THE CORRELATION OF METABOLIC AND PLATELET INDICATORS IN PATIENTS WITH HEREDITARY THROMBOPHILIA

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ABSTRACT — Patients with the A2 polymorphism of the subunit gene of glycoprotein IIb/IIIa platelet receptors showed an increased level of endothelial dysfunction markers and increased free-radical reactions, as well as an increase in the geometric parameters of platelets, an increase in their functional activity and the appearance of young cells in peripheral blood. A correlation was found between the parameters of platelet hemostasis and the severity of free-radical reactions in women patients with thrombophilia who have A2 polymorphism in the gene of the subunits of the platelet receptor glycoprotein IIb/IIIa.

KEYWORDS — gene polymorphism, free radical reactions, platelets, thrombophilia.

INTRODUCTION

It has now been established that genetic abnormalities of hemostasis are found in patients with thrombosis in 80–90% of cases. Mutation of factor V Leiden is detected in 20% of patients, other defects in the anticoagulant system (antithrombin III deficiency, protein C and S deficiency) in 20% of cases, sticky platelet syndrome (Tp) in 14% of cases, etc. [1, 9]. In modern literature there are scattered data on the relationship of the genetic polymorphism of the Tr glycoprotein (GP) IIb/IIIa receptor gene with the development of thrombosis but the information on the relationship of this polymorphism with the development of typical obstetric pathology is very contradictory [1].

Thrombophilic complications of pregnancy and childbirth are currently considered from the standpoint of the development of polyorgan failure syndrome which is based on systemic endothelial dysfunction [2, 8, 10]. And oxidant stress acts as a provoking factor resulting in the formation of lipid peroxidation products — free radicals and atomic oxygen damaging the endothelium [7, 11, 12].

The presented mechanisms of the relationship of coagulation, angiopathy and the development of oxidative stress from the standpoint of modern concepts and their violation in patients with genetic thrombophilia (TF) have not been studied.

Defects in the Tr GP IIb/IIIa receptor gene lead to an increase in the functional properties of Tr and they contribute to an increase in the thrombogenic properties of the endothelium [3, 4, 5, 6].

The purpose of the study is a comprehensive assessment and establishment of the relationship between the level of free-radical reactions, the state of the vascular wall and changes in the parameters of platelet hemostasis in pregnant women with the genetic A^2 polymorphism of the Tr GP IIb/IIIa receptor subunit gene.

MATERIALS AND RESEARCH METHODS

226 women in the third trimester of pregnancy were treated at the obstetric department of the pathology of pregnancy at the Stavropol city hospital. The age of patients ranged from 20 to 35 years with an average of 25.2±0.6 years. Only 59 of these patients had various obstetric complications associated with TP (thrombophilia), in 152 women the pregnancy was unremarkable.

Blood sampling was carried out with the consent of the attending physician while observing the rules of the preanalytical phase of the study.

All surveyed were divided into the following groups:

I — women with physiological pregnancy, genetic carrier of the normal variant of the Tr GP IIb/IIIa receptor subunit gene (Pl^{A1}/Pl^{A1}) (n = 128) — control group;

II — healthy pregnant women with a heterozygous mutation variant (Pl^{A1}/Pl^{A2}) (n = 24);

III — patients with TP and heterozygous polymorphism (Pl^{A1}/Pl^{A2}) (n = 35);

IV — patients with TP and homozygous Pl^{A2}/Pl^{A2} polymorphism (n = 39).

Patients with other confirmed mutations in the hemostatic system genes were excluded from the study.

The determination of the genotype by the Pl^{A1}/Pl^{A2} polymorphism was carried out by an amplification-restrictional method. DNA isolation was carried out by the sorption method using the kit of DNA-Sorb B. Amplification mixtures were prepared on the basis of a universal set of reagents and preparations for PCR (polymerase chain reaction) *Ampli Sens-200-1* apparatus manufactured by CSUE (central scientific university of epidemiology) of *Rospotrebnadzor*. The primers were synthesized at Liteh LLC in accordance with the sequences described by Bojesen S.E. et al., 2000 [5].

