УДК 618.3:612.017.1

The structure of antiphospholipid antibodies circulation investigated in pregnancy I and II trimester

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The article presents the results of a prospective study on the presence of antiphospholipid antibodies in healthy primagravida with the analysis of further gestational complications.
The objective: **to determine the serum circulation of antibodies to different types of phospholipids in the first and second gestational trimester and to study the incidence of obstetric complications depending on this circulation.**
Materials and methods**. Determination of antibodies to phospholipids in 11–12 and 18–20 gestational weeks by immunoassay analysis. The study included 150 primagravida (11-12 weeks) without reproductive impairment in history, a re-study was performed in 148 women, since 2 cases of spontaneus abortion were observed. The relative risk of development of major obstetric complications in women with the appearance of antibodies to phospholipids in the second trimester of pregnancy is calculated.**
Results**. At the first inspection, the frequency of detection of antibodies to phospholipids ranged from 3 to 4%, which corresponds to global data on the prevalence of antibody circulation in a healthy population. However, in 18-20 weeks, a statistically significant increase in the frequency of antibody circulation, mainly to phosphatidylserine (21,6%) and phosphatidylethanolamine (17,4%), was detected. The peculiarities of the structure of the cell membrane are asymmetry of the location of phospholipids, in which negatively charged molecules, including phosphatidylserine and phosphotadylethanolamine, are located dominantly in a inner layer. The emergence of antibodies precisely to these molecules in the dynamics of pregnancy is evidence of their externalization, which may be the result of a violation of the normal functioning of the endothelium. Analysis of the course of the second half of pregnancy in patients with detected antibody subtypes showed an increase in the relative risk of severe preeclampsia, placental dysfunction and premature labor.**
Conclusions: **1. The APA circulation frequency by healthy primagravida in first gestational trimester is in accordance to world population. 2. In the second trimester an increasing of APA detection is registered, exactly for phosphatidylserin (21,6%) and phosphatidyletanolamin (17,4%) antibodies 3. Appearance of antibodies to negative phospholipids in the second pregnancy trimester lets propose destabilization of endothelium membranes in these patients, what is confirmed by a higher frequency of main obstetrics complications prospectively.**
Key words: *antibodies to phospholipids, phosphatidylserine antibodies, phosphatidylethanolamine antibodies, endothelial dysfunction.*

The circulation of antiphospholipid antibodies (APA) is considered to be a result of an immune response against the components of cell membranes – phospholipids [9]. A lot of investigations were devoted to the problem of antiphospholipid syndrome (APS) in the pathogenesis of different obstetrics and nonobstetrics complications [1]. The objective point of view requires twice detection of three or more types of APA in plasma in 12 weeks interval and 1 or more clinical signs to confirm APS diagnosis [8]. This position is discussable, because speaking about current pregnancy and possible life threatening complications, the 12 week interval between investigation is too long. APA are detected by either a solid phase or a liquid phase test. An enzyme-linked immunosorbent assay (ELISA) is used in the solid phase test to , is most widely used to detect anti-cardiolipin antibody (ACL), anti-β_2 glycoprotein-I (β_2GPI) antibody and anti-prothrombin (aPT) antibody. Lupus anticoagulants (LA) as a reason of phospholipid-dependent clotting time prolongation is detected in the liquid phase test by a clot-based functional assay.

One of the first criteria classification for true APS-Sapporo system (1998) supposed presence one of clinical signs (thrombosis or pregnancy loss) and one of laboratory signs – ACL IgG or M circulation or positive LA test, measured twice or more in 6 weeks [2]. In 2006 in Sydney this classification was revised, the laboratory criteria were added by detection of IgG or M β_2GPI and follow-up term of investigation 12 weeks.

