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EARLY DIAGNOSIS OF ANTIPHOSPHOLIPID SYNDROME IN PREGNANT WOMEN

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Abstract. Antiphospholipid syndrome (APS) is a significant autoimmune disorder characterized by the presence of antiphospholipid antibodies (aPL), which disrupt normal hemostatic balance and contribute to thrombotic complications. In autoimmune thrombophilia, the concentration of aPL often exceeds that of phospholipids and glycoproteins bound to negatively charged phospholipid membranes. These immunological changes lead to endothelial dysfunction, thrombocytopenia, and activation of platelet aggregation, resulting in a disturbed coagulation cascade. Consequently, local macro- and microthromboses form within the microvasculature, causing impaired placental vascularization and recurrent pregnancy loss associated with placental insufficiency.

This article presents the findings of our study conducted among women who experienced recurrent miscarriage due to antiphospholipid syndrome, emphasizing the importance of early diagnosis and pre-gravid preparation to prevent pregnancy complications.

Keywords: recurrent pregnancy loss, antiphospholipid syndrome, autoimmune thrombophilia, placental insufficiency, early diagnosis

РАННЯЯ ДИАГНОСТИКА АНТИФОСФОЛИПИДНОГО СИНДРОМА У БЕРЕМЕННЫХ ЖЕНЩИН

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Резюме. Антифосфолипидный синдром (АФС) представляет собой значительное аутоиммунное заболевание, характеризующееся наличием антифосфолипидных антител (АФА), которые нарушают нормальное состояние системы гемостаза и способствуют развитию тромботических осложнений. При аутоиммунной тромбофилии концентрация АФА нередко превышает уровень фосфолипидов и гликопротеинов, связанных с отрицательно заряженными фосфолипидными мембранами. Эти иммунологические изменения приводят к эндотелиальной дисфункции, тромбоцитопении и активации агрегации тромбоцитов, вызывая нарушение коагуляционного каскада. В результате формируются локальные макро- и микротромбозы в микрососудистом русле, что приводит к нарушению васкуляризации плаценты и повторным самопроизвольным выкидышам, связанным с плацентарной недостаточностью.

В данной статье представлены результаты исследования, проведённого среди женщин, перенёсших повторные выкидыши вследствие антифосфолипидного синдрома. Особое внимание уделено значению ранней диагностики и предгравидарной подготовки для профилактики осложнений беременности.

Ключевые слова: привычное невынашивание беременности, антифосфолипидный синдром, аутоиммунная тромбофилия, плацентарная недостаточность, ранняя диагностика

Relevance. Miscarriage is a complex, multifactorial condition, and one of its main pathogenetic mechanisms involves congenital or acquired disorders of the hemostatic system [2,4,8,9,10]. Despite extensive research, many aspects remain unclear, and there is no definitive consensus regarding the specific contribution of individual factors to recurrent pregnancy loss, fetal growth restriction,

preeclampsia, gestational hypertension, and spontaneous abortion [1,3,7,11,12]. A growing body of evidence indicates that antiphospholipid syndrome (APS) plays a critical role in the development of these adverse gestational outcomes. Physiological activation of the coagulation system during early pregnancy facilitates blastocyst implantation and prevents peri-implantation hemorrhage during endovascular invasion of the trophoblast [5,6,8,13,18]. However, in clinical practice, the rising incidence of extragenital vascular diseases, especially those associated with APS, contributes to systemic hemodynamic and microcirculatory disturbances, including uterine circulation abnormalities [14,5,16,17]. Excessive fibrin deposition at the implantation site and microthrombosis of spiral arteries and arterioles disrupt normal placental invasion, potentially leading to early pregnancy loss or primary placental insufficiency, which predisposes to further obstetric complications.

Purpose of the study. To evaluate the diagnostic value of hemostatic system markers and antiphospholipid antibodies in the early detection of structural and functional placental abnormalities.

Materials and methods. This study included 70 pregnant women at risk of miscarriage and 35 women with normal pregnancies, all of whom were observed at the Bukhara Regional Perinatal Center between 2020 and 2022. Venous blood and serum samples were collected for hematological and biochemical analyses. The study employed general clinical, ultrasound, Doppler, biochemical, hematological, and statistical methods, with data analysis performed using the Fisher–Student test. Information from each participant was recorded in a specially designed questionnaire and statistically processed. Participants were categorized by age: 19–25 years (21 women, 20%), 26–35 years (68 women, 64.7%), and over 35 years (16 women, 15.2%). The majority (72 women, 69%) resided in urban areas (mainly Bukhara), while 33 (31%) were from rural regions.

Employment analysis revealed that most participants were temporarily unemployed (13.3%) or housewives (42.8%), while 7.6% performed manual labor and 36% were engaged in intellectual work. The mean age at menarche was 13.5 ±

0.4 years in the study group and 13.8 ± 0.2 years in the control group ($p \leq 0.05$). The menstrual cycle duration ranged from 26 to 32 days, averaging 4.3 days in the main group and 4.1 days in the control group ($p \leq 0.05$).

