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Studies on Blood Coagulation and Fibrinolysis in Pregnancy, during Delivery and in the Puerperium

I. Normal Condition

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Abstract. Blood coagulation and fibrinolytic variables were investigated with recently developed laboratory methods during normal pregnancy, at delivery and during puerperium. The state of hypercoagulability was registered by an increase in activity of factors XII, X and VIII, in VIII antigen, in ratio between VIII antigen and activity as well as in fibrinogen. Activation of blood coagulation at delivery results in decreasing levels of factors XII and XI and an increase in fibrinopeptide A. However, normal mean values of antithrombin and platelets were observed. An increase is noted in the plasminogen level as well as in urokinase inhibitor during pregnancy. In addition α_2-antiplasmin and prekallikrein were studied; however, no marked changes were noted.

Introduction

Thromboembolism and bleeding complications are two major causes of mortality during pregnancy, delivery and puerperium. Changes in blood coagulation and fibrinolysis take place during pregnancy to create a situation of hypercoagulability (3, 13, 17). This phenomenon is due to hormonal changes and will protect the woman from life-threatening bleeding during delivery, but it will also make the woman predisposed to thromboembolism. In order to be able to monitor women with coagulation complications which may occur at thromboembolism, toxicosis and intrauterine growth retardation, normal laboratory values during normal pregnancy and deliv-

ery should be known. Such studies have been performed earlier (3, 13, 17, 22). However, new laboratory methods useful in this respect have been developed and, therefore, must be evaluated.

Materials

Blood coagulation and fibrinolysis were studied in 9 healthy women, mean age 28 (22–33) years, during pregnancy, at delivery and during puerperium. 3 women had blood group A Rh+ and 6 had 0 Rh+. All women were closely observed during the pregnancy and delivery. No hypertension, proteinuria, growth retardation or ablatio placentae were noted. Normal weight gain was observed in all women. The management of the pregnancy was according to routine at the Department of Obstetrics and Gynecology, Karolinska Hospital.

Blood samples were drawn from the women in week 15, 20, 28, 32, 34, 36 and 38 of pregnancy, at delivery (no later than 12 h post partum) and 1 and 8 weeks after delivery. The last sample was considered to reflect the normal state of the woman. 7 of the women breast-fed their children at this time. The women were fat-starving at blood sampling.

Methods

Blood Sampling

After the first 3–5 ml blood were discarded, blood was allowed to run directly into an anticoagulant or antifibrinolyticum (see ref. for respective methods), mixed thoroughly and centrifuged at 2,500 *g* for 20 min. The plasma was drawn off and deep frozen at −70 °C until the tests were performed. Platelet count was carried out immediately on fresh blood and ethanol gelation test was performed on fresh plasma. All other tests were made on frozen samples in duplicate or triplicate. Pooled human plasma from healthy men, collected in the same way, was used as standard. Platelet count, fibrinogen level, ethanol gelation test and thrombin and Reptilase times were analyzed as earlier described (8).

Factor XII and XI levels were measured in a one-stage recalcification system after addition of kaolin (20). Prekallikrein, factor X, antithrombin, antiplasmin and inhibitors to urokinase were anayzed with the chromogenic peptide substrate S-2302, S-2222, S-2238, S-2251 and S-2444, respectively (1, 2, 5, 12, 18). Factor VIII activity (VIII:C) was measured by both one- and two-stage methods (7, 16).

Factor VIII antigen (VIIIR:Ag) was analyzed by immunoelectrophoretic assay (8). Fibrinopeptide A (FPA) was measured by radioimmunological assay (10).

Fibrinogen degradation products (FDP) and plasminogen were analyzed immunologically (11, 12, 14). In the latter, partigen plates from Behringwerke were used. Plasminogen was also analyzed using the chromogenic substrate S-2251 (6). Student's t test for dependent values was used for statistical analysis; significant level $p < 0.05$. When changes in pregnancy were statistically analyzed, the values from the sampling 8 weeks after delivery served as control, as the women could not be checked before pregnancy. Selected coagulation parameters from a group of 20 normal fertile women were used as reference (19).