Nevertheless, dealing with a patient, very often doctor needs to explain results of APA-analysis. In most cases this investigation is performed after pregnancy loss, in first weeks or by planning next conception. There are a lot of publications, devoted to the frequency of APA by different obstetrics complications, such as pregnancy loss, preterm labor, placenta abruption, preeclampsia and so on [9]. Such a direct clinical and laboratory dependence tempted many researchers to form a monofactor explanation of certain obstetric complications by APA circulating antiphospholipid antibodies [1]. But curruently we do not have argued data, what influence a circulation of APA in asymptomatic patient will have on the pregnancy complications and results. Pathogenetic treatment of antiphospholipid syndrom prior to pregnancy or in the first and second half actually demonstrates effective results, mainly for prevention of miscarriage [6]. But this treatment should be completely argued, because it is long, expensive and some dangerous side-effects are possible. So we proposed to follow up the detection of APA different classes in first and second pregnancy trimester to check their variability during gestation.

The goal of the study was to detect different APA classes in serum of pregnant women in first and second gestational trimester and to analyze dependence of obstetric complications on this circulation.

MATERIALS AND METHODS

150 women were included in investigation, that was prospective. These women came for their first consultation in 11–12 gestational weeks. By working with design of research we included only primagravida with singletone pregnancy, without history of infertility. So all our participants didn't have any history of pregnancy loss, preterm labor, severe preeclampsia

etc. All patients were informed about the goal of research, their possible benefits and very low risk, associated only with blood test. The first test was performed by first visit, after discussion and getting an agreement, the second probe – in 18–20 weeks, on this stage we have only 148 patients, because of 2 miscarriage cases. Using ELISA method the concentration of APAs of different subtypes – Ig class G to cardiolipin (ACL IgG), phosphatidylserine (PS IgG), phosphatidylethanolamine (PE IgG), phosphatidylglycerol (PG IgG) and phosphatidylcholine (PC IgG) was determined. The reference values for all immunoglobulins was a concentration of 50 MPL, the higher result was considered positive

After the second test all patients were divided in two groups, according to the detection of APA in their serum, the first one - without any class APA circulation, the second group with one or more APA class circulation. In these groups the gestational complications were calculated – preterm labor, fetal growth restriction, severe preeclampsia. The structure and frequency of these complications were compared using Cholmogorov-Smirnov method and Student criteria. The relative risk (RR) of each complication according to APA in first and second trimester detection also was calculated.

RESULTS AND DISCUSSION

According to world data, the frequency of APA detection is about 5–6%. Much more publications are devoted to the epidemiology of APS exactly, they propose evidence of 50 cases of syndrome on 100 000 USA population [5]. Table 1 contains results of APA testing in first pregnancy trimester.

Results of this test confirms the population frequency of APA circulation – not more 5–6%, the most popular class of APA in serum of healthy primagravida is ACL-antibody. The detection of other investigated subclasses in this term is sporadic.

Phospholipids are structurally similar molecules formed by a glycerol skeleton with phosphodiester groups, coupled with alcoholic polar groups and two esterified glycerol fatty acids. In human cells, alcoholic groups are formed by nitrogenous bases (choline, ethanolamine, serine), glycerol or inositol. Accordingly, phospholipids are called phosphatidylcholine (the old name is lecithin, PC), phosphatidylethanolamine (the old name is kefalin, PE), phosphatidylserine (PS), phosphatidylglycerin (PG), phosphatidylinositol and diphosphatidylglycerin (cardiolipin). The uniqueness of the cardiolipin consists in the presence in its composition of two diether phosphate groups, connected with the molecule of glycerol.

The chemical structure of the polar «head» determines the final electric charge and the ionic state of the phospholipid. PC and PG have a negatively charged phosphate group and a positively charged amino group, therefore, are electrically neutral, so are also called neutral phospholipids. These two phospholipids are metabolically linked to each other and are the principal lipids that provide the so-called lamellar configuration, typical for all cell membranes, when the phospholipids are arranged in two layers, where hydrophobic fatty acid chains are oriented in the middle of the membrane and the hydrophilic polar groups are outside. PS, PE, phosphatidylinositol and Cl are electrically negative, or anionic phospholipids, have a negatively charged phosphate group, phosphatidylinositol does not have an amino group at all [2].

