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The Peculiarities of Vascular-Platelet Hemostasis at Different Stages of Physiological Pregnancy

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Abstract

The article presents the allowed value ranges of platelet aggregation, activity and amount of von Willebrand factor and ADAMTS-13 during physiological pregnancy. The obtained data clarify the mechanisms of platelet activation and can be used as reference values in the study of the hemostatic system in pregnant women and formation of groups at risk for bleeding and thrombosis.

Keywords: platelet aggregation, von Willebrand factor, metalloproteinase ADAMTS-13, physiological pregnancy.

Introduction

The hemostatic system during pregnancy can be represented as a model of balanced reactions with increasing thrombogenicity at the end of pregnancy (Holmes and Wallace 2005). It is obvious that the formation of a "thrombogenicity model" by the mother's body was intended to reduce blood loss during delivery. The shift of the balance of this model towards the coagulation increase leads to the development of venous thrombosis of different localizations and severities in the mother's body (for example: pulmonary embolism in pregnant women is in 4-50 times higher than in non-pregnant women). Many types of fetal pathology (miscarriages, intrauterine growth retardation, premature birth, pathophysiological syndromes: microcirculatory dysfunction of uterine and fetoplacental blood flow) are associated with activation of coagulation. Due to low or reduced activation of coagulation massive uterine bleeding may be developed which is a critical state for both a mother and a baby with blood loss more than 1.5% of body weight in labor. The study of hemostatic system is of great importance. Publications describe some hemostatic parameters in pregnancy without any identified pathologies (Brenner 2004, Franchini 2006, O'riordan and Higgins 2003, Thornton 2010, Abbassi-Ghanavati et al. 2009, Klajnbard et al. 2010, Ramsay 2010, Szecsi et al. 2010). However, in the available publications we failed to determine the role of such factors of vascular-platelet hemostasis – platelets, von Willebrand factor, metalloproteinase ADAMTS-13, platelet factor 4 (PF4), endothelin-1 in blood at different stages of pregnancy. We consider that our study of these analytes is clinically important for the formation of groups at risk for thrombosis and/or bleedings during pregnancy and delivery.

Objective

The objective is to determine allowed value ranges of the parameters characterizing the participants of vascular-platelet hemostasis (platelet function, von Willebrand factor activity (vWF:RCoF) and antigen (vWF:Ag), ADAMTS-13 antigen and activity, the level of platelet factor 4 - PF4 and endothelin-1) in pregravid period, during pregnancy without obvious signs of a pathology and 2-3 days after delivery.

Materials and Methods

The study involved data obtained from 301 Caucasian (white) women who provided their informed consent. The study was approved by the local Research Ethics Committee of the Altai Medical University (Protocol № 14, 11.12.2013). There were the following exclusion criteria: age under 18 and over 35 years, women with the history of obstetric and gynecological disorders, personal history of hemorrhage or thrombosis, administration of combined oral contraception for at least 3 months before pregnancy, assisted reproductive technologies, operative delivery. Besides, exclusion criteria

included the presence of extragenital pathology including diabetes mellitus, bronchial asthma, endocrine disorder, mesenchymal dysplasia, heart valve or vessels prostheses, acute pyelonephritis, cystitis or its exacerbation, malignant neoplasms, human immunodeficiency virus carrier state, viral hepatitis type B or C carrier state. Women with drug administration during pregnancy affecting the hemostatic system (lowering platelet aggregation, nonsteroidal anti-inflammatory drug, anticoagulants) were also excluded. Deviations in the results of laboratory examinations: homocysteine level in blood serum (before pregnancy) more than 15 μmol, hemoglobin more than 140 g/l in non pregnant women, platelet count less than 150 or more than $450x10^9$/l, white blood count more than $12x10^9$/l; factor V Leiden mutation carrier state (1691G>A) and/or FII mutation carrier state (20210G>A), rare homozygous MTHFR (677C>T), as well as three or more polymorphisms of MTHFR (677C>T), PAI 1 (-675 5G>4G) fibrinogen-FGB (–55G>A) platelet receptors GP IIIA (1565T>C) and GP IA (807C>T); antiphospholipid syndrome and other autoimmune states were also considered as exclusion criteria.

The following endpoints were selected to study hemostatic system, taking into account "critical" stages of pregnancy: pregravid period, 6-8 weeks, 12-13 weeks, 22-24 weeks, 34-36 weeks and 2-3 days after delivery (Table 1). All women enrolled to the study were primarily examined once.

