Antibodies against Fibrinogen in Pregnant Women, in Post Delivery Women and in the Newborns

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Summary

Pregnancy and delivery have measurable effects on haemostatic and immunological changes. Degradation of fibrinogen induces significant structural and conformational modulation and leads to the progressive loss of antigenic sites present on the parent molecule but also exposes some new sites. These neoantigens may be recognized by the immune system and may be elicited by the autologous host manifested by the production of autoantibodies. Therefore in the present study, in pregnant and post delivery women and in the newborns, levels of antibodies against fibrinogen, fibrinogen degradation products (FDP) and fibrin degradation products (D-dimer) were examined.

Enzyme immunoassay (ELISA) was used to detect and quantitate autoantibodies against fibrinogen (class G immunoglobulin) in human sera. In all sera there were found varying concentrations of autoantibodies and their levels were significantly higher in all pregnant women in comparison with non-pregnant ones. Significantly higher levels were found in Rh immunized and clinically complicated pregnancies.

The level of autoantibodies, coagulation and fibrinolytic system components were higher in post delivery women than in normal pregnant women. Also antibodies to fibrinogen were studied in cord serum of newborns in different terms of delivery. The low levels of antibodies in all newborns raise questions of possible foetal-maternal immunological interactions. Positive correlation between mothers and newborns was demonstrated after delivery at gestational age from 34th to 41st week, and negative in 42nd and more week. There were no significant differences in antibody level among the newborns delivered by the same mothers.

It was found that autoantibodies bind selectively to the fibrinogen and fibrinogen fragments X, Y and D. These autoantibodies may represent a new interface between the coagulation and the immune systems which may be significant in controlling the pathologic activities of the cleavage fragments.

Introduction

Normal pregnancy induces marked changes in the coagulation mechanism and fibrinolytic system and creates a state of hypercoagulability. Complicated pregnancy is often accompanied by some abnormalities of the coagulation and fibrinolytic systems and by the occurrence of fibrin deposits or microthrombosis in the kidneys, liver and placenta (1, 2). Also state of prolonged pregnancy is characterized by intensified degenerative changes of the placental tissue which finally lead to disruption of the structure of villi and an increased thickening of the fibrinoid layer. Common to these conditions is the fact that they stimulate fibrinogen catabolism. As a result fibrin monomers and fibrinogen degradation products (FDP) might appear in the maternal blood (3).

Fibrinogen, the focal compound of the coagulation system, is a major homodimeric 340 kDa glycoprotein of plasma (4). The catabolism of fibrinogen induces significant structural and conformational modulations and is associated with modification of native antigenic expression and the exposure of cleavage-associated neoantigenic sites on the derivative molecular fragments. The presentation of such cleavage-associated neoantigenic expressions to a host endowed with immunocompetent lymphocytes could potentially recruit an autoimmune response manifested by the production of autoantibodies with specificity for the cleavage fragments (5, 6).

Autoantibodies against fibrinogen were described for the first time by Brönnimann in a patient with congenital afibrinogenemia after several blood and plasma transfusions (7). Low levels of autoantibodies were found in normal subjects (8). High levels of autoantibodies were found in subjects with complicated pregnancy and various diseases (9, 10). It is suggested that these autoantibodies could play a role in the catabolism of fibrinogen and sometimes produce pathologic effects.

During pregnancy a number of immunological changes occur. The main change appears to be an increase in immunological potential of T and B lymphocytes rather than an increase in their activity. Immunoglobulin production from peripheral blood lymphocytes was elevated in normal pregnancy and spontaneous abortion (11).

The present study was designed to investigate the levels of antibodies against fibrinogen in pregnant women by comparing normal, Rh immunized and clinically complicated pregnancies with reference to normal non-pregnant women. It also included investigation on the level of antibodies in post delivery women and in the newborns.

Patients, Materials and Methods

Patients

Four groups of subjects were included in this study. The first group included 100 healthy normal pregnant women at mean age of 27.3 years (SD 4.2) in range from 19 to 35 years, attending routine antenatal examinations. No proteinuria, hypertension, growth retardation, diabetes mellitus occurred during their whole pregnancy period. The same women were studied again immediately after delivery. The second group included 70 Rh immunized primigravidae. Their mean age was 25.8 years (SD 3.4) in range from 20 to 33 years. These women were evaluated with monthly anti-D titres by the direct and indirect antiglobulin test of the mother's sera. Titer of anti-D antibodies remained low, 1:4 to 1:16 (critical titre), and the pregnancy was continued to term without other intervention. The third group included 70 women with clinically complicated pregnancies (threatened abortion) hospitalized for the monitoring of their...
pregnancy. Their mean age was 31.2 years (SD 4.4) in range from 24 to 38 years. The fourth group included 120 healthy non-pregnant volunteers (control group) recruited from laboratory personnel and medical students. Their mean age was 27.4 years (SD 3.5) in range from 20 to 39 years.