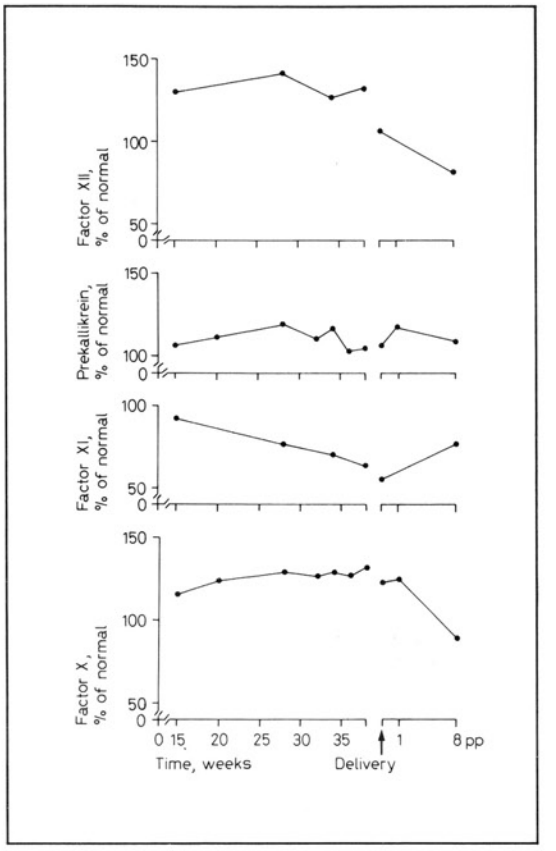

Fig. 1. Mean values for factor XII, prekallikrein, factor XI and X during pregnancy, after delivery and during puerperium (pp).

Results

All women delivered healthy children, 7 at full term and 2 in the 36th week of pregnancy. The weight of the children at birth was mean 3,745 g (2,920–4,880) and no fetal growth retardation was noted. No asphyxia was noted. The mean blood loss at delivery was 520 ml including 1 woman who was given 2 units of blood due to retention of placenta. This woman developed superficial migrating thrombophlebitis 2 days after delivery. 1 woman had positive glucosuria and was treated with insulin during the last trimester of pregnancy.

Table I. Blood coagulation factors XII, XI, X and prekallikrein during pregnancy, at delivery and during puerperium

Method		Week of pregnancy							At delivery	Week after delivery		Reference values
		15	20	28	32	34	36	38		1	8	
Factor XII,	$\bar{X}$	130	–	142	–	127	–	133	107	–	82	95
% of normal	SD	24		43		28		24	27		18	19
	n	8		9		9		7	9		9	20
	p	<0.001		<0.001		<0.001		<0.01	<0.01		–	
Prekallikrein,	$\bar{X}$	107	111	119	110	116	103	108	106	117	109	100[1]
% of normal	SD	19	17	15	42	20	17	20	20	16	15	15
	n	8	7	9	9	8	7	7	9	9	8	25
	p	NS	NS	NS	NS	NS	NS	NS	NS	NS		
Factor XI,	$\bar{X}$	93	–	77	–	71	–	64	56	–	76	94
% of normal	SD	23		18		11		8	14		10	15
	n	8		9		9		7	9		9	20
	p	NS		NS		NS		<0.05	<0.01		–	
Factor X,	$\bar{X}$	115	125	129	125	129	126	131	123	124	88	86
% of normal	SD	12	18	23	14	15	18	17	27	16	8	13
	n	8	9	9	9	9	7	7	9	9	9	20
	p	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	–	

[1] This normal material was randomly selected (see ref. 19).