Table 1. *Characteristics of the Examined Women and Born Babies in Present Pregnancy (n=301)*

Feature	**Sample Time**				
	6-8 week (n=54)	*12-13 week (n=43)*	*22-24 week (n=59)*	*34-36 week (n=40)*	*After delivery (n=51)*
Mean age (years±SD)	27,02±4,44	27,21±3,76	26,64±3,69	27,20±4,44	25,84±4,43
Primigravida women [n (%)]	32 (59,3)	25 (58,1)	33 (55,9)	29 (72,5)	21 (41,2)
Multigravida women [n (%)]	22 (40,7)	18 (41,9)	26 (44,1)	11 (27,5)	30 (58,8)
The mean gestational age in peripartum period (days±SD)	274,9±21,6	279,1±11,8	277,0±15,6	281,8±9,3	279,1±7,4
Mean weight of a baby at birth (grams±SD)	3468,0±385,3	3538,9±436,5	3384,3±371,9	3510,6±425,9	3478±418,4

Statistical Analysis

The statistical analysis of data were done by using the programs SPSS 16.0 for Windows (*SPSS Inc.*, USA) and STATISTICA 6.1 (Statsoft Inc., Tulsa, USA). To test whether our data was normally distributed, we used the Kolmogorov-Smirnov and the Shapiro-Wilk tests. The data from different

laboratory methods were found to be not of normal distribution. Nonparametric tests (Kruskal Wallis and Mann-Whitney U tests) were used for comparative analysis. Reference intervals (2.5^{th} and 97.5^{th} percentiles with Confidential Interval 95%) were calculated for each test in each time point. Spearman rank correlation coefficients were used. The differences were considered statistically significant when the p value was < 0.05.

Blood Collection

Venous blood collection was carried out from the cubital vein to the test tubes VACUETTE with buffer solution of sodium citrate in a ratio of 9:1 (9NC Coagulation sodium citrate 3.2%). Blood was centrifuged at 1400g for 15 min at room temperature resulted in platelet poor plasma. To study platelet function, platelet enriched plasma was obtained by blood centrifugation at 160 g for 7 min at the same temperature conditions. Plasma samples were studied during the first two hours. Prior to enzyme immunoassays or endogenous thrombin potential assessment by thrombin generation test, plasma was stored in 1.5 ml Eppendorf tubes filled to ¾ at temperature -40^{o}C in the time interval from one day to one month in MDF-192 Ultra low temperature freezer (Sanyo).To determine homocysteine level, blood serum obtained by vacuum tubes Improvacuter (Lind-Vac) was used without filler. Platelet counts were determined in stabilized EDTA blood using vacuum tubes Improvacuter (Lind-Vac) with dissolved stabilizer - EDTA K3.

In the study the following equipment was used: Hematology analyzer MEK-7222 J/K (Nihon Kohden); optical aggregometer AggRAM (Helena Laboratories) and a microplate photometer Bio-Rad 680 (Bio-Rad Laboratories).

Results

Platelet aggregation in platelet enriched plasma used as an agonist of low dose (0.1 μmol) disodium salt of adenosine diphosphate (ADP) increased during pregnancy compared to women in the pregravid period (Figure 1a). This increase was observed in median from early pregnancy (12-13 weeks, by 1.9 times) and reached its maximum in late pregnancy. In contrast, if the dose of ADP was changed into 2.0 μmol or other reagents (adrenalin, collagen or ristocetin) were used to stimulate platelet aggregation, the differences in the results between pregnant and non-pregnant women were not observed (Figure 1b, Table 2). It should be noted that the level of PF4 in blood plasma in pregnant women was not different compared to its content in the pregravid period (Figure 1h).

Platelet activation was accompanied by the gradual increase of von Willebrand factor level that was measured by two different kits of reagents (Figures 1c, 1d). The obtained results correlated with each other (correlation coefficient 0.34; $p<0.001$) but they had different reference intervals at different stages of pregnancy. Ristocetin cofactor activity (RCoF) also grew

up from 12-13 weeks of pregnancy and increased in median compared to baseline values (pregravid period) by 1.18 times at 22-24 weeks and by 1.41 times at 34-36 weeks (Figure 1e). The correlation coefficient between the platelet aggregation activity (ADP in a dose of 0.1 μmol/) and RCoF or VWF Ag was 0.17 ($p=0.025$) and 0.34 ($p<0.001$), respectively.

