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Immunological and biochemical markers in preeclampsia

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ABSTRACT

A basic precondition for the development of preeclampsia is the presence of placental trophoblast cells in the maternal blood circulation. On the other hand, while trophoblast cells are present in the blood of all pregnant women, preeclampsia occurs in only 2–5% of them. Evidently, other factors play a crucial role. The aim of this study was to compare a set of selected immunological factors (anti-cardiolipin autoantibodies, trophoblast-induced cell-mediated immunity, C3 and C4 complement components) and biochemical factors (serum immunoglobulins IgA, IgG, IgM) among three groups of women with uncomplicated pregnancy, gestational hypertension, or preeclampsia. Blood samples were taken 2–12 h before delivery. In the preeclampsia group, there was a significantly higher number of women positive for anti-cardiolipin autoantibodies, trophoblast-induced cell-mediated immunity was elevated, serum IgG was elevated and C4 complement component was reduced. We conclude that both elevated autoimmune reactivity and the higher immune reactivity to trophoblast may contribute to the onset of preeclampsia.

1. Introduction

Pre-eclampsia is relatively common complication, affecting about 3% of pregnancies (Redman and Sargent, 2010). It is the major cause of maternal mortality (15–20% in developed countries) and morbidity (acute and long-term), perinatal deaths, preterm delivery, and intrauterine growth restriction (Sibai et al., 2005). Leading clinical symptoms in mother are of vascular origin – hypertension, proteinuria, and edema. Thus, the first theories emphasized vascular pathophysiology of preeclampsia (placental asphyxia, leading to increased systemic vascular resistance, enhanced platelet aggregation, activation of the coagulation system, and endothelial-cell dysfunction). On the other hand, immunologists built an immunological theory of preeclampsia, pointing out the fact that the presence of fetal tissue – trophoblast – is responsible for the onset and duration of clinical symptoms of this disease. Chaouat et al. (2003) proposed that both theories are correct and could be integrated. According to him, activated immune cells produce cytokines that are endowed with pleiotropic properties, of which the action on the vascular endothelium and smooth muscle, as well as the influence on blood coagulation, are the most relevant for the development and symptoms of preeclampsia. It has been assumed (Redman

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and Sargent, 2010) that dysregulated immunity at the feto-maternal interface in the very beginning of pregnancy leads to the poor placentation and subsequently to the onset of clinical symptoms of preeclampsia.

There is no doubt that the presence of fetal trophoblast is the essential factor which starts the chain of pathologic mechanisms in preeclampsia and maintains the disease. Symptoms of preeclampsia terminate with the elimination of trophoblast, but not the fetus, and consequently even ectopic persistence of trophoblast (trophoblastic disease) prolongs the symptoms of preeclampsia. However, particles of invasive trophoblast are regularly found in the blood circulation of all pregnant women and a great majority of them do not develop preeclampsia. Evidently, other co-factor(s) must play a role (Bonney, 2007).

Our study is a retrospective cohort type. The aim is to investigate the hypothesis that additional factors which enhance maternal immunity, such as disposition to autoimmune reactivity and/or elevated sensibility of cell-mediated immunity to trophoblast-derived antigens, play a role in the onset of preeclampsia. We compare clinical symptoms of preeclampsia (blood pressure, proteinuria, edema) with both biochemical markers (serum concentration of blood proteins, C3 and C4 component of complement) and immunological markers (activated cell-mediated immunity, anticardiolipin autoantibodies, circulating immunocomplexes).

2. Patients and methods

Women delivered in the Institute for the Care of Mother and Child were included in the study. Blood samples were taken shortly (2–12 h) before termination of pregnancy. Described tests were either a part of routine diagnostics, or obtained by an analysis of residual materials taken for routine tests. No unnecessary blood sampling was performed.