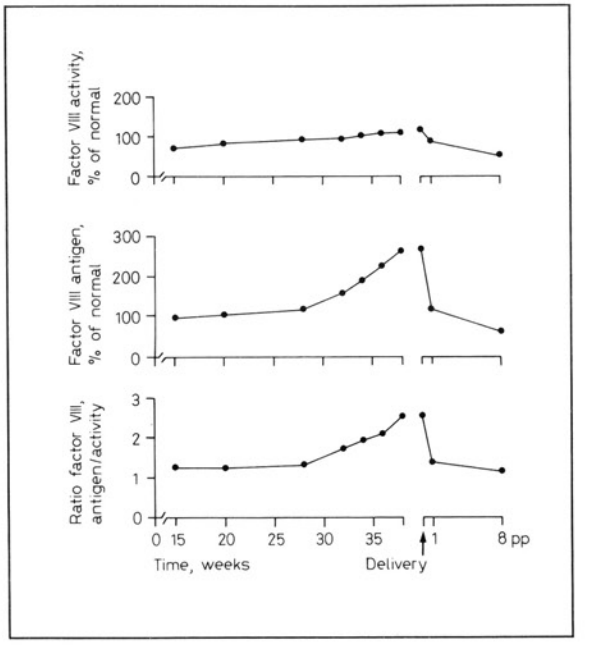

Fig. 2. Mean values for factor VIII activity, factor VIII antigen and ratio antigen/activity during pregnancy, at delivery and during puerperium (pp).

With regard to blood coagulation variables, an increase was found for factor XII and a slight decrease for factor XI during late pregnancy (table I; fig. 1). The level of both factors was normal 8 weeks after delivery. The level of prekallikrein was unchanged during the whole observation period. Factor X increased in the early stage of pregnancy and remained elevated until at least 1 week after delivery.

Factor VIIIR:Ag increased pronouncedly during the last trimester (table II; fig. 2). There was also a significant increase in factor VIII:C. However, the latter increase was not as prominent as that of factor VIIIR:Ag. These findings show that there is a steady increase in the ratio between factor VIII antigen and activity, from 1.1 in 15th week of pregnancy to 2.5 in the last part of the third trimester and at delivery. 2 months after delivery the ratio was found to be normal again. In 13 samples drawn on different occasions from 8 of the women, the factor VIII activity measured by the two-stage method was compared with factor VIII activity analyzed with a recalcification method. The latter in all cases gave higher values resulting in

Table II. Factor VIII-clotting activity, factor VIII-related antigen and ratio antigen/clotting activity during pregnancy, at delivery and during puerperium

Method		Week of pregnancy							At delivery	Week after delivery		Reference values
		15	20	28	32	34	36	38		1	8	
Factor VIII:C,	$\bar{X}$	71	84	91	91	101	107	110	113	90	54	69[1]
% of normal,	SD	18	24	20	27	20	23	30	24	21	13	15
2-stage method	n	8	9	9	9	9	7	7	9	9	9	20
	p	<0.01	<0.001	<0.001	<0.001	<0.001	<0.001	<0.01	<0.001	<0.01	–	–
Factor VIIIR:Ag,	$\bar{X}$	96	101	118	152	188	225	264	269	117	60	75
% of normal	SD	36	26	37	63	75	81	80	96	48	18	34
	n	8	9	9	9	9	7	7	9	9	8	20
	p	<0.05	<0.001	<0.001	<0.01	<0.001	<0.001	<0.001	<0.001	<0.01	–	–
VIIIR:Ag/VIII:C	$\bar{X}$	1.25	1.24	1.31	1.70	1.92	2.09	2.51	2.53	1.33	1.15	1.06
	SD	0.30	0.26	0.27	0.37	0.43	0.59	1.07	0.66	0.32	0.29	0.34
	n	8	9	9	9	9	7	7	9	9	9	20
	p	NS	NS	NS	<0.001	<0.01	<0.01	<0.01	<0.001	<0.05	–	–

[1] Mean value for 80 normals (40 men, aged 24–61 years, and 40 women, aged 21–61 years) was 77%.