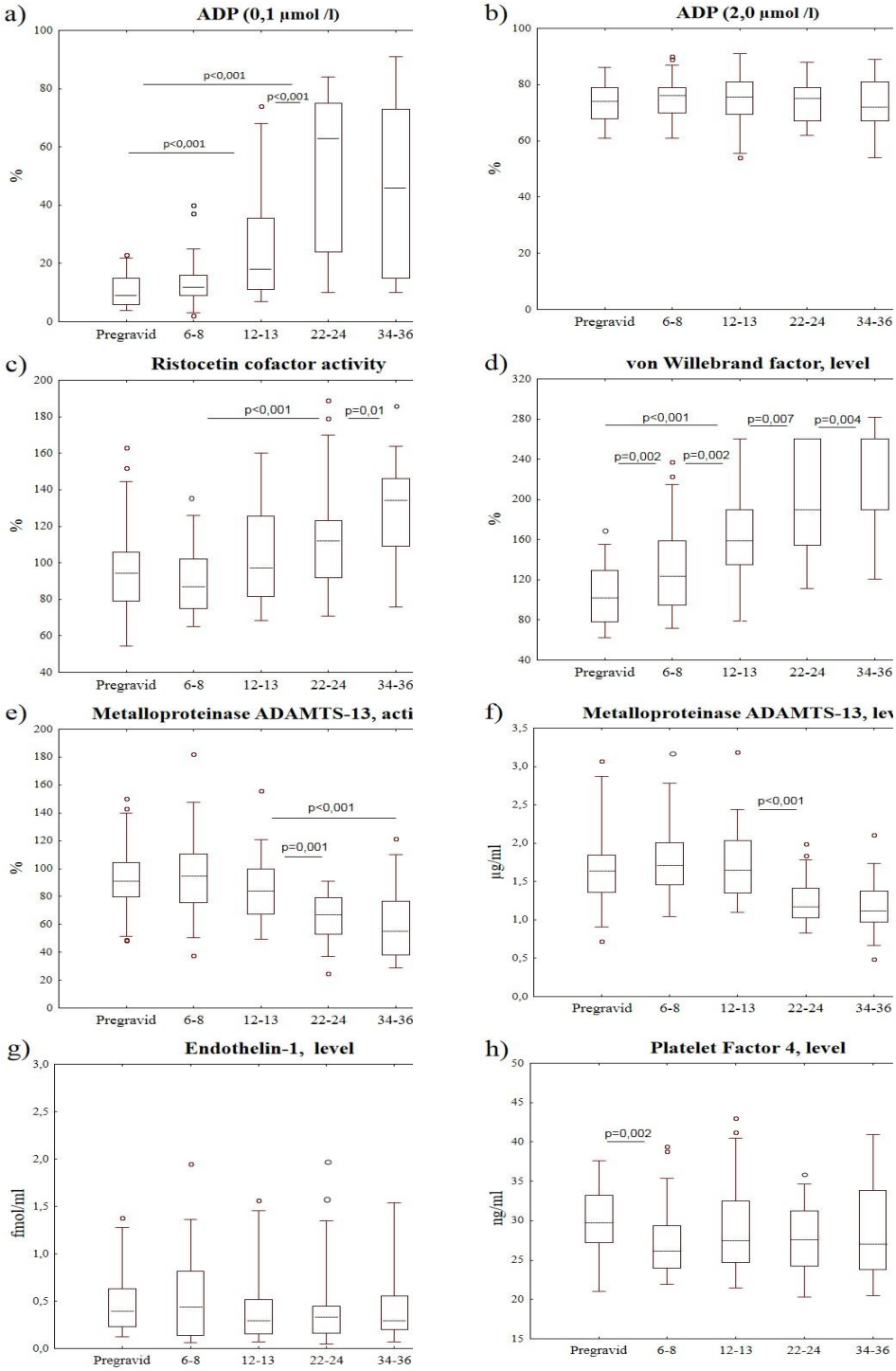
The obtained results also indicate the stable level and activity of metalloproteinase ADAMTS-13 [metalloproteinase which cleaves von Willebrand factor ("a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13") at 6-8 weeks and 12-13 weeks, which were not different from the results of women in pregravid period. Then starting from 22nd week to the late pregnancy noticeable reduction of this metalloproteinase was observed -by 28.7-31.7% (antigen) and by 26.4-39.2% (activity) compared to the data in pregravid period.

In the performed studies correlation coefficient between RCoF and ADAMTS-13 activity was -0.27 ($p<0.001$) and between antigens vWF and metalloproteinase ADAMTS-13 (with reagent vWF AG) was -0.32 ($p<0.001$). In Annex, we present in details all the results, in table format.