The first group of patients comprised 26 women suffering from preeclampsia in the third trimester of gestation. Preeclampsia was defined as blood pressure higher than 140/90 and proteinuria higher than 300 mg per 24 h. Lack of edema was not taken as a contradiction to the diagnosis of preeclampsia. A second group of 16 women with hypertension was characterized by blood pressure higher than 140/90, measured at least twice within 24 h. Neither elevated proteinuria nor edemas were present in this group. The control group comprised 25 women with spontaneous term delivery and no pathology evident during pregnancy (Table 1). Women suffering from arterial hypertension before pregnancy, or any chronic disease before or during pregnancy, were not included in the study.

Concentrations of immunoglobulins IgG, IgM, IgA and complement components were measured on Roche Integra analyzer, using the manufacturer's chemicals and procedures. Bindazyme human anti-cardiolipin IgG/IgM combi EIA kits from The Binding Site (Birmingham) were used for detection of anti-cardiolipin autoantibodies. The cut-off level was established for each set according to the manufacturer's manual. Results were expressed as "negative" (below the cut-off level) and "positive" (above the cut-off level).

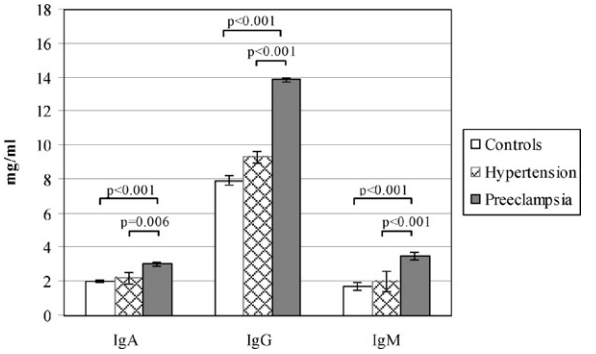

Fig. 1. Concentration of serum immunoglobulins IgA, IgG, IgM. Values are expressed in mg per ml. Simultaneous statistical significances between groups (Control, Hypertension, Preeclampsia) were tested by one-way ANOVA/$F(2, 63) = 8.940$, $p < 0.001$ for IgA; $F(2, 63) = 46.586$, $p < 0.001$ for IgG; $F(2, 73) = 49.253$, $p < 0.001$ for IgM. The significant p-values for Fisher LSD post hoc pair comparisons are given in Fig. 1.

Trophoblast-induced cell-mediated immunity (TI-CMI) was measured by the use of method of inhibition of leukocyte migration in the presence of specific antigen in one-step modification of the classic two-step method of Clausen (Clausen, 1973; Dimitrov et al., 1992). White blood cells were separated from venous blood by sedimentation in dextran gradient, washed twice and resuspended in tissue culture medium to the concentration of 35×10^6 cells per ml. The cell suspension was placed into the holes (2 mm diameter) in an agarose-gel either without antigen (control holes) or with antigen prepared from JAR choriocarcinoma cells (active holes). After a 16-h incubation (37 °C, 5% CO_2, humid chamber) the preparations were fixed using formaldehyde solution and areas of migration were measured by computerized image analysis. The percentage of inhibition of migration (control hole = 100%) indicated the intensity of cell-mediated immunity induced by the presence of trophoblastic antigen. The results were expressed as migration inhibition index (MII), where zero means no inhibition and 100 means complete inhibition of migration.

Statistical analysis: All dependent indicators (IgA, IgG, IgM, C3, C4 and TI-CMI) were successfully attested to normality in comparison with theoretical normal values of skewness and kurtosis and by Kolmogorov–Smirnoff test. Hence, the influence of one fixed factor (in the control, hypertension and preeclampsia groups) on the mean level of each parameter was proved by one-way ANOVA (followed by Fisher LSD post hoc tests for multiple comparisons). The standard Fisher chi-square test was also used for evaluation of difference from the control group.

3. Results

Concentrations of serum immunoglobulins of the IgA, IgG and IgM classes were elevated in both pregnancy pathologies, when compared with normal pregnancies. While in the IgA class the differences are not statistically significant, serum levels of IgG were markedly higher in the preeclampsia group and slightly higher also in women with gestational hypertension. IgM-class immunoglobulins were also elevated in the preeclampsia group (Fig. 1).