Ig – immunoglobuline

TABLE 3
STATISTICAL CARACTERISTICS OF ANTIPHOSPHOLIPID ANTIBODIES IN PATIENT WITH SECUNDARY ANTIPOSPHOLIPD SYNDROME

	LA	IgGaCL	IgMaCL	IgAaCL
Arithmetic mean	1.503	26.092	33.959	9.738
Median	1.420	8.995	6.575	4.760
Interkvartil	0.480	19.160	16.660	14.850
Standard deviation	0.387	38.047	68.608	9.330
Variation coefficient	25.749	145.819	202.068	95.810
Skewness	1.037	3.194	3.005	1.210
Kurtosis	0.891	11.975	8.695	0.347

LA – lupus anticoagulant, aCL –anticardiolipin antibody, Ig – immunoglobuline

During determination of correlation between pregnancy duration and four analysed variables (LA, IgG aCL, IgM aCL and IgA aCL) obtained results showed negative medium strong correlation between LA and IgM aCL variables. Positive medium strong correlation existed between IgM aCL and IgA aCL. Correlation coefficients describing connection between these variables were statistically significant on the level ($p<0.05$, Table 5).

There was no statistically significant correlation between antibody type and level and pregnancy duration. All pregnant patients with APS delivered live babies. Average pregnancy lasted 37.06 ± 0.707 weeks, the shortest lasted 29 weeks (1 patient), and the longest lasted 41 weeks (2 patients). Considering gestation age and children's ability to breed independently, and taking into account classification criteria, LDA and LMWH therapy efficiency was in our trial 97.22%.

TABLE 4
RESULTS OF TESTING THE HYPOTHESIS OF THE EQUALITY OF ARITHETIC CENTRES OF FIVE ANALYSED VARIABLES IN PATIENTS WITH PRIMARY ANTIPHOSPHOLIPID SYNDROME AND PATIENTS WITH SECONDARY ANTIPHOSOHOLIPID SYNDROME USING t-TEST

Variable	Arithmetic mean (PAPS)	Arithmetic mean (SAPS)	t-ratio	p-value
LA	1.461	1.503	–0.522	0.604
IgG aCL	16.905	26.092	–1.038	0.303
IgM aCL	8.112	33.953	–2.114	0.039
IgA aCL	4.228	9.738	–2.883	0.005

LA – lupus anticoagulant, aCL – anticardiolipin antibody, Ig – immunoglobuline PAPS – primary antiphospholipid syndrome, SAPS – secundary antiphospholipid syndrome

Discussion

Women with antiphospholipid antibodies have unusually high pregnancy loss percentage during fetal period (10 or more weeks of gestation)[8,9]. On the other hand, unselected women with history of sporadic or recurrent miscarriages, more often suffer pregnancy loss in preembryonic period (less than 6 weeks of gestation) or embryonic period (6 to 9 weeks of gestation). Pregnant women with antiphospholipid antibodies can manifest complications such as early delivery caused by gestational hypertension as well as placental insufficiency[10,11].

TABLE 5
CORRELATION COEFFICIENT BETWEEN THE VARIABLES REPRESENTING PREGNANCY DURATION, LA, IgG aCL, IgM aCL, IgA aCL WITH p-VALUES, BROUGHT FROM TESTING THE HYPOTHESIS OF CORRELATION COEFFICIENTS SIGNIFICANCE

	Pregnancy duration	LA	IgG aCL	IgM aCL	IgA aCL
Pregnancy duration	1.000	–0.104 p=0.577	–0.075 p=0.689	0.015 p=0.935	0.170 p=0.361
LA	–0.104 p=0.577	1.000	–0.225 p=0.223	–0.409 p=0.022	–0.076 p=0.684
IgG aCL	–0.075 p=0.689	–0.225 p=0.223	1.000	0.304 p=0.096	0.311 p=0.089
IgM aCL	0.015 p=0.935	–0.409 p=0.022	0.3040 p=0.096	1.000	0.531 p=0.002
IgA aCL	0.170 p=0.361	–0.076 p=0.684	0.311 p=0.089	0.531 p=0.002	1.000

LA – lupus anticoagulant, aCL – anticardiolipin antibody, Ig – immunoglobuline

Unfavourable pregnancy outcomes in women with positive antiphospholipid antibodies can be result of inadequate placental perfusion caused by localized thrombosis[12] possibly due to interference with trophoblastic anexin V connected with antiphospholipid antibodies[13]. Anexin V is an anticoagulant protein in blood that protects anion phospholipids[14]. Anion phospholipids exposed to blood coagulant proteins initiate thrombosis. Antiphospholipid antibodies may diminish throphoblastic invasion and hormonal production and by that initiate not only preembryonic and embryonic loss, but fetal loss and placental insufficiency as well[15]. aCL antibody prevalence in general obstetric population is not high and therefore screening of otherwise healthy pregnant women is not needed[16]. Since aCL and LA antibodies are connected to pregnancy loss, without complete concordance, both antibodies have to be tested if there is any suspicion of APS. Predictive value of antiβ_2GP1 in identifying women with potential risk of pregnancy loss is questionable. Other antiphospholipid antibodies like antiphosphatidilserine and antiphosphatidilethanolamine do not identify such patients[17,18].