Table 2. *Allowed Value Range of Platelet Aggregation in Blood (% Aggregation) with ADP (Final Concentration is 0.1 μmol)*

Gestational age	**Me**	**Reference interval (2,5‰-97,5‰)**
Pregravid period (n=48)	10,0	4,0-22,98
6-8 week (n=50)	13,0	3,0-36,7
12-13 week (n=4)	19,0	6,5-98,75
22-24 week (n=58)	64,0	10,0-86,25
34-36 week (n=38)	47,0	9,6-91,6
2-3 days after delivery (n=31)	38,0	12,0-77,9

Figure 1. *Box Plots of Reference Intervals in Pregravid Period, Different Stages of Pregnancy and after Delivery for a) ADP (0.1 µmol /L), b) ADP (2.0 µmol/L), c) Ristocetin Cofactor Activity Von Willebrand Factor, d) Von Willebrand Factor Level, e) Metalloproteinase ADAMTS-13, Activity, f) Metalloproteinase ADAMTS-13, Level, g) Endothelin -1, Level, h) Platelet Factor 4, Level*



Note: Figures represent the range of data from the 25th to 75th percentiles, while the bar in the middle of each box plot represents the median value obtained excluding outliers. Circles indicate outliers (1.5 × the interquartile range) and extreme values (3.0 × the interquartile range) outside the central box, respectively.

Discussion

In the present study prothrombotic manifestations in vascular-platelet hemostasis include the increase of platelet aggregation activity in response to low dose of ADP (0.1 μmol), the increase of vWF Ag and vWF RCoF with the simultaneous decrease of a potential of metalloproteinase ADAMTS-13. During physiological pregnancy platelet activation and the increase of their ability to aggregate was described earlier. The latter was explained by the increase of a number of aggregates of circulating platelets (O'Brien et al. 1986) as well as by the increase of a base level of a component of platelet α-granules CD63 (Holthe et al. 2004). According to this publication, including observations of 325 women at the age from 14 to 41 years old, the decrease of ADAMTS-13 activity was registered in up to 65% compared to the values in pregravid period (17-23 weeks), 61% (24-28 weeks), 65% (29-35 weeks), 58% (36-40 weeks), and 52% (first two days after delivery). Moreover, they identified the dependence between the increase of vWF antigen and the decrease of the amount of enzyme ADAMTS-13 (Pearson correlation coefficient -0.22, p=0.0003) during pregnancy. It is suggested that the increase of vWF Ag during pregnancy is associated with hyperestrogenemia and activation of its synthesis by endothelium of blood vessels (Sánchez-Luceros et al. 2004, Szecsi et al. 2010, Mannucci et al. 2001).

vWF is known to be a complex multimeric adhesive glycoprotein (in monomeric form 280 kDa) synthesized by endothelial cells and megalokaryocytes (Moake 2007). A special role of this factor in hemostatic system is determined by its structure in the form of a series of heterogeneous multimers (from 0.5 to 20 mln Da) consisting of repetitive subunits with binding domains of glycoprotein platelet receptors (GPIb and IIb/IIIa), collagen, heparin, and factor VIII. It mediates platelet adhesion to subendothelium through the interaction with platelet receptor – glycoprotein Ib (Arya et al. 2002). Large vWF multimers are cleaved by metalloproteinase ADAMTS-13 during their release from endothelial cells under the influence of proinflammatory cytokines (Bernardo et al. 2004).

Ultralarge vWF multimers with low activity of this enzyme initiate platelet activation and are able to lead to thrombocytopenia and multiple formation of hyaline (thrombocytic) thrombi in microcirculation vessels in thrombotic thrombocytopenic purpura – TTP (Moake and McPherson 1989). It can be stated that vWF RCoF during pregnancy is in opposition to functional activity of metalloproteinase ADAMTS-13 released by endothelium of blood vessels and cleaved vWF. Failure to degrade large multimers, vWF is associated with inherited and idiopathic types of TTP or predispose to similar abnormalities (Moake and McPherson 1989). TTP is more common in women (66-78%) and 12-25% of the episodes are related to pregnancy (Levy et al. 2001) mostly in the III trimester and in postpartum period (George 2003, Vesely et al. 2003).

The work by Lattuada and et al. (2003) is of great interest. It describes the reduction of the enzyme ADAMTS-13 activity in patients with HELLP-syndrome (in median 31% with the ranging 12-43%) compared to women in the III trimester of physiological pregnancy (in median 71 ranging 48-

105%) and non-pregnant women (in median 101% ranging 45-152%). ADAMTS- 13 activity often falls below threshold level in antiphospholipid syndrome (Austin et al. 2008). Moreover, the association between the decrease of ADAMTS-13 activity and the increase of RCoF and vWF Ag in pregnant women with pre-eclampsia was recently shown (Aref and Goda 2013). In general, it can be assumed that the increase of RCoF and vWF Ag is not that significant for pregnancy as the decrease of ADAMTS-13 activity and antigen potentially contributing to the increase of this factor multimerization and the increase of platelet activation before delivery.