But in the end of second gestational trimester we found absolutely different results – table 2.

Comparing by use of Student's criteria the difference between cohorta's results in first and second pregnancy trimester were significant – $p<0,05$. Calculating by Cholmogorov-Smirnov criteria also demonstrates, that frequency of positive tests in 18-20 gestational weeks is more, then in 11–12 ($\alpha_{emp}>\alpha_{crit}$).

After the first stage of research we had only 8 patients from 150 (5,3%), that have positive ELISA for one of investigated APA subtypes. 6 of them by follow test were negative. So, we can surely say, that majority of positive results for APA in second pregnancy trimester are positive *de novo*. So, during first half of pregnancy these patients have some features, those led to APA synthesis.

The cellular membrane of virtually all cells has a pronounced asymmetry in the distribution of phospholipids of different classes in the outer and inner layers. Derivative of choline neutral phospholipids sphingomyelin and PC are localized on the outer surface of the membrane together with a small portion of PE. The internal (cytosolic) surface is formed mainly by PE and a small portion of PS. CL normally is absent on plasma membranes, on which up to 50–60% of the total pool of phospholipids is sphingomyelin and PC, 20–30% is PE, 10–15% is PS. It was found that preservation of phospholipid asymmetry is supported by a complex process associated with the activity of ATP and the enzyme aminophospholipid transloxase, which shifts aminophospholipids towards the inner membrane layer [4, 10]. That is, in general, anionic phospholipids on the outer surface of biomembranes are absent. The loss of a normal structure by a cell will necessarily lead to loss of asymmetry of the membrane phospholipid layers and will lead to the externalization of anionic phospholipids. This process plays an important physiological role in the development of a local blood clotting reaction. Usually the appearance of an anionic phospolipids on the outer side of the membrane stimulates the rapid removal of such cells from the circulation or from cells layer, that is known as apoptosis [7].

In particular, the formation of APA is an immune reaction, so for it the appearance of antigenic determinants on the outer surface of the membrane is required, which are anionic phospholipids. To illustrate this phenomenon comfortably through the procoagulant activity of platelet phospholipids, which is based on the similar externalization process.

Taking into account the participation in the processes of coagulation of phospholipids, the platelet membranes are divided into 2 groups:

1) deprived of procoagulant activity of choline – phosphatidylcholine and sphingomyelin,

Table 1

APA circulation in first pregnancy trimester

Antibody Subtype	Number of positive patients, n (%). Total – 150
ACL IgG	6 (4,0)
PS IgG	3 (2,0)
PE IgG	3 (2,0)
PG IgG	-
PC IgG	-

Table 2

APA circulation in second pregnancy trimester

Antibody Subtype	Number of positive patients, n (%). Total – 148	Number of de novo positive patients, n (%). Total -140
ACL IgG	8 (5,4)	7 (5,0)
PS IgG	32 (21,6)	31 (22,1)
PE IgG	25 (17,4)	25 (17,9)
PG IgG	2 (1,4)	2 (1,4)
PC IgG	5 (3,5)	5 (3,6)

Table 3

Complications of pregnancy and labor

Complication	Group 1, n (%) Total n=99	Group 2, n (%) Total n=41
Severe preeclampsia	3 (3,0)	7 (17,1)*
Placental dysfunction	2 (2,0)	5 (12,2)*
Growth fetal restriction	4 (4,0)	6 (14,6)*
Preterm labor	3 (3,0)	8 (19,5)*
Fetal distress in labor	7 (7,1)	10 (24,4)*
Placental abruption	1 (1,0)	-

*p<0,05 comparing to group 1.

Table 4

Relative risk of pregnancy complication

Complication	Circulation PS	Circulation PE
Severe preeclampsia	5,7	4,5
Placental dysfunction	5,3	4,6
Growth fetal restriction	3,5	2,4
Preterm labor	5,9	4,6
Fetal distress in labor	2,5	1,3

2) procoagulant active: neutral phosphatidylethanolamine, and acid phosphatidylserine and phosphatidylinositol.