Results. Women who experienced recurrent miscarriages at 9–10 weeks of gestation showed markedly elevated antiphospholipid antibody levels. The mean number of gestational losses was 3.76 ($P < 0.001$). Serum concentrations of IgM anti-phospholipid and IgG anti-β2-glycoprotein antibodies were increased 15-fold ($P < 0.001$) and 11.8-fold ($P < 0.001$), respectively, compared to the control group. These findings confirm that recurrent pregnancy loss at 9–10 weeks of gestation is strongly associated with antiphospholipid syndrome.

Table 1. Indicators for antiphospholipid syndrome in recurrent miscarriage, M ± m

Index	**Physiological pregnancy, 9-10 week, n = 35**	**Misscarriage 9-10 week, n = 35**
VA, conventional unit	0,50±0,04	1,88±0,06***
IgM anti-FL, U/ml	1,17±0,11	23,43±1,00***
IgG anti-β2-GP	1,33±0,14	15,74±1,01***

Note: * - differences between the control group and patients with APS are significant (P<0.05), ** - P<0.01 and * - P<0.001.**

It should be noted that when interpreting the results of the VA indicator, it is necessary to pay attention to the degree of its increase, i.e.: if its value is about 1.2-1.5 conventional units - the average change, if it is 1.5-2.0 conventional units - the formation of blood clots in the veins, if it is above 2.0, then the danger is strong. In our study, VA was 1.88 ± 0.06 units, which is due to the formation of macro- and microthrombosis in the fetal tract, impaired fetoplacental circulation, and the absence of habitual miscarriage.

It is known from the literature that antibodies to APL slow down the synthesis of prostacyclin in endothelial cells by inhibiting phosphorylase A2 and protein S and contribute to the formation of blood clots. The resulting antibodies bind to the phospholipids of cell membranes and lead to conformational and metabolic changes in the membranes. This leads to their dysfunction, slowing down and cessation of blood flow in capillaries and venules, as well as to thrombosis. However, it should be noted that antibodies to APL are also present to some extent in the blood serum of healthy people, and this is associated with cell renewal.

In our study, women who had recurrent miscarriage were 15 ($P < 0.001$) higher than those in the control group, indicating the presence of APS. Such a sharp increase leads to the connection of platelets and vascular endothelium and, as a result, to their destruction, the development of thrombosis and thromboembolism. At the same time, we observed a 12-fold ($P<0.001$) increase in serum anti-β2-GP IgG. The oxidized form of the β2-GP protein binds to dendritic cells, the activation of which can accelerate the formation of antibodies.

In turn, they lead to disruption of the complement system, as well as to the activation of the hemostasis system, a complex course of the disease, a sharp increase in thrombosis in the fetoplacental microcirculation system and fetal death. As noted above, long-term circulation of APL antibodies in plasma, free lipid vesicles in plasma, as well as in the endothelium, platelets and other cellular phospholipid compounds. This, in turn, reduces the platelet resistance of endothelial cells, activates platelets and leads to an imbalance in the coagulation hemostasis system. For this reason, we have identified some indicators of hemostasis in women with recurrent miscarriage.

Platelets are involved in primary hemostasis and in the first stage of stagnation. At this stage, platelets become active, and platelets activate platelets by exposing them to plasma factors, which in turn increase platelet aggregation. At the third stage of coagulation, a prothrombinase complex is formed on the surface

of active platelets, which, in turn, increases the production of thrombin. Thrombin is involved in the conversion of fibrinogen to fibrin. At the same time, platelets provide thrombus retraction and complete the blood clotting process.

To study the vascular-platelet stage of hemostasis, the number of platelets in the general blood test was counted. Studies have shown that the amount of hemoglobin, erythrocytes and platelets in women who have undergone RPL tends to decrease compared to the group of women who are physiologically pregnant. In particular, the amount of hemoglobin in the main and control groups was 91.93±3.68 and 108.11±1.04 g/l, the number of erythrocytes was 3.17±0.06 and 3.57±0.08x1012/l , leukocytes - 7.31±0.15 and 6.48±. 0.34x109/l, platelet count - 211.70±3.82 and 234.23±4.40x109/l, ECG - 18.23±0.69 and 10.83±0.66 mm/h. In other words, in the group of women who had a normal abortion, there was a partial decrease in the number of platelets and a statistically significant increase in ESR.

Coagulation hemostasis consists of a cascade of reactions involving plasma factors. Coagulation hemostasis was studied at all three stages of coagulation: according to the Morawitz method of clotting time (MAC) and active partial thromboplastin time (APTT) (1 stage of coagulation); prothrombin time (PT), prothrombin index (PTI) and international normalization coefficient (INR) (2 stages of blood coagulation); the amount of fibrinogen (3 stages of blood coagulation).

In the group of women who had recurrent miscarriage, a significant reduction in bleeding time was observed (see Table 3.2). In this group, the start time of blood clotting was 151.00 ± 6.82 seconds, and the end time of blood clotting was 190.54 ± 5.47 seconds. In the control group, these values were as follows: the onset of bleeding 148.69±3.73 sec, the end 205.75±4.48 sec. That is, a 1.5-fold reduction in bleeding time ($P<0.01$) in the control group of women who had experienced habitual miscarriage indicated a pronounced hypercoagulability in plasma hemostasis.