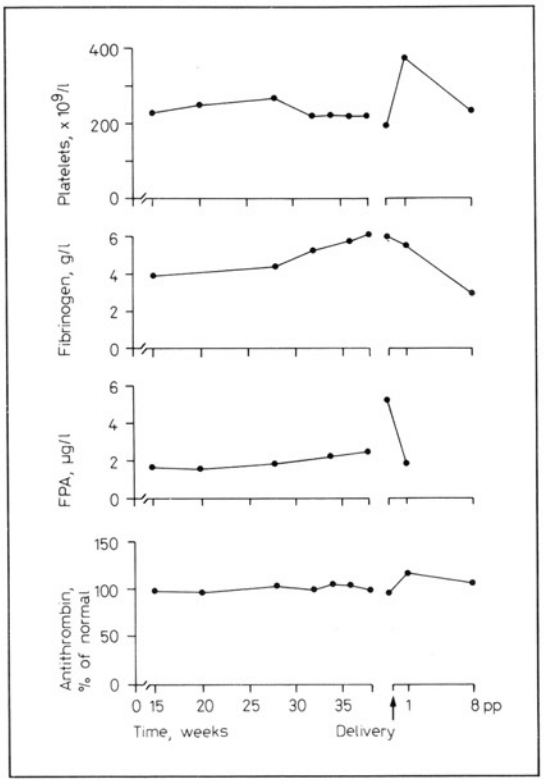

Fig. 3. Mean values for platelet counts, fibrinogen, fibrinopeptide A (FPA) and antithrombin during pregnancy, at delivery and during puerperium (pp).

a lower ratio (68 ± 7% of the ratio calculated when factor VIII:C was analyzed with the two-stage method). However, there was still a good correlation (r = 0.8) between both calculated ratios.

Platelet count was above 200 × 10⁹/l in all women during pregnancy (table III, fig. 3). 1 week after delivery an increase to a mean of 380 × 10⁹/l was noted.

An increase in fibrinogen level was noted during pregnancy. FPA increased slightly at delivery (table III; fig. 3). There was no correlation (r = 0.47) between the ratio of VIII:R Ag to VIII:C and FPA when the values from all the women were analyzed. However, when each woman was studied individually, there seemed to be a good correlation in 4 of them (r = 0.9).

Table III. Platelet count, fibrinogen, FPA and antithrombin during pregnancy, at delivery and during puerperium

Method		Week of pregnancy							At delivery	Week after delivery		Reference values
		15	20	28	32	34	36	38		1	8	
Platelet count,	$\bar{X}$	228	249	266	224	225	219	220	196	381	241	278[1]
$\times 10^9$/l	SD	56	56	42	49	31	38	67	40	110	64	41
	n	8	8	8	8	9	7	7	9	9	9	20
	p	NS	NS	NS	NS	NS	NS	NS	NS (0.1–0.05)	<0.01	–	
Fibrinogen,	$\bar{X}$	3.9	–	4.3	5.2	–	5.7	6.1	6.0	5.6	2.9	3.2
g/l	SD	1.0		0.7	0.5		0.5	1.1	1.6	1.4	0.5	0.6
	n	8		9	9		7	7	9	9	9	20
	p	<0.05		<0.001	<0.001		<0.001	<0.001	<0.001	<0.001	–	
FPA, µg/l	$\bar{X}$	1.7	1.5	1.8	–	2.2	–	2.5	5.2	1.9	–	<2.0
	SD	0.8	0.8	1.2		0.8		1.4	3.3	0.7		
	n	8	9	9		9		7	9	9		
	p	NS	NS	NS		NS		NS	<0.02	–		
Antithrombin,	$\bar{X}$	97	96	103	99	104	103	99	97	119	107	103
% of normal	SD	11	11	9	11	12	17	13	18	11	9	11
	n	8	9	9	9	9	7	7	9	9	9	20
	p	NS	<0.02	NS	<0.05	NS	NS	NS	NS	<0.001	–	

[1] Normal material 10 men and 10 women.