Phospholipids of the first group are distributed on both surfaces of the cell membrane of unactivated platelets. Phospholipids of the second group in unactivated platelets are localized mainly on the inner surface of the membrane. In the process of platelet activation, the concentration of PE, PS, and phosphatidylinositol from the outside of the membrane increases significantly and forms the procoagulant surface necessary for fixation, activation and interaction of plasma hemostasis proteins. In addition, such a redistribution changes the cellular membrane's load, which is also important for hemostatic reactions. The acidic (anionic) phospholipids of platelet membranes PS and PG are called factor 3 of thrombocytes, or thrombocyte thromboplastin [4].

That is, as for the external coagulation pathway, for the beginning of antibody formation, conditions are required in which the components of the inner surface of the cell membranes are exposed outside (externally). The next essential circumstance is the intensity of this externalization, simplified by the scale of damage to the membranes and the duration of the influence of the pathogenic factor [10].

Making analogy with endothelium, which function and regulation is critically important for normal pregnancy development, we observed the second half of pregnancy, depending on positive test to different APA classes. For statistical clear we excluded 8 patients with first positive test. The rest 140 women were divided in 2 groups – 99 with all negative tests (group 1) and 41 with one or more detected APA subtype (group 2). The pregnancy and labor complications are demonstrated in table 3.

It is not new to confirm, that pregnant with APA circulation have greater risk of listed gestational complications. Nevertheless, we considered only new cases of this circulation, that usually are not available to detection. The main explanation we propose for it is more active process of anionic phoshpolipid moleculas externalization, that can be a marker of endothelial dysfunction.

Speaking about placental abruption, it's frequency is not so high in population, but we have found authors, that are sure in a role of APA circulation like a prognostication factor of this very dangerous condition [3]. Absence of this thesis confirmation in our research is a result of not so big quantity of patients.

We have also calculated relative risk of each complication according to detection of most popular APA – PS and PE (table 4).

The greatest increasing of RR is registered for severe preeclampsia. Modern conception of preeclampsia pathogenesis is based on early endothelium dysfunction in placental vessels, that leads to system angiospasm and proteinuria, so the process of cell membrane asymmetry loss can be a part of this scenario. The clinically manifesting placental dysfunction also has beginning in cell regulation disorders in the period of placental formation, the period, when APA circulation was revealed.

The RR of preterm labor by APA circulation is also statistically increased. Today placental dysfunction is considered to be one of the preterm labor reasons, together with cervical insufficiency and infection. RR of fetal distress in labor also is increased, but not statistically (is less 3), because this complication can have a lot of reasons, first of all – of intranatal period. But the revealed trend may be explained by decreased tolerance of a fetus to hypoxia, caused by placental factors.

These data cannot have great practical results, because routine investigation for APA of healthy primagravida is not advisable, only with the prognostication goals. Nevertheless, theoretical value of this investigation is very significant. Future researches should argue the instability of endothelial cell, distortion of phospholipid structure, that can be a reason of great obstetric syndrome, as it considered now to join most dangerous obstetric complications. The APA circulation in this situation should be discussed not as pathogenic mechanism of endothelial disfunction, because this circulation is really variable, but like a marker of cell membrane instability.

CONCLUSIONS

1. The APA circulation frequency by healthy primagravida in first gestational trimester is in accordance to world population.

2. In the second trimester an increasing of APA detection is registered, exactly for phosphatidylserin (21,6%) and phosphatidyletanolamin (17,4%) antibodies.

3. Appearance of antibodies to negative phospholipids in the second pregnancy trimester lets propose destabilization of endothelium membranes in these patients, what is confirmed by a higher frequency of main obstetrics complications prospectively.

Структура виявлених антифосфоліпідних антитіл у I та II триместрах вагітності

І.Б. Венцківська, С.Ст. Леуш, О.С. Загородня

У статті викладено результати проспективного дослідження з вивчення наявності антифосфоліпідних антитіл у здорових першовагітних з аналізом подальших гестаційних ускладнень.