According to results from our population of pregnant women with APS, LA antiphospholipid antibodies had higher incidence in PAPS group, than in SAPS group. That may result from influence of other autoimmune processes, which can be expected in patients with APS and another autoimmune disease. It is well known that aCL and LA incidence is less than 1% in general population, on the other hand their incidence shows manifold increase in patients with autoimmune diseases. Almost one third of patients with SLE have positive aCL, and just 15% of them have positive LA. IgG aCL class is, by many authors, better predictor for thrombosis occurrence than IgM aCL class which is increasing along with antibody level[19]. Such phenomenon in our sample showed higher incidence in SAPS group, with statistically significant difference in average level of IgM aCL class between PAPS and SAPS group ($p<0.05$). Low values of IgG aCL and IgM aCL titre as well as IgA aLC antibodies are connected to low complication incidence. It has been ascertained that women with pregnancy complications and APS have high risk of thrombosis development later in life. Erkan and al showed in his study that almost 60% of women developed thrombosis within 10 years after delivery[20].

Big controversy presents introduction and choice of therapy. Current standard therapy for obstetric complications related to APS is LDA and LMWH. Farquharson's results from 2002 showed that LMWH therapy added to LDA did not significantly improve pregnancy outcome[21]. However, results of other authors show significantly greater success of heparin addition to therapy concerning pregnancy outcome. Malinowski's results from 2003 showed that LMWH therapy alone was successful in 81.1% of patients, low dose of acetylsalicylic acid therapy alone had success in 89.3% of patients, and combina- tion of these two therapies brought success in 92.5% of patients[5,22,23].

Other authors also have similar results[21,22]. Our experience is similar, all babies were born alive, only one was born before 34th week of gestation. Considering high risk of thrombosis development during pregnancy in women with positive antiphospholipid antibodies, as preventive measure, LDA therapy can be introduced. Such therapy has not only anti-aggregating effect on platelets, and thus prevents first step in thrombogenesis, but also initiates interleukin 3 (IL-3) production that regulates placental growth. Inhibition of cyclooxygenasis changes arahidonic acid metabolism towards excessive leucotrien production that can stimulate IL-3 production[7]. All pregnant women with other clinical manifestations such as vascular thrombosis and positive antiphospholipid antibodies, fulfilling clinical criteria for APS need to receive LDA and LMWH therapy. Some authors suggest that aspirin can be valuable in preventing future non–gravid vascular thrombosis in patients with APS and the history of previous pregnancy problems[24]. Women who had APS diagnosed due to recurrent miscarriages should receive heparin and LDA for at least 3 to 5 days after delivery, especially if they had other risk factors. Women with APS and previous vascular thrombosis should receive prophylaxis for 6 weeks after delivery[25].

Conclusion

Despite greater domination of LA as the predictor of pregnancy complications, which are, according to our results, primarily typical for PAPS, for APS diagnosis it is of great importance to search for all antiphospholipid antibodies (aPL). Regardless to its lower prevalence, positive anti-beta2GP1 can be of great importance for early identification of patients with the risk of pregnancy loss or other obstetric complications related to APS. Suggestion to introduce LDA therapy to all aPL positive patients, and add LMWH therapy if they also have previous bad reproductive anamnesis, should be taken into consideration. Bearing in mind that the population of women with such problems have future disposition to thrombosis development, permanent LDA therapy should also be considered along with regular laboratory controls of antibody level and profile. Intravenous immunoglobuline therapy, systemic corticosteroides, cytostatic therapy and plasmapheresis should be saved for cases of refractivity of standard therapy or for case of catastrophic APS (CAPS). Since in our trial neither pregnant woman had vascular thrombosis as a clinical criterion for APS, they received LMWH therapy for 5 days after delivery. If patient with APS should have vascular thrombosis as a clinical criterion, pregnancy should be planned carefully; if patient received warfarin, LMWH should be introduced in 6th week of pregnancy. Heparin prophylaxis should be continued during delivery and for 4–6 weeks after that, after that warfarin can be introduced again.

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PROFIL PROTUTIJELA TRUDNICA S ANTIFOSFOLIPIDNIM SINDROMOM I ISHOD NJIHOVIH TRUDNOĆA TERAPIJOM NISKOMOLEKUALNIM HEPARINOM I ASPIRINOM NISKE DOZE

SAŽETAK

Cilj nam je bio prikazati naša iskustva u dijagnozi i liječenju antifosfolipidnog sindroma (APS) u trudnica. 36 trudnih žena sa sumnjom na APS je uključeno u studiju: 32 s primarnim antifosofolipdinim sindromom (PAPS) i 4 žene sa sekundarnim antifosfolipidnim sindromom(SAPS). Sve trudnice su imale terapiju nisko-molekularnim heparinom (LMWH) i apsirinom niske doze(LDA). U kontrolnoj skupini imali smo 26 žena sa SAPS i prijašnjom nepovoljnom reproduktivnom anamnezom. Prosječno je trudnoća trajala 37.06±0.707 tjedana. Terapija LMWH i LDA polučila je uspjeh u 97.22%. Lupus antikoagulans (LA) imao je češću pojavu u PAPS (71.87%). Antikardiolipinska protutijela su bila češća u SAPS (26,66%). U tri pacijenta dijagnoza se postavila temeljem pozitivnosti antibeta2.glikoproteina 1 (antiβ2-GP1) (3.37%). Od velike je važnosti kod sumnje na APS tražiti sav spektar antifosfolipidnih protutijela. LMWH i LDA bi trebali biti prvi lijek izbora.