With regard to the antithrombin level, normal mean levels were noted during pregnancy (table III; fig. 3). In some women, there was a decrease during delivery to a minimum value of 58%, and even on other single occasions some women had low levels of minimum 74%. An increase was seen 1 week post partum to a mean level of 119% of normal plasma.

Ethanol gelation test was normal, and fibrinogen degradation products were below 25 mg/l throughout the observation period. An increase was found in thrombin times in 3 women in week 38, in 6 women after delivery and in 5 one week later. Although an increase in fibrinogen degradation products is said to prolong thrombin time, this correlation was not seen in these cases. An increase in Reptilase time was also noticed. In 1 woman, there was a prolongation during the whole observation period until 1 week after delivery. No analyses were made 8 weeks after delivery.

Plasminogen levels, as measured by the radial immunodiffusion technique, were significantly increased during the whole pregnancy, with a maximum at week 28.

The levels returned to normal 8 weeks post partum. The levels were also measured by a chromogenic substrate method in 33 of the samples, drawn from 7 of the women. There does not seem to be any significant difference between the results. However, there is a tendency to lower values for the substrate method.

Antiplasmin levels increased slightly in the first trimester when compared with the values obtained 8 weeks after delivery. They begin to return to normal after week 34 and until delivery. A significant increase was noted 1 week after delivery. After delivery, some women exhibited low levels of antiplasmin with a minimum level of 73% of normal. The inhibitory capacity of urokinase increased significantly after the 32nd week of pregnancy, but as compared to nonpregnant women, the level seemed increased as early as in the 20th week of pregnancy. The level returned to normal 1 week after delivery (table IV; fig. 4).

The woman with diabetes mellitus or the one with thrombophlebitis did not differ from the other 7 with respect to all variables. The most striking changes seen in blood coagulation at delivery were the decreases in the levels of the early contact activation factors XII and XI. The activation of the blood coagulation system was also observed as increased levels of FPA. With these findings in mind, it is astonishing that the factor X level did not decrease. The inhibiting capacity of the fibrinolytic system, i.e. the urokinase inhibitors, decreased (see tables I–IV; fig. 1–4).

Table IV. 'Fibrinolytic' parameters, i.e. plasminogen, antiplasmin and urokinase inhibitor during pregnancy, at delivery and during puerperium

Method		Week of pregnancy							At delivery	Week after delivery		Reference values
		15	20	28	32	34	36	38		1	8	
Plasminogen,	$\bar{X}$	144	149	155	141	143	140	139	131	141	113	118
% of normal	SD	16	17	14	15	12	12	10	21	11	10	18
	n	8	9	8	9	9	7	7	9	9	9	20
	p	<0.001	<0.001	<0.001	<0.001	<0.001	<0.01	<0.01	<0.05	<0.001	–	
Antiplasmin,	$\bar{X}$	109	109	108	109	104	106	101	98	116	100	111
% of normal	SD	6	8	6	10	6	6	7	12	9	7	8
	n	8	9	9	9	9	7	7	9	9	9	20
	p	<0.05	<0.05	<0.05	<0.05	NS	NS	NS	NS	<0.01	–	
Urokinase	$\bar{X}$	96	117	125	196	199	197	191	162	105	–	88
inhibitor,	SD	16	17	26	49	46	48	55	55	21		10
% of normal	n	7	7	4	6	9	6	7	9	9		20
	p	NS	NS	NS	<0.001	<0.001	<0.001	<0.001	<0.001	–		