***Мета дослідження*:** визначення циркуляції у сироватці антитіл до різних типів фосфоліпідів у I та II гестаційних триместрах та вивчення частоти розвитку акушерських ускладнень залежно від цієї циркуляції.

***Матеріали та методи*.** Проведено визначення антитіл до фосфоліпідів в 11–12 та 18–20 тиж вагітності методом імуноферментного аналізу. До дослідження включено 150 першовагітних (11–12 тиж) без розладів репродуктивної функції в анамнезі, повторне дослідження виконано у 148 жінок, оскільки у 2 відбулось мимовільне переривання вагітності. Обчислено відносний ризик розвитку основних акушерських ускладнень у жінок з появою антитіл до фосфоліпідів у II триместрі вагітності.

***Результати*.** Під час першого обстеження частота виявлення антитіл до фосфоліпідів коливалася від 3% до 4%, що відповідає світовим даним щодо поширеності циркуляції антитіл у здоровій популяції. Проте вже у 18–20 тиж виявлено статистично вірогідне підвищення частоти циркуляції антитіл, головним чином – до фосфатидилсерину (21,6%) та фосфатидилетаноламіну (17,4%). Особливості будови клітинної мембрани полягають в асиметрії розташування фосфоліпідів, за якої негативно заряджені молекули, серед яких – фосфатидилсерин та фосфатидилетаноламін, розташовано переважно у внутрішньому шарі. Поява антитіл саме до цих молекул у динаміці вагітності є свідченням їхньої екстерналізації, що може бути наслідком порушення нормального функціонування ендотелію. Аналіз перебігу другої половини вагітності у пацієнток з виявленими субтипами антитіл продемонстрував зростання відносного ризику тяжкої прееклампсії, плацентарної дисфункції та передчасних пологів.

***Заключення*.** 1. Частота виявлення антифосфоліпідних антитіл у здорових першовагітних у I триместрі не відрізняється від даних світової популяції. 2. У II триместрі вагітності зареєстровано зростання частоти виявлення антитіл до фосфатидилсерину (21,6%) та фосфатидилетаноламіну (17,4%). 3. Поява антитіл до негативно заряджених фосфоліпідів у II триместрі вагітності дозволяє припустити дестабілізацію ендотеліальних мембран у цих пацієнток, що проспективно підтверджується високою частотою акушерських ускладнень у них.

Ключеві слова: *антитіла до фосфоліпідів, антитіла до фосфатидилсерину, антитіла до фосфатидилетаноламіну, ендотеліальна дисфункція.*

Структура выявленных антифосфолипидных антител в I и II триместрах беременности

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В статье изложены результаты проспективного исследования по изучению наличия антифосфолипидных антител у здоровых первобеременных с анализом дальнейших гестационных осложнений.

***Цель исследования*:** определение циркуляции в сыворотке антител к различным типам фосфолипидов в I и II гестационных триместрах и изучение частоты развития акушерских осложнений в зависимости от этой циркуляции.

***Материалы и методы*.** Проведено определение антител к фосфолипидам в 11–12 и 18–20 нед беременности методом иммуноферментного анализа. В исследование включено 150 первобеременных (11–12 нед) без расстройств репродуктивной функции в анамнезе, повторное исследование выполнено у 148 женщин, поскольку у 2 произошло самопроизвольное прерывание беременности. Вычислен относительный риск развития основных акушерских осложнений у женщин с появлением антител к фосфолипидам во II триместре беременности.