Willebrand's factor (WF) activity level was used as an indicator characterizing the state of the endothelium of the vascular wall. The determination of the levels of WF was performed on the analyzer hemostasis *STA Compact* designed by the company *Diagnostica Stago* (France) with the help of reagents *vWF STA LIATEST* by the method of latex agglutination with monoclonal antibodies to the factor.

Malonic dialdehyde (MDA) indicators were used as markers of oxidative stress. The study was performed in serum by the method of Jagi K., Nishigaki I., Chama N. and platelets by the method according to J. Smith methods.

The quantitative indices of Tr were determined using the automatic hematology analyzer *Advia 2120 i* designed by Simens company (Germany). Four platelet parameters were analyzed: PLT — total Tp in peripheral blood and platelet indices (MPV, PCT, PDW). The study of functional activity of Tr was performed in Tr rich plasma by the Born G.V.R. method with graphic registration on a *BIOLA LA-23* laser aggregometer (Russia). ADP (200 μM), collagen (0.2 mg/ml), ristocetin (5 μM/ml) were used as inducers. To study the morphometric parameters of Tr in blood smears a computer morphometric installation *MECOS-C* (Medical Computer Systems CJSC, Moscow) was used. During the study the following geometric and color-brightness characteristics of Tr were analyzed: cell area, cell diameter, form factor, blue and red color in the preparation, the index of rejuvenation of Tr (IRTr).

The degree of reliability of differences in the studied parameters was determined by the t-criterion, the level of significance was considered reliable at $p < 0.05$.

THE RESULTS OF THE STUDY AND THEIR DISCUSSION

We judged the changes in free-radical oxidation and the state of the vascular wall in women by the indices of MDA in the serum and Tr. and the level of WF.

These changes in metabolic parameters are presented in Table 1.

A comparative analysis of the data revealed that in women without clinical manifestations of TP (groups I and II), the indicators did not go beyond the reference boundaries, however, in patients with heterozygous polymorphism a significant increase in the level of MDA Tp was found ($p < 0.001$).

Analysis of data from patients with TP revealed an increase in serum lipid peroxidation and Tp in all women compared to the control group. MDA Tp in women with the homozygous polymorphism variant (Pl^{A2}/Pl^{A2}) was most changed. The difference of this indicator in comparison with the I group was 202%. Serum MDA was increased by 110%. In the group of women with TP and heterozygous polymorphism, the indicators of serum MDA and Tp were higher by 57.1% and 103%. In healthy women with heterozygous polymorphism a significant ($p < 0.001$) increase of the MDA Tr index was also noted.

The level of WF was significantly different only in patients with TP and A^2 polymorphism ($p < 0.001$). The greatest changes in this indicator were observed in group of women IV. From the indicator of group I it was different by 88.8%.

The factual data obtained indicates that in patients with TP and A^2 polymorphism the intensification of free-radical reactions indicates a complex nature of humoral and hemostasiological disorders.

Identification of significant changes in indicators in healthy pregnant women of group II shows the effect of A^2 polymorphism on the development of signs of endothelial dysfunction even without the presence of clinical manifestations of TP which may indicate a high sensitivity of these indicators to the presence of a genetic defect.

In order to assess the effect of genetic polymorphism GP IIb / IIIa on the state of platelet hemostasis a study was made of total Tp in peripheral blood (PLT) and platelet indices (MPV, PCT, PDW), Tp aggregation activity and morphometric analysis indicators. The obtained data are presented in Table 2.

When conducting a comparative analysis of the quantitative parameters of Tr it was found that in women without clinical manifestations of thrombophilia (groups I and II) the values of Tr and platelet indices practically did not differ from each other.

Comparing the quantitative parameters of Tp in women without clinical manifestations of TP with a carrier of the normal version of the Tp GP IIb/IIIa receptor subunit gene and women with a heterozygous variant of polymorphism (Pl^{A1}/Pl^{A2}) — group III there was a significant change in the MPV and PDW, which amounted to an average of 10.9 fl and 18.2%, respectively ($p \leq 0.01$).