Conclusion

In the article, a number of laboratory parameters is studied which characterize the peculiarities of vascular-platelet hemostasis at different stages of physiological pregnancy and at first days after vaginal delivery compared to the values of these parameters in pregravid period. Reference ranges of different study methods related to the hemostatic system in Caucasian women are presented. They might be different between racial groups, regions of the world as well as due to the peculiarities of pre-analytical and analytical phases of laboratory testing.

According to the obtained results and taking into account the opinions of other authors, we consider that:

- the assessment of the excessive platelet activation during pregnancy can be performed in the study of aggregation of this cells with minimum dose of ADP. This method along with others can be used in order to get objective reasons to prescribe medicinal drugs reducing platelet function;
- the decrease of metalloproteinase ADAMTS-13 activity and level from 12-13 weeks of gestation can be significant for platelet activation and risk of thrombotic microangiopathy.

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Annex

Table A1. *Allowed Value Range of Von Willebrand Factor-Ristocetin Cofactor (%)*

Gestational age	Me	Reference interval (2,5‰-97,5‰)
Pregravid period (n=48)	94,5	53,8-152,28
6-8 week (n=50)	87,0	64,15-128,55
12-13 week (n=4)	97,0	67,9-160,0
22-24 week (n=58)	112,0	70,1-178,1
34-36 week (n=38)	134,0	74,8-167,45
2-3 days after delivery (n=31)	138,5	89,0-178,88

Table A2. *Allowed Value Range of Von Willebrand Factor Antigen in Blood Plasma (%)*

Gestational age	Me	Reference interval (2,5‰-97,5‰)
Pregravid period (n=48)	102,0	62,0-157,33
6-8 week (n=50)	123,5	71,18-221,6
12-13 week (n=4)	159,0	79,0-260,0
22-24 week (n=58)	190,0	103,75-260,0
34-36 week (n=38)	260,0	121,0-282,2
2-3 days after delivery (n=31)	260,0	169,58-291,0

Table A3. *Allowed Value Range of ADAMTS-13 Activity in Blood Plasma (%)*

Gestational age	Me	Reference interval (2,5‰-97,5‰)
Pregravid period (n=48)	90,8	49,6-142,33
6-8 week (n=50)	94,55	44,87-151,7
12-13 week (n=4)	83,75	49,6-126,46
22-24 week (n=58)	66,8	31,7-92,0
34-36 week (n=38)	55,25	28,67-110,93
2-3 days after delivery (n=31)	58,2	40,45-149,85

Table A4. *Allowed Value Range of ADAMTS-13 Antigen (Mg/Ml)*

Gestational age	Me	Reference interval (2,5‰-97,5‰)
Pregravid period (n=48)	1,64	0,89-2,88
6-8 week (n=50)	1,71	1,04-3,04
12-13 week (n=4)	1,65	1,09-2,54

22-24 week (n=58)	1,17	0,79-1,82
34-36 week (n=38)	1,12	0,65-1,76
2-3 days after delivery (n=31)	1,02	0,70-2,24

Table A5. *Allowed Value Range of Plate Factor 4 (PF4) Antigen in Blood Plasma (Ng/Ml)*

Gestational age	**Me**	**Reference interval (2,5‰-97,5‰)**
Pregravid period (n=48)	29,76	20,88-38,55
6-8 week (n=50)	26,09	21,73-38,08
12-13 week (n=4)	27,47	21,38-41,27
22-24 week (n=58)	27,6	20,0-35,76
34-36 week (n=38)	27,07	20,35-40,95
2-3 days after delivery (n=31)	26,87	21,88-36,88

Table A6. *Allowed Value Range of Endothelin-1 Antigen in Blood Serum (Fmol/L)*

Gestational age	**Me**	**Reference interval (2,5‰-97,5‰)**
Pregravid period (n=48)	0,39	0,1-1,31
6-8 week (n=50)	0,44	0,06-1,86
12-13 week (n=4)	0,29	0,06-1,6
22-24 week (n=58)	0,33	0,05-1,54
34-36 week (n=38)	0,3	0,07-1,68
2-3 days after delivery (n=31)	0,31	0,05-1,24