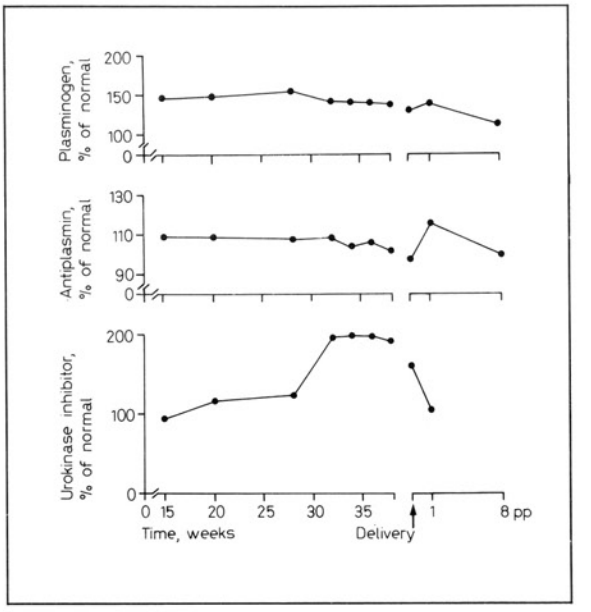

Fig. 4. Mean values for plasminogen, antiplasmin and urokinase inhibitor during pregnancy, at delivery and during puerperium (pp).

In 1 woman, values indicative of disseminated intravascular coagulation were seen without clinical signs. In this case, a decreased level of prekallikrein (65% of normal), increased level of FPA (10.4 μg/l) and decreased levels of antithrombin (58%) and antiplasmin (73%) were noted. She recovered without any specific treatment.

Discussion

The hypercoagulability noted in pregnancy is reflected by several changes in the blood coagulation and fibrinolytic systems.

In the present study some recent methods for monitoring changes in blood coagulation and fibrinolysis have been used to study a number of women during normal pregnancy, at delivery and during puerperium. The women were in addition followed with some more conventional methods.

The intrinsic system appears to be activated during delivery. There is a decrease in the elevated factor XII level at delivery and factor XI decreases

below the normal level already during late pregnancy. During delivery the vasculature in the placenta bed degenerates; thus, collagen may be exposed and may activate the intrinsic system.

The levels of factor VIII activity, and factor VIII-related antigen increase during pregnancy. The increase of the latter is much more pronounced than that of the former. The ratio between antigen and activity has increased significantly in week 32 and is almost normalized 1 week post partum. This phenomenon has been said to be related to activation by thrombin and/or plasmin, and according to *Denson* (4) such activation seems to occur during diffuse intravascular coagulation. In the present study, however, no correlation between the level of FPA, which gives an indication of thrombin activation, and the factor VIII antigen-activity ratio was seen as a mean. However, in 4 women there seems to be a correlation ($r = 0.9$). The increased ratio has also been used as a discriminator for fetal growth retardation (21). However, in our material all children had normal birth weight and an increased ratio was registered in all women. A possible explanation could have been that in the referred study, where no increase was seen in ratio during pregnancy a one-stage method was used for determination of the factor VIII activity. However, on the 13 occasions when our results were controlled with another one-stage recalcification method, we could still demonstrate an increase in ratio.

As can be understood from the present study, an evaluation concerning the hemophilia A carrier state should not be made during pregnancy, particularly during its late phase. Since the main diagnostic tool for such evaluation is the VIII:Ag/VIII:C ratio (23), it would be recommended that such studies be done very early in pregnancy or more preferably in the nonpregnant state.

The presence, close to delivery, of increased, most probably local, intravascular coagulation has been verified (3, 4, 15). Thus, the level of FPA is significantly increased. This may reflect the well-known activation concomitant with the separation of placenta which, together with mechanical constriction, is a physiological event to avoid bleeding from the placenta bed. However, no severe consumption in platelet number or of fibrinogen and antithrombin were registered, except in 1 woman. More studies around the time of delivery are probably needed before the usefulness of the methods can be correctly evaluated. Otherwise overdiagnosis and unnecessary treatment for disseminated intravascular coagulation may occur.

The increase in urokinase inhibitor may indicate a decrease in fibrinolytic capacity which can be related to placenta, as the level of this inhibitor

returns to normal soon after the separation of the placenta. *Holmberg* et al. (9) have demonstrated high levels of urokinase inhibitors in the human placenta.

The blood coagulation and fibrinolytic systems seem to be normalized 2 months after delivery even with lactation.

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