***Результаты*.** При первом обследовании частота выявления антител к фосфолипидам колебалась от 3% до 4%, что соответствует мировым данным о распространенности циркуляции антител в здоровой популяции. Однако уже в 18–20 нед выявлено статистически достоверное увеличение частоты циркуляции антител, главным образом – к фосфатидилсерину (21,6%) и фосфатидилэтаноламину (17,4%). Особенности строения клеточной мембраны состоят в асимметрии расположения фосфолипидов, при которой отрицательно заряженные молекулы, среди которых – фосфатидилсерин и фосфатидилетаноламин, расположены преимущественно во внутреннем слое. Появление антител именно к этим молекулам в динамике беременности является свидетельством их экстернализации, что может быть следствием нарушения нормального функционирования эндотелия. Анализ хода второй половины беременности у пациенток с выявленными субтипами антител продемонстрировал рост относительного риска тяжелой преэклампсии, плацентарной дисфункции и преждевременных родов.

***Заключение*.** 1. Частота выявления антифосфолипидных антител у здоровых первобеременных в I триместре не отличается от данных мировой популяции. 2. Во II триместре беременности зарегистрирован рост частоты выявления антител к фосфатидилсерину (21,6%) и фосфатидилэтаноламину (17,4%). 3. Появление антител к отрицательно заряженным фосфолипидам во II триместре беременности позволяет предположить дестабилизацию эндотелиальных мембран у этих пациенток, что проспективно подтверждается высокой частотой акушерских осложнений у них.

Ключевые слова: *антитела к фосфолипидам, антитела к фосфатидилсерину, антитела к фосфатидилетаноламину, эндотелиальная дисфункция.*

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REFERENCES

1. Загородня О.С. Вплив антифосфоліпідного синдрому на перебіг пологів /О.С. Загородня// Український журнал клінічної та лабораторної медицини. – 2014. – № 4. – С. 23–27.

2. Bobba R. A review of the Sapporo and revised Sapporo criteria for the classification of antiphospholipid syndrome. Where do the revised sapporo criteria add value?/ R. Bobba, S. Johnson, A. Davis//J Rheumatol. – 2007. – N 34 (7). – P. 1522-7.

3. Costedoat-Chalumeau N. Pregnancy and antiphospholipid syndrome /N. Costedoat-Chalumeau, V. Leguern, G. Leroux et al.// Rev Med Interne. – 2012. – N 33 (4). – P. 209-16. doi: 10.1016/j.revmed.2012.01.003

4. Devaux P. Protein involvement in transmembrane lipid asymmetry/ P. Devaux // Ann. Rev. Biophys. Biomol. Struct. – 1992. – Vol. 21. – P. 417–439.

5.6. Duarte-Garcia A. Epidemiology of Antiphospholipid Syndrome: A Population-Based Study /A. Duarte-Garcia, M. Pham, C. Crowson et al.// Arthritis Rheumatol. – 2017. – N 69 – P. 34–45.

6. McNeil H. Immunology and clinical importance of antiphospholipid antibodies/H. McNeil, C. Chesterman, S. Krilis// Adv. Immunol. – 1991. – Vol. 49. – P. 193–280.

7. Mulla M. Antiphospholipid antibodies induce a pro-inflammatory response in first trimester trophoblast via the TLR4/ MyD88 pathway /M. Mulla, J. Brosens, L. Chamley et al. // Am J Reprod Immunol. – 2009. – N 62 (2). – P. 96–111.

8.3. Park S. Clinical Application of Revised Laboratory Classification Criteria for Antiphospholipid Antibody Syndrome: Is the Follow-Up Interval of 12 Weeks Instead of 6 Weeks Significantly Useful? /S. Park, S. Jang, C. Park, H. Chi // Biomed Res Int. – 2016. – N 16. – P. 64–75. doi: 10.1155/2016/2641526

9. Pengo V. Confirmation of initial antiphospholipid antibody positivity depends on the antiphospholipid antibody profile/ V. Pengo, A. Ruffatti, T. Del Ross et al.// J Thromb Haemost. – 2013. – N 11 (8). – P. 1527-31. doi: 10.1111/jth.12264.

10. Schroit A. Transbilayer movement of phospholipids in red cell and platelet membrane/ A. Schroit, R. Zwaal// Biochem. Biophys. Acta. – 1991. – Vol. 1071. – P. 313–329.

Статья поступила в редакцию 23.05.2019