The women from the first group delivered vaginally 105 live healthy newborns. Twenty-four women delivered 26 newborns at gestational week 34 to 37. Sixty-two women delivered 65 newborns at gestational week 38 to 41. The examinations were carried out on 14 women with confirmed after-term pregnancy. They delivered 14 newborns at 42nd and more week. Gestational age was estimated from obstetric history and on the basis of postmature symptoms of newborns. The average birth weight of all newborns was 3450 g (range 2050-4200 g) and the Appgar score was 7 or more after 1 min. There were no significant differences in sex and birth weight distribution in newborns.

All patients were asked for informed consent prior to entry into the study. Blood was collected from the antecubital veins of pregnant women at different stages of gestation (first, second and third trimester) and women immediately after delivery. The blood samples of the newborns were collected from the cut umbilical cord. For measurement of antibodies to fibrinogen and FDP serum was needed. To prepare serum, the blood was collected into empty tubes, allowed to coagulate and centrifuged at 2500 × g for 20 min at room temperature. For measuring fibrinogen and D-dimer citrated blood was collected (9 parts of blood : 1 part of 0.11 M trisodium citrate) and was thoroughly mixed and centrifuged at 2500 × g for 20 min at room temperature to obtain plasma. When the assays were not done immediately, the serum and plasma were stored at –70° C until used.

Materials

Purified fragments D (Fg-D) and E (Fg-E) of human fibrinogen came from Calbiochem – Novabiochem AG, Lucerne, Switzerland. Reference mixtures of fibrinogen degradation products were obtained exactly as described (12). Fibrinogen (10 mg/ml) (Sigma Chemical Co., St. Louis, MO, USA) suspended in 30 ml of 0.15 mol/l Tris-HCl buffer, pH 7.8, containing 0.005 mol/l CaCl_2 was digested with human plasminogen activated by streptokinase. Usually 1.5-2.0 mg of plasminogen were preincubated with 10,000 IU of streptokinase overnight at 4° C onto 0.45 μm nitrocellulose membrane (18). The residual binding capacity of membranes was blocked by incubation in 1% (w/v) casein in TBS (20 mM Tris-HCl, 500 mM sodium chloride, pH 7.5). The strips, each of which contained the fibrinogen and mixture of fibrinogen degradation products and purified Fg-D and Fg-E, were reacted for 2 h at room temperature with the human serum diluted in TBS from 1 : 25 to 1 : 100. Then the strips were incubated with goat anti-human IgG conjugated with horseradish peroxidase diluted 1 : 150 in TBS. The blot was developed by submerging it in a solution of 4-chloro-1-naphtol (50 mg) in absolute methanol/45 ml 10 mM Tris-HCl, pH 7.4, and 50 μl 30% H_2O_2. The reaction was terminated by washing with water. The blot was then dried between filter papers and stored protected from light.

Statistical Analysis

The Mann-Whitney U, Student’s t-test and correlation coefficient were used for statistical evaluations. The data are presented as mean ± SD, p ≤0.05 was statistically significant.

Results

Autoantibodies to Fibrinogen in Pregnant Women

In all pregnant women autoantibodies against fibrinogen levels were significantly higher in comparison with non-pregnant women (p ≤0.0001). There was significant difference between the Rh immunized and clinically complicated and normal pregnant women (p ≤0.0001). There was no significant difference between the Rh immunized and clinically complicated pregnancies. In relation to fi-
brinogen, FDP and D-dimer levels were found to be significantly higher in all pregnant in comparison to non-pregnant women (p ≤0.0001). The highest levels of these parameters were in clinically complicated pregnancy (Table 1).

The levels of autoantibodies against fibrinogen in pregnant women in consecutive trimesters are presented in Fig. 1. By comparing the same trimesters in all groups of pregnant women, it was found that in the first, second and the third trimester autoantibody levels were significantly higher in Rh immunized and clinically complicated pregnancies compared with the normal pregnancy (p ≤0.05). No significant change could be found between Rh immunized and clinically complicated pregnancies. No significant negative correlation between gestational age and level of autoantibodies against fibrinogen was found.

**Antibodies to Fibrinogen in Post Delivery Women and Newborns**

The autoantibodies against fibrinogen were significantly increased when comparing the post delivery women (3.88 ± 1.00 µg/ml) to the normal pregnant women (p ≤0.0001). Fibrinogen (4.46 ± 0.62 g/l), D-dimer (662.37 ± 304.12 ng/ml) and FDP (10.34 ± 6.16 µg/ml) levels were significantly higher in post delivery women than in the normal pregnant women (p < 0.0001, p < 0.05, respectively).

