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Autoantibodies to endothelial cells in patients with hypertensive disorders during pregnancy

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1. Introduction

About 10% of all pregnant women experience hypertensive disorders, and these are responsible for many prenatal, perinatal and postnatal deaths [1,2]. Pre-existing chronic arterial hypertension (CAH) is one of the risk factors for preeclampsia (PE) and eclampsia, preterm delivery, placental abruption, intrauterine growth restriction (IUGR) and other obstetric complications [1,3]. Moreover, patients with hypertensive disorders in pregnancy (HDP) have an increased risk of further cardiovascular diseases [4–6].

The aetiology and pathogenesis of HDP remain controversial. Researchers have failed so far to identify a universal mechanism for their development. A combination of genetic, immunological, hormonal, placental and neurogenic factors seems to be involved [7,8]. However, the common feature of all hypertensive conditions is endothelial activation with subsequent endothelial dysfunction [9,10]; this process is accompanied by a systemic inflammatory reaction (SIR) of varying degrees of severity [11,12]. A SIR manifests with activation of the immune system and is a generalised (systemic) response. Phenotypical changes in endothelial cells and production of several immune factors can be a consequence of endothelial activation [8,9,13,14].

A humoral response to neo-antigens (arising due to inflammation), and to allo-antigens (of transplant or foetal origin) comprises production of antibodies to endothelial cells [15]. Large numbers of anti-endothelial cell antibodies (AECAs) are produced in response to vasculitis, inflammatory and autoimmune diseases and after organ transplantations [16–18]. High levels of AECAs in patients with masked hypertension [19] and idiopathic [20] or complicated pulmonary hypertension [21] have also been reported. Some studies of AECAs in pregnancy have shown an increased level in severe pre-eclampsia (PE) and a cytotoxic effect of IgG AECAs on endothelial cells [22–24].

Because AECAs are potential regulators of endothelial cell function [25] and are also supposed to be activators of endothelial damage [15],

Abbreviations: AECAs, anti-endothelial cell antibodies; BMI, body mass index; BP, blood pressure; CAH, chronic arterial hypertension; DBP, diastolic blood pressure; FCM, flow cytometry; GAH, gestational arterial hypertension; HDP, hypertensive disorders in pregnancy; HUVECs, human umbilical vein endothelial cells; Ig, immunoglobulin; IUGR, intrauterine growth restriction; mPE, moderate PE; mPE-CAH, moderate PE developing under preexisting CAH; nAECAs, natural AECAs; NP, normal pregnancy; PE, pre-eclampsia; RBC, red blood cell; SBP, systolic blood pressure; sPE, severe PE; sPE-CAH, severe PE under preexisting CAH; SIR, systemic inflammatory reaction; SLE, systemic lupus erythematosus

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they seem to contribute to the development of HDP. The aim of this study was to evaluate the level and diagnostic value of AECAs in patients with HDP.

2. Material and methods

2.1. Study design

A total of 686 pregnant women were admitted to the Research Centre for Obstetrics, Gynaecology and Perinatology, Moscow, Russia, between 28 April 2010 and 15 March 2015, for delivery, or chronic concomitant disease, or PE treatment. The center is a level IV facility; it has federal status and specialises in the management of patients with severe obstetric pathology. One hundred fifty-three out of 686 patients with pregnancies at 28–40 weeks met the inclusion criteria and were selected for participation in the study. The main group consisted of 123 patients with HDP; 30 healthy women formed the normal pregnancy (NP) control group.

The main group was divided into three subgroups: group 1, 26 patients with chronic arterial hypertension (CAH); group 2, 16 patients with gestational arterial hypertension (GAH); and group 3, 81 patients with PE. Group 3 was divided into four subgroups: 3A, 29 patients with moderate PE (mPE); 3B, 28 patients with severe PE (sPE); 3C, 15 patients with moderate PE developing under pre-existing CAH (mPE-CAH); and 3D, 9 patients with severe PE under pre-existing CAH (sPE-CAH). The study design is shown in Fig. 1.

For a different set of analyses, a group of patients with IgG^+ AECAs (n = 29) was identified within the main group (Fig. 1).

2.2. Inclusion and exclusion criteria

Inclusion criteria were spontaneous singleton pregnancy and age 18–42 years.

Inclusion criteria for the control group were: spontaneous singleton normal pregnancy; absence of any chronic gynaecological or somatic disease; no threat of abortion, of early toxicosis, or of inflammatory disease, PE or intrauterine growth restriction (IUGR); no medical therapy (except for vitamins or mineral supplements); normal vaginal flora; and normal ultrasonography and Doppler ultrasonography during current pregnancy; term vaginal delivery with newborns of normal weight and length and Apgar score 9–10, without any congenital abnormalities or signs of prenatal foetal distress.