However, in the group of women who had recurrent miscarriage, plasma APTT increased to 42.8±1.26 (P<0.001) sec. In the control group, APTT was 28.26±0.15 sec. It should be noted that an increase in the APTT level of 1.7 (P<0.001) indicates the presence of LA in women. In fact, our study showed that the VA value was 3.76 times (P < 0.005) higher than normal, indicating a correlation between the two.

Table 2. Assessment of the first stage of blood coagulation coordination in recurrent miscarriage, M ± m

Indicators	**Physiological pregnancy, 9-10 weeks, n = 35**	**Habitual miscarriage, 9-10 weeks, n=35**
Start VSK, sec	148,69±3,73	151,00±6,82
Completion of VSC, sec	205,75±4,48	190,54±5,47
APTT, sec	28,26±0,15	42,8±1,26***

Note: * - differences between the indicators of the studied control group and patients with APS are significant (R < 0.05), ** - R < 0.01 and *** - R < 0.001.

It has been shown that significant disturbances in blood clotting time and partial thromboplastin time are associated with hypercoagulability at the first stage of coagulation hemostasis in habitual miscarriage. To characterize the second stage of plasma hemostasis, prothrombin time, prothrombin index and INR were studied. The study of prothrombin time showed that the hemostasis system is significantly shifted towards hypercoagulation in patients in groups of women who experienced RPL in relation to the control group. So, if in the main group PT was 16.49 ± 0.20 s (see Table 3), then in the control group it was 17.69 ± 0.28 s.

Table 3. Assessment of the second stage of blood coagulation by recurrent miscarriage, M±m

Indicators	**Physiological pregnancy, 9-10 weeks,**	**Habitual miscarriage, 9-10**

	n = 35	**weeks, n=35**
PV, sec	16,49±0,20	17,69±0,28
PTI, %	84,4±0,40	96,23±1,48*
ON	1,16±0,01	1,20±0,01
MNO	1,05±0,01	1,25±0,05*

Note: * - differences between the indicators of the studied control group and patients with APS are significant (R < 0.05), ** - R < 0.01 and *** - R < 0.001.

The prothrombin index was calculated using a special formula and amounted to 96.23 ± 1.48% in the main group and 84.4 ± 0.40% in the control group. This indicated the presence of hypercoagulation in women with recurrent miscarriage (see Table 3). The table shows that the international normalized ratio was 1.25 ± 0.05 in all women who underwent RPL, and 1.05 ± 0.01 in the control group. The study of indicators of the second stage of blood coagulation showed a significant shift of blood towards hypercoagulability in women of the main group.

The level of fibrinogen, plasma tolerance to heparin, thrombotest and thrombin time, which characterize the third stage of blood coagulation, were determined. Fibrinogen is the first coagulation factor synthesized in the liver. The study of the fibrinogen level showed a significant increase in the concentration of fibrinogen, which indicated a strong hypercoagulable shift. The content of fibrinogen in the group of women with RPL was 4334.2±148.7 mg/ml (R<0.001), in the control group this figure was 2677.14±28.91 mg/l. Thus, the study of the third stage of blood coagulation showed the presence of pronounced hypercoagulability relative to the control group in women with RPL.

Currently, one of the main markers of activation of the hemostasis system is an increase in the amount of D-dimer in the blood. The formation of fibrin D-dimer is one of the factors indicating both its breakdown and the development of

thrombosis and is important in the diagnosis of DIC. A gradual increase in D-dimer in blood plasma begins in the early stages of pregnancy and ends 3-4 times higher than normal. Such changes are especially observed in preeclampsia. **Table 4. The amount of fibrinogen and D-dimer in women with recurrent miscarriage, M±m**

Indicators	**Physiological pregnancy, 9-10 weeks, n = 35**	**Habitual miscarriage 9-10 weeks, n = 35**
Fibrinogen, mg/ml	2677,14±28,91	4334,2±148,7***
D-dimer, ng/ml	45,09±2,94	577,74±44,5***

Note: * - differences between the indicators of the studied control group and patients with APS are significant (R < 0.05), ** - R < 0.01 and *** - R < 0.001.

Therefore, we estimated the amount of D-dimer in the blood plasma of women with APS who had RPL. The study showed that the frequency in this group of women was 12.81 (R<0.001) times higher than in the control group, and amounted to 577.74±44.5 ng/ml. In the control group, its content was 45.09±2.94 ng/ml.

Conclusion. Thus, based on the results obtained, women with RPL develop hypercoagulable syndrome in the blood at 8-9 weeks of gestation due to the presence of APS. This is due, in our opinion, to the fact that antibodies are formed not only against phospholipids and the b2-GP protein, but also against all proteins associated with negatively charged phospholipids. To date, antibodies to AF have also been shown to be present in prothrombin, thrombin, protein S, protein C, thrombomodulin, annexin II and annexin V. Most of them have procoagulant effects and have thrombogenic effects.

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