The antibodies to fibrinogen levels were studied in serum of post delivery women and in cord serum of newborns in different terms of delivery (Table 2). The significantly higher levels of autoantibody were in women after delivery between 34th and 37th week and in women who delivered in 42nd and more week in comparison to women who delivered between 38th and 41st week (p ≤0.05, p ≤0.0001, respectively).

In cord serum antibody levels were higher in the newborns who were delivered between 38th and 41st week and the highest in the newborns who were delivered in 42nd and more week. The antibody levels were significantly lower in the newborns delivered in 34th-37th week of gestation (p ≤0.05), while significantly higher in newborns delivered in 42nd and more week of gestation in comparison with the newborns delivered in 38th-41st week (p ≤0.0001). The antibody levels in cord serum were significantly lower in all 105 newborns than in all 100 post delivery women (p ≤0.0001). No significant positive correlation...
between mothers and newborns was demonstrated after delivery at gestational age from 34th to 37th week ($r = 0.44$, $p = 0.0299$), significantly positive in 38th to 41st week ($r = 0.50$, $p \leq 0.0001$) and not significant negative in 42nd and more week ($r = -0.64$, $p = 0.0462$). There were not significant differences in antibody levels between the newborns delivered by the same mothers.

**Epitope Specificity of Autoantibodies to Fibrinogen**

The correlation between autoantibodies to fibrinogen levels and fibrin(ogen) degradation products and D-dimer levels were studied. There was observed positive non-significant correlation between the autoantibodies levels and FDP levels in serum of normal ($r = 0.17$, $p = 0.0937$), Rh immunized ($r = 0.02$, $p = 0.8870$) and clinically complicated pregnancies ($r = 0.02$, $p = 0.8620$). No significant positive correlation was demonstrated between the autoantibodies and D-dimer levels in normal ($r = 0.01$, $p = 0.9585$), Rh immunized ($r = 0.11$, $p = 0.3829$) and clinically complicated pregnancies ($r = 0.08$, $p = 0.4974$). In Rh immunized pregnant women there was found no significant correlation between autoantibodies against fibrinogen and antibodies to antigen D (Rh) ($r = 0.18$, $p = 0.1371$).

The specific reactions of autoantibodies against fibrinogen were confirmed by competitive inhibition of the ELISA assay after incubation of human sera samples used at the dilution of 1:200 with competitive inhibitors added to the final concentration which ranged from $1 \times 10^{-11}$ to $1 \times 10^{-6}$ M. The reactions were significantly inhibited by preincubation with fibrinogen and Fg-D. No inhibition was observed with Fg-E, human albumin and class G, A and M immunoglobulins (Fig. 2).

Finally, when the affinity of autoantibodies to fibrinogen was assayed by immunoblotting, it was found to bind the strips in which native fibrinogen was electrophoresed, to mixed of fibrinogen degradation products (containing fragments X, Y, D) with range of antigens including a fibrinogen and to the fibrinogen fragment D (Fig. 3). The results strongly suggest that the autoantibodies bind selectively to antigenic determinants on the fibrinogen, fragments X, Y and D in plasmin lystate fibrinogen and antigenic determinants Fg-D which are available on native fibrinogen as well. No antibody to the Fg-E was detected.

**Discussion**

Pregnancy and delivery have measurable effects on essentially every organ system in a woman’s body. It is generally agreed that haemostatic changes during normal pregnancy are multiple and complicated. The results of various investigations indicate that a number of immunological changes occur in normal pregnancy. The main change appears to be an increase in immunological potential of T and B lymphocytes as measured by the release of IgG and IgM in response to mitogenic stimulation. Immunoglobulin production from peripheral blood lymphocytes was elevated in normal pregnancy and spontaneous abortion (19). The mechanisms behind these physiologic changes are complex and not always well understood. Therefore in the present study, in pregnant and non-pregnant women, autoantibodies to fibrinogen, FDP and D-dimer levels were examined and comparison among normal, Rh immunized and clinically complicated pregnancies was made.

All coagulation and fibrinolytic factors were significantly higher in pregnant women than in non-pregnant ones. It is known from the literature that coagulation factors are increased during pregnancy which is the most striking in the case of plasma fibrinogen concentration. With progress of pregnancy there is an elevation of the fibrinolytic activity. It is possible that the source of a systemic elevation in the serum FDP during late pregnancy may be the placenta itself and the local mechanisms, perhaps related to repair following subliminal immune damage rather than placental infarction. FDP have been found in small amounts in normal pregnancy and in larger amounts in complicated pregnancy (20). Our data clearly demonstrate that pregnant women have increased plasma concentrations of FDP as compared to healthy non-pregnant women. The significantly higher level was found in clinically complicated pregnancies.

Van Wersch and Ubachs (21) demonstrated increasing levels of D-dimer throughout normal pregnancy. The occurrence of increased levels of fragment D-dimer during pregnancy is indicative of a compensating, low-grade intravascular coagulation state, which may be more pronounced in patients with complicated pregnancy (22). The presence of D-dimer documents the occurrence of both thrombin generation and plasmin formation. In our study, fragment D-dimer levels were significantly higher in clinically complicated pregnancies as compared to Rh immunized and normal pregnancies.

In this study, the values for the components of the coagulation and fibrinolytic system were higher in post delivery women than normal.
pregnant women. This is in agreement with previously published data, too. Bremer et al. (23) reported that the FDP concentration increases greatly after delivery, presumably because of the increased plasma fibrinolytic activity and local fibrinolysis occurring in the placental bed. Also the enhanced fibrinolytic capacity of cord blood of normal newborns is well documented in the literature. The fibrinogen degradation products in newborn babies may be generated during hyperfibrinolytic state which may be induced by the stressful conditions at birth (24).

Fibrinogen, the focal point of the coagulation system, is the source of a large number of pathophysiologically significant derivatives. It is well known, that the degradation of fibrinogen induces significant structural and conformational modulation and leads to the progressive loss of antigenic sites present on the parent molecule but also exposes some new sites. When new antigenic expressions are generated these neoantigens may be recognized by the immune system and may elicit production of autoantibodies by the autologous host (25, 26).

A simple enzyme immunoassay (ELISA) was done to detect and quantitate autoantibodies against fibrinogen, class IgG immunoglobulins, in human serum. Autoantibodies against fibrinogen were found in varying concentrations in all pregnant women and non-pregnant women sera. Only low levels were found in normal pregnancies, while the highest levels were found in clinically complicated pregnancies. The autoantibodies were found in post delivery women, too. IgG is immunoglobulin which actively crosses the placenta by Fc binding to specific receptors on trophoblastic cells. As described, at birth the serum of normal term neonates contains low levels of IgM and almost undetectable levels of IgA but approximate to adult levels of IgG (27). Therefore autoantibodies against fibrinogen were determined in cord blood of newborns. It was found that in all newborns antibody levels were decreased in comparison to their mothers. Low levels of antibodies against fibrinogen in all newborns raise interesting questions of possible foetal-maternal immunologic interactions.

Reports associated with formation of antibodies to fibrinogen are extremely rare. Plow and Edgington (8) reported in most normal sera autoantibodies interacting with X, Y, D fragments of fibrinogen which were apportioned among IgA, IgG and IgM. They reported that in sera containing intermediate or terminal cleavage fragments, antibodies to the fragments were not detected and speculated upon immune complex formation leading to depletion of antibody. Whitaker et al. (9) reported autoantibodies directed against at least several cryptic antigens which appear during fibrin(ogen) degradation and some appear to be directed specifically against cross-linked fibrin derivatives in normal human prior to infective, thrombotic, inflammatory or traumatic disorders. They suggested that these autoantibodies may contribute to the catabolism of fibrinogen derivatives, provide a marker of thrombosis and sometimes produce pathologic effects and were evidently of mixed immunoglobulin class. The data presented here and that of Whitaker et al. (9) did not confirm any relationship between low autoantibody level and raised FDPs.

There have been a few reports of a possible relation of such antibodies to clinical manifestations in men (28). Anti-fibrinogen antibodies in patients with congenital afibrinogenemia following fibrinogen transfusions (29, 30) and in one patient with migratory thrombophlebitis migrans were reported (31). Antibodies interfering with the release of fibrinopeptide A and B by thrombin (32), in serum of blood recipients with post-transfusion reactions (33) and inhibiting fibrin polymerization (34-37) have been described.

The veterinary literature reports similar autoantibodies in a variety of experimental infections. These include trypanosomiasis (38), malaria and babesiosis (39) in rodents. In these instances it has been postulated, that infection triggers disseminated intravascular coagulation and secondary fibrinolysis producing fibrin degradation products which stimulate the rise in autoantibodies.

Specificity of autoantibodies against fibrinogen was evidenced by competitive inhibition of the ELISA analysis employing human sera in which fibrinogen, Fg-X, Fg-Y and Fg-D produced very significant inhibition while the fragment E did not react. The heterogeneity of antibody observed in Western blotting in most sera is consistent with a polyclonal immune response to the cleavage fragments. The presence of low autoantibody level in all subjects suggests a physiologic role. It is possible that circulating autoantibodies could bind cleavage fragments and in situ deposits of fibrin appears to be a new interface between the coagulation and immune systems which may be significant in controlling the numerous pathologic activities of the cleavage fragments. The low levels of antibodies in all newborns raise questions of possible foetal-maternal immunologic interactions. It is also possible that generation of immune complexes between fibrinogen/fibrin derivatives and autoantibody could contribute to complement activation and sometimes produce pathologic effects.

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References