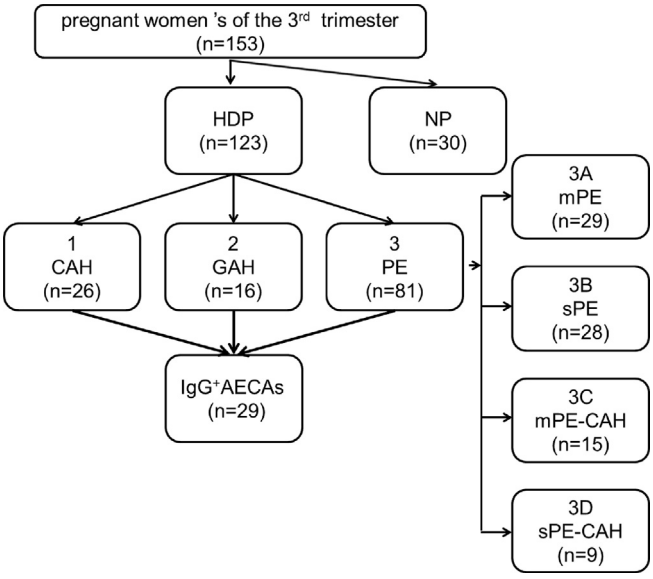

Fig. 1. Study design.

Exclusion criteria for all groups were: multiple pregnancy; chromosomal abnormalities in the parents or newborns; severe somatic diseases; autoimmune diseases, including antiphospholipid syndrome; acute and chronic inflammatory diseases; oncologic diseases; RBC sensitisation; history of blood transfusion or organ transplantation; immunoglobulin therapy; and use of drugs which affect antibody production and bioavailability, including low-molecular-weight heparins.

2.3. Diagnostic evaluation of hypertensive disorders

Inclusion in the patient subgroups was according to the specific hypertensive disorder meeting the criteria of the International Society for the Study of Hypertension in Pregnancy (ISSHP) and Russian guidelines [26–28]. CAH was defined as essential hypertension without a known cause, with blood pressure greater than or equal to 140 mmHg systolic and/or 90 mmHg diastolic confirmed before pregnancy or before 20 completed weeks of gestation. GAH was defined as hypertension after the 20th week of gestation without any maternal or foetal features of PE, followed by return of blood pressure to normal within 3 months of giving birth. The diagnostic criteria for PE were hypertension after the 20th week of gestation, proteinuria ≥ 0.3 g/L, edema and polyorganic/polysystemic failure. Criteria for PE in patients with CAH were proteinuria ≥ 0.3 g/L appearing after 20 weeks of gestation, significant increase in the level of proteinuria, progressing arterial hypertension or signs of polyorganic failure under pre-existing CAH. The criteria for moderate PE were systolic blood pressure (SBP) ≥ 140 mmHg or diastolic blood pressure (DBP) $\geq$ 90 mmHg appearing after the 20th week of gestation in women with no history of hypertension, proteinuria ≥ 0.3 g/L. The criteria for severe PE were the following: SBP $\geq$ 160 mmHg or DBP $\geq$ 110 mmHg in two measurements at rest 6 h apart; proteinuria ≥ 5 g/L in daily urine, or > 3 g/L in a series of urine samples taken 6 h apart; oliguria < 500 ml for 24 h; vision impairment, cerebral symptoms, pulmonary oedema, cyanosis, liver dysfunction, thrombocytopenia ($< 100 \times 10^6$/l), and IUGR [28,29].

2.4. Clinical and laboratory studies

Questioning, anthropometric examination, BP and pulse measurements, gynaecological and external obstetric examination were carried out with all patients included in the study. Laboratory tests included complete blood count, chemistry panel, coagulogram and double urinalysis in accordance with the Directive of the Ministry of Health of the Russian Federation of 12 November 2012 №. 572n “On approval of the Order of healthcare delivery in obstetrics and gynaecology”. If PE was suspected, the patient’s urine was tested daily for protein.

2.5. Ethics statement

The study was approved by the local ethics committee. All patients gave written informed consent for participation in the study.

2.6. Detection of AECAs

For AECAs detection, EA.hy 926 cells (provided by Dr. C.-J. Edgell, Chapel Hill, North Carolina) derived through hybridisation of human umbilical vein endothelial cells with lung carcinoma cells, A-549, were used [30]; these cells bear all the main morphological, phenotypical and functional characteristics of endothelial cells of the macrocirculation.

Binding of AECAs in the M and G immunoglobulin classes with endothelial cells (defined as the AECA binding activity) was measured by flow cytometry (FCM) with FACS Calibur (Becton, Dickinson and Co., USA), as described earlier [31]. The cells were incubated with the test serum samples and subsequently treated with fluorescence-labelled secondary antibodies specific to the Fc fragment of human IgG or to the μ-chain of the human IgM (Sigma, USA). The samples were washed and