Table 1. *Humoral indices of pregnant women (X ± m; p≤0.001)*

Indicators, units of measure	Groups of pregnant women			
	I (n=128)	II (n=24)	III (τ=35)	IV (n=39)
MDA of serum, μmol/l	3,29±0,15	3,31±0,24	5,17±0,28*	6,91±0,37*
MDA Tr, μmol /10^{9}Tp	1,82±0,09	2,61±0,19*	3,7±0,16*	5,5±0,29*
WF,%	112,6±2,34	116,9±2,54	148,7±2,73*	212,6±2,27*

Note: ** — significance of differences compared with group I patients*

Table 2. *Changes in platelet counts of healthy pregnant women and patients with thrombophilia (X ± m; p≤0.05)*

Indicators, units measuring	Patient groups			
	I (n=128)	II (n=24)	III (n=35)	IV (n=39)
PLT,109/l	265,7±7,6	271,8±7,9	289,8±11,8	234,1±12,3*
MPV, fl	9,2±0,18	9,5±0,20	10,9±0,25*	11,2±0,27*
PCT, %	0,25±0,008	0,24±0,008	0,25±0,007	0,25±0,008
PDW, %	16,9±0,17	16,8±0,18	18,2±0,17*	18,4±0,17*
Aggregation with ADP, %	46,0±0,69	51,1±1,71*	78,4±2,97*	79,4±3,68*
Aggregation with collagen, %	42,0±0,83	45,0±1,96	65,3±2,74*	68,0±2,77*
Aggregation with ristotsetin, %	47,5±0,74	51,2±1,82	86,3±2,36*	89,1±2,11*
The average diameter of Tp, μm	2,24±0,10	2,31±0,15	2,66±0,13*	2,81±0,12*
Area Tr, μm 2	3,65±0,41	3,96±0,44	5,48±0,44*	6,33±0,44*
Form factor, cu	12,8±0,15	13,5±0,17*	15,12±0,17*	16,18±0,18*
The proportion of blue color, c.u.	0,32±0,002	0,37±0,007*	0,45±0,007*	0,52±0,007*
The proportion of red color, c.u.	0,42±0,002	0,39±0,007*	0,34±0,007*	0,31±0,007*
Common index of rejuvenation of Tr (IR Tr) c.u.	0,76±0,005	0,94±0,07*	1,32±0,07*	1,67±0,11*

Note: ** — the differences are significant compared with group 1*

In patients with a homozygous variant of the Tr GP IIb/IIIa receptor gene (Pl^{A2}/Pl^{A2}) mutations in the quantitative indices of Tr are most pronounced. There is a significant (p≤0.05) decrease in the number of Tp in peripheral blood (234.1±12.3 · 10^{9}/l, p≤0.05) an increase in the average volume of Tp (11.2±0.27 fl) and Tp anisocytosis index (18.4±0.16%) in comparison with the data of healthy puerperal (p≤0.001) of group I.

Analysis of the aggregatogram data showed that the groups of healthy puerperal significantly differed in aggregation induced by ADP (p ≤ 0.01) and there was a tendency for an increase in the degree of aggregation with collagen and ristocetin in the group of women with a heterozygous mutation in the Tr GP IIb / IIIa receptor gene. In patients with TP and Tp GP IIb / IIIa Pl^{A1}/Pl^{A2} receptor gene polymorphism there was an increase in Tg aggregation for 66% for ADP, 81% for ristocetin and 80% for collagen compared with the healthy women with normal genotype. In the group of women with the $P1^{A2}/P1^{A2}$ polymorphism the aggregation activity induced by ADP, ristocetin and collagen at the indicated concentrations which was higher by 68%, 87% and 61%.

An analysis of the morphometric parameters of Tr of healthy women revealed an increase in the functional activity of Tr in group II as evidenced by a significant (p≤0.001) increase in the index of the form factor. In addition, an increase in the number of young Tp was observed, which was manifested in an increase in the share of blue color and a decrease in the share of red color in the preparation. IR Tr was significantly higher in group II and amounted to 0.94 c.u (in group I — 0.76 in. e.), which reflects the activation of Tp in patients with Pl^{A1}/Pl^{A2} polymorphism compared with women with normal genotype. There was a tendency to an increase in the indices of the diameter and area Tr.

In patients with clinical manifestations of TP the mean diameter of Tp, cell area form factor and IR Tr were significantly increased (p≤0.001).

Thus, all geometrical and color-bright parameters changed most vividly in patients with clinical manifestations of TP and a homozygous variant of polymorphism (Pl^{A2}/Pl^{A2}). At the same time, the difference between the average diameter of Tp expressed as a percentage between patients of group V and healthy women of group I was 25.4%, between indicators of the area Tp — 73.4%, form factor — 26.4%. In patients with the Pl^{A1}/Pl^{A2} polymorphism the cell diameter was 18.7% larger, the form factor 18.1%, and the Tp area was increased by 50.9%. In women with the A1A1 genotype the diameter, area, and form factor indices were increased by 9.8%, 50.9% and 10.2% in comparison with the data of women of the first group.

The assessment of the intensity of *rejuvenation* Tr was carried out according to the degree of change in the IR Tr. The appearance of young Tp was detected in all groups of women with clinical manifestations of TP but to the greatest extent in patients with a homozygous mutation (1.67 in). In groups of women with a normal genotype and a heterozygous mutation this indicator was 1.31 c/u and 1.32 c.u.

Thus, in patients with Pl^{A2}/Pl^{A2} polymorphism there was a significant (p≤0.001) increase in geometric parameters with greater functional activity of Tp and the appearance of young cells in peripheral blood. In the groups of puerperal with the normal genotype and the polymorphism of Pl^{A1}/Pl^{A2}, similar changes were revealed but the degree of increase in the indicators was slightly lower than in patients with the polymorphism of Pl^{A2}/Pl^{A2}.

The important role of the genetic defect of the Tr GP IIb/IIIa receptor subunit gene in the development of thrombotic complications of pregnancy and childbirth is indicated by the fact that the most pronounced changes in the quantitative, functional and morphometric parameters of Tp were observed in a group of patients with homozygous polymorphism of this gene (Pl^{A2}/Pl^{A2}).

Since the heterozygous mutation variant (Pl^{A1}/Pl^{A2}) was also encountered in healthy women without clinical manifestations of TP it can be assumed that the genetic polymorphism of the Tr GP IIb/IIIa receptor subunits does not necessarily lead to the occurrence of the disease. It is possible that other provoking factors contribute to this situation. Detection of mutations and polymorphism in genes is an indication for monitoring the state of the hemostasis system since the presence of polymorphism in the Tr GP IIb/IIIa receptor subunit gene and especially when combined with other gene defects it may be associated with the risk of developing thrombosis.

There is the fact that the presence of A^2 dysmorphism of the Tp GP IIb/IIIa receptor subunit gene in pregnant women leads to an increase in free-radical reactions led us to conclude that the effects of the above disorders are indicators of platelet hemostasis in women with A^2 polymorphism.

To confirm this conclusion we carried out a correlation analysis of humoral and platelet indicators (see Table 3).

Table 3. *Correlation of humoral and platelet indicators in patients with thrombophilia*

Metabolic indicators	Hemostatic Indicators	Correlation Coefficient
MDA serum	PDW	R=0,33
	Aggregation with ADP	R=0,35
	Form Factor Tr	R=0,34
MDA Tr	MPV	R=0,42
	PDW	R=0,55
	Aggregation with ADP	R=0,61
	Area Tr	R=0,53
	Form factor Tr	R=0,42
	IR Tr	R=0,45

During the correlation analysis we established positive correlation links between an increase in serum MDA level and an increase in the heterogeneity index of the Tp population (R = 0.33) increased aggregation with ADP (R = 0.35) and an increase in the Tp form factor (R = 0.34). The indicators of lipid peroxidation (LP) in the Tr membranes were closely correlated with the parameters of hemostasis. The most significant were the association of increased LP with an increase in functional properties of Tr, as evidenced by the presence of positive correlations between the increase in Tr, the heterogeneity of the platelet population and the increased aggregation with ADP, an increase in the Tr form factor and IR Tr.

Based on the correlation analysis the dependence of the parameters of platelet hemostasis on the severity of free-radical reactions in women with TP with A^2 polymorphism in the Tr GP IIb/IIIa receptor subunit gene was revealed which is confirmed by significant correlations between metabolic and platelet